Universidad Michoacana de San Nicolás de Hidalgo

Programa Institucional de Doctorado en Ciencias Biológicas

Facultad de Medicina Veterinaria y Zootecnia
Centro Multidisciplinario de Estudios en Biotecnología

Instituto de Investigaciones Químico-Biológicas

Caracterización química, toxicidad y efecto antipsoriásico anti-IL17 del extracto acuoso, etanólico y de aceites pirolíticos de plantas usadas en la etnomedicina Purépecha

Tesis

Para obtener el grado de:
Doctor en Ciencias Biológicas
Opción: Biotecnología Molecular Agropecuaria

Presenta:
M.C. Roberto Esquivel García

Directora:
Dra. Martha Estrella García Pérez

Codirectora:
Dra. Alejandra Ochoa Zarzosa

Sinodales:
Dra. Rosa Elva Norma Del Río Torres
Dr. Joel Edmundo López Meza
Dr. Manuel García Pérez

Morelia, Michoacán. Agosto de 2020

DRA. ESPERANZA MELÉNDEZ HERRERA
COORDINADORA GENERAL DEL PROGRAMA INSTITUCIONAL DE DOCTORADO EN CIENCIAS BIOLÓGICAS
P R E S E N T E

Por este conducto nos permitimos comunicarle que después de haber revisado el manuscrito final de la Tesis Titulada: "Caracterización química, toxicidad y efecto antipsoriásico anti-IL17 del extracto acuoso, etanólico y de aceites pirolíticos de plantas usadas en la etnomedicina Purépecha" presentado por el M.C. ROBERTO ESQUIVEL GARCÍA con Número de Matrícula 0526857B, consideramos que reúne los requisitos suficientes para ser publicado y defendido en Examen de Grado de Doctor en Ciencias.

Sin otro particular por el momento, reiteramos a usted un cordial saludo.

A T E N T A M E N T E
Morelia, Michoacán, a 06 de agosto de 2020

MIEMBROS DEL COMITÉ TUTORAL

Dra. Martha Estrella García Pérez
Directora de Tesis

Dra. Alejandra Ochoa Zarzosa
Co directora

Dr. Joel Edmundo López Meza

Dra. Rosa Elva Norma del Río Torres

Dr. Manuel García Pérez

El presente trabajo se realizó en el Instituto de Investigaciones Químico-Biológicas y el Centro Multidisciplinario de Estudios en Biotecnología de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad Michoacana de San Nicolás de Hidalgo, bajo la dirección de la Dras. Martha Estrella García Pérez y Alejandra Ochoa Zarzosa. Además, se realizó una estancia de investigación en la Universidad del Estado de Washington, campus Pullman.

DEDICATORIA

A mi familia, porque hay estrellas que guían mis pasos, iluminando el camino y hacen que esta vida me parezca maravillosa y perfecta

AGRADECIMIENTOS

A mi esposa Elena, gracias por escucharme, por tus consejos y paciencia, por darme lo mejor de ti, sin dobleces ni egoísmos, con alegría y dulzura; manifestándolo en nuestras hijas Vanessa e Itzi Ximena, quienes nos impulsan a mantener la determinación de alcanzar nuestros sueños. Mi orgullo por ustedes aumenta sin cesar, ¡las amo!

A mi padre Roberto†, por guiarme sobre el camino de la educación y el trabajo. A Martha mi madre; por tu amor, apoyo y ejemplo que me permiten sentir que puedo lograr lo que me proponga. A Claudia, Martha, Ana, Beatriz y Mercedes por ser excelentes hermanas y ayudarme con mis obligaciones para facilitar el cumplimiento de muchas metas, ¡los quiero mucho!

A mis suegros Lucila y Humberto, por el apoyo incondicional y la confianza que han depositado en mí, así como a Martha, Alejandra y Guadalupe, sin su ayuda la dificultad de alcanzar esta meta hubiera sido gigantesca.

A la Dra. Martha E. García, gracias por dirigirme en este proyecto de investigación, usted depositó su confianza en mí para integrarme a su equipo de investigación y siempre me ha motivado para afrontar con determinación los retos que se presentan en la vida del investigador, agradezco sus criticas llenas de verdad, sus consejos y la gran paciencia que me ha tenido durante todos estos años. A la Dra. Alejandra Ochoa, gracias por dirigirme en este proyecto de investigación, el cual sin su apoyo hubiera sido difícil realizar, agradezco por su atención y sus comentarios acertados que contribuyeron a mejorar la calidad de la investigación y a mi formación profesional.

Agradezco a la Dra. Rosa E. del Río, al Dr. Joel E. López y al Dr. Manuel García por formar parte de mi comité tutorial, por dedicar su tiempo para evaluar y corregir mi proyecto de investigación y por sus contribuciones que fueron esenciales para realizar el presente trabajo. Dr. Manuel gracias por permitirme realizar una estancia de investigación en la WSU, por su apoyo incondicional en todo lo que me fue requerido y por la gran experiencia de trabajar con su grupo.

A los pobladores Purépecha que compartieron sus conocimientos de medicina tradicional. Al Dr. Emmanuel Pérez del INECOL, por sus consejos y por realizar la identificación de las especies para la realización del estudio etnofarmacológico. A los doctores Rosalio Mercado y Héctor Martínez por permitirme trabajar en sus respectivos laboratorios de la Facultad de QFB. A la Dra. Eréndira Valencia y a la Mtra. Eunice Tranquilino por su ayuda en el trabajo de laboratorio y de campo. Al Dr. Omar Guzmán por su apoyo en mi etapa de evaluación predoctoral. A Anamaria Paiva, Dra. Ayca Seker, Dorota Wilk, Evan Terrel, Jonathan Lomber, Dr. José Martínez, Kalidas Mainali, Dr. Michael Apasiku, Sohrab Haghighi, Yaime Jefferson, Dr. Yinlgei Han y demás compañeros de la WSU por todo el apoyo que me brindaron durante mi estancia de investigación. A la Dra. Eva M. Salinas de la UAA y al Dr. Bruno Rivas del IMSS por facilitarme la línea de cultivo celular.

A todos mis amigos y familiares que estuvieron conmigo compartiendo tantas experiencias y triunfos. Especialmente a quienes me ayudaron a afrontar grandes retos sin dudas ni condiciones: Andrea Falcón, Beatriz Bautista, Blanca Tetitla, Esteban Ramírez, Efigenia Carrillo, Esther Tadeo, Gabriela Flores, Guadalupe Salgado, Ildefonso Sánchez, Judith Prieto, Linda Hurtado, Marco Barajas, Margarita Aviña, María Hernández, Marisol Báez, Mónica Sánchez, Nancy Alejandre, Paola Jiménez, Rosa García, Salvador Padilla, Susana Bautista, Teresa Arceo e Yvette Resendiz. No olvidaré sus enseñanzas ni la agradable convivencia.

¡Gracias!

CONTENIDO

Página

DEDICATORIA I

AGRADECIMIENTOS II

LISTA DE FIGURAS VI

LISTA DE TABLAS VIII

LISTA DE ABREVIATURAS IX

RESUMEN 1

ABSTRACT 3

I. INTRODUCCIÓN 5

I.1. Psoriasis 5
I.1.1. Interleucina-17 y la patogénesis de la psoriasis 7
I.1.2. El desarrollo de fármacos para la psoriasis 10
I.2. La etnofarmacología y el desarrollo farmacéutico en la psoriasis 13
I.3. Uso de extractos acuosos y etanólicos de plantas como candidatos terapéuticos para el tratamiento de la psoriasis 16
I.4. Estructura química de los compuestos fenólicos extraíbles 18
I.5. Caracterización química de la lignina 21
I.6. La pirólisis: un medio para obtener aceites pirolíticos ricos en compuestos fenólicos 23
I.7. Uso de productos pirolíticos para el tratamiento de la psoriasis 26

II. JUSTIFICACIÓN 28

III. HIPÓTESIS 30

IV. OBJETIVOS 31

IV.1. Objetivo general 31
IV.2. Objetivos particulares 31

V. ESTRATEGIA EXPERIMENTAL 32

VI. RESULTADOS 33

CAPÍTULO 1. Flora etnomedicinal utilizada para el tratamiento de afecciones dermatológicas en la Meseta Purépecha, Michoacán, México 34

VI.1. PREFACIO 35
VI.2. ARTÍCULO 36
VI.2.1. Abstract 36
VI.2.2. Resumen 36
VI.2.3. Introduction 37
VI.2.4. Materials and methods 38
VI.2.5. Results 41

VI.2.6. Discussion 43
VI.2.7. Conclusions 48
VI.2.8. Author contributions 49
VI.2.9. Financing 49
VI.2.10. Acknowledgment 49
VI.2.11. Literature cited 49
VI.2.12. Appendix 1. Plants used by inhabitants from Purépecha Plateau for the treatment of dermatological conditions: use value, fidelity level, modes of preparation and ethnomedicinal studies. 58
CAPÍTULO 2. Extracto etanólico, fracciones y bio-aceites de partes aéreas de *Urtica subincisa*: un análisis comparativo de su composición química, toxicidad y actividad anti-IL17 en queratinocitos HaCaT 74
VI.3. PREFACIO 75
VI.4. ARTÍCULO 76
VI.4.1. Abstract 77
VI.4.2. Introduction 78
VI.4.3. Material and methods 80
VI.4.4. Results and Discussion 85
VI.4.5. Conclusions 102
VI.4.6. Author contributions 103
VI.4.7. Conflict of interest 104
VI.4.8. Acknowledgements 104
VI.4.9. References 104
CAPÍTULO 3. Los aceites pirolíticos de la corteza de *Amphipterygium adstringens* inhiben la producción de IL-8 en queratinocitos HaCaT estimulados con IL-17. 108
VI.5. PREFACIO 109
VI.6. ARTÍCULO 110
VI.6.1. Abstract 110
VI.6.2. Introduction 110
VI.6.3. Material and methods 111
VI.6.4. Results and Discussion 114
VI.6.5. Conclusions 118
VI.6.6. Credit authorship contribution statement 118
VI.6.7. Declaration of Competing Interest 118
VI.6.8. Acknowledgements 118
VI.6.9. References 118
CAPÍTULO 4. Caracterización química, toxicología y actividad antiinflamatoria del extracto acuoso de la corteza de *Amphipterygium adstringens* y sus fracciones en queratinocitos HaCaT. 120
VI.7. PREFACIO 121
VI.8. ARTÍCULO 122
VI.8.1. Abstract 123
VI.8.2. Introduction 124

VI.8.3. Material and methods 126
VI.8.4. Results and Discussion 129
VI.8.5. Conflict of interest statement 138
VI.8.6. Authors' contributions 138
VI.8.7. References 139
VII. DISCUSIÓN GENERAL 142
VIII. CONCLUSIÓN GENERAL 150
IX. PERSPECTIVAS 151
X. BIBLIOGRAFÍA 152
XI. ANEXOS 159
XI.1. ANEXO 1 - La psoriasis: de la investigación básica y clínica al desarrollo de nuevos tratamientos 159
XI.1.1. PREFACIO 159
XI.1.2. ARTÍCULO 160
XI.1.3. Resumen 160
XI.1.4. Abstract 160
XI.1.5. Introducción 161
XI.1.6. Conceptos etiopatogénicos actuales de la psoriasis 161
XI.1.7. De la investigación básica y clínica al desarrollo de tratamientos antipsoriásicos 163
XI.1.8. Nuevos fármacos antipsoriásicos en desarrollo 164
XI.1.9. Perspectivas futuras en el desarrollo de nuevos medicamentos antipsoriásicos 164
XI.1.10. Bibliografía 165

LISTA DE FIGURAS

Página

Fig. 1. Circuitos inflamatorios en la psoriasis. 6
Fig. 2. Vía de señalización de IL-17.....8
Fig. 3. Esquema clásico de desarrollo farmacéutico. 11
Fig. 4. Proceso de evaluación científica en etnofarmacología. 15
Fig. 5. Estructuras químicas de polifenoles encontrados frecuentemente en las plantas. 20
Fig. 6. Monómeros precursores de la lignina. 22
Fig. 7. Pirólisis de una partícula de biomasa. 24
Fig. 8. Estrategia metodológica empleada en el presente trabajo.....32
Fig. 9. Resumen gráfico.....35
Fig. 10. Major steps depicted in this paper.....81
Fig. 11. Pyrograms of the dried aerial parts and residual biomass of *U. subincisa*.....86
Fig. 12. TGA (A) and DTG (B) curves of the crude extract, its fractions, and pyrolytic products from *U. subincisa.* 90
Fig. 13. FTIR spectra of the crude extract, its fractions, and bio-oils from *U. subincisa.* 92
Fig. 14. UV-Fluorescence spectra of the crude extract, its fractions, and bio-oils from *U. subincisa.* 93
Fig. 15. GC-MS chromatograms of the crude extract, its fractions, and bio-oils from *Urtica subincisa*. 95
Fig. 16. Effect of the crude extract, phases A-C, and bio-oils on the IL-8 production in keratinocytes HaCaT stimulated with IL-17. 100
Fig. 17. Graphical abstract.....121
Fig. 18. Py-GC/MS analysis chromatogram of dry bark powder from *A. adstringens.* 130
Fig. 19. TGA (A) and DTG (B) curves for the dry bark, crude aqueous extract and its fractions from *A. adstringens*. 132
Fig. 20. FTIR spectra of the dry bark, aqueous crude extract and fractions from *A. adstringens*. 133
Fig. 21. Chromatograms obtained by GC-MS in aqueous bark extract from *A. adstringens* and its solvent fractions. 134
Fig. 22. UV-fluorescence spectra of the crude extract and its fractions from *A. adstringens* bark. 136
Fig. 23. Effect of crude extract and phases A-C from *A. adstringens* bark on the IL-17-induced production of IL-8 on HaCaT keratinocytes.....137
Fig. 24. Esquema representativo de los resultados obtenidos en este trabajo. 149

Figuras en el Capítulo 1

Página

Fig. 1. Study area……39
Fig. 2. Characteristics of the participants in the ethnopharmacological study on the Purépecha Plateau……40
Fig. 3. Family distribution of medicinal plants used on the Purépecha Plateau……..42
Fig. 4. Number of plant species used for treating dermatological conditions on the Purépecha Plateau……44

Fig. 5. Plant parts and preparation modes used by inhabitants of Purépecha Plateau for treating dermatological disorders……44
Fig. 6. Plants with higher use values (UV) for treating dermatological ailments on the Purépecha Plateau……45

Figuras en el Capítulo 3

Página

Fig. 1. Schematic representation of mayor tasks described in this paper……...111
Fig. 2. System used for pyrolytic collection of oil-1 and oil-2……..112
Fig. 3. TGA (A) and DTG (B) curves for the residual biomass and pyrolytic products from *A. adstringens* bark……...112
Fig. 4. FTIR spectra of residual biomass and pyrolytic fractions from *A. adstringens* bark…..113
Fig. 5. Py-GC-MS analysis chromatogram of residual biomass from *A. adstringens*……...113
Fig. 6. GC-MS analysis chromatograms of pyrolytic oils from *A. adstringens*…….115
Fig. 7. UV-fluorescence spectra of the oil samples from *A. adstringens*……...116
Fig. 8. HPLC-DAD profiles of liquid pyrolysis samples from *A. adstringens*……..117
Fig. 9. Effect of pyrolytic oils on the IL-17-induced production of IL-8 on HaCaT keratinocytes……....117

Figuras en el Anexo 1

Página

Fig. 1. Patogénesis de la psoriasis e implicación de los nuevos fármacos en desarrollo……..162

LISTA DE TABLAS

Página

Tabla. 1. Estudios que utilizan extractos acuosos y etanólicos de plantas empleados por la medicina tradicional para el tratamiento de la psoriasis. 17
Tabla. 2. Porcentaje de los diferentes monolignoles presentes en la lignina de diversas plantas. 23
Tabla 3. Tipos de pirólisis y productos principales. 25
Table 4. Compounds identified by Py-GC/MS in *U. subincisa* aerial parts and residual biomass. 86
Table 5. Yields of the crude extract, its fractions, and pyrolytic products from aerial parts from *U. subincisa.* 88
Table 6. Proximate and elemental analysis of the crude extract, its fractions, and pyrolytic products from *U. subincisa.* 89
Table 7. Compounds identified by GC-MS in the crude extract, phases A-C and bio-oils obtained from aerial parts of *U. subincisa*. 95
Table 8. Total phenols and initial toxicity values (IC_{90}) of different fractions of *U. subincisa*. 98
Table 9. Py-GC/MS characterization of dry bark from *A. adstringens*. 130
Table 10. Proximate and elemental analysis of *A. adstringens* bark, crude extract and fractions. 132
Table 11. Compounds identified by GC-MS in the crude aqueous extract from *A. adstringens* bark and its fractions. 135

Tablas en el Capítulo 1

Página

Tabla 1. Consensus factor values for different dermatological affection categories on the Purépecha Plateau……..……....43

Tablas en el Capítulo 3

Página

Tabla 1. Pyrolytic products yield of residual biomass from *A. adstringens*……112
Tabla 2. Proximate and elemental analyses of *A. adstringens* samples……...112
Tabla 3. Py-GC-MS characterization of the main compounds found in the residual biomass from *A. adstringens*…….……..114
Tabla 4. GC-MS characterization of the main compounds found in the pyrolytic oils from *A. adstringens*……...116
Tabla 5. HPLC-DAD quali-quantitative analysis of phenolic compounds in *A. adstringens* oils……117

Tablas en el Anexo 1

Página

Tabla 1. Nuevos medicamentos en desarrollo para el tratamiento de la psoriasis……...165

LISTA DE ABREVIATURAS

AhR	Receptor de hidrocarburos de arilo
CCL	Ligando de quimiocina CC
CXCL	Quimiocinas
FTIR	Espectroscopía infrarroja por transformada de Fourier
GC-MS	Cromatografía de gases acoplada a espectrometría de masas
HaCaT	Línea celular de queratinocitos humanos espontáneamente transformados
IL	Interleucina
IL-17R	Receptor de interleucina 17
INF	Interferón
MAPK	Cinasas de proteínas activadas por mitógenos
NF-κB	Factor nuclear potenciador de las cadenas ligeras kappa de la célula B activada
PGE2	Prostaglandina E2 (dinoprostona)
PY-GC-MS	Pirólisis - cromatografía de gases acoplada a espectrometría de masas
S100A7	Psoriasina
STAT	Activadores de las señales de transcripción
TGF	Factor de crecimiento transformante
Th	Linfocito T
TNF	Factor de necrosis tumoral
UV	Radiación ultravioleta

RESUMEN

La psoriasis es una enfermedad dermatológica incurable caracterizada por la presencia de placas rojizas bien delimitadas que afecta aproximadamente a 3 millones de mexicanos. Aunque no es considerada una enfermedad mortal sus repercusiones sobre la calidad de vida de los pacientes se han comparado con las del cáncer y la hipertensión. En la actualidad no se conocen con exactitud sus bases etiopatogénicas, si bien se considera a la interleucina (IL) 17 como un mediador clave en su desarrollo y perpetuación. De hecho, los medicamentos biotecnológicos (secukinumab, ixekizumab, brodalumab) dirigidos a inactivar esta citocina o su receptor, han mostrado una elevada eficacia, aunque sus altos costos, la imposibilidad de ser administrados por vía tópica y su toxicidad han propiciado la búsqueda incesante por parte de las compañías farmacéuticas de nuevas terapias. El presente trabajo se enmarca en una visión de desarrollo farmacéutico, donde se siguió un enfoque etnofarmacológico para la búsqueda de nuevos candidatos terapéuticos de origen natural con posible actividad antipsoriásica. La Meseta Purépecha se seleccionó como área de estudio para la realización del estudio etnofarmacológico de partida, lo que permitió la identificación de dos especies candidatas (*Urtica subincisa* y *Amphipterygium adstringens*) con alto valor de uso por esta población. Noventa y siete especies de plantas pertenecientes a 47 familias fueron documentadas por vez primera para el tratamiento de 19 afecciones dermatológicas en la Meseta Purépecha, siendo la familia Asteraceae la principal entre las plantas colectadas (20.61%). La comparación de los resultados con la literatura etnomedicinal reveló que 8.25% de las plantas utilizadas en la Meseta Purépecha se registraron por primera vez para tratamiento de afecciones dermatológicas. El extracto etanólico de *U. subincisa*, acuoso de *A. adstringens*, sus fracciones (A-Hexano; B-Acetato de etilo; C-Agua) así como los aceites pirolíticos 1 y 2 derivados de la biomasa extraída fueron químicamente caracterizados (análisis proximal, elemental, FTIR, Fluorescencia-UV, Py-GC-MS, GC-MS) además de que se determinó su toxicidad y capacidad para inhibir la producción de IL-8 en queratinocitos HaCaT estimulados con IL-17. Los resultados del análisis químico permitieron constatar que tanto la composición química de los extractos como de los aceites pirolíticos de ambas especies fue variable. En el caso de *U. subincisa*, el extracto etanólico y las fases A y B mostraron ser ricas en ésteres, mientras que en la fase C y el aceite pirolítico-2 prevalecieron los compuestos nitrogenados. En contraste, el aceite pirolítico-1 de esta especie

mostró una preponderancia de fenoles. El extracto acuoso de *A. adstringens*, así como sus fracciones A y B, presentaron un predominio de fenoles y ácidos, mientras que la fase C mostró una gran abundancia de ácidos. En los aceites pirolíticos 1 y 2 de *A. adstringens* los fenoles representaron el grupo dominante de moléculas identificadas. Tanto para *U. subincisa*, como para *A. adstringens* la muestra con mayor toxicidad estuvo representada por el aceite pirolítico-2, mientras que los extractos obtenidos por extracción convencional presentaron una menor toxicidad sobre los queratinocitos HaCaT. Respecto a la capacidad de inhibir la acción proinflamatoria de la IL-17, pudo constatarse diferencias en ambas especies. En el caso de *U. subincisa*, los extractos mostraron un efecto generalmente proinflamatorio, mientras que el aceite pirolítico-2 a una concentración de 7.5 μg/mL provocó una disminución significativa de la producción de IL-8, en forma comparable a la dexametasona. En cambio, para *A. adstringens* el extracto crudo y la fase C, mostraron una acción anti-IL-17 significativamente mayor que la de la dexametasona y los aceites pirolíticos ($p<0.05$). Aunque los resultados de este trabajo permitieron demostrar por vez primera el potencial antinflamatorio de los aceites pirolíticos de *A. adstringens* y *U. subincisa*, se consideró al extracto acuoso de *A. adstringens* como el candidato más prometedor, dado su alto rendimiento de extracción, baja toxicidad y elevada acción anti-IL17.

Palabras clave: bio-aceites, extractos, fenoles, IL-17, psoriasis, toxicidad

ABSTRACT

Psoriasis is an incurable dermatological disease characterized by well-defined reddish plaques affecting around 3 million Mexicans. Although it is not considered a fatal disease, its repercussions on the patients' quality of life are comparable to those of cancer and hypertension. At present, its etiopathogenic bases have not been elucidated, although interleukin (IL) 17 is considered as a key mediator in its development and perpetuation. In fact, biological drugs (secukinumab, ixekizumab, brodalumab) aimed to inactivate this cytokine or its receptor, have shown high efficacy, although their high costs, the impossibility of being administered topically and their toxicity have led to an incessant search by pharmaceutical companies for new therapies. The present work follows a vision of pharmaceutical development, where an ethnopharmacological study was performed to identify new therapeutic candidates of natural origin with possible antipsoriatic activity. The Purépecha Plateau was selected as the study area for the ethnomedical research, which allowed the identification of two candidate species (*Urtica subincisa* and *Amphipterygium adstringens*) according to the high use value reported by this population. Ninety-seven plant species belonging to 47 families were documented for the first time for treating 19 dermatological conditions at the Purépecha Plateau, Asteraceae was the main family of the collected plants (20.61%). The ethanolic extract from *U. subincisa*, the aqueous extract from *A. adstringens*, their solvent fractions (A-Hexane; B-Ethyl acetate; C-Water) as well as the pyrolytic oils 1 and 2 derived from the extracted biomass were chemically characterized (proximal and elemental analysis, FTIR, Fluorescence-UV, Py-GC-MS, GC-MS) in addition to determining their toxicity and ability to inhibit IL-8 production in HaCaT keratinocytes stimulated with IL-17. The results of the chemical analysis confirmed that the chemical composition of the extracts and the pyrolytic oils of both species was variable. In the case of *U. subincisa,* the ethanolic extract and phases A and B showed to be rich in esters, whereas in phase C and oil-2, nitrogenous compounds prevailed. In contrast, oil-1from this species showed a preponderance of phenols. The aqueous extract of *A. adstringens*, as well as its fractions A and B, presented a preponderance of phenols and acids, while phase C showed high abundance of acids. In oils 1 and 2 from *A. adstringens*, phenols represented the dominant group. For both, *U. subincisa* and *A. adstringens*, the sample with the highest toxicity was represented by pyrolytic oil-2, while the extracts obtained by conventional extraction were

generally less toxic. Regarding the ability to inhibit the pro-inflammatory action of IL-17, differences were found in both species. As to *U. subincisa*, the extractives showed a proinflammatory effect, while pyrolytic oil-2 at 7.5 μg/mL caused a significant decrease in the production of IL-8, comparable to that of dexamethasone. On the other hand, for *A. adstringens* the crude extract and phase C, showed a significantly higher anti-IL-17 action than that of dexamethasone and pyrolytic oils ($p < 0.05$). Although the results of this work demonstrated for the first time the anti-inflammatory potential of the pyrolytic oils from *A. adstringens* and *U. subincisa*, the aqueous extract from *A. adstringens* was considered the most promising candidate, given its high extraction yield, low toxicity and significative anti-IL17 activity.

Key words: bio-oils, extracts, phenols, IL-17, psoriasis, toxicity

I. INTRODUCCIÓN

I.1. Psoriasis

Las enfermedades dermatológicas constituyen un motivo principal de consulta en la atención primaria de salud, destacándose entre ellas la psoriasis que es una enfermedad incurable de la piel caracterizada por la presencia de placas rojizas bien delimitadas y escamas secas de color blanco-plateado (Boehncke & Schön, 2015). La prevalencia mundial de esta patología varía de 0.51 a 11.43% en adultos (Michalek et al., 2017); y en México, pese a que hay pocos estudios epidemiológicos sobre las enfermedades cutáneas, se ha estimado una prevalencia de 2.9%; lo que equivaldría a que alrededor de 3 millones de mexicanos estarían afectados (International Psoriasis Council, 2009). Se estima que del 5 al 30% de los pacientes desarrollan artritis psoriásica, una enfermedad inflamatoria dolorosa que afecta mayoritariamente las articulaciones. La psoriasis tiene un impacto negativo en la calidad de vida de los pacientes, fundamentalmente de aquellos con psoriasis moderada o severa (25-35%), al punto que las repercusiones sobre sus funciones físicas y psicológicas se han comparado con las del cáncer, la hipertensión, las enfermedades cardiovasculares, la diabetes o la depresión (Rapp et al., 1999).

La etiología de la psoriasis no está completamente elucidada, pero la mayoría de los conceptos aceptados indican que su patogénesis está impulsada por una interacción compleja entre los queratinocitos y las células inmunes en donde las citocinas y quimiocinas juegan un papel crucial (**Fig. 1**) (Lowes et al., 2014). Una de las hipótesis aceptadas hasta ahora propone que la proliferación acelerada de los queratinocitos psoriásicos comparativamente a los normales (7-10 días vs. 28-50 días) y la diferenciación incompleta de estas células, están relacionadas con un aumento de la infiltración de linfocitos T (Th1, Th17, Th22) y con la liberación de compuestos inmunomoduladores y quimioatrayentes en la dermis y la epidermis que generan circuitos inflamatorios complejos responsables de perpetuar la enfermedad (**Fig. 1**) (Cai et al., 2012; Lowes et al., 2007).

Las terapias actuales, dirigidas fundamentalmente hacia el manejo de los síntomas, incluyen productos tópicos, fototerapia y tratamientos sistémicos. Estos se encaminan a: a) inhibir la hiperproliferación de los queratinocitos (*ciclosporina A, retinoides*); b) normalizar el proceso

de diferenciación aberrante de los queratinocitos (*análogos de la vitamina D, retinoides*); c) disminuir el reclutamiento y la activación de inmunocitos (*ciclosporina A, medicamentos biotecnológicos*) y d) neutralizar citocinas proinflamatorias (*medicamentos biotecnológicos*) (García-Pérez et al., 2012). Desafortunadamente, ninguno de estos medicamentos es curativo, por lo que la industria farmacéutica continúa con la búsqueda incesante de nuevos candidatos terapéuticos para el manejo de la enfermedad (véase **Anexo 1**).

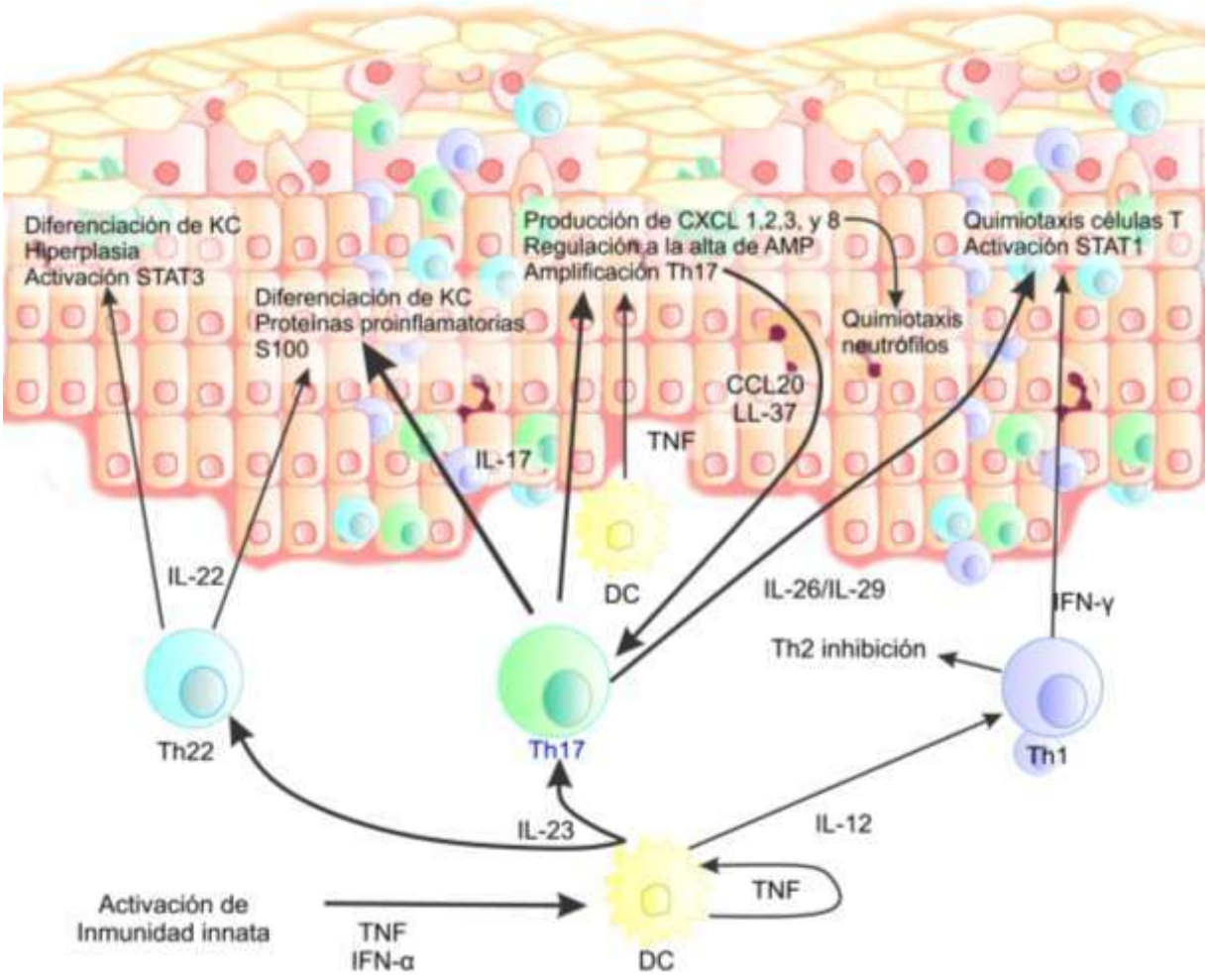

Fig. 1. Circuitos inflamatorios en la psoriasis.
La psoriasis implica la interacción compleja entre neutrófilos, células dendríticas (DC), queratinocitos y linfocitos Th1 y Th17 quienes producen mediadores inflamatorios (IL-17A, IL-17F, IL-22, TNF-α e IFN-ɣ) que estimulan la generación de quimiocinas (CXCL y CCL), citocinas (TNF, IL-26,IL-29), proteínas proinflamatorias (S100) que contribuyen a la activación de factores de transcripción (NF-κB, STATs) en los queratinocitos (KC), con la consecuente producción de una mayor cantidad de citocinas y quimiocinas que finalmente contribuyen al reclutamiento de más inmunocitos en la piel.

I.1.1. Interleucina-17 y la patogénesis de la psoriasis

Numerosas evidencias sugieren que la activación de la respuesta inmunitaria mediada por linfocitos Th1/Th17 participa en el desencadenamiento y perpetuación de la psoriasis. En la piel psoriásica, los niveles de interleucina (IL)-17 son hasta 6 veces superiores que en la piel normal y estos correlacionan con el grado de severidad de la enfermedad (Johansen et al., 2009). De hecho, la eficacia clínica con medicamentos biotecnológicos que bloquean la IL-17 o su receptor, no dejan dudas sobre el impacto de la IL-17 sobre esta patología.

En el contexto de la piel, la IL-17 es producida, entre otros, por linfocitos Th17, células linfoides innatas, neutrófilos, y mastocitos. La familia IL-17 está integrada por 6 miembros (IL-17A-F), siendo la IL-17A la isoforma más estudiada. La IL-6 y el TGF β promueven la diferenciación inicial de las células Th0 a Th17, mientras que la IL-23, producida por las células dendríticas dérmicas y los macrófagos, estabiliza y expande las células Th17. La IL-23, es un heterodímero constituido por las subunidades p19 y p40, esta última compartida con la IL-12. Al unirse a su receptor, la IL-23 activa factores de transcripción como RORγt y STAT3 presentes en las células Th17, desencadenando la liberación de la IL-17A-F (Sakkas & Bogdanos, 2017). La familia de receptores de IL-17 posee cinco miembros: IL-17RA, IL-17RB, IL-17RC, IL-17RD e IL-17RE. La IL-17A se unen al heterodímero IL-17RA/IL-17RC, favoreciendo la activación de la proteína adaptadora (ACT1), quien activa al factor nuclear κB (NF-κB). Una vez que el NF-κB está completamente activado, participa en la regulación de varios genes diana en las células epidérmicas y dérmicas, modulando la expresión de citocinas inflamatorias (TNF, IL-1, IL-6), quimiocinas (IL-8), receptores inmunes y moléculas de adhesión en la superficie celular (ICAM-1) (**Fig. 2**). Además, la IL-17 también activa otras vías como las relacionadas con las MAPKs quinasas como la quinasa N-terminal c-jun (JNK), la quinasa regulada por señal extracelular (ERK), así como la ruta JAK/STAT (quinasa Janus/el transductor de señal y el activador de la transcripción) y la de la fosfoinositol 3 quinasa (PI3K) induciendo la formación de péptidos antimicrobianos (β-defensina), citocinas y metaloproteinasas de la matriz extracelular (Adami et al., 2014).

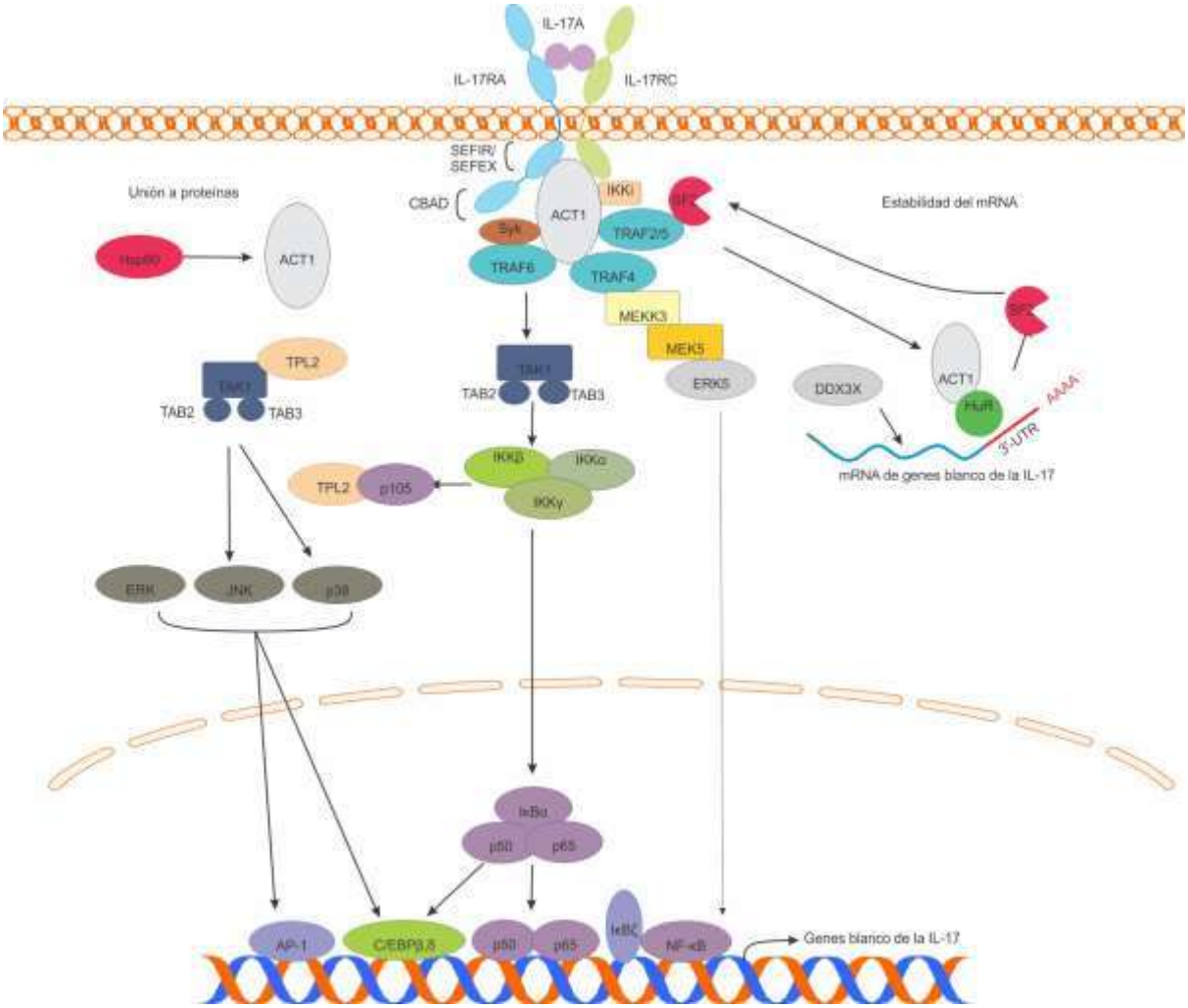

Fig. 2. Vía de señalización de IL-17.

La señalización de IL-17 comienza con la unión de las citocinas IL-17 a sus receptores IL-17RA e IL-17RC. Tras la unión del ligando, Act1 activa múltiples vías de señalización independientes que median a través de diferentes proteínas TRAF. La activación de TRAF6 da como resultado la activación de las vías NF-κB, C/EBPβ, C/EBPδ y MAPK. El complejo IL-17R-Act1 también se asocia con MEKK3 y MEK5 a través de TRAF4, lo que resulta en la activación de ERK5. Si bien la señalización de IL-17 mediada por TRAF6 y TRAF4 da como resultado la transcripción de genes inflamatorios, la señalización de IL-17 a través del complejo ACT1-TRAF2-TRAF5 da como resultado el control de la estabilidad del ARNm de los genes diana de IL-17 (Hsp: proteína de choque térmico, TRAF: factor asociado al receptor de TNF, TAK1: quinasa 1 activada por TGF-β, IKK: inhibidor de la quinasa kappa B, ERK: quinasa relacionada con la señal extracelular, JNK: quinasa Janus, HuR: antígeno humano R, SF2: factor de empalme 2. Modificado de Amatya et al., 2017).

Los queratinocitos, son células epidérmicas que desempeñan un papel crucial en el desarrollo de la psoriasis mediado por la IL-17. Un estudio reciente en un modelo animal de psoriasis inducida por imiquimod demostró que la eliminación de los receptores IL-17RA en células T o mieloides no tuvo un impacto significativo en el desarrollo de la psoriasis, en cambio la carencia

de este receptor en los queratinocitos resultó en una disminución del engrosamiento epidérmico y la inflamación en los animales estudiados (Moos et al., 2019). La IL-17 no solo es fuertemente proinflamatoria, sino que también estimula la hiperproliferación y la diferenciación aberrante de los queratinocitos dentro de la epidermis (Ekman et al., 2019). Los efectos de la IL-17 sobre los queratinocitos no se presentan en forma aislada, sino que actúa en sinergismo con otras citocinas relevantes en la psoriasis como el factor de necrosis tumoral (TNF-α), induciendo la expresión de numerosos genes proinflamatorios característicos de la enfermedad (psoriasina S100A7, β-defensina DEFB4, IL-8, CCL20, IL-23 (p19) y CXCL1). Aunque se ha sugerido que esta acción puede deberse a la activación conjunta de NF-κB, también es necesaria la participación de otros mecanismos post-transcripcionales que no han sido dilucidados (Chiricozzi et al., 2011).

Una quimiocina fuertemente inducida por la IL-17 en los queratinocitos es la IL-8, también conocida como CXCL-8 que actúa atrayendo a los leucocitos, fundamentalmente a los neutrófilos, hacia la piel psoriásica, mediante quimiotaxis (Duan et al., 2001). La acumulación de los neutrófilos en la piel, se considera un evento temprano de gran relevancia en la formación de las placas psoriásicas, ya que estas células activadas pueden influir no solo en el crecimiento y la diferenciación de los queratinocitos epidérmicos, sino también en el estado de activación de los linfocitos T (Terui et al., 2000). La producción de IL-8 es mayor en el suero y la piel de pacientes con psoriasis (Bai et al., 2017), y sus niveles se correlacionan con la severidad de la enfermedad (Arican et al., 2005). Recientemente se ha sugerido que la variación de los niveles de IL-8 en pacientes con psoriasis podría ser utilizado como un marcador de respuesta terapéutica (Bai et al., 2017). Los marcadores de respuesta terapéutica son de gran utilidad durante el desarrollo temprano de medicamentos, ya que facilitan la toma de decisiones respecto a estudios ulteriores con el candidato terapéutico de interés.

El descubrimiento de productos biotecnológicos dirigidos a inactivar a la interleucina IL-17 o a su receptor (secukinumab, ixekizumab y brodalumab), han demostrado una eficacia antipsoriásica superior a la de inhibidores del TNF-α (Griffiths et al., 2010); aunque los eventos adversos, los altos costos, la imposibilidad de administración tópica y la falta de respuesta en algunos pacientes pueden limitar su uso (Bissonnette et al., 2014; Lv et al., 2018).

Para alcanzar el éxito terapéutico la adherencia al tratamiento es esencial, desafortunadamente en el caso de la psoriasis hay una gran insatisfacción que conduce a los pacientes al uso de medicina complementaria o alternativa donde los extractos de plantas y los productos pirolíticos como el alquitrán de hulla son ampliamente utilizados (Moscaliuc et al., 2013; Svendsen et al., 2017; Talbott & Duffy, 2015). Sin embargo, muchos de estos extractos y productos pirolíticos no están caracterizados químicamente o bien no cuentan con pruebas toxicológicas o farmacodinámicas pudiendo contribuir a la aparición de eventos adversos y a la exacerbación de la enfermedad (Fleischer et al., 1996). Por lo tanto, la búsqueda de agentes terapéuticos anti-IL-17 más asequibles, efectivos y seguros sigue en curso (véase **Anexo 1**).

I.1.2. El desarrollo de fármacos para la psoriasis

El desarrollo farmacéutico puede definirse como un conjunto ordenado de fases que conducen al desarrollo de un medicamento (King et al., 2010). El esquema clásico de desarrollo de fármacos está conformado convencionalmente por el traslado de conocimientos desde la investigación básica hacia la investigación clínica en un sentido unidireccional, involucrando varias fases: 1) Fase de descubrimiento; 2) Fase de investigación preclínica; 3) Fase de investigación clínica; 4) Fase de registro y aprobación, y la 5) Fase de desarrollo químico-farmacéutico (**Fig. 3**). Estas fases cuentan a su vez con las etapas siguientes: a) establecimiento de hipótesis etiopatogénicas de la enfermedad; b) identificación de dianas farmacológicas; c) descubrimiento y selección de candidatos terapéuticos; d) optimización química de los compuestos seleccionados; e) pruebas preclínicas en animales de experimentación; f) evaluación de la seguridad en seres humanos y el establecimiento de las propiedades farmacocinéticas (fase I); g) las pruebas de eficacia (prueba de concepto; fase II) y finalmente f) los ensayos clínicos multicéntricos a mayor escala para evaluar la seguridad y eficacia (fase III) (Turner, 2010). La vigilancia posterior a la comercialización del medicamento, también conocida como farmacovigilancia, continúa vigente ante cualquier eventualidad que se presente con el uso no controlado de los fármacos (Turner, 2010).

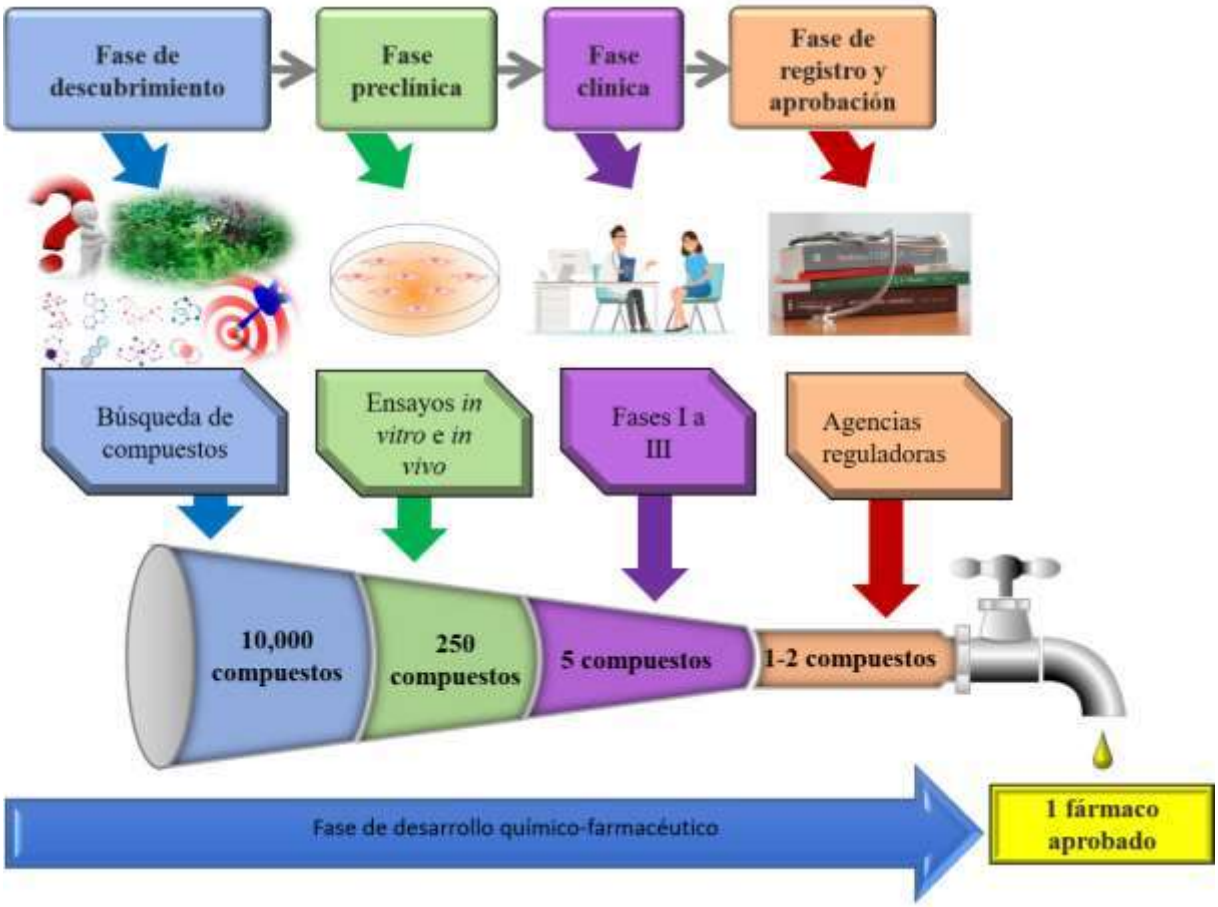

Fig. 3. Esquema clásico de desarrollo farmacéutico.
El esquema clásico de desarrollo farmacéutico se inicia por la fase de descubrimiento y termina con la fase clínica que brindan la evidencia para el registro de aprobación ante las agencias reguladoras. Pocos fármacos son aprobados anualmente y estos continúan bajo vigilancia durante su comercialización.

En los últimos años ha nacido el término "medicina traslacional", que se refiere a un nuevo paradigma basado en la necesidad de integrar los conocimientos científicos que se generan durante la investigación básica al desarrollo de tratamientos con impacto clínico (bench-to-bedside). En su acepción más amplia el concepto de medicina traslacional puede entenderse como un proceso bidireccional, donde además del traslado de conocimientos desde la ciencia básica a la clínica, los nuevos hallazgos derivados de la investigación clínica sirven de base para la generación de hipótesis científicas utilizadas en ciencia fundamental (bedside-to-bench) (Guttman-Yassky & Krueger, 2007). La psoriasis constituye un excelente ejemplo de la importancia de la medicina traslacional para el desarrollo de nuevos tratamientos. De hecho, los primeros tratamientos antipsoriásicos, como la fototerapia o la ciclosporina, se desarrollaron a

partir de una aproximación empírica que partió de la evidencia clínica y se trasladó rápidamente a nuevos conceptos en ciencia básica (bedside-to-bench).

La relevancia de los enfoques de medicina traslacional se evidencia en descubrimientos que han sacudido los paradigmas etiopatogénicos de la enfermedad. Un ejemplo de ello es la reconocida importancia de la IL-17 y el desarrollo ulterior de medicamentos biotecnológicos. Investigaciones de ciencia básica combinados con comprobaciones clínicas (bench-to-bedside/bedside-to-bench) permitieron el establecimiento del nuevo paradigma en la fisiopatología de la enfermedad relacionado con el eje IL-23/ IL-17. La consecuencia lógica de estas investigaciones fue el desarrollo de dos anticuerpos contra la subunidad p40: el ustekinumab y briakinumab, así como de inhibidores de la IL-17 o de su receptor (secukinumab, ixekizumab, brodalumab) (véase **Anexo 1**).

En la actualidad, la psoriasis es una de las enfermedades que presenta mayor desarrollo farmacéutico a escala global (Cervantes-Durán et al., 2020; Esquivel-García et al., 2018a). Sin embargo, son numerosos los retos a enfrentar para llevar con éxito este proceso, dentro de los que se destaca: 1) la identificación de biomarcadores predictivos de toxicidad, farmacocinética y eficacia farmacológica; b) el desarrollo de modelos *in vitro* e *in vivo* más representativos de la enfermedad, así como c) la integración de aspectos farmacogenómicos al proceso del descubrimiento de fármacos (Esquivel-García et al., 2018a).

Una vez que los fármacos son desarrollados, estos se integran en esquemas clínicos que deben ajustarse a las características y necesidades de los pacientes. Ello implica que durante el desarrollo farmacéutico se tenga en cuenta la población a la que va a ser administrado el producto de interés. La individualización de las terapias para la psoriasis encuentra algunos desafíos, ya que cada paciente tiene su propia forma de psoriasis, e incluso dentro del mismo paciente, las características de la psoriasis pueden variar considerablemente (van de Kerkhof, 2008). De hecho, se han descrito diferencias inter-paciente en términos del inicio de la enfermedad, la cronicidad, su ubicación anatómica, los factores desencadenantes y las comorbilidades existentes. Además, los polimorfismos en los genes que codifican proteínas importantes para la farmacocinética y farmacodinámica de los medicamentos antipsoriásicos podrían explicar la variabilidad encontrada en la respuesta a los tratamientos (Lebwohl, 2016; Woolf & Smith, 2010). Aunque el uso de biotecnológicos anti-IL-17 ha sido bien aceptado por

pacientes con psoriasis severa, su administración parenteral, elevados costos y presencia de reacciones de inmunogenicidad constituyen limitaciones para individuos con psoriasis leve y moderada (aproximadamente el 80% de los pacientes) (Armstrong et al., 2013), quienes prefieren el uso de medicamentos tópicos (Kim et al., 2017). El desarrollo de medicamentos anti-IL17 administrados en la piel mejor adaptados a las necesidades de personas con psoriasis leve y moderada sería una estrategia, que, de lograrse permitiría elevar la adherencia a las terapias y el éxito de los tratamientos con el consecuente incremento de la calidad de vida de estos individuos.

I.2. La etnofarmacología y el desarrollo farmacéutico en la psoriasis

Varias son las estrategias que se utilizan durante la fase de descubrimiento de nuevos medicamentos para la psoriasis, destacándose el uso de moléculas o mezclas de moléculas obtenidas por síntesis química, a partir de procesos biotecnológicos o bien utilizando fuentes naturales. Los productos naturales presentan numerosas ventajas para el desarrollo de nuevos medicamentos antipsoriásicos ya que poseen una amplia gama de farmacóforos con alta complejidad estereoquímica (Harvey et al., 2015), lo que favorece la unión sobre dianas complejas que implican interacciones proteína-proteína como las que se presentan en los circuitos inflamatorios mediados por citocinas. Otra ventaja del uso de productos naturales para la psoriasis se relaciona con el hecho de provenir directamente de la naturaleza, ya que se ha sugerido que numerosos compuestos exitosos como fármacos tienen la propiedad de "semejanza de metabolitos" ("metabolite-likeness"), que significa que tales compuestos no solo son biológicamente activos, sino que también pueden ser sustratos para uno o más sistemas transportadores, facilitando la cesión de los compuestos activos a su sitio de acción intracelular (Hert et al., 2009).

La elección de la fuente natural sobre la cual se estructurará el desarrollo farmacéutico es de crucial importancia para la obtención de candidatos terapéuticos relevantes. Este proceso puede seguir varias estrategias: a) estrategia al azar; b) estrategia quimiotaxonómica; c) estrategia etnofarmacológica; d) estrategia ecológica (Abreu Guirado & Cuéllar Cuéllar, 2008). La estrategia etnofarmacológica ha sido una de las más eficaces en el descubrimiento de nuevos medicamentos, siendo la etnofarmacología considerada como "la exploración científica interdisciplinaria de productos biológicamente activos utilizados u observados por el hombre"

(Bruhn & Helmstedt, 1981). En este enfoque cobran extraordinaria importancia las tradiciones que se consideran como entidades dinámicas de conocimiento experimental en continua evolución a medida que las comunidades y los individuos descubren nuevas técnicas que transforman el uso de las plantas (Patwardhan, 2005).

La etnofarmacología como un campo de investigación bien definido tiene una historia de desarrollo que se inicia en el siglo XX (Heinrich & Gibbons, 2001). Sin embargo, mediante su interacción con otras áreas del conocimiento como la antropología, la botánica y la farmacología se ha transformado en una herramienta esencial para la búsqueda de nuevos candidatos terapéuticos a través del uso, entre otros, de plantas medicinales relevantes para la medicina tradicional de distintas regiones del mundo (Heinrich et al., 2009).

Siguiendo el enfoque etnofarmacológico las especies elegidas para el desarrollo farmacéutico son aquellas que han demostrado una eficacia terapéutica como consecuencia de su utilización tradicional por la población (Abreu Guirado & Cuéllar Cuéllar, 2008). A partir de estos estudios se inicia un proceso de validación científica (**Fig. 4**), en donde las moléculas de origen natural que se consideran candidatos para el desarrollo de nuevos fármacos son sometidas a rigurosas pruebas para contar con la evidencia de seguridad y eficacia requerida para su uso en una patología determinada (Fabricant & Farnsworth, 2001; Gertsch, 2009). En la psoriasis este proceso cobra extraordinaria importancia al tratarse de una enfermedad caracterizada por la presencia de circuitos inflamatorios complejos donde las moléculas naturales podrían ofrecer alternativas terapéuticas dada su complejidad estructural y acción multidiana (Patwardhan, 2005).

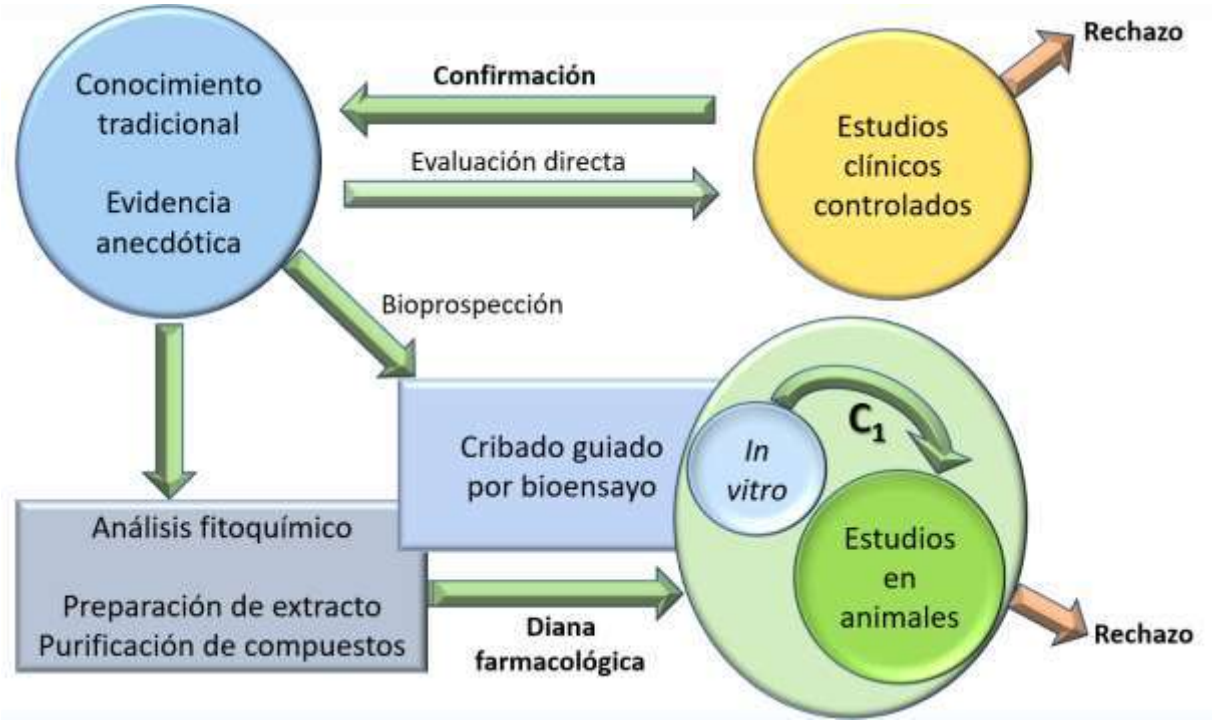

Fig. 4. Proceso de evaluación científica en etnofarmacología.
Fases de validación en el proceso de evaluación etnofarmacológica. Los medicamentos tradicionales a base de plantas pueden evaluarse directamente en ensayos clínicos, utilizarse como base para la bioprospección o someterse a estudios *in vitro* relacionados con la indicación etnofarmacológica. Las hipótesis farmacológicas pueden ser refutadas en base a estudios *in vitro* y en animales. Sin embargo, solo los estudios clínicos aleatorizados, doble ciego y controlados con placebo permiten la confirmación o refutación de la evidencia anecdótica (conocimiento tradicional. Modificado de Gertsch, 2009).

La medicina tradicional mexicana es reconocida a nivel mundial por su gran riqueza cultural, alta diversidad florística, y por su contribución en el desarrollo de medicamentos de gran impacto en la terapéutica (Heinrich et al., 2014). Los estudios etnofarmacológicos realizados en el territorio mexicano no solo muestran que la práctica de la medicina tradicional continúa vigente (Alonso-Castro et al., 2017; Heinrich et al., 2014), sino que también permiten resguardar el conocimiento etnomedicinal que desafortunadamente se pierde en el paso de generación a generación (Calvo et al., 2011).

Una de las regiones de interés etnofarmacológico en México es la meseta Purépecha, la cual es un rico reservorio de biodiversidad (Bello-González et al., 2015; Medina, 2003). La participación de la medicina tradicional como parte de los servicios de salud en esta región es muy importante, principalmente en aquellos lugares donde el sistema de salud pública aún es deficiente (BDMTM, 2009; Gallardo Ruiz, 2002). A pesar del vasto conocimiento tradicional

de la flora medicinal de los terapeutas Purépechas desde tiempos precortesianos, no hay estudios etnofarmacológicos disponibles que reporten el uso de plantas para el tratamiento de afecciones dermatológicas por esta población. Por lo tanto, se considera que la documentación de este conocimiento podría contribuir a la identificación de especies de interés para el desarrollo de nuevos candidatos terapéuticos o preparaciones herbarias antipsoriásicas.

Uno de los objetivos de la etnofarmacología durante el proceso de validación científica es realizar el análisis fitoquímico de las especies de interés (Gertsch, 2009). Dicho análisis debe ser congruente con la forma de preparación reportada en la medicina tradicional (Gupta et al., 2010). Dada la baja toxicidad de los solventes empleados en las culturas tradicionales, es frecuente el uso de preparaciones acuosas, alcohólicas e hidroalcohólicas de las plantas para tratar padecimientos de la piel. Estos solventes polares permiten extraer diversos grupos de metabolitos como polifenoles, azúcares, terpenos y compuestos nitrogenados, implicados en los sistemas de defensa de las plantas. Muchos de estos metabolitos se han empleado como candidatos terapéuticos para el tratamiento de la psoriasis (Edeas, 2008).

Una vez que se completa el análisis fitoquímico, los extractos deben evaluarse desde el punto de vista farmacológico y toxicológico usando modelos preclínicos *in vitro* o *in vivo* relevantes para la patología de interés. Esta evaluación es de extraordinaria importancia ya que permite corroborar el uso tradicional, y pone en evidencia los riesgos del consumo para la población. En caso de obtenerse una buena relación beneficio/riesgo durante el desarrollo preclínico, las preparaciones analizadas deben ser probadas como parte de estudios clínicos controlados en seres humanos. Finalmente, un elemento esencial que distingue al desarrollo de medicamentos siguiendo el enfoque etnofarmacológico debe ser una comprensión sólida de la base sociocultural del grupo étnico y una integración de la información recopilada a lo largo de los estudios científicos con el contexto sociocultural de partida (Heinrich et al., 2009).

I.3. Uso de extractos acuosos y etanólicos de plantas como candidatos terapéuticos para el tratamiento de la psoriasis

El uso de extractos acuosos y etanólicos para el tratamiento de la psoriasis en diversos sistemas médicos tradicionales en todo el mundo está bien documentado en la literatura científica. La **Tabla. 1** muestra los extractos y las especies que han encontrado mayor aplicación para esta enfermedad basado en su uso en la medicina tradicional.

Tabla. 1. Estudios que utilizan extractos acuosos y etanólicos de plantas empleados por la medicina tradicional para el tratamiento de la psoriasis.

Especie	Parte de la planta	Tipo de extracto	Resultados	Referencia
***Cassia auriculata* L.**	Flores	Etanólico	Reducción del grosor epidérmico y disminución de la severidad de las placas (eritema, enrojecimiento y descamación) en la piel psoriásica de un modelo animal.	(Vijayalakshmi et al., 2019)
***Gentiana lutea* L.**	Raíz	Etanólico	Modulación de la síntesis de ceramidas en cultivos de queratinocitos.	(Gendrisch et al., 2020)
***Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thomson; *Curcuma longa* L.**	Tallos Raíz	Acuoso Etanólico	Mejora la apariencia de la piel en un modelo animal de psoriasis inducida con Imiquimod, disminuye los niveles de ARNm de IL-17, IL-23, IL-1 y TNF en la piel y la sangre de los animales tratados.	(Arora et al., 2016)
***Dillenia indica* L.**	Frutos	Etanólico	Reducción de la ortoqueratosis y paraqueratosis en un modelo animal de psoriasis.	(Kviecinski et al., 2016)
***Mahonia aquifolium* (Pursh) Nutt.**	Cortezas Frutos	Acuoso Etanólico	Inhibidor de la proliferación de los queratinocitos. En estudios clínicos se demostró una mejoría de las lesiones psoriásicas con un perfil favorable de eventos adversos.	(Gulliver & Donsky, 2005; Müller et al., 1995)
***Aloe vera* (L.) Burm. f.**	Hojas	Etanólico	En un modelo de cola de ratón, el extracto estimuló la diferenciación de la epidermis. La actividad antipsoriásica fue del 81.95% comparativamente al 87.94% del grupo que recibió el Tazaroteno como control positivo.	(Dhanabal et al., 2012)
***Melissa officinalis* L.**	Partes aéreas	Acuoso	Alta capacidad antioxidante. Reestablecimiento de la hidratación de la piel y la función de barrera epidérmica en un modelo murino de psoriasis inducida.	(Dimitris et al., 2020)
***Picea mariana* (Mill.) Britton, Sterns & Poggenb.**	Cortezas	Acuoso	Disminución de la producción de IL-8, IL-6, fractalkina, óxido nítrico y PGE2 en queratinocitos normales y psoriásicos estimulados con TNF-α vía la inhibición de la activación de NF-κB.	(García-Pérez et al., 2014)
***Artemisia capillaris* Thunb.**	Partes aéreas	Etanólico	Disminución de la severidad de la psoriasis en un modelo animal de psoriasis inducida con Imiquimod. Disminución del engrosamiento epidérmico y de la expresión de Ki67. Efectos antiproliferativos en queratinocitos HaCaT.	(Lee et al., 2018)

La elección de estos solventes, además de ser aceptables desde el punto de vista toxicológico, y por lo tanto de gran uso en la medicina tradicional, permiten extraer numerosos metabolitos

secundarios, siendo los fenoles, dada su polaridad, uno de los grupos de compuestos bioactivos mayoritariamente presentes en este tipo de extractos.

El uso de compuestos fenólicos, extraídos de manera convencional con solventes polares a partir de plantas medicinales (Edeas, 2008; Stevanovic et al., 2009), ha quedado plasmado en la medicina tradicional de distintas regiones del mundo, y actualmente diferentes formulaciones que los contienen son utilizadas en las medicinas convencional, complementaria y alternativa para el tratamiento de placas psoriásicas (Svendsen et al., 2017; Talbott & Duffy, 2015). Aunque estos estudios demuestran el potencial farmacológico de estos extractos, las plantas más utilizadas son en su mayoría provenientes de sistemas médicos tradicionales de otros países, mientras que falta documentación sobre las propiedades antipsoriásicas de plantas nativas de México.

Se ha demostrado que los polifenoles del té verde pueden inducir la expresión de la caspasa 14, una proteasa cuya expresión aparece disminuida en la piel psoriásica y que participa en la diferenciación terminal de los queratinocitos y la formación del *stratum corneum* (Hsu et al., 2007). Además, polifenoles como la curcumina, presente en la planta *C. longa*, pueden disminuir la expresión de citocinas proinflamatorias como la IL-6 e IL-8 en los queratinocitos (Miquel et al., 2002).

Los polifenoles de *P. mariana* han demostrado ser potentes antinflamatorios, bloqueando la acción del TNF-α en queratinocitos psoriásicos, con una actividad incluso superior a la dexametasona, un fármaco utilizado para el tratamiento de esta enfermedad (García-Pérez et al., 2014). Aunque estos estudios indican el potencial antinflamatorio de estas moléculas para el tratamiento de la psoriasis, su efecto farmacológico en otras dianas terapéuticas como la IL-17, de importancia capital para la patogénesis de esta enfermedad, no ha sido elucidado.

I.4. Estructura química de los compuestos fenólicos extraíbles

Los polifenoles son un extenso grupo de compuestos que se utilizan como agentes antipsoriásicos a partir de diversas modalidades terapéuticas y que son considerados como extraíbles en las plantas ya que pueden ser obtenidos por extracción convencional con solventes polares. Se trata de una gran variedad de metabolitos secundarios que se caracterizan por contener uno o más grupos fenólicos y reciben el nombre de polifenoles, compuestos fenólicos o bien de fenilpropanoides.

Desde el punto de vista de la estructura química, son un grupo diverso que comprende desde moléculas sencillas como los fenoles simples y ácidos fenólicos hasta polímeros complejos como los taninos y la lignina, aunque esta última no puede ser extraída con solventes por métodos de extracción convencional y no pertenece al grupo de los extraíbles. En el grupo también se encuentran los flavonoides, estilbenos y lignanos, muchos de los cuales están implicados en las interacciones planta-herbívoro (Schultz et al., 1992). En combinación con fototerapia, el psoraleno es uno de los fenoles más representativos para el tratamiento de la psoriasis (Lapolla et al., 2011).

La ruta del ácido shikímico es responsable de la biosíntesis de la mayoría de los fenoles de plantas. A partir de eritrosa-4-P y de ácido fosfoenolpirúvico se inicia una secuencia de reacciones que conduce a la síntesis del ácido shikímico y, derivados de éste, como los aminoácidos aromáticos (fenilalanina, triptófano y tirosina). La mayoría de los compuestos fenólicos derivan de la fenilalanina. Esta ruta está presente en plantas, hongos y bacterias, pero no en animales (García & Carril, 2011).

Los fenoles simples, los aldehídos y los ácidos fenólicos presentan estructuras químicas simples, y poseen un peso molecular bajo respecto a los taninos. Los fenoles simples están representados por una estructura C_6 formada por un anillo bencénico y sus derivados. Dentro de este subgrupo se encuentra el pirocatecol, resorcinol y el orcinol (**Fig. 5**). Las estructuras C_6-C_1 constituida por un anillo bencénico al cual se une un átomo de carbono, son características de múltiples aldehídos fenólicos presentes en el reino de las plantas como la vainillina, el salicilaldehído e hidroxibenzaldehído, pero también de ácidos fenólicos que se han considerado bioactivos como los ácidos gálico y salicílico (**Fig. 5**) (Stevanovic et al., 2009).

Entre los compuestos fenólicos con estructuras C_6-C_2 se encuentran compuestos relacionados con el ácido fenilacético y las acetofenonas, dentro de los que se encuentran los derivados de la p-hidroxiacetofenona que han sido identificados en plantas como *Populus balsamífera* L. (Stevanovic & Perrin, 2009). Los ácidos hidroxicinámicos, por su parte tienen una estructura C_6-C_3 y han sido reconocidos por sus propiedades antioxidantes y antiinflamatorias, dentro de los que se encuentran los ácidos clorogénico, cafeico y ferúlico (**Fig. 5**) (Stevanovic et al., 2009).

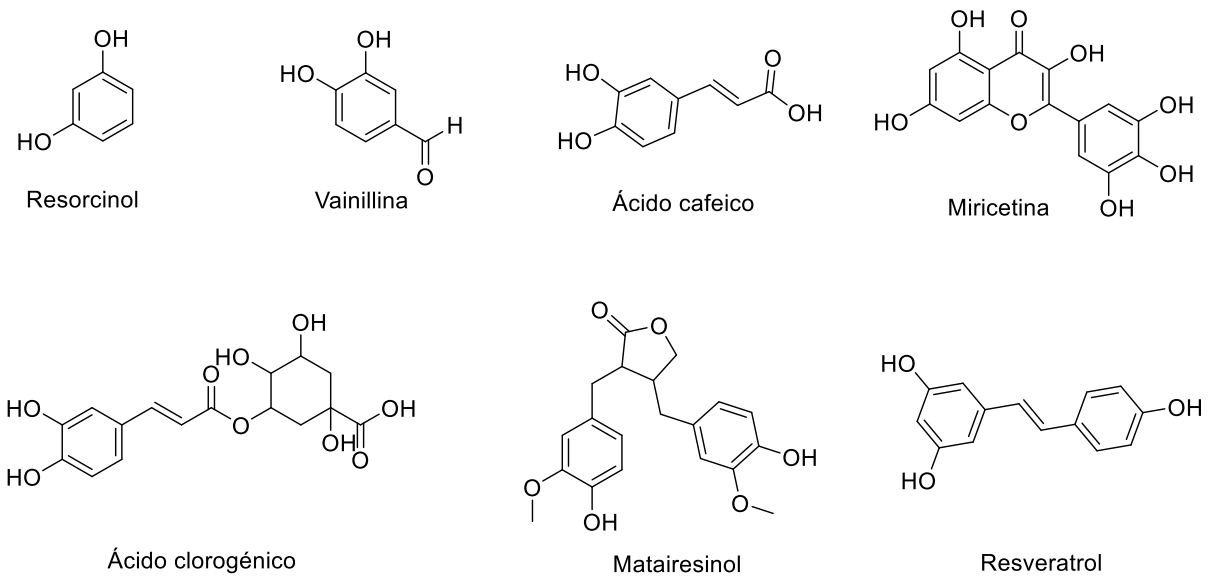

Fig. 5. Estructuras químicas de polifenoles encontrados frecuentemente en las plantas.

Los estilbenos son otro subgrupo de compuestos fenólicos que contienen muchas moléculas bioactivas, siendo el resveratrol (**Fig. 5**) uno de los más reconocidas (Shen et al., 2009). Se sintetizan mediante la adición de 1 a 3 moléculas de malonil-CoA a los ácidos cinámicos. La estructura más simple de los estilbenos está representada por el 1,2-difeniletano. Además, existen también derivados hidroxilados. Juntamente con los bibencilos, que también tienen un esqueleto C6-C2-C6, forman la familia de los estilbenoides (Stevanovic & Perrin, 2009).

Los lignanos y los neolignanos son dímeros naturales de fenilpropano que también son reconocidos por sus propiedades biológicas. Cuando las unidades de fenilpropano están unidas por enlaces C-C en las posiciones 8 y 8', el compuesto se considera un lignano. Si los dímeros de fenilpropano están unidos por otro tipo de enlaces, es un neolignano. Por lo tanto, hay neolignanos que contienen enlaces C-C 8-O-4', 3-O-4' (oxineolignanos), 3-3', 8-3', 1-3', etc. (Moss, 2000). El (-)-matairesinol (**Fig. 5**) es un intermediario central en la biosíntesis de muchos lignanos, dentro de los que se encuentra la podofilotoxina, un agente antiviral con propiedades anticancerígenas obtenido de los rizomas y raíces de plantas perennes del género *Podophyllum* L. (X. Zhang et al., 2018). La podofilotoxina es el ingrediente activo de Condylox® o Condyline®, utilizado clínicamente para el tratamiento de las verrugas genitales.

Los flavonoides son uno de los grupos de polifenoles más distribuidos en el reino de las plantas y están representados por compuestos que tienen esqueletos C6-C3-C6, es decir, una parte basada en el fenilpropano. Su estructura molecular se caracteriza por un esqueleto de carbono del tipo difenil 1,3-propano que comprende 15 átomos de carbono distribuidos en dos anillos de benceno, denominados A y B, unidos entre sí por la estructura de cromano con tres carbonos. Existen varios subgrupos de flavonoides según el grado de insaturación y oxidación del heterociclo: flavonas, flavanonas, flavanos e isoflavonas (Stevanovic & Perrin, 2009). Los flavonoides generalmente existen en la naturaleza en forma hidroxilada, aunque la presencia de carbohidratos o grupos metilo en hidroxilos fenólicos es muy frecuente. Dentro de este grupo se encuentran compuestos como el galato de epigalocatequina y la miricetina (**Fig. 5**) con propiedades antipsoriásicas (Lee & Lee, 2016; Zhang et al., 2016).

Los taninos son compuestos polifenólicos, solubles en agua, cuyas masas molares están entre 500 y 3000 Da. Además de exhibir las reacciones características de los fenoles, pueden precipitar alcaloides, gelatina y otras proteínas. Existen dos tipos de taninos, en función de su estructura y propiedades: taninos hidrolizables y taninos condensados. Los taninos hidrolizables se caracterizan por una parte central que consiste en un poliol (con mayor frecuencia glucosa), cuyas funciones hidroxilo están esterificadas con ácido gálico o elágico (Stevanovic et al., 2009). Las proantocianidinas o taninos condensados son oligómeros o polímeros de flavan-3-ols y flavan-3,4-dioles. Las proantocianidinas se pueden clasificar en dos categorías según su grado de polimerización de unidades monoméricas: oligómeros (desde dímeros a pentámeros) y polímeros (a partir de hexámeros) (Stevanovic & Perrin, 2009). Los taninos actúan como repelentes alimenticios y también son reconocidos por sus propiedades beneficiosas sobre la salud humana.

Aunque la lignina es también considerada un compuesto fenólico, no puede ser obtenida por métodos de extracción convencional y sus características químicas varían respecto a los compuestos fenólicos de bajo peso molecular.

I.5. Caracterización química de la lignina

La lignina es un polímero heterogéneo de alto peso molecular y constituido por unidades de fenilpropano que comparte la misma vía biosintética que los polifenoles extraídos por métodos convencionales (Stevanovic et al., 2009). Durante la evolución, la lignificación de las paredes

vegetales estuvo relacionada al paso de las plantas del medio acuático al terrestre, de hecho, especies acuáticas como las algas no contienen lignina como constituyente químico (Stevanovic, 2005). El desarrollo del sistema de lignificación estuvo influenciado por la necesidad de un sistema excretor mejorado en las plantas superiores. La lignina constituye uno de los componentes vegetales de la pared celular de las células vegetales, en estrecha asociación con la celulosa y la hemicelulosa. Los tejidos lignificados presentan una resistencia superior contra los ataques biológicos, sobre todo de microorganismos, lo que asegura la longevidad de las plantas lignosas.

La lignina no es exclusiva de los árboles, sino que también se presentan en las plantas herbáceas. Los monómeros (monolignoles) precursores de la lignina son derivados del alcohol cinámico: a) el alcohol coniferílico; b) el alcohol sinapílico y, c) alcohol p-cumarílico, cuyas estructuras se muestran en la **Fig. 6**. Estos se forman en el citoplasma a través de la ruta del ácido shikímico que produce fenilalanina como intermediario clave. Los monolignoles se generan mediante reacciones de desaminación, hidroxilación, reducción y metilación catalizadas por diversas enzimas. Estos monolignoles reaccionan en la pared celular, a través de reacciones de oxidación catalizadas por peroxidasas (intermedios radicalarios) para formar finalmente polímeros de lignina.

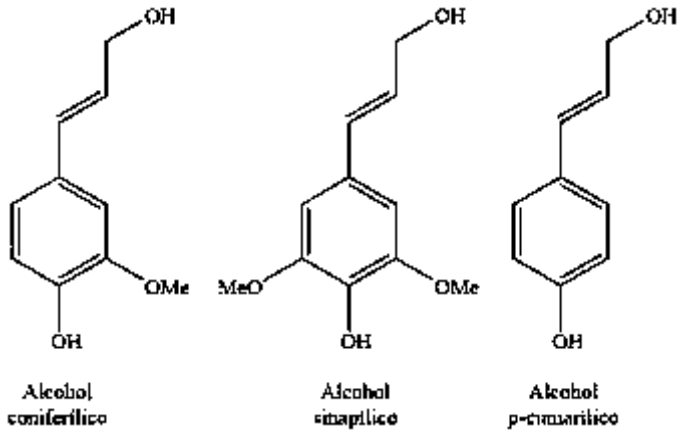

Fig. 6. **Monómeros precursores de la lignina.**

Los monolignoles son dirigidos (temporal y espacialmente) a diferentes tipos de regiones de la pared celular, en las que polimerizan formando biopolímeros con propiedades biofísicas características, los cuales en conjunto refuerzan la pared celular. Las ligninas son consideradas

mezclas racémicas, como se evidencia por análisis de diversos fragmentos diméricos tales como, (±)-pinoresinoles y (±)-siringoresinoles (Chávez-Sifontes & Domine, 2013).

En dependencia del tipo de especie, los porcentajes de distribución de los monolignoles varían (véase **Tabla. 2**). Por ejemplo, el monolignol más abundante de las maderas de especies coníferas es el alcohol coniferílico, que puede llegar a superar el 95% del total de monolignoles presentes, en cambio, en las maderas provenientes de especies latifoliadas coexisten los alcoholes coniferílico y sinapílico (Hon & Shiraishi, 2000). En el caso de plantas herbáceas, puede haber proporciones similares de los tres monolignoles principales. Además, el contenido de lignina también varía entre árboles y las plantas herbáceas, siendo menor en estas últimas (Novaes et al., 2010).

Tabla. 2. **Porcentaje de los diferentes monolignoles presentes en la lignina de diversas plantas.**

Tipo de planta		**Monolignol (%)**		
		Alcohol p-coumarílico	Alcohol coniferílico	Alcohol sinapílico
Gimnospermas	Coníferas	<5	>95	0
Angiospermas	Latifoliadas	0-8	25-50	45-75
	Herbáceas	5-35	35-80	20-55

(Modificado de Gellerstedt & Henriksson, 2008).

La lignina, debido a su alto peso molecular, no puede penetrar las membranas biológicas y consecuentemente no puede modular la activación/desactivación de vías de señalización implicadas en la patogénesis de enfermedades. Sin embargo, esta puede ser degradada térmicamente por pirólisis para producir fenoles de bajo peso molecular (mono y oligo-fenoles), también conocidos como fenoles pirolíticos (Liaw et al., 2013). Estos fenoles pirolíticos pueden ser separados fácilmente por precipitación en agua (Scholze & Meier, 2001). La producción de polifenoles por vía térmica es un medio de obtención de estas moléculas a muy bajo precio.

I.6. La pirólisis: un medio para obtener aceites pirolíticos ricos en compuestos fenólicos

La pirólisis consiste en la descomposición térmica de un sólido que ocurre en ausencia de oxígeno o agentes oxidantes, resultando en la producción de gases, líquidos (aceites pirolíticos o alquitrán) y un carbonizado. Durante el proceso, la temperatura se incrementa localmente, por

lo que primeramente ocurre la evaporación de la humedad (etapa de secado), mientras que posteriormente ocurre la liberación de volátiles (primera etapa de la pirólisis). Estos compuestos volátiles se producen a partir de la ruptura térmica de los enlaces químicos de la celulosa, la hemicelulosa y la lignina, así como los extractivos, cada uno de los cuales tiene sus propias características cinéticas (Oasmaa et al., 2016). La **Fig. 7** muestra las reacciones de descomposición que tienen lugar en una partícula de biomasa sometida a pirólisis.

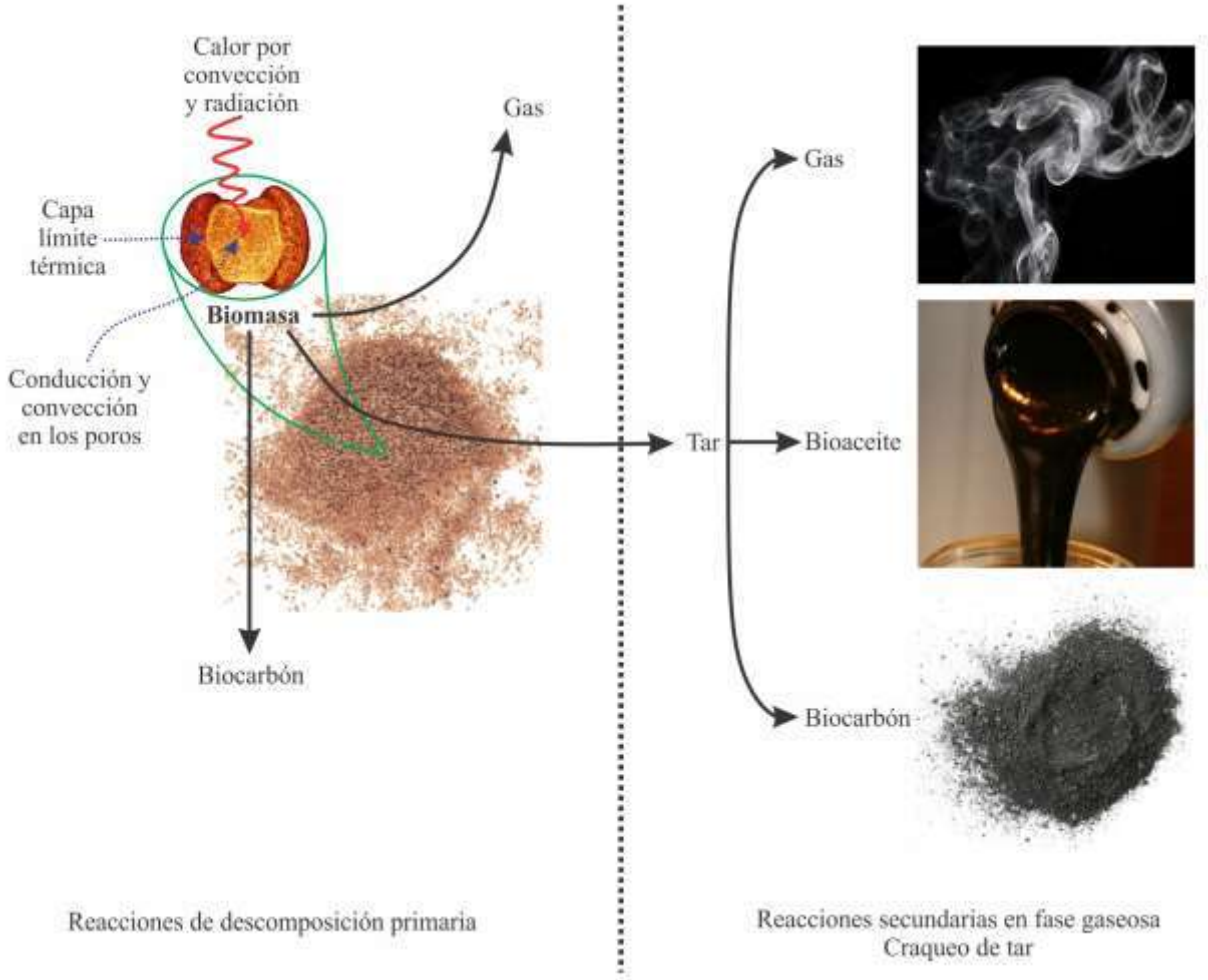

Fig. 7. Pirólisis de una partícula de biomasa.

En el esquema se pueden observar los principales mecanismos de calentamiento, descomposición y los productos del proceso de pirolisis de biomasa (Modificado de Montoya Arbeláez et al., 2013).

Considerando la temperatura y la velocidad de calentamiento, la pirólisis puede subdividirse en: lenta o convencional, rápida, carbonización y torrefacción. La **Tabla 3** muestra las características de cada uno de los tipos antes mencionados.

Tabla 3. Tipos de pirólisis y productos principales.

Tipo de pirólisis	Condiciones	Líquido (aceites pirolíticos)	Sólido	Gas
Lenta/intermedia	Temperatura del reactor: 400-500ºC Tasas de calentamiento: 1-1000 ºC/s Tiempos de residencia: 1-10 s	50%	25%	25%
Rápida	Temperatura del reactor: 500 ºC Tasas de calentamiento: >1000 ºC/s Tiempos de residencia: ~ 1s	75%	12%	13%
Torrefacción	Temperatura del reactor: 290 ºC Tasas de calentamiento: 1ºC/s Tiempos de residencia: ~ 30 min	0-5%	77%	23%
Carbonización	Temperatura del reactor: 400-500ºC Tasas de calentamiento: 1 ºC/s Tiempos de residencia: horas, días	30%	35%	35%

(Tomado de Montoya Arbeláez et al., 2013).

Los aceites pirolíticos son mezclas multicomponentes que contienen diferentes tipos de moléculas primarias derivadas de las reacciones de despolimerización y fragmentación de la celulosa, la hemicelulosa y la lignina. Una de las aplicaciones industriales más importantes de estos aceites es el desarrollo de biocombustibles. El contenido de oxígeno de los aceites pirolíticos suele ser del 35-40% (Czernik & Bridgwater, 2004). La presencia de oxígeno en los aceites pirolíticos es la razón principal de las diferencias en las propiedades y el comportamiento observados entre los combustibles fósiles y los bio-aceites. El alto contenido de oxígeno da como resultado un bajo poder calorífico, por lo que esta característica podría considerarse una desventaja para el uso de aceites pirolíticos como biocombustibles. El oxígeno está presente en más de 300 compuestos identificados en los aceites pirolíticos (Czernik & Bridgwater, 2004), siendo los compuestos fenólicos de los compuestos oxigenados más importantes (2-20% w/w), los que se generan principalmente de la despolimerización de lignina y que están representados por mono fenoles (~5-10 %) y oligómeros (~6-15%) (Oasmaa et al., 2016).

Si bien para el desarrollo de combustibles la presencia de estas moléculas en los aceites pirolíticos puede ser un problema y, por lo tanto, se han utilizado múltiples estrategias de desoxigenación, podría ser ventajoso para el desarrollo de nuevos fármacos. La pirólisis es una forma muy económica de obtener fenoles a partir de lignina con altos rendimientos. Un aspecto

interesante es que puede llevarse a cabo después de la extracción convencional, lo que permite el uso integral de la matriz vegetal extraída, frecuentemente considerada como un desecho.

I.7. Uso de productos pirolíticos para el tratamiento de la psoriasis

La utilización de aceites pirolíticos en el desarrollo de medicamentos como fuentes valiosas de moléculas bioactivas ha sido soslayada por la comunidad científica. Sin embargo, el uso de otros productos pirolíticos como el alquitrán para tratar afecciones dermatológicas se remonta a más de 2000 años, mientras que el alquitrán de hulla, generado a partir de la pirólisis lenta del carbón, se ha utilizado precisamente para la psoriasis durante más de 100 años (Zeichner, 2010).

El régimen de Goeckerman, actualmente utilizado para la psoriasis, que consiste en la exposición a la radiación ultravioleta B combinada con la aplicación de alquitrán de hulla, es un ejemplo de la importancia que los productos pirolíticos pueden tener como terapias antipsoriásicas eficaces. Introducido por primera vez en la terapéutica en 1925, el régimen de Goeckerman sigue siendo una de las opciones de tratamiento más antiguas y confiables para pacientes con psoriasis moderada a severa (Zhu et al., 2016). La ventaja de usar alquitrán y fototerapia juntos es que el alquitrán es un fotosensibilizador y cuando se combina con la luz UVB actúa sinérgicamente para producir mejores resultados que cuando se usan cualquiera de los tratamientos solos. En comparación con otras modalidades de tratamiento, como los agentes biotecnológicos, los medicamentos sistémicos y los tratamientos tópicos, la terapia Goeckerman sigue siendo extremadamente efectiva con relativamente pocos efectos secundarios. Esto la convierte en una excelente alternativa para pacientes que pueden haber fracasado previamente con otras terapias, incluyendo ancianos, pacientes embarazadas, niños e individuos con inmunodepresión (Zhu et al., 2016).

Se ha reportado que el 100% de los pacientes que reciben el régimen de Goeckerman durante un período de 12 semanas logran una mejora del 75% o más en sus lesiones de psoriásicas (Lee & Koo, 2005). Otra ventaja de este tratamiento es el largo período de remisión después de la finalización de la terapia, que puede durar entre 8 meses y más de un año, en dependencia del tipo de paciente (Koo & Lebwohl, 1999). Los estudios también han demostrado que la terapia de Goeckerman puede aumentar significativamente la satisfacción de los pacientes hacia su tratamiento, mejorando su calidad de vida (Chern et al., 2011). Además, puede usarse para tratar otras enfermedades de la piel, como eccema, prurigo nodular y prurito.

El alquitrán de hulla consta de miles de componentes químicos, muchos de los cuales no están identificados. De estos ingredientes, se ha concedido mayor importancia farmacológica a los hidrocarburos de arilo policíclicos. Se cree que estos hidrocarburos, particularmente el carbazol, son responsables de la eficacia del alquitrán como agente antipsoriásico (Sekhon et al., 2018). Aunque su mecanismo de acción no está completamente dilucidado, se presume que el alquitrán de hulla activa el receptor de hidrocarburos de arilo (AhR), lo que resulta en la inducción de la diferenciación epidérmica. La eliminación de AhR en ratones revirtió completamente este efecto, destacando la importancia de este receptor en la homeostasis cutánea (Bogaard et al., 2013). Un estudio ulterior demostró que los ligandos activadores de AhR como el alquitrán reducen la inflamación en la piel psoriásica, mientras que los antagonistas de AhR aumentan la inflamación (Di Meglio et al., 2014).

Sin embargo, mucho queda aún por conocer acerca de la señalización mediada por este receptor y se desconoce si otros productos pirolíticos como los aceites pirolíticos derivados de nuevas fuentes naturales, podrían tener un potencial antiinflamatorio sobre dianas relevantes para la psoriasis, como la IL-17.

La presente investigación siguió una estrategia de desarrollo farmacéutico, usando el enfoque etnofarmacológico con el fín de identificar especies mexicanas prometedoras para tratar la psoriasis, a partir de la etnomedicina Purépecha. Los aceites pirolíticos y extractos acuoso y etanólico de especies relevantes fueron químicamente caracterizados y analizados respecto a su toxicidad y capacidad de inhibir la producción de IL-8 en queratinocitos HaCaT estimulados con IL-17. La investigación presentada aporta elementos que sirven para validar la información etnomédica reportada por la medicina tradicional Purépecha y proponer futuras aplicaciones para el desarrollo de nuevos tratamientos para la psoriasis.

II. JUSTIFICACIÓN

La psoriasis es una enfermedad dermatológica inflamatoria incurable que afecta a 120 millones de personas a nivel mundial y a más de 3 millones de mexicanos. La psoriasis tiene un impacto negativo en la calidad de vida de los pacientes, tanto que sus repercusiones en las funciones físicas y psicológicas se han comparado con las que genera el cáncer, la diabetes o la depresión.

Los tratamientos antipsoriásicos actuales se orientan a tratar la sintomatología, pero su uso a largo plazo se ve limitado por su falta de eficacia, costo y toxicidad. Por consiguiente, existe una búsqueda incesante por parte de la industria farmacéutica de nuevos candidatos terapéuticos. Durante este proceso, la identificación de dianas farmacológicas prometedoras resulta crucial. En los últimos años, se ha considerado que la inhibición de la IL-17 o su receptor representa una opción para tratar la psoriasis, y en consecuencia han sido diseñados medicamentos biotecnológicos novedosos, con alta eficacia. Sin embargo, los eventos adversos, los altos costos y la falta de respuesta en algunos pacientes limitan su uso. Por lo tanto, la búsqueda de agentes terapéuticos anti-IL17 más asequibles, efectivos y seguros está en curso. El desarrollo de nuevos productos naturales tópicos que muestren efectos anti-IL-17 ofrece varias ventajas en comparación con los productos biotecnológicos con respecto a su administración, ya que estos últimos, a diferencia de las moléculas naturales, no pueden cruzar el estrato córneo, lo que impide su uso en formulaciones tópicas, preferidas por los pacientes que tienen psoriasis leve y moderada (~80 %). En consecuencia, deben identificarse nuevos productos naturales siguiendo esquemas de desarrollo farmacéutico con vistas a garantizar la caracterización química rigurosa, eficacia y seguridad de los candidatos en estudio.

Una de las estrategias más usadas para este fin, utiliza el enfoque etnofarmacológico para la identificación de especies de interés. Siguiendo esta estrategia, las especies elegidas son aquellas que han demostrado eficacia terapéutica como consecuencia de su utilización tradicional por la población. México es un país con una impresionante riqueza cultural y alta diversidad florística. Diversas regiones del territorio nacional, como la Meseta Purépecha, son reconocidas por su conocimiento ancestral de prácticas de la Medicina Tradicional, lo que representa una oportunidad única para la identificación de plantas medicinales como candidatas terapéuticas para la psoriasis. A pesar de la importancia de la medicina tradicional Purépecha, ningún estudio

etnofarmacológico ha sido reportado sobre el uso de plantas para tratar afecciones dermatológicas por esta población. Por lo tanto, se requiere documentar este conocimiento etnomedicinal y preservarlo para las futuras generaciones. En los sistemas tradicionales, son frecuentes el uso de extractos acuosos y etanólicos para tratar patologías dermatológicas dada la baja toxicidad y asequibilidad de los solventes involucrados. Estos solventes permiten extraer una pléyade de metabolitos secundarios, dependiendo de la planta de que se trate, destacándose los compuestos fenólicos. Aunque las propiedades biológicas antipsoriásicas de los extractos etanólicos y acuosos de múltiples plantas usadas en la medicina tradicional a nivel mundial son reconocidas, las plantas nativas de México de importancia etnomedicinal para tratar afecciones dermatológicas continúan sin ser suficientemente exploradas.

Los compuestos fenólicos pueden ser obtenidas usando métodos de extracción convencional con solventes polares o ser generados como parte de bioaceites a partir de la pirólisis de la lignina. El uso de aceites pirolíticos como fuente valiosa de moléculas bioactivas para el desarrollo de nuevos tratamientos para la psoriasis ha sido soslayado por la comunidad científica, a pesar de que el alquitrán de hulla, obtenido a partir de la pirólisis lenta del carbón, se ha utilizado precisamente para esta enfermedad durante más de 100 años. Hoy día, las propiedades antiinflamatorias sobre dianas relevantes para la psoriasis, como la IL-17, de los aceites pirolíticos y de extractos provenientes de plantas mexicanas, son casi desconocidas, aunque es probable que sean comparables, pero aún se necesitan más investigaciones multidisciplinarias para demostrarlo.

III. HIPÓTESIS

Los extractos etanólico, acuoso y los aceites pirolíticos de una planta herbácea y un árbol, nativos de México, de alto valor de uso por la etnomedicina Purépecha, poseen, sobre los queratinocitos humanos, un potencial antinflamatorio anti-IL17, una diana farmacológica implicada en la patogénesis de la psoriasis.

IV. OBJETIVOS

IV.1. Objetivo general

Determinar el potencial antinflamatorio anti-IL17 en queratinocitos humanos del extracto etanólico, acuoso y de aceites pirolíticos de una planta herbácea y un árbol con alto valor de uso por la etnomedicina Purépecha para el tratamiento de enfermedades dermatológicas.

IV.2. Objetivos particulares

IV.2.1. Identificar plantas utilizadas por la etnomedicina Purépecha para el tratamiento de afecciones dermatológicas y seleccionar una especie herbácea y un árbol de alto valor de uso nativos de México como candidatos antipsoriásicos.

IV.2.2. Realizar la caracterización química del extracto acuoso, etanólico, sus fracciones, así como de los aceites pirolíticos de las especies seleccionadas.

IV.2.3. Determinar la toxicidad y el potencial antiinflamatorio anti-IL17 de las muestras obtenidas en queratinocitos HaCaT y seleccionar el candidato terapéutico más prometedor.

V. ESTRATEGIA EXPERIMENTAL

La estrategia experimental llevada a cabo en el presente trabajo de investigación se representa en la **Fig. 8**.

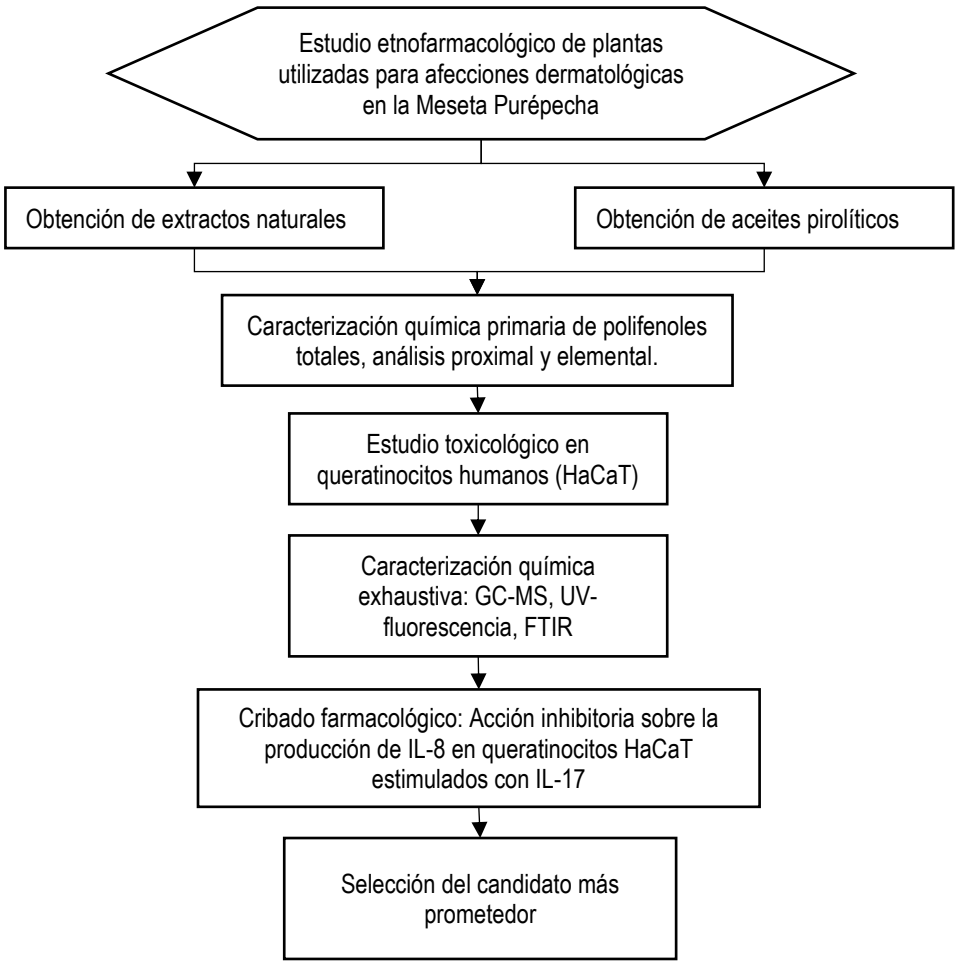

Fig. 8. Estrategia metodológica empleada en el presente trabajo.

VI. RESULTADOS

Los resultados generados con la realización del presente trabajo se presentan en los capítulos siguientes:

- Capítulo 1. Flora etnomedicinal utilizada para el tratamiento de afecciones dermatológicas en la Meseta Purépecha, Michoacán, México. (Artículo publicado)

- Capítulo 2. Extracto etanólico, fracciones y bio-aceites de las partes aéreas de *Urtica subincisa*: un análisis comparativo de su composición química, toxicidad y actividad anti-IL17 en los queratinocitos HaCaT.

- Capítulo 3. Aceites pirolíticos de la corteza de *Amphipterygium adstringens* inhiben la producción de IL-8 en queratinocitos HaCaT estimulados por IL-17. (Artículo publicado)

- Capítulo 4. Caracterización química, toxicología y actividad antiinflamatoria del extracto acuoso de la corteza de *Amphipterygium adstringens* y sus fracciones en queratinocitos HaCaT.

Además, se presenta una revisión de la literatura que complementa el trabajo desarrollado:

- Anexo 1 - La psoriasis: de la investigación básica y clínica al desarrollo de nuevos tratamientos. (Artículo publicado)

CAPÍTULO 1. Flora etnomedicinal utilizada para el tratamiento de afecciones dermatológicas en la Meseta Purépecha, Michoacán, México

VI.1. PREFACIO

Este capítulo es el tema de un artículo titulado: “Ethnomedicinal plants used for the treatment of dermatological affections on the Purépecha Plateau, Michoacán, Mexico”. El cual fue publicado en *Acta Botanica Mexicana*, 125: 95-132. Su identificador de objeto digital es: https://doi.org/10.21829/abm125.2018.1339

El capítulo se dirige a dar respuesta al objetivo particular # 1, relacionado con la identificación de plantas utilizadas en la etnomedicina Purépecha para el tratamiento de afecciones dermatológicas y permitió la selección de una especie herbácea (*Urtica subincisa*) y un árbol (*Amphipterygium adstringens*) nativos de México de alto valor de uso como candidatos antipsoriásicos, los que se utilizaron en el resto de las investigaciones.

Fig. 9. Resumen gráfico.

Acta Botanica Mexicana 125: 95-132 October 2018

Research article

Ethnomedicinal plants used for the treatment of dermatological affections on the Purépecha Plateau, Michoacán, Mexico

Flora etnomedicinal utilizada para el tratamiento de afecciones dermatológicas en la Meseta Purépecha, Michoacán, México

Roberto Esquivel-García[1], Emmanuel Pérez-Calix[2], Alejandra Ochoa-Zarzosa[3], Martha-Estrella García-Pérez[1,4]

1 Universidad Michoacana de San Nicolás de Hidalgo, Facultad de Químico Farmacobiología, Morelia, Michoacán, Mexico.
2 Instituto de Ecología, A.C., Red de Diversidad Biológica del Occidente Mexicano, Pátzcuaro, Michoacán, Mexico.
3 Universidad Michoacana de San Nicolás de Hidalgo, Centro Multidisciplinario de Estudios en Biotecnología-Facultad de Medicina Veterinaria y Zootecnia, Tarímbaro, Michoacán, Mexico.
4 Author for correspondence: margarc@live.ca

Received: January 8, 2018.
Reviewed: February 13, 2018.
Accepted: April 24, 2018.
Online first: August 8, 2018.
Published: October 3, 2018.

To cite as:
Esquivel-García, R., E. Pérez-Calix, A. Ochoa-Zarzosa and M.-E. García-Pérez. 2018. Ethnomedicinal plants used for the treatment of dermatological affections on the Purépecha Plateau, Michoacán, Mexico. Acta Botanica Mexicana 125: 95-132. DOI: 10.21829/abm125.2018.1339

DOI:
10.21829/abm125.2018.1339

Abstract:

Background and Aims: Inhabitants of the Purépecha Plateau preserve an excellent ancestral knowledge on medicinal plants used for dermatological affections, which has not been documented. An ethnopharmacological survey was carried out in this region to gather information on the use of medicinal plants and herbal preparations for treating dermatological affections, to disseminate the Purépecha indigenous knowledge and identifying promising plants for developing new formulations for cutaneous conditions.
Methods: The study was conducted in the 21 municipalities that compose the Purépecha Plateau. A total of 86 local inhabitants (62 women and 24 men) were interviewed. The data were quantitatively analyzed through the determination of the use value, fidelity level and informant consensus factor.
Key results: A total of 97 plant species belonging to 47 families were documented for treating 19 dermatological conditions on the Purépecha Plateau. Asteraceae was the leading family among the collected medicinal plants (20.61%), followed by Lamiaceae (13.40%) and Solanaceae (5.15%). The largest number of plants was used for the treatment of cuts (40.20%), skin inflammation (37.11%) and rash (37.11%). The aerial parts were the most commonly used (34.75%). The medicinal plant species with larger use values were *Heterotheca inuloides* (0.53), *Aloe vera* (0.37) and *Oenothera rosea* (0.21). The comparison of results with ethnomedicinal literature worldwide revealed that 8.25% of plants used on the Purépecha Plateau were recorded for the first time for the treatment of dermatological affections.
Conclusions: This study provides new information on medicinal plants used on the Purépecha Plateau to treat cutaneous diseases. Future pharmacological and toxicological investigations are required to demonstrate the efficacy and safety of these species for treating dermatological affections.
Key words: dermatological diseases, ethnomedicine, herbal preparations, Purépecha, skin.

Resumen:

Antecedentes y Objetivos: Los habitantes de la Meseta Purépecha conservan un excelente conocimiento ancestral sobre plantas medicinales utilizadas para tratar afecciones dermatológicas que no ha sido documentado. Un estudio etnofarmacológico fue realizado en esta región para recopilar información sobre el uso de plantas medicinales y preparaciones herbarias utilizadas en la zona para el tratamiento de afecciones dermatológicas, con el objetivo de difundir la medicina tradicional Purépecha e identificar plantas prometedoras en el desarrollo de tratamientos para afecciones cutáneas.
Métodos: El estudio se realizó en los 21 municipios que componen la Meseta Purépecha. Se entrevistó a un total de 86 habitantes locales (62 mujeres y 24 hombres). Los datos se analizaron cuantitativamente mediante la determinación del valor de uso, nivel de fidelidad y el factor de consenso informante.
Resultados clave: En total 97 especies de plantas pertenecientes a 47 familias fueron documentadas para el tratamiento de 19 afecciones dermatológicas en la Meseta Purépecha. Asteraceae fue la familia principal entre las plantas colectadas (20.61%), seguida de Lamiaceae (13.40%) y Solanaceae (5.15%). La mayor cantidad de plantas se utilizó para tratamiento de heridas (40.20%), inflamación de la piel (37.11%) y erupciones cutáneas (37.11%). La principal parte utilizada de la planta fue la aérea (34.75%). Las plantas medicinales con mayores valores de uso fueron *Heterotheca inuloides* (0.53), *Aloe vera* (0.37) y *Oenothera rosea* (0.21). La comparación de los resultados con la literatura etnomedicinal reveló que 8.25% de las plantas utilizadas en la Meseta Purépecha se registraron por primera vez para tratamiento de afecciones dermatológicas.
Conclusiones: Este estudio proporciona nueva información sobre plantas medicinales utilizadas en la Meseta Purépecha para tratamiento de enfermedades cutáneas. Se requieren futuras investigaciones farmacológicas y toxicológicas para demostrar la eficacia y seguridad de estas especies para el tratamiento de afecciones dermatológicas.
Palabras clave: enfermedades dermatológicas, etnomedicina, preparaciones herbarias, Purépecha, piel.

INTRODUCTION

Skin diseases have been considered as the fourth leading cause of nonfatal disease burden worldwide (Hay et al., 2014). These conditions are widespread, being one of the most prevalent and disabling diseases and a source of considerable loss of life quality of patients. In rural populations, they are supposed to be one of the most common ailments (Policepatel and Manikrao, 2013). Skin affections have been related to clinical depression, anxiety and suicidal ideation, which represent a major additional problem with a significant negative impact for health systems (Dalgard et al., 2015).

Although in Mexico there are no extensive studies about the prevalence of skin diseases, the main reasons for dermatological consultation in the country are related with acne vulgaris, followed by psoriasis and melasma (HGM-SSA, 2012). In rural zones, the most frequent skin diseases are parasitic, viral and fungal infections as well as dermatitis and eczema (Paek et al., 2012).

Low adherence to treatments has been described for skin ailments (Storm et al., 2008; Augustin et al., 2011). Consequently, people frequently use natural products, mainly in undeveloped areas in which traditional herbal medicines play an important role in the management of these conditions (Sharma et al., 2014). In addition, many dermatological diseases have no cure (psoriasis, melanoma, pemphigus, etc.), so it is important to identify promising plants used in traditional medical systems to develop new therapeutic candidates. The ethnomedical studies remain very important in the discovery of new drugs and in the development of improved herbal preparations for use by local people (Heinrich and Gibbons, 2001).

The state of Michoacán, bordered by the states of Colima, Jalisco, Guanajuato, Querétaro, México and Guerrero is one of the regions of Mexico with the greatest diversity of plant species, many of which used for medicinal purposes (Medina, 2003). In pre-Hispanic times, this region was the home of the Purépecha Empire, which was centered on the Pátzcuaro lake basin. At that time, the Purépechas used copper agricultural tools in an exceptional way, compared to other Mesoamerican cultures (Arnézcua Luna and Sánchez Díaz, 2015). They had a social stratification represented by the king, followed by knights, priests, and communities of farmers, fishermen, artisans and merchants (Arnézcua Luna and Sánchez Díaz, 2015). Nowadays, the Purépecha people live mostly in the highlands of Central Michoacán, with a very important number of them on the Purépecha Plateau. The Purépechas preserve their language, traditions and customs, including an excellent ancestral knowledge of medicinal plants to treat various human health problems (INEGI, 2004).

The Purépecha Plateau is a rich reservoir of biodiversity which includes woody and herbaceous plants, the most important belonging to the genera *Quercus* L., *Salvia* L., *Gnaphalium* L., *Senecio* L., *Eupatorium* L., *Bidens* L., *Tagetes* L., *Stevia* Cav., *Pinus* L., *Cirsium* Mill., *Adiantum* L. and *Dalea* L. (Bello-González et al., 2015). Today, the participation of traditional Purépecha therapists in health services in this region is very important, mainly in those places where the public health system is still deficient (BDMTM, 2009).

Despite the importance of folk medicine by Purépechas, no ethnopharmacological studies are available about the use of plants for the treatment of dermatological conditions by this population. Therefore, it is considered that the documentation of Purépecha ethnomedicinal knowledge is required to identify promising plants for developing new therapeutic candidates or improved herbal preparations in the future.

Keeping the previously mentioned facts in mind, the present study was undertaken to scientifically enumerate medicinal plants and herbal preparations used by inhabitants of the Purépecha Plateau for the treatment of dermatological affections. Additionally, the ethnomedicinal studies performed on these plants are discussed to know whether other communities worldwide use the plants here reported for treating skin conditions, to estimate the contribution of this investigation to the global ethnopharmacological knowledge. Additionally, this study allows for the identification of plants to treat skin pathologies, which opens new perspectives for performing future phytoche-

mical, toxicological and pharmacological investigations with extracts or pure compounds from natural origin.

Materials and Methods

Study area

The state of Michoacán, formally "Michoacán de Ocampo", is situated in the western part of Mexico between 20°23'27" and 17°53'50"N latitude; 100°03'32" and 103°44'49"W longitude, with a surface of 59,864 km², which represents 3.04% of the Mexican territory (Mercado-Vargas and Palmerín Cerna, 2012). The state is very rich in biological diversity with around 5000 plant species described, several of them with medicinal properties (Medina, 2003). Additionally, it possesses a unique cultural heritage regarding the use of different natural preparations for many illnesses.

The present study was conducted on the Purépecha Plateau, which is located in the central-northwestern part of Michoacán, belonging to the Trans-Mexican Volcanic Belt. The area includes mountains and volcanoes such as Paricutin, Tancítaro, San Marcos and Comburindos. Its height ranges from 1700 to 3200 m. The annual mean temperature varies from 12-18 °C, with rainfall concentrated in the summer (Orduña Trejo et al., 1999). The dominant vegetation of this zone is mainly composed by *Pinus* and species of *Quercus* and *Abies* Mill. In the northern part and in the northeastern area of the Purépecha Plateau, tropical deciduous forests are formed by species belonging to *Bursera* Jacq. ex L., *Acacia* Mill., *Opuntia* Mill., *Tagetes*, *Melampodium* L. and *Euphorbia* L., among others (Orduña Trejo et al., 1999).

Twenty-one municipalities compose the Purépecha Plateau (surface area, 7348.64 km²): (1) Charapan, (2) Cherán, (3) Chilchota, (4) Erongaricuaro, (5) Los Reyes, (6) Nahuatzen, (7) Nuevo Parangaricutiro, (8) Paracho, (9) Pátzcuaro, (10) Peribán, (11) Quiroga, (12) Tancítaro, (13) Tangamandapio, (14) Tangancícuaro, (15) Tingambato, (16) Tingüindín, (17) Tocumbo, (18) Tzintzuntzan, (19) Uruapan, (20) Zacapu, and (21) Zinacuarétiro. All of them were visited to record the ethnomedicinal information for this study (Fig. 1). The most important reasons that were considered to choose this zone were: a) the important presence of the Purépecha population on the Plateau (78.86% of its inhabitants) (CDI, 2010) with an inherited knowledge of medicinal plants, b) the high variety of plant species in this region, c) the significant impact of folk medicine on the health services, and d) the fact that no ethnopharmacological studies related to the treatment of dermatological conditions had been performed in the zone.

Studied population characteristics

Thirty trips were undertaken all over the Purépecha Plateau from May 2015 to October 2016, to collect information on ethnomedical plants. This period included both dry and rainy seasons. The people from this region were very accessible. Among municipalities, there is variation in the way of dressing, although some elements prevail. It has been noted that the traditional clothing for a woman consists in the use of an apron, often embroidered, as well as an embroidered back strap, woven belt, pleated skirt, embroidered blouse, and a mantle ("rebozo"). Indeed, the "rebozo" is more than a clothing to protect Purépecha women from the cold, its diversity (colorful, embroidered or frayed) is a symbol of their culture (Anázcua Luna and Sánchez Díaz, 2015). The man's outfit consists of a hand-woven sash, a blanket suit, and a hat for daily use. The most important economic activities of this region are the agriculture, forestry and the craft industry (BD-MTM, 2009). On the Plateau, 75% of habitants are bilingual, speaking both Spanish and Purépecha, and 25% speak Purépecha only (Anázcua Luna and Sánchez Díaz, 2015). This language has been classified as isolated, as no similitudes have been found with other Mesoamerican languages (Cambell, 1997).

Although there are public health services within the Plateau, most Purépechas trust traditional medicine as having positive effects on health. Currently, there are two organizations of Purépecha physicians: indigenous doctors from the 11 towns (Acachuén, Carapan, Chilchota, Etúcuaro, Huáncito, Ichán, Santo Tomás, Tacuro, Tanaquillo, Urén y Zopoco). The first institution, based in Cherán, is the "Clínica del Instituto Nacional Indigenista"

Figure 1: Study area. The diamond-shaped symbols indicate the location where ethnopharmacological surveys were conducted.

(Gallardo Ruiz, 2002); while the second association, currently located in Pátzcuaro, is the "Unidad de Medicina Tradicional". In descending order of importance for Purépechas, these traditional doctors are: healers, midwives, masseurs, sorcerers, bonesetters, herbalists and persons exclusively dedicated to the treatment of baby´s fallen fontanelle (BDMTM, 2009).

Ethnomedical survey and plant collection

The identification of "key informants" was performed as suggested by Bhat et al. (2014), considering the information obtained after discussion with local village people, who recommended these individuals, based on their knowledge regarding the use of plants for treating dermatological affections. Participants were clearly informed about the objec-

tive of the study and the survey was achieved only when they verbally consented to participate. All of them were also informed about their right to withdraw from the study at any time. Participants were asked about the tissue, method of preparation and mode of administration of plants used for the treatment of skin conditions using a semi-structured questionnaire. Information about age, sex and occupation of informants was compiled and analyzed as shown in Figure 2. Considering that all participants were bilingual and nobody requested to be interviewed in the Purépecha language, the interviews were conducted in Spanish.

The plant specimens collected during the survey were taxonomically identified, and properly labeled with the following data: habitat and size of the organism, flower and fruit color, date of collection, locality, municipality, collection site, altitude, geographical coordinates, plant uses, collector and collection number.

Taxonomic identification was realized based on specialized literature on the sampled taxa and floras of central and western Mexico. In support of identification, the specimens were compared to samples of the herbarium IEB of the Instituto de Ecología, A.C., in Pátzcuaro, Michoacán. Samples identified in the study were deposited in this herbarium with their respective voucher number (Appendix).

Statistical analysis and literature review

The use value (UV) was calculated for each of the recorded plant species, as proposed by Ferreira et al. (2009). This method allows quantitative evaluation of the relative importance of a determined medicinal plant, considering its use among informants (Ferreira et al., 2009). Higher UV values mean that participants attribute a great significance to a plant, with the advantage that it does not de-

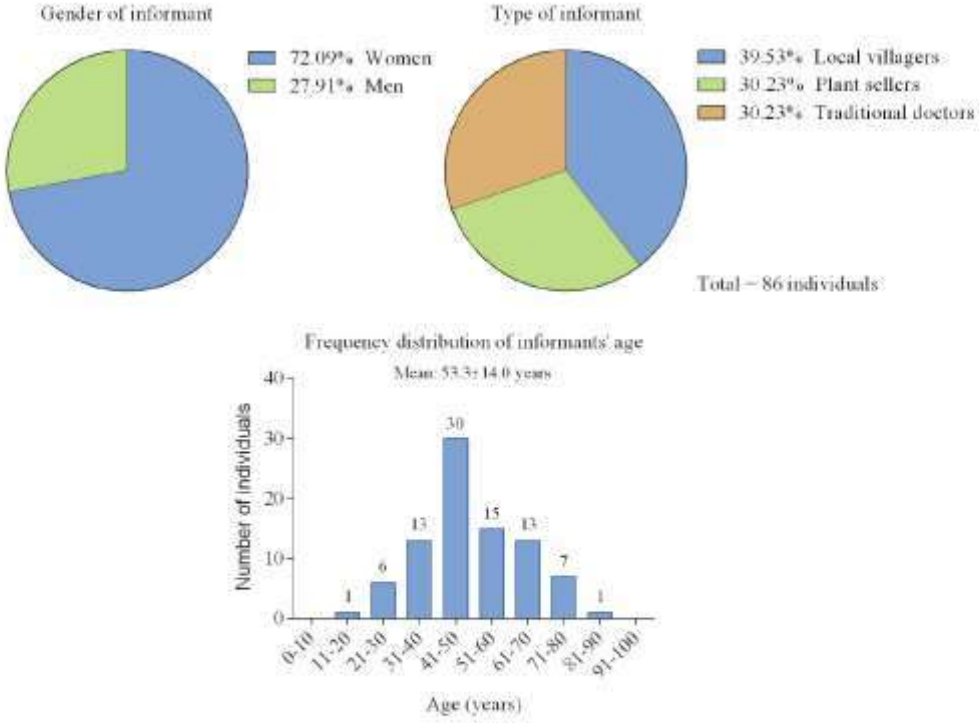

Figure 2: Characteristics of the participants in the ethnopharmacological study on the Purépecha Plateau.

pend on the researcher opinion. The following equation shows the formula for the evaluation of this parameter, where U is the number of times a species is cited and n the informant number.

$$UV = \sum \frac{U}{n}$$

To know how representative a plant was for the treatment of a particular dermatological condition, the fidelity level (FL) was calculated as shown in the next equation (Sharma et al., 2014):

$$FL\,(\%) = \frac{Ip}{Iu} \times 100$$

where Ip is the number of informants who independently indicated the use of a plant for the same particular dermatological condition and Iu the number of informants who mentioned the species for any dermatological condition (Sharma et al., 2014). A plant with high FL (close to 100) is used by traditional healers and people in the community for treating a particular disease. In contrast, a plant with low FL (close to 0.0) indicates that the plant is used to treat diverse diseases (Neamsuvan and Bunmee, 2016).

To estimate the level of agreement between all participants along the Purépecha Plateau about the plant used for each category, the informant consensus factor (F_{ic}) was determined as proposed by Ferreira et al. (2009). Higher values of this factor, close to 1, were related to a greater consensus between participants (Ferreira et al., 2009). Low values of F_{ic} indicate that the participants disagree on the species to be used for treating a particular dermatological affection. The F_{ic} was calculated as follows:

$$F_{ic} = \frac{(n_{ur} - n_t)}{(n_{ur} - 1)}$$

where n_{ur} is the number of reports concerning a dermatological category and n_t the number of species used for a dermatological affection by all the informants.

When all information was compiled, a detailed review was performed to know whether plants reported in this study were native of Mexico or not (Villaseñor, 2016). Moreover, their use in other ethnomedicinal studies worldwide for the treatment of dermatological disorders was also recorded. Literature was searched from various scientific databases such as Google Scholar, Science direct, Scopus and PubMed. Besides these bibliographic sources, the Digital Library of Traditional Mexican Medicine was also consulted to know the contribution of this work to the ethnopharmacological knowledge in Mexico (BDMTM, 2009).

Results

The ethnomedicinal information on plants used to treat dermatological affections was collected from 86 individuals of the Purépecha Plateau; detailed information on these persons is shown in Figure 2. They mentioned 731 plant uses and the specimens of collecting plants corresponded to 97 species belonging to 87 genera and 47 families, which were documented for the first time for the treatment of dermatological conditions in this area. Families frequently used by Purépecha Plateau inhabitants were Asteraceae (20 taxa), Lamiaceae (13) and Solanaceae (5) (Fig. 3), with the genera *Salvia* (4 spp.), *Tagetes* (4), *Allium* L. (2), *Artemisia* L. (2), *Euphorbia* (2), and *Solanum* L. (2) the most represented.

The dermatological conditions were classified into 19 categories according to the symptoms referred by participants as suggested by Sharma et al. (2014). These included blemishes, burns, calluses, cuts, fungal infections, hair fall, hematomas, infections with abscesses, skin inflammation, insect bites, pruritus, psoriasis, rash, skin bumps, skin care, superficial infections, varicella, varicose veins and warts. In this study, F_{ic} values were found to range between 0.14-0.75 (Table 1). The highest values were linked with hematomas (0.75), skin inflammation (0.73), varicose veins (0.73) and cuts (0.70), whereas lowest values were found for psoriasis (0.16) and blemishes (0.14). The highest numbers of plants were used for treating cuts (39 spp.), skin inflammation (36) and rash (36), whereas the treatment of warts (3) and calluses (2) was less reported among participants (Fig. 4).

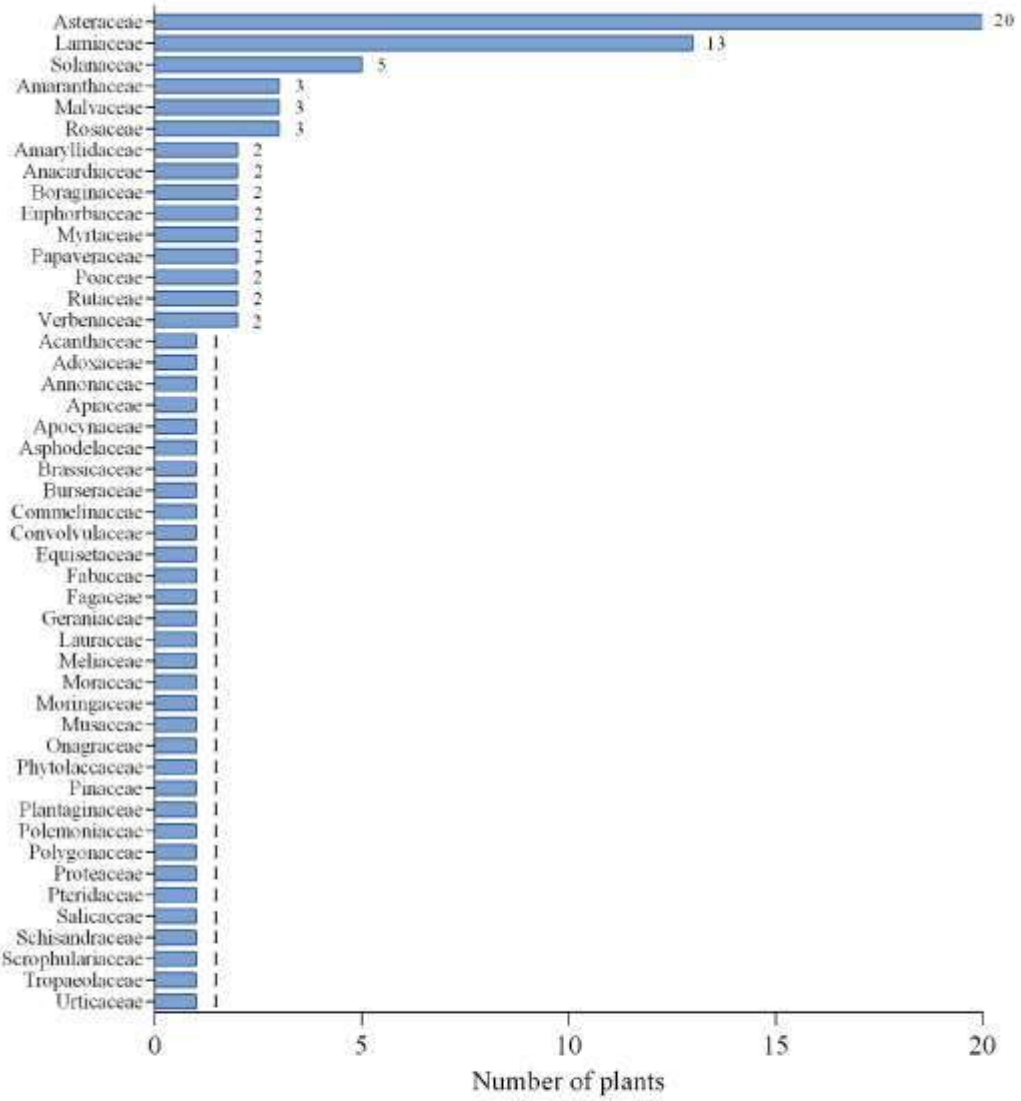

Figure 3: Family distribution of medicinal plants used on the Purépecha Plateau.

As revealed in Figure 5, the aerial parts which consist of a heterogeneous mixture of flowers, leaves and stems were preferred by participants (34.75%), followed by leaves (27.3%) and flowers (13.24%). In relation to the preparation modes, the most important were infusions (52.36%) and decoctions (15.13%). The present study also reports the use of common salt (sodium chloride) in small proportions for the preparation of pastes, particularly with the species *Senecio salignus* DC. and *Sida kaenkeana* C. Presl. Both plants are employed for treating cuts, hematomas, skin inflammation and skin bumps (Appendix).

Native and introduced plants (56.7 and 43.3%, respectively) are a valuable resource for local population for treating dermatological ailments (Appendix). According to

Table 1: Consensus factor values for different dermatological affection categories on the Purépecha Plateau.

Dermatological affection categories	F_{ic}
Hematomas	0.75
Skin inflammation	0.73
Varicose veins	0.73
Cuts	0.70
Calluses	0.67
Warts	0.67
Hair fall	0.66
Rash	0.61
Burns	0.60
Skin bumps	0.59
Skin care	0.57
Fungal infections	0.50
Superficial infections	0.48
Insect bites	0.48
Pruritus	0.46
Infections with abscesses	0.40
Varicella	0.40
Psoriasis	0.16
Blemishes	0.14

the UV, the five most preferred plant species to treat dermatological disorders on the Purépecha Plateau were *Heterotheca inuloides* Cass. (0.53), *Aloe vera* (L.) Burm. f. (0.37), *Oenothera rosea* L'Hér. ex Aiton (0.21), *Calendula officinalis* L. (0.17) and *Urtica subinclusa* Benth. (0.17) (Fig. 6).

Appendix shows the fidelity levels (FL) for each plant recorded in the study. The FL indicates which species are mostly preferred for the study population to treat a given disease. In the present study, 39 species were determined to have the highest fidelity level (FL=100%) for different skin ailments (Appendix). For cuts these plants were *Azadirachta indica* A. Juss., *Commelina diffusa* Burm. f., *Chenopodium ambrosioides* L., *Eriobotrya japonica* (Thunb.) Lindl., *Geronium seemannii* Peyr., *Leonotis nepetifolia* (L.) R. Br., *Piqueria trinervia* Cav., *Sonchus oleraceus* L., *Tagetes lucida* Cav. and *Tagetes remotiflora* Kunze, for fungal infections *Zea mays* L., for hair fall *Buddleja sessiliflora* Kunth and *Verbena carolina* L., for infections with abscesses *Ipomoea murucoides* Roem. & Schult. and *Lepidium virginicum* L., for insect bites *Bresine interrupta* Benth. and *Tanacetum parthenium* (L.) Sch. Bip., for rash *Ceiba aesculifolia* (Kunth) Britten & Baker f., *Euphorbia nutans* Lag., *Lactuca sativa* L. *Montanoa tomentosa* Cerv., *Origanum majorana* L., *Physalis pubescens* L., *Prunus persica* (L.) Batsch, *Psidium sartorianum* (O. Berg) Nied. and *Stevia serrata* Cav., for skin bumps *Euphorbia pulcherrima* Willd. ex Klotzsch, *Ficus carica* L., *Illicium verum* Hook. f. and *Sida haenkeana* C. Presl, for skin care *Salvia leucantha* Cav., for treating skin inflammation *Aloysia citrodora* Palau, *Artemisia ludoviciana* Nutt., *Artemisia mexicana* Willd. ex Spreng., *Eryngium beecheyanum* Hook. f. & Arn., *Marrubium vulgare* L. and *Moringa oleifera* Lam., and finally for superficial infections *Argemone ochroleuca* Sweet, *Bursera bipinnata* (DC.) Engl. and *Eriobotrya japonica*.

The literature review related to ethnopharmacological studies worldwide, focusing on dermatological conditions (96 published articles) and the information contained in the Digital Library of Traditional Mexican Medicine (Appendix), shows that several plants here reported (91.25%) are used by other populations for treating skin affections. Additionally, this study provides new ethnopharmacological information about plants used for treating skin ailments in the study area, as well as at national and global level, as no ethnomedicinal reports were found for *Eryngium beecheyanum*, *Tagetes remotiflora*, *Tournefortia mutabilis* Vent., *Equisetum hyemale* L. var. *affine* (Engelm.) A.A. Eaton, *Chenopodium macrostemum* (Moc. & Sessé ex Benth.) Kuntze, *Salvia leucantha*, *Sida haenkeana* and *Urtica subinclusa* for managing dermatological conditions, which represent 8.25% of the reported plants (Appendix).

Discussion

Mexico is considered one of the countries with the highest floristic richness in the world, only preceded by Brazil.

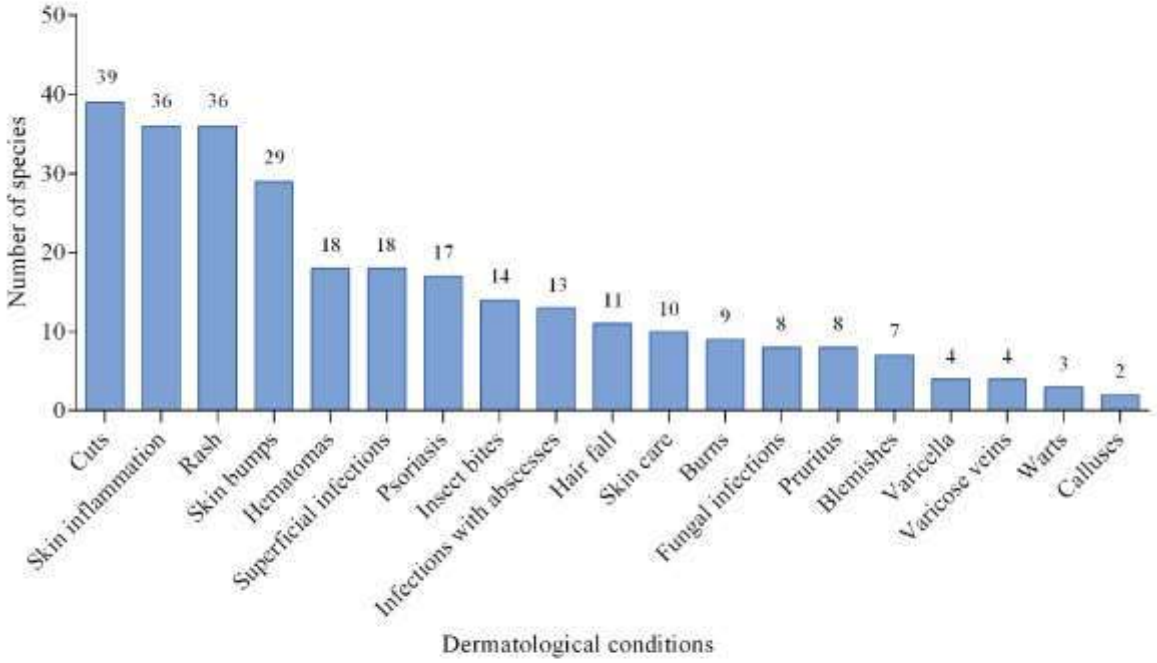

Figure 4: Number of plant species used for treating dermatological conditions on the Purépecha Plateau.

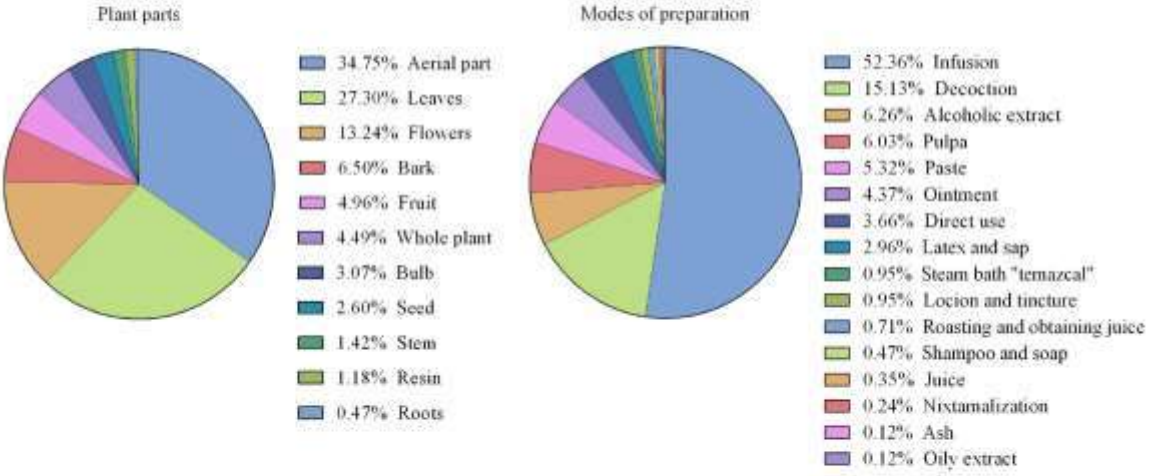

Figure 5: Plant parts and preparation modes used by inhabitants of the Purépecha Plateau for treating dermatological disorders.

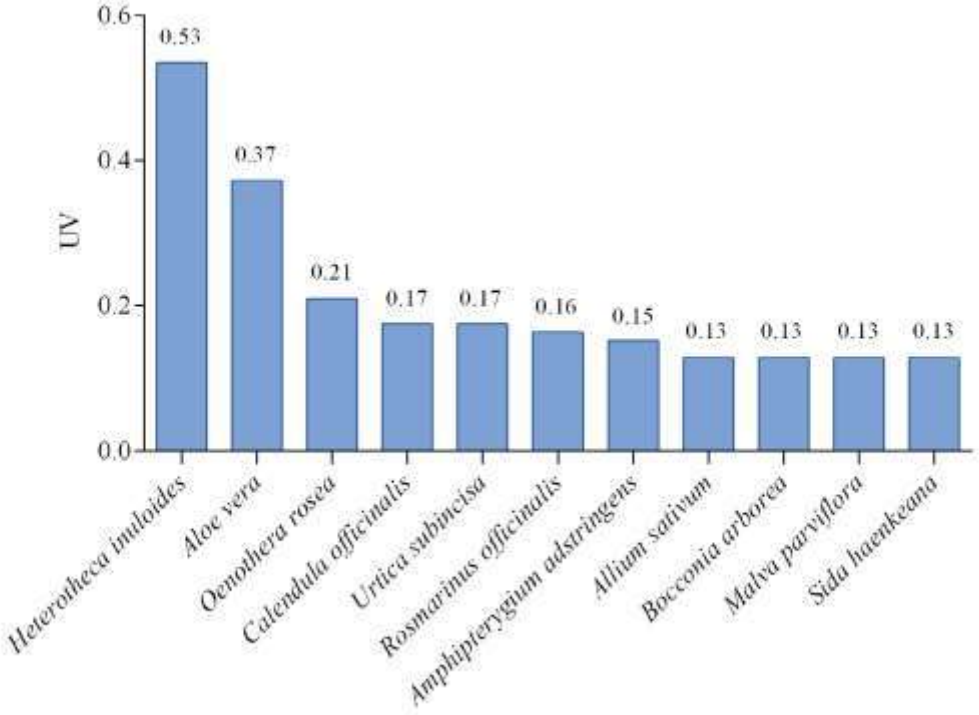

Figure 6: Plants with highest use values (UV) for treating dermatological ailments on the Purépecha Plateau.

China, Colombia and South Africa (Villaseñor, 2016). In this context, the study of ethnomedical systems, including the identification of herbal medicines, is of great importance in addressing health problems of traditional communities. On the Purépecha Plateau in the Mexican state of Michoacán, the knowledge about medicinal plants not only has an anthropological significance, it also allows that people with economic problems solve their health issues.

It is now recognized that the transmission of knowledge regarding the use of medicinal plants from old to new generations is declining (Calvo et al., 2011). This fact is also a reality in indigenous communities because of globalization and the use of orthodox medicine. Therefore, it is crucial to rescue this knowledge to prevent the loss of ethnomedical traditions, which is a precious scientific and cultural heritage for future generations.

The results indicate that among the inhabitants of the Purépecha Plateau, middle-aged people are the ones who best preserve the ethnopharmacological knowledge. It is suggested that there is a tendency towards the loss of these traditions among young persons of less than 30 years (Fig. 2). Juárez-Vázquez et al. (2013) noted that this is a common trend in Mexico. It is also striking that almost all knowledge about plants was obtained from women. This is related to the fact that based on the villager's recommendations, most of the contributors to this study were female. This was not unexpected, because it has been previously reported that among Purépechas, there is a clear quantitative predominance of women engaged in activities related to the use of medicinal plants (Gallardo Ruiz, 2002; BDMTM, 2009).

The present investigation has revealed the medicinal plants for treating skin affections and their modes of

preparation on the Purépecha Plateau, Michoacán, Mexico. The most prominent families were Asteraceae, Lamiaceae and Solanaceae (Fig. 3). These results agree with a previous study performed in the indigenous community of Nuevo Parangaricutiro, a municipality of the Purépecha Plateau, which reported Asteraceae and Lamiaceae as the most important families used for medical purposes in this region (Bello-González and Salgado-Garciglia, 2013). Other ethnomedicinal studies performed in Mexico have also suggested that Asteraceae are an important source of medicinal plants in the country (Andrade-Cetto, 2009; Alonso-Castro et al., 2012), probably due to the high diversity of its phytochemical constituents.

The highest F_{ic} values were linked with hematomas, skin inflammation, varicose veins and cuts, whereas lowest values were found for psoriasis and blemishes (Table 1). It is considered that higher values of this factor are related to prevalent illness in the region, which lead people to exchange information about their knowledge concerning plant therapy for a longer time (Gazzaneo et al., 2005). As previously mentioned, among dermatological conditions, a high morbidity has been reported in the state of Michoacán for venous insufficiency (100.88 per 100,000 population) and wounds (DGE, 2015), which could explain the higher values obtained for the consensus factor in these categories. Lower F_{ic} values for psoriasis and blemishes indicate that plants used for these ailments were chosen randomly or no information about them was exchanged among participants (Neamsuvan and Bunmee, 2016).

According to the information referred by the participants, the higher number of reported plants was used for treating cuts, skin inflammation and rash (Fig. 4). Literature review, regarding ethnopharmacological studies worldwide for dermatological conditions, also shows that many plants are used for cuts, in comparison with other skin ailments (Sharma et al., 2014; Neamsuvan and Bunmee, 2016). A recent study performed among the Zoque indigenous people of Chiapas and Oaxaca, Mexico, also found that among dermatological conditions, most plant remedies are used for wound treatment (Geck et al., 2016). Another possible reason that could explain the preponderance of the use of plants in the region for cuts is the high incidence in Michoacán of morbidities associated with this ailment due to gunshots and dog bites (54.26 and 82.11 per 100,000 population respectively) (DGE, 2015).

Unexpectedly, some participants (mainly sellers and healers) referred during semi-structured interviews to the use of plants to treat specific diseases such as psoriasis (Fig. 4). Psoriasis is considered the second reason for dermatological consultation in Mexico (HGM-SSA, 2012). As it is an incurable disease, characterized by a low satisfaction of patients with treatments (García-Pérez et al., 2012), it is likely that the inhabitants of the Purépecha Plateau search natural alternatives for its treatment, leading to sellers and healers to know about this ailment. Unfortunately, no epidemiological studies were found about the incidence of this illness in this particular population.

For treating of dermatological disorders, the aerial parts were preferred by participants, followed by leaves and flowers (Fig. 5). This result agrees with previous studies which have reported the use of aerial parts to cure skin disorders due to their ease access in natural environments (Sharma et al., 2014; Neamsuvan and Bunmee, 2016). It is interesting to note that unlike other ethnopharmacological studies focusing on skin ailments (Njoroge and Bussmann, 2007; Abbasi et al., 2010; Afolayan et al., 2014; Sharma et al., 2014), Purépechas prefer the use of infusions and decoctions taken orally instead of topical administration, applying crushed plants, pastes and juices. This could be explained by the fact that among Purépechas there is a belief that dermatological disorders are manifestations of blood contamination (BDMTM, 2009). Therefore, they believe that cleansing the blood using herbal remedies by oral route would lead to improve skin problems. Another peculiar aspect to be highlighted is the fact that the population under study uses the "temazcal" (0.95%) for the treatment of cutaneous conditions. This is a steam bath of pre-Hispanic origin used in Mexico for curative, preventive, hygienic and religious purposes (Aparicio Mena, 2006). Its use is supported by the belief that the heat of the patient´s body is fundamental for hea-

ling, since the disease is eliminated through sweat (BDMTM, 2009). The present study also reports the use of common salt in small proportions for the preparation of pastes. Additionally, Sharma et al. (2014) found the same trend in a study focusing on the use of herbal preparations for dermatological disorders in India. These authors argued that the possible reason behind the use of salt would be related with the fact that it inhibits the activity of bacteria and fungi, thereby helping the skin to heal (Sharma et al., 2014).

Native (56.7%) and introduced (43.3%) plants were valuable resources for local population for treating dermatological ailments (Appendix). Although in the context of medicinal plants, both introduced and native plants are important, it has been reported that in rural areas of Mexico, practitioners depend on the natural environment rather than cultivated areas to obtain medicinal plants (Andrade-Cetto, 2009; Alonso-Castro et al., 2012). Indeed, 53.6% of the collected plants of this study were gathered from the wild. The extensive use of native species by Purépechas could not only be related to a higher availability of these plants through the seasons, but also to the inherited knowledge about their medicinal properties from the ancestors. In fact, it is known that among Purépechas the transmission of knowledge related to traditional medicine is performed in more than 50% by means of relatives (BDMTM, 2009). However, a non-negleciable amount of exotic plants was also reported in the study area. The contribution of these species is very important as they amplify the spectrum of therapeutic alternatives for treating dermatological conditions on the Purépecha Plateau, reinforcing the local plant formulary.

Five plants with the highest UV to treat dermatological disorders on the Purépecha Plateau were *Heterotheca inuloides*, *Aloe vera*, *Oenothera rosea*, *Calendula officinalis* and *Urtica subnuda* (Fig. 6). *Heterotheca inuloides* is known as Mexican arnica and is extensively used for dermatological problems in Mexico. Additionally, this plant is used alone or in combination with other herbs, for diminishing the pain associated to rheumatism, stomach ulcers and other painful conditions associated with inflammatory processes. It is also reported in the management of kidney disease, urinary infections, cough, lung diseases, vaginal infections, gastrointestinal disorders and cancer (Rodríguez-Chávez et al., 2017).

According to the present study, Purépechas used this species for cuts, hematomas, infections with abscesses, skin inflammation, insect bites, rash, skin bumps, skin care, superficial infections, varicella, and varicose veins (Appendix). Its anti-inflammatory and antimicrobial properties in Purépecha traditional medicine are supported by pharmacological studies. Sesquiterpenes isolated from this plant exert activity against *Bacillus subtilis* var. *niger* Smith et al. (ATCC® 9372™), *Brevibacterium ammoniagenes* (Cooke and Keith) Breed (ATCC® 6872™), *Propionibacterium acnes* (Gilchrist) Douglas and Gunter (ATCC® 11827™), *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 12598™), *Staphylococcus aureus* subsp. *aureus* (ATCC® 33591™), *Streptococcus mutans* Clarke (ATCC® 25175™), and *Trichophyton mentagrophytes* (Robin) Blanchard (ATCC® 18748™) with minimal inhibitory concentrations ranged from 6.23 to 12.5 mg/mL (Kubo et al., 1994). Infusions of *Heterotheca inuloides* inhibit the activity of cyclooxygenase enzyme by 40%, suggesting an anti-inflammatory effect of polar metabolites of this plant (Muñoz-Velázquez et al., 2012).

Aloe vera has been widely used for treating skin diseases across the globe. This plant is also very popular for managing cutaneous conditions in other Mexican states, such as Oaxaca and Chiapas (Geck et al., 2016). *Aloe vera* has demonstrated pro-cicatrizing effects by increasing collagen content in wounds and the degree of collagen cross linking (Heggers et al., 1996). Additionally, it possesses a protective effect against skin damage induced by radiation, anti-inflammatory activity, moisturizing and anti-aging effects (Choi and Chung, 2003). These pharmacologic properties justify its extensive use in traditional Purépecha medicine. *Oenothera rosea* is a native plant of Mexico that stimulates the proliferation of normal human fibroblasts in vitro (Takahashi et al., 2012), which could be related to its use for cuts by Purépechas. This species also reduces chronic and acute skin inflammation in rats (Villena and Arro-

yo, 2012), so it can function as anti-inflammatory agent, as referred by the population under study. *Calendula officinalis* has been employed in folk therapy for treating skin ailments worldwide. On the Purépecha Plateau, this plant is used for multiple skin affections including blemishes, burns, cuts, skin inflammation, insect bites, pruritus, psoriasis, rash, skin bumps and superficial infections (Appendix). Pharmacological studies suggest that extracts from this plant can protect skin against ultraviolet-induced damage (Fonseca et al., 2010). Moreover, this plant is highly effective for the prevention of acute dermatitis of grade 2, which occurs after postoperative irradiation for breast cancer, demonstrating its anti-inflammatory properties (Pommier et al., 2004). The extract of the flowers of this plant is also broadly recognized by its wound-healing promoting effects (Preethi and Kuttan, 2011). The multiplicity of pharmacological activities of this species on the skin agrees with its wide use on the Purépecha Plateau for dermatological conditions. Surprisingly, Purépechas frequently reported the use of *Urtica subincisa* for skin inflammation, skin bumps and varicose veins (Appendix). To the best of our knowledge, this native plant has not been previously reported neither in Mexico nor in the world for treating cutaneous disorders. Further phytochemical, toxicological and pharmacological studies with this species are required to establish its importance for treating skin ailments.

Thirty-nine plants were the most important, according to their FL (100%) for the treatment of 10 of the 19 dermatological conditions informed by the participants. These plants offer very interesting clues as to their pharmacological potential for the treatment of a specific skin ailment (Appendix). Additionally, this study provides new ethnopharmacological information about plants used for treating skin ailments on the Purépecha Plateau, Mexico and the world (Appendix). Apart from *Tagetes remotiflora*, all these plants are native of Mexico. *Eryngium beecheyanum* is a herbaceous plant which has been reported by peasants of the Sierra de Huautla, Morelos, Mexico, for kidney inflammation (Maldonado-Almanza, 1997). According to its fidelity level, it seems to be a promising candidate to treat skin inflammation (FL=100%). *Tagetes remotiflora* has been reported in the community of Nuevo Parangaricutiro by its antidiarrheic effect, as well as against vomiting, nerves and blood pressure (Bello-González et al., 2015). Its highest FL for cuts (FL=100%) indicates that it could also be employed for this affection. *Tournefortia mutabilis* has been reported to have antimicrobial properties (Sharma et al., 2017), which could be related to its use in superficial skin infections by Purépechas (FL=66.7%). In traditional Mexican medicine, *Equisetum hyemale* var. *affine* is mainly used for kidney conditions, pain, stomach inflammation, gastritis, ulcers and reproductive woman disorders (BDMTM, 2009). Although its use for treating skin conditions has not been reported, results obtained in this study indicate that it could probably be effective for hair fall and skin inflammation (FL=50.0%). *Salvia lemantha* is used in Mexico for stomach pain, respiratory ailments, reproductive disorders and diabetes (BDMTM, 2009; Romero-Cerecero et al., 2009). Our results point out that this species could also be used for skin care as the highest FL was obtained in this category. Although no reports were found regarding the use of *Gnaphalium macrostemum* for dermatological disorders, its use for gastrointestinal affections and respiratory diseases has been documented in Mexico (INEGI, 2014). As its higher FL was obtained for skin inflammation (FL=83.3%), it would be interesting that further studies be performed to ratify this activity using in vitro and in vivo relevant models. To the best of our knowledge, *Sida haenkeana* and *Urtica subincisa* are reported for the first time as medicinal plants. According to their FL, *Sida haenkeana* seems to be a promising candidate to treat skin bumps (FL=100%), whereas *Urtica subincisa* was catalogued as anti-inflammatory agent (FL=80.0%). Traditional uses of the medicinal flora from the Purépecha Plateau can serve as a starting point for further scientific analysis, which may provide novel compounds or improved herbal preparations for treating skin diseases.

Conclusions

This study demonstrates that herbal remedies play an important role in healing skin by inhabitants of the Purépecha

Plateau, state of Michoacán, Mexico. A total of 97 species were identified to treat 19 skin ailments. The quantitative analysis performed through the determination of the use value, fidelity level and the informant consensus factor allowed to identify plants as promising candidates for developing new dermatological formulations. This investigation is also a guide to the conservation of folk medicinal knowledge from indigenous people of Mexico, through a vision of sustainable use of natural resources. From the ethno-pharmacological point of view, it provides new information about plants whose medicinal properties for the skin had been unknown up to now. Additionally, it allows for dissemination of the inherited knowledge of Purépechas regarding herbal preparations for cutaneous diseases. The identification of plants used by this population to treat skin pathologies also opens new perspectives for performing phytochemical, toxicological and pharmacological investigations that allow the development of new herbal preparations or new drugs addressed to improve the quality of life of patients suffering from these diseases.

Authors contribution

MEGP, AOZ and REG designed the study. REG, MEGP and EPC conducted interviews and collected the plants referred by participants. EPC identified the specimens and deposited them in the herbarium IEB. REG drafted the manuscript under the supervision of AOZ, EPC and MEGP. All authors contributed to the review and approval of the final manuscript.

Financing

We thank the "Consejo Nacional de Ciencia y Tecnología" (CONACYT, Mexico) for the financial support of REG (Doctoral Fellowship).

Acknowledgment

The authors are very grateful to traditional healers, sellers and local inhabitants from the Purépecha Plateau for providing the valuable information regarding medicinal plants for skin ailments and kindly sharing their ancestral knowledge with us.

Literature cited

Abbasi, A. M., M. A. Khan, M. Ahmad, M. Zafar, S. Jahan and S. Sultana. 2010. Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province, Pakistan. Journal of Ethnopharmacology 128(2): 322-335. DOI: https://doi.org/10.1016/j.jep.2010.01.052

Acharya, E. and B. Pokhrel. 2006. Ethno-Medicinal plants used by Bantar of Bhandaha, Morang, Nepal. Our Nature 4(1): 96-103. DOI: https://doi.org/10.3126/on.v4i1.508

Afolayan, A. J., D. S. Grierson and W. O. Mbeng. 2014. Ethnobotanical survey of medicinal plants used in the management of skin disorders among the Xhosa communities of the Amathole District, Eastern Cape, South Africa. Journal of Ethnopharmacology 153(1): 228-232. DOI: https://doi.org/10.1016/j.jep.2014.02.023

Akhtar, N., A. Rashid, W. Murad and E. Bergmeier. 2013. Diversity and use of ethno-medicinal plants in the region of Swat, North Pakistan. Journal of Ethnobiology and Ethnomedicine 9(1): 25. DOI: https://doi.org/10.1186/1746-4269-9-25

Al-Khalil, S. 1995. A survey of plants used in Jordanian traditional medicine. International Journal of Pharmacognosy 33(4): 317-323. DOI: https://doi.org/10.3109/13880209509065385

Alonso-Castro, A. J., J. J. Maldonado-Miranda, A. Zarate-Martínez, M. R. Jacobo-Salcedo, C. Fernández-Galicia, L. A. Figueroa-Zuñiga, N. A. Rios-Reyes, M. A. de León-Rubio, N. A. Medellín-Castillo, A. Reyes-Munguía, R. Méndez-Martínez and C. Carranza-Álvarez. 2012. Medicinal plants used in the Huasteca Potosina, Mexico. Journal of Ethnopharmacology 143(1): 292-298. DOI: https://doi.org/10.1016/j.jep.2012.06.035

Argüelles Luna, J. and G. Sánchez Díaz. 2015. P'urhépecha. Comisión Nacional para el Desarrollo de los Pueblos Indígenas. México, D.F., México. Pp. 1-176.

Amin, M. S. and M. R. Joharchi. 2013. Ethnobotanical investigation of traditional medicinal plants commercialized in the markets of Mashhad, Iran. Avicenna Journal of Phytomedicine 3(3): 254-271. DOI: https://doi.org/10.22038/ajp.2013.487

Andrade-Cetto, A. 2009. Ethnobotanical study of the medicinal plants from Tlanchinol, Hidalgo, México. Journal of Ethnopharmacology 122(1): 163-171. DOI: https://doi.org/10.1016/j.jep.2008.12.008

Aparicio Mena, A. J. 2006. El temazcal en la cultura tradicional de salud y en la etnomedicina mesoamericana. Gazeta de Antropología 22: 1-11.

Augustin, M., B. Holland, D. Dartsch, A. Langenbruch and M. A. Radtke. 2011. Adherence in the treatment of psoriasis: a systematic review. Dermatology 222(4): 363-374. DOI: https://doi.org/10.1159/000329026

Ávila-Uribe, M. M., S. N. García-Zárate, A. S. Sepúlveda-Barrera and M. A. Godínez-Rodríguez. 2016. Plantas medicinales en dos poblados del municipio de San Martín de las Pirámides, Estado de México. Polibotánica 42: 215-245. DOI: https://doi.org/10.18387/polibotanica.42.11

Balangcod, T. D. and A. K. D. Balangcod. 2011. Ethnomedical knowledge of plants and healthcare practices among the Kalanguya tribe in Tinoc, Ifugao, Luzon, Philippines. Indian Journal of Traditional Knowledge 10(2): 227-238.

Barrett, B. 1994. Medicinal plants of Nicaragua's Atlantic coast. Economic Botany 48(1): 8-20. DOI: https://doi.org/10.1007/BF02901375

BDMTM. 2009. Biblioteca Digital de la Medicina Tradicional Mexicana. Universidad Nacional Autónoma de México. México, D.F., México. http://www.medicinatradicionalmexicana.unam.mx/index.php (consulted November, 2017).

Bello-González, M. Á., S. Hernández-Muñoz, M. B. N. Lara-Chávez and R. Salgado-Garciglia. 2015. Plantas útiles de la comunidad indígena Nuevo San Juan Parangaricutiro, Michoacán, México. Polibotánica 39: 175-215. DOI: https://doi.org/10.18387/polibotanica.39.10

Bello-González, M. Á. and R. Salgado-Garciglia. 2013. Plantas medicinales de la comunidad indígena Nuevo San Juan Parangaricutiro, Michoacán, México. Biológicas 9(1): 126-138.

Benítez, G., M. R. González-Tejero and J. Molero-Mesa. 2010. Pharmaceutical ethnobotany in the western part of Granada province (southern Spain): ethnopharmacological synthesis. Journal of Ethnopharmacology 129(1): 87-105. DOI: https://doi.org/10.1016/j.jep.2010.02.016

Bhandary, M. J. and K. R. Chandrasheker. 2011. Herbal therapy for herpes in the ethno-medicine of Coastal Karnataka. Indian Journal of Traditional Knowledge 10(3): 528-532.

Bhardwaj, S. and S. K. Gakhar. 2005. Ethnomedicinal plants used by the tribals of Mizoram to cure cuts & wounds. Indian Journal of Traditional Knowledge 4(2): 75–80.

Bhat, P., G. R. Hegde, G. Hegde and G. S. Mulgund. 2014. Ethnomedicinal plants to cure skin diseases-An account of the traditional knowledge in the coastal parts of Central Western Ghats, Karnataka, India. Journal of Ethnopharmacology 151(1): 493-502. DOI: https://doi.org/10.1016/j.jep.2013.10.062

BIOAGRICOOP. 1998. Bioagricoop México, A.C. Etnobotánica y herbolaria de la Meseta P'urepecha. http://bioagricoop.mx.tripod.com/etnobotanica.pdf (consulted November, 2017).

Cáceres, A., L. M. Girón, S. R. Alvarado and M. F. Torres. 1987. Screening of antimicrobial activity of plants popularly used in Guatemala for the treatment of dermatomucosal diseases. Journal of Ethnopharmacology 20(3): 223-237. DOI: https://doi.org/10.1016/0378-8741(87)90050-X

Calvo, M. I., S. Akerreta and R. Y. Cavero. 2011. Pharmaceutical ethnobotany in the Riverside of Navarra (Iberian Peninsula). Journal of Ethnopharmacology 135(1): 22-33. DOI: https://doi.org/10.1016/j.jep.2011.02.016

Campbell, L. 1997. American Indian Languages: The historical linguistics of Native America. Oxford Studies in Anthropological Linguistics. Oxford University Press. New York, USA. Pp. 1-528.

Canales-Martínez, M., I. Hernández-Delgado, C. Flores-Ortiz, A. Durán-Díaz, A. M. García-Bores and G. Ávila-Acevedo. 2005. Antimicrobial activity of *Alternanthera caracasana*. Pharmaceutical Biology 43(4): 305-307. DOI: https://doi.org/10.1080/13880200590951685

Cassar, M. and E. Attard. 2016. An ethno botanical survey of medicinal plants used in the Island of Gozo. Studies on Ethno-Medicine 10(2): 269-281. DOI: https://doi.org/10.1080/09735070.2016.11905497

CDI. 2010. Cédulas de información básica de los pueblos indígenas de México. Comisión Nacional para el

Desarrollo de los Pueblos Indígenas. México. http://www.cdi.gob.mx/cedulas/ (consulted November, 2017).

Chase, P. and O. P. Singh. 2013. Ethnomedicinal plants used by Angami tribe of Nagaland, India. Indian Journal of Tropical Biodiversity 21(1): 29-42.

Choi, S. and M.-H. Chung. 2003. A review on the relationship between *Aloe vera* components and their biologic effects. Seminars in Integrative Medicine 1(1): 53-62. DOI: https://doi.org/10.1016/S1543-1150(03)00005-X

Cuevas Figueroa, X. M. 2005. A revision of the genus *Amphipterygium* (Julianaceae). Ibugana 13(1): 27-47.

Dalgard, F. J., C. Gieler, J. Tomas-Aragones, L. Lien, F. Poot, G. B. E. Jemec, I. Misery, C. Szabo, D. Linder, F. Sampogna, A. W. M. Evers, J. A. Halvorsen, F. Balkema, J. Szepietowski, D. Romanov, S. E. Marron, I. K. Altunay, A. Y. Finlay, S. S. Salek and J. Kupfer. 2015. The psychological burden of skin diseases: a cross-sectional multicenter study among dermatological out-patients in 13 European countries. Journal of Investigative Dermatology 135(4): 984-991. DOI: https://doi.org/10.1038/jid.2014.530

Das, T., S. B. Mishra, D. Saha and S. Agarwal. 2012. Ethnobotanical survey of medicinal plants used by ethnic and rural people in Eastern Sikkim Himalayan region. African Journal of Basic & Applied Sciences 4(1): 16-20.

De Feo, V., R. Aquino, A. Menghini, E. Ramundo and F. Senatore. 1992. Traditional phytotherapy in the Peninsula Sorrentina, Campania, southern Italy. Journal of Ethnopharmacology 36(2): 113-125. DOI: https://doi.org/10.1016/0378-8741(92)90010-O

DGE. 2015. Anuario de Morbilidad 2015. Dirección General de Epidemiología – Secretaría de Salud, México. http://www.epidemiologia.salud.gob.mx/anuario/html/anuarios.html (consulted November, 2017).

Egharevba, R. K. A. and M. I. Ikhatua. 2008. Ethno-medical uses of plants in the treatment of various skin diseases in Ovia North East, Edo State, Nigeria. Research Journal of Agriculture and Biological Science 4(1): 58-64.

El-Ghazali, G. E., K. S. Al-Khalifa, G. A. Saleem and E. M. Abdallah. 2010. Traditional medicinal plants indigenous to Al-Rass province, Saudi Arabia. Journal of Medicinal Plants Research 4(24): 2680-2683. DOI: https://doi.org/10.5897/JMPR09.556

El Mansouri, L., A. Ennabih and D. Bousta. 2011. Interés socioeconómico y valorización de plantas medicinales del oasis de Rissani (SE de Marruecos). Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 10(1): 30-45

Fernández, E. C., Y. E. Sandi and L. Kokoska. 2003. Ethnobotanical inventory of medicinal plants used in the Bustillo Province of the Potosi Department, Bolivia. Fitoterapia 74(4): 407-416. DOI: https://doi.org/10.1016/S0367-326X(03)00053-4

Ferreira, F. S., S. V. Brito, S. C. Ribeiro, W. O. Almeida and R. R. N. Alves. 2009. Zootherapeutics utilized by residents of the community Poço Dantas, Crato-CE, Brazil. Journal of Ethnobiology and Ethnomedicine 5: 1-21. DOI: https://doi.org/10.1186/1746-4269-5-21

Fonseca, Y. M., C. D. Catini, F. T. M. C. Vicentini, A. Nomizo, R. P. Gerlach and M. J. V. Fonseca. 2010. Protective effect of *Calendula officinalis* extract against UVB-induced oxidative stress in skin: evaluation of reduced glutathione levels and matrix metalloproteinase secretion. Journal of Ethnopharmacology 127(3): 596-601. DOI: https://doi.org/10.1016/j.jep.2009.12.019

Forgione, G., L. De Martino and V. De Feo. 2008. Medicinal plant in Samnium popular medicine. Pharmacology online 1: 90-98.

Frei, B., M. Baltisberger, O. Sticher and M. Heinrich. 1998. Medical ethnobotany of the Zapotecs of the Isthmus-Sierra (Oaxaca, Mexico): documentation and assessment of indigenous uses. Journal of Ethnopharmacology 62(2): 149-165. DOI: https://doi.org/10.1016/S0378-8741(98)00051-8

Gallardo Ruiz, J. 2002. Medicina tradicional P'urhépecha. El Colegio de Michoacán. Zamora, Michoacán, México. Pp. 1-342.

García-Pérez, M. E., J. Jean and R. Pouliot. 2012. Antipsoriatic drug development: challenges and new emerging therapies. Recent Patents on Inflammation & Allergy Drug Discovery 6(1): 3-21. DOI: https://doi.org/10.2174/187221312798889248

Gazzaneo, L. R. S., R. F. P. de Lucena and U. P. de Albuquerque. 2005. Knowledge and use of medicinal plants by local specialists in a region of Atlantic Forest in the state of Pernambuco (Northeastern Brazil). Journal of Ethnobiology and Ethnomedicine 1: 1-9. DOI: https://doi.org/10.1186/1746-4269-1-9

Gebrezgabiher, G., S. Kalayou and S. Sahle. 2013. An ethnoveterinary survey of medicinal plants in woredas of Tigray region, Northern Ethiopia. International Journal of Biodiversity and Conservation 5(2): 89-97.

Geck, M. S., A. J. Reyes García, L. Casa and M. Leonti. 2016. Acculturation and ethnomedicine: a regional comparison of medicinal plant knowledge among the Zoque of southern Mexico. Journal of Ethnopharmacology 187: 146-159. DOI: https://doi.org/10.1016/j.jep.2016.04.036

Gómez Aizu, A. 2013. Quemada: reproducción humana y enfermedades culturales en la sierra Otomí-Tepehua, Hidalgo. Estudios de Antropología Biológica 15(1): 273-313.

Grosso, L. 2010. El uso popular de las plantas medicinales en Uruguay. la experiencia de los pequeños productores agroecológicos. ZooBioDi - Associazione Italiana di Zootecnia Biologica e Biodinamica. Bergamo. Italia. Pp. 1-78

Guarrera, P. M. 2005. Traditional phytotherapy in Central Italy (Marche, Abruzzo, and Latium). Fitoterapia 76(1): 1-25. DOI: https://doi.org/10.1016/j.fitote.2004.09.006

Guarrera, P. M., G. Forti and S. Marignoli. 2005. Ethnobotanical and ethnomedicinal uses of plants in the district of Acquapendente (Latium, Central Italy). Journal of Ethnopharmacology 96(3): 429-444. DOI: https://doi.org/10.1016/j.jep.2004.09.014

Halberstein, R. A. 1997. Traditional botanical remedies on a small Caribbean island: Middle (Grand) Caicos, West Indies. Journal of Alternative and Complementary Medicine 3(3): 227-239. DOI: https://doi.org/10.1089/acm.1997.3.227

Harsha, V. H., S. S. Hebbar, V. Shripathi and G. R. Hegde. 2003. Ethnomedicobotany of Uttara Kannada District in Karnataka, India-plants in treatment of skin diseases. Journal of Ethnopharmacology 84(1): 37-40. DOI: https://doi.org/10.1016/S0378-8741(02)00261-1

Hay, R. J., N. E. Johns, H. C. Williams, I. W. Bolliger, R. P. Dellavalle, D. J. Margolis, R. Marks, L. Naldi, M. A. Weinstock, S. K. Wulf, C. Michaud, C. J. L. Murray and M. Naghavi. 2014. The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. Journal of Investigative Dermatology 134(6): 1527-1534. DOI: https://doi.org/10.1038/jid.2013.446

Heggers, J. P., A. Kucukcelebi, D. Listengarten, J. Stabenau, F. Ko, L. D. Broemeling, M. C. Robson and W. D. Winters. 1996. Beneficial effect of *Aloe* on wound healing in an excisional wound model. Journal of Alternative and Complementary Medicine 2(2): 271-277. DOI: https://doi.org/10.1089/acm.1996.2.271

Heinrich, M. and S. Gibbons. 2001. Ethnopharmacology in drug discovery: an analysis of its role and potential contribution. Journal of Pharmacy and Pharmacology 53(4): 425-432. DOI: https://doi.org/10.1211/0022357011775712

HGM-SSA. 2012. Boletín estadístico de dermatología 2002-2011. Hospital General de México-Secretaría de Salud. México, D.F., México. http://www.hgm.salud.gob.mx/descargas/pdf/planeacion/boletin_derma_02_11.pdf (consulted November, 2017).

Hurtado-Rico, N. E., C. Rodríguez-Jiménez and A. Aguilar-Contreras. 2006. Estudio cualitativo y cuantitativo de la flora medicinal del municipio de Copándaro de Galeana, Michoacán, México. Polibotánica 22: 21-50.

Ijaz, F., Z. Iqbal, I. U. Rahman, J. Alam, S. M. Khan, G. M. Shah, K. Khan and A. Afzal. 2016. Investigation of traditional medicinal floral knowledge of Sarban Hills, Abbottabad, KP, Pakistan. Journal of Ethnopharmacology 179: 208-233. DOI: https://doi.org/10.1016/j.jep.2015.12.050

INEGI. 2014. Sistema de consulta de especies y productos agropecuarios, forestales y pesqueros. Instituto Nacional de Estadística y Geografía. http://www.inegi.org.mx/est/contenidos/proyectos/aspectosmetodologicos/clasificadoresycatalogos/catalogo_especies.aspx (consulted November, 2017).

INEGI. 2004. La población hablante de lengua indígena de Michoacán de Ocampo. Instituto Nacional de Estadística y Geografía e Informática. Aguascalientes, México. Pp. 1-140.

Jarić, S., Z. Popović, M. Mačukanović-Jocić, L. Djurdjević, M. Mijatović, B. Karadžić, M. Mitrović and P Pavlović. 2007. An ethnobotanical study on the usage of wild medicinal herbs from Kopaonik Mountain (Central Serbia). Journal of Ethnopharmacology 111(1): 160-175. DOI: https://doi.org/10.1016/j.jep.2006.11.007

Jiofack, T., C. Fokunang, V. Kemeuze, E. Fongnzossie, N. Tsabang, R. Nkuinkeu, P. M. Mapongmetsem and B. A. Nkongmeneck. 2008. Ethnobotany and phytopharmacopoea of the South-West ethnoecological region of Cameroon. Journal of Medicinal Plants Research 2(8): 197-206.

Joshi, A. R. and K. Joshi. 2000. Indigenous knowledge and uses of medicinal plants by local communities of the Kali Gandaki Watershed Area, Nepal. Journal of Ethnopharmacology 73(1-2): 175-183. DOI: https://doi.org/10.1016/S0378-8741(00)00301-9

Juárez-Vázquez, M. C., C. Carranza-Álvarez, A. J. Alonso-Castro, V. F. González-Alcaraz, E. Bravo-Acevedo, F. J. Chamarro-Tinajero and E. Solano. 2013. Ethnobotany of medicinal plants used in Xalpatlahuac, Guerrero, Mexico. Journal of Ethnopharmacology 148(2): 521-527. DOI: https://doi.org/10.1016/j.jep.2013.04.048

Kala, C. P. 2005. Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. Journal of Ethnobiology and Ethnomedicine 1: 11. DOI: https://doi.org/10.1186/1746-4269-1-11

Kamatenesi-Mugisha, M., H. Oryem-Origa, O. Odyek and D. W. Makawiti. 2008. Medicinal plants used in the treatment of fungal and bacterial infections in and around Queen Elizabeth Biosphere Reserve, western Uganda. African Journal of Ecology 46(s1): 90-97. DOI: https://doi.org/10.1111/j.1365-2028.2008.00935.x

Kasolo, J. N., G. S. Bimenya, L. Ojok, J. Ochieng and J. W. Ogwal-okeng. 2010. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. Journal of Medicinal Plants Research 4(9): 753-757.

Khabbach, A., M. Libiad, A. Ennabili and D. Bousta. 2012. Medicinal and cosmetic use of plants from the province of Taza, Northern Morocco. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 11(1): 46-60.

Kinghorn, A. D. 2001. Stevia: The genus *Stevia*. Taylor & Francis Inc. New York, USA. Pp. 1-202.

Kubo, I., H. Muroi, A. Kubo, S. K. Chaudhuri, Y. Sanchez and T. Ogura. 1994. Antimicrobial agents from *Heterotheca inuloides*. Planta Medica 60(3): 218-221. DOI: https://doi.org/10.1055/s-2006-959462

Lal, B. and K. N. Singh. 2008. Indigenous herbal remedies used to cure skin disorders by the natives of Lahaul-Spiti in Himachal Pradesh. Indian Journal of Traditional Knowledge 7(2): 237-241.

Lans, C. 2007. Comparison of plants used for skin and stomach problems in Trinidad and Tobago with Asian ethnomedicine. Journal of Ethnobiology and Ethnomedicine 3: 3. DOI: https://doi.org/10.1186/1746-4269-3-3

Leporatti, M. L. and P. M. Guerrera. 2007. Ethnobotanical remarks in Capitanata and Salento areas (Puglia, Southern Italy). Ethnobiologia 5(1): 51-64.

Lev, E. 2006. Ethno-diversity within current ethnopharmacology as part of Israeli traditional medicine - A review. Journal of Ethnobiology and Ethnomedicine 2: 4. DOI: https://doi.org/10.1186/1746-4269-2-4

Luna-José, A. L., L. Montalvo-Espinosa and B. Rendón-Aguilar. 2003. Los usos no leñosos de los encinos en México. Boletín de la Sociedad Botánica de México 72: 107-117.

Mahbubur Rahman, A. H. M., S. Akter, R. Rani and A. K. M. Rafiul Islam. 2015. Taxonomic study of leafy vegetables at Santahar Pouroshova of District Bogra, Bangladesh with emphasis on medicinal plants. International Journal of Advanced Research 3(5): 1019-1036.

Muiza, K., V. Hamuniche and F. Maiza-Benabdesselam. 2011. Traditional medicine in North Sahara: the "delil". Life Sciences Leaflets 16: 551-560.

Maldonado-Almanza, B. J. 1997. Aprovechamiento de los recursos florísticos de la Sierra de Huautla, Morelos, México. Tesis de maestría. Facultad de Ciencias, Universidad Nacional Autónoma de México. México, D.F., México. Pp. 1-149.

Malla, B. and R. B. Chhetri. 2009. Indigenous knowledge on ethnobotanical plants of Kavrepalanchowk District,

Kathmandu University Journal of Science, Engineering and Technology 5(2): 96-109.

Manzur-ul-Kadir Mia, M., M. Fahim Kadir, M. Shahadat Hossan and M. Rahmatullah. 2009. Medicinal plants of the Garo tribe inhabiting the Madhupur forest region of Bangladesh. American-Eurasian Journal of Sustainable Agriculture 3(2): 165-171.

Martínez, G. J. and G. E. Barboza. 2010. Natural pharmacopoeia used in traditional Toba medicine for the treatment of parasitosis and skin disorders (Central Chaco, Argentina) Journal of Ethnopharmacology 132(1): 86-100. DOI: https://doi.org/10.1016/j.jep.2010.07.049

Medina, C. 2003. La flora: riqueza, diversidad y sus relaciones fitogeográficas. In: Velázquez, A., A. Torres and G. Bocco (eds.). Las Enseñanzas de San Juan. Instituto Nacional de Ecología-Secretaría de Medio Ambiente y Recursos Naturales, México, D.F., México. Pp. 165-200.

Mercado-Vargas, H. and M. Palmerín Cerna. 2012. El Estado de Michoacán y sus regiones turísticas. Fundación Universitaria Andaluza Inca Garcilaso. Málaga, España. Pp. 1-139.

Mollik, M. A. H., M. S. Hossan, A. K. Paul, M. Taufiq-Ur-Rahman, R. Jahan and M. Rahmatullah. 2010. A comparative analysis of medicinal plants used by folk medicinal healers in three districts of Bangladesh and inquiry as to mode of selection of medicinal plants. Ethnobotany Research and Applications 8: 195-218. DOI: https://doi.org/10.17348/era.8.0.195-218

Monroy-Ortiz, C., E. García-Moya, A. Romero-Manzanares, C. Sánchez-Quintanar, M. Luna-Cavazos, E. Ucsanga-Mortera, J. S. Flores-Guido and V. González-Romero. 2013. Plants of local interest for medicinal and conservation purposes in Morelos, Mexico. Studies on Ethno-Medicine 7(1): 13-26. DOI: https://doi.org/10.1080/09735070.2013.11886443

Monzote, L., M. García, A. A. Montalvo, R. Linares and R. Scull. 2009. Effect of oral treatment with the essential oil from *Chenopodium ambrosioides* against cutaneous leishmaniasis in BALB/c mice, caused by *Leishmania amazonensis*. Research in Complementary Medicine 16(5): 334-338. DOI: https://doi.org/10.1159/000237741

Muñoz-Velázquez, E. E., K. Rivas-Díaz, M. G. F. Linarez-Piña, S. Mendoza-Díaz, R. Reynoso-Camacho and M. Ramos-Gómez. 2012. Comparación del contenido fenólico, capacidad antioxidante y actividad antiinflamatoria de infusiones herbales comerciales. Revista Mexicana de Ciencias Agrícolas 3(3): 481-495.

Nath, M. and M. D. Choudhury. 2010. Ethno-medico-botanical aspects of Hmar tribe of Cachar district, Assam (Part I). Indian Journal of Traditional Knowledge 9(4): 760-764.

Navarrete-Heredia, J. L. and V. H. Gómez Flores. 2005. Aspectos etnoentomológicos acerca de *Paederus* sp. (Coleoptera: Staphylinidae) en Mascota, Jalisco, México. Dugesiana 12(1): 9-18.

Navarro Pérez, L. C. and S. Avendaño Reyes. 2002. Flora útil del municipio de Astacinga, Veracruz, México. Polibotánica 14: 67-84.

Navarro, V., M. L. Villarreal, G. Rojas and X. Lozoya. 1996. Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. Journal of Ethnopharmacology 53(3): 143-147. DOI: https://doi.org/10.1016/0378-8741(96)01429-8

Neamsuvan, O. and P. Bunmee. 2016. A survey of herbal weeds for treating skin disorders from Southern Thailand: Songkhla and Krabi Province. Journal of Ethnopharmacology 193: 574-585. DOI: https://doi.org/10.1016/j.jep.2016.09.048

Nicholson, M. S. 1991. The market medicinal plants of Monterrey, Nuevo León, Mexico. Master Thesis. Eastern Illinois University, Illinois, USA. Pp. 1-188.

Njoroge, G. N. and R. W. Bussmann. 2007. Ethnotherapeutic management of skin diseases among the Kikuyus of Central Kenya. Journal of Ethnopharmacology 111(2): 303-307. DOI: https://doi.org/10.1016/j.jep.2006.11.025

Okuma, C. H., A. M. Pereira, A. L. P. de Souza, F. M. Tasca, J. V. Visentainer, J. Mazucheli, J. C. Palazzo de Mello, T. Ueda-Nakamura, B. P. Dias-Filho and C. V. Nakamura. 2011. In vitro melanogenic activity of *Macadamia integrifolia* oil and clinical pilot study of a pharmaceutical formulation in patients with vitiligo. Latin American Journal of Pharmacy 30(7): 1336-1341.

Oliveira, D. F., A. C. Pereira, H. C. P. Figueiredo, D. A. Carvalho, G. Silva, A. S. Nunes, D. S. Alves and H. W. P. Carvalho. 2007. Antibacterial activity of plant extracts from Brazilian southeast region. Fitoterapia 78(2): 142-145. DOI: https://doi.org/10.1016/j.fitote.2006.09.027

Ong, H. C. and M. Nordiana. 1999. Malay ethno-medico botany in Machang, Kelantan, Malaysia. Fitoterapia 70(5): 502-513. DOI: https://doi.org/10.1016/S0367-326X(99)00077-5

Ong, H. C., R. M. Zuki and P. Milow. 2011. Traditional knowledge of medicinal plants among the Malay Villagers in Kampung Mak Kemas, Terengganu, Malaysia. Studies on Ethno-Medicine 5(3): 175-185. DOI: https://doi.org/10.1080/09735070.2011.11886407

Ordaña Trejo, C., A. Castro Campillo and J. Ramírez Pulido. 1999. Mammals from the Tarascan Plateau, Michoacán, México. Revista Mexicana de Mastozoología 4(1): 53-68.

Ouhaddou, H., H. Boubaker, F. Msanda and A. El Mousadik. 2015. An ethnobotanical study of medicinal plants of the Agadir Ida Ou Tanane province (southwest Morocco). Journal of Applied Biosciences 84(1): 7707-7722. DOI: https://doi.org/10.4314/jab.v84i1.5

Paek, S. Y., A. Koriakos, S. Saxton-Daniels and A. G. Pandya. 2012. Skin diseases in rural Yucatán, Mexico. International Journal of Dermatology 51(7): 823-828. DOI: https://doi.org/10.1111/j.1365-4632.2011.05190.x

Palmese, M. T., R. E. Uncini Manganelli and P. E. Tomei. 2001. An ethno-pharmacobotanical survey in the Sarrabus district (south-east Sardinia). Fitoterapia 72(6): 619-643. DOI: https://doi.org/10.1016/S0367-326X(01)00288-X

Panda, A. and M. K. Misra. 2011. Ethnomedicinal survey of some wetland plants of South Orissa and their conservation. Indian Journal of Traditional Knowledge 10(2): 296-303.

Parvez Rana, M., M. Shawkat Islam Sohel, S. Akhter and M. Jahurul Islam. 2010. Ethno-medicinal plants use by the Manipuri tribal community in Bangladesh. Journal of Forestry Research 21(1): 85-92. DOI: https://doi.org/10.1007/s11676-010-0015-1

Pascual, L. M. 1994. Estudios etnobotánicos en la provincia de Castellón. Naturaleza Medicatrix 37: 22-29.

Pieroni, A. and M. E. Giusti. 2008. The remedies of the folk medicine of the Croatians living in Ćićarija, Northern Istria. Collegium Antropologicum 32(2): 623-627.

Pieroni, A., M. E. Giusti, H. Münz, C. Lenzarini, G. Turković and A. Turković. 2003. Ethnobotanical knowledge of the Istro-Romanians of Žejane in Croatia. Fitoterapia 74(7-8): 710-719. DOI: https://doi.org/10.1016/j.fitote.2003.06.002

Pohcepatel, S. S. and V. G. Mamkrao. 2013. Ethnomedicinal plants used in the treatment of skin diseases in Hyderabad Karnataka region, Karnataka, India. Asian Pacific Journal of Tropical Biomedicine 3(11): 882-886. DOI: https://doi.org/10.1016/S2221-1691(13)60173-2

Pollio, A., A. De Natale, E. Appetiti, G. Aliotta and A. Touwaide. 2008. Continuity and change in the Mediterranean medical tradition: *Ruta* spp. (Rutaceae) in Hippocratic medicine and present practices. Journal of Ethnopharmacology 116(3): 469-482. DOI: https://doi.org/10.1016/j.jep.2007.12.013

Pommier, P., F. Gomez, M. P. Sunyach, A. D'Hombres, C. Carrie and X. Montbarbon. 2004. Phase III randomized trial of *Calendula officinalis* compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer. Journal of Clinical Oncology 22(8): 1447-1453. DOI: https://doi.org/10.1200/JCO.2004.07.063

Prashantkumar, P. and G. M. Vidyasagar. 2008. Traditional knowledge on medicinal plants used for the treatment of skin diseases in Bidar district, Karnataka, Indian Journal of Traditional Knowledge 7(2): 273-276.

Preethi, K. C. and R. Kuttan. 2011. Wound healing activity of flower extract of *Calendula officinalis*. Journal of Basic and Clinical Physiology and Pharmacology 20(1): 73-80. DOI: https://doi.org/10.1515/JBCPP.2009.20.1.73

Priya, K. and S. Sharma. 2014. Ethno-botanical importance of some tree species in Jammu District, J&K. International Journal of Science and Research 3(11): 2795-2798.

Ramya, S., C. Rajasekaran, R. Sivaperumal, A. Krishnan and R. Jayakumararaj. 2008. Ethnomedicinal perspectives of botanicals used by Malayali Tribes in Vattal Hills of Dharmapuri (TN), India. Ethnobotanical Leaflets 12: 1054-1060.

Regassa, R. 2013. Assessment of indigenous knowledge of medicinal plant practice and mode of service delivery in Hawassa city, southern Ethiopia. Journal of Medicinal Plants Research 7(9): 517-535. DOI: https://doi.org/10.5897/JMPR12.1126

Rexhepi, B., B. Mustafa, A. Hajdari, J. Rushidi-Rexhepi, C. L. Quave and A. Pieroni. 2013. Traditional medicinal plant knowledge among Albanians, Macedonians and Gorani in the Sharr Mountains (Republic of Macedonia). Genetic Resources and Crop Evolution 60(7): 2055-2080. DOI: https://doi.org/10.1007/s10722-013-9974-3

Rodríguez-Chávez, J. L., V. Egas, E. Linares, R. Bye, I. Hernández, F. J. Espinosa-García and G. Delgado. 2017. Mexican arnica (*Heterotheca inuloides* Cass. Asteraceae: Astereae): ethnomedical uses, chemical constituents and biological properties. Journal of Ethnopharmacology 195: 39-63. DOI: https://doi.org/10.1016/j.jep.2016.11.021

Romero-Cerecero, O., H. Reyes-Morales, L. Aguilar-Santamaría, M. Huerta-Reyes and J. Tortoriello-García. 2009. Use of medicinal plants among patients with Diabetes mellitus type 2 in Morelos, Mexico. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 8(5): 380-388.

Saboor, M. and S. S. Ahmad. 2009. Exploring the folk medicinal flora of Abbotabad City, Pakistan. Ethnobotanical Leaflets 13: 810-833.

Sarma, S. K. and M. Saikia. 2010. Utilization of wetland resources by the rural people of Nagaon district, Assam. Indian Journal of Traditional Knowledge 9(1): 145-151.

Shah, B., F. Sheth and M. Parabia. 2011. Documenting Grandmas' prescriptions for skin ailments in Valsad district, Gujarat. Indian Journal of Traditional Knowledge 10(2): 372-374.

Sharma, A., R. D. C. Flores-Vallejo, A. Cardoso-Taketa and M. I. Villarreal. 2017. Antibacterial activities of medicinal plants used in Mexican traditional medicine. Journal of Ethnopharmacology 208: 264-329. DOI: https://doi.org/10.1016/j.jep.2016.04.045

Sharma, J., S. Gairola, Y. P. Sharma and R. D. Gaur. 2014. Ethnomedicinal plants used to treat skin diseases by Tharu community of district Udham Singh Nagar, Uttarakhand, India. Journal of Ethnopharmacology 158(Part A): 140-206. DOI: https://doi.org/10.1016/j.jep.2014.10.004

Silja, V. P., K. S. Varma and K. V. Mohanan. 2008. Ethnomedicinal plant knowledge of the Mullu kurtuma tribe of Wayanad district, Kerala. Indian Journal of Traditional Knowledge 7(4): 608-612.

Singh, K. K. 1997. Studies on native medicine of Jaunsari Tribe of Dehradun District, Uttar Pradesh, India. International Journal of Pharmacognosy 35(2): 105-110. DOI: https://doi.org/10.1076/phbi.35.2.105.13289

Storm, A., E. Benfeldt, S. E. Andersen and J. Serup. 2008. A prospective study of patient adherence to topical treatments: 95% of patients underdose. Journal of the American Academy of Dermatology 59(6): 975-980. DOI: https://doi.org/10.1016/j.jaad.2008.07.039

Tahvilian, R., S. Shahriari, A. Faramarzi and A. Komasi. 2014. Ethno-pharmaceutical formulations in Kurdish ethno-medicine. Iranian Journal of Pharmaceutical Research 13(3): 1029-1039.

Takahashi, M., Y. Asiku, K. Takara and K. Wada. 2012. Screening of medicinal and edible plants in Okinawa, Japan, for enhanced proliferative and collagen synthesis activities in NB1RGB human skin fibroblast cells. Bioscience, Biotechnology, and Biochemistry 76(12): 2317-2320. DOI: https://doi.org/10.1271/bbb.120478

Tolossa, K., E. Debela, S. Athanasiadou, A. Tolera, G. Ganga and J. G. Houdijk. 2013. Ethno-medicinal study of plants used for treatment of human and livestock ailments by traditional healers in South Omo, Southern Ethiopia. Journal of Ethnobiology and Ethnomedicine 9: 32. DOI: https://doi.org/10.1186/1746-4269-9-32

Tomé, M. C. 2013. A medicina tradicional na América do Sul e as relações complexas entre os valores culturais, espirituais e terapêuticos das plantas. Revista de Geografia (Recife) 30(3): 6-23.

Uddin, M. Z., M. A. Hassan, M. Rahman and K. Arefin. 2012. Ethno-medico-botanical study in Lawachara National Park, Bangladesh. Bangladesh Journal of Botany 41(1): 97-104. DOI: https://doi.org/10.3329/bjb.v41i1.11087

Ugulu, I. 2012. Fidelity level and knowledge of medicinal plants used to make Therapeutic Turkish Baths. Studies

on Ethno-Medicine 6(1): 1-9. DOI: https://doi.org/10.1080/09735070.2012.11886413

Van der Nat, J. M., W. G. van der Sluis, K. T. D de Silva and R. P Labadie. 1991. Ethnopharmacognostical survey of *Azadirachta indica* A. Juss (Meliaceae). Journal of Ethnopharmacology 35(1): 1-24. DOI: https://doi.org/10.1016/0378-8741(91)90131-V

Villaseñor, J. L. 2016. Checklist of the native vascular plants of Mexico. Revista Mexicana de Biodiversidad 87(3): 559-902. DOI: https://doi.org/10.1016/j.rmb.2016.06.017

Villena, C. A. and J. L. Arroyo. 2012. Efecto antiinflamatorio del extracto hidroalcohólico de *Oenothera rosea* (yawar sococ) en ratas con inducción a la inflamación aguda y crónica. Ciencia e Investigación 15(1): 15-19.

Vivekraj, P. and V. Anandgideon. 2015. Medico-ethno botany: a survey of Topsengattupatti Pachamalai Hills of eastern ghats in Tamilnadu, South India. Advances in Applied Science Research 6(12): 59-63.

Zacchino, S., M. G. Derita and M. J. Svetaz. 2012. Discovery of antifungal plants in Argentinean San Luis Province: ethnomedical information or random selection? Revista Eletrônica de Farmácia 9(1): 18-39. DOI: https://doi.org/10.5216/ref.v9i1.17775

Appendix: Plants used by inhabitants from the Purépecha Plateau for the treatment of dermatological conditions: use value (UV), fidelity level (FL), modes of preparation and ethnomedicinal studies.

Scientific name (voucher specimen number in the herbarium IEB)	Vernacular name	Native plant of Mexico	Use Value Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purepecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
PTERIDOPHYTA						
Pteridaceae						
Adiantum capillus-veneris L. (2568671)	culantrillo	yes	0.03 rash (66.7), skin bumps (66.7)	The infusion of the whole plant is used for washing the affected area.	North-West Frontier Province, Pakistan, for measles treatment.	Abbasi et al., 2010
GYMNOSPERMAE						
Pinaceae						
Pinus lenophylla Schiede ex Schltdl. & Cham. (2568852)	pino	yes	0.05 skin inflammations (75), hematomas (25), psoriasis (25), skin bumps (25)	The alcoholic extract of its bark is used in fermentation form on the affected area. The oil obtained from the distillation of its resin is used to prepare ointments.	Mexico, for treating dermatitis	BDMTM, 2009.
ANGIOSPERM						
Acanthaceae						
Justicia spicigera Schltdl. (2568899)	muicle	yes	0.12 skin inflammations (50), rash (30), blemishes (20), insect bites (20), varicose veins (10)	The infusion or decoction of the flowers and leaves is used for the washing on affected area. They are also used as ferments. The leaves infusion is taken orally.	Mexican medicine, to purify the blood as well as in erysipelas, syphilis, scabies, rinoses, pimples and skin inflammations. In Guatemala, for treating eruptions and erysipelas.	Cáceres et al., 1987 Andrade-Cetto, 2009. BDMTM, 2009. Ávila-Uribe et al., 2016
Adoxaceae						
Sambucus nigra L. (2568844)	saúco, ciruelenilla	yes	0.05 cuts (50), bites (25), pruritus (25), rash (25)	The leaves are rubbed on the affected area. The decoction of leaves and bark is used to wash the lesions. With the aqueous bark extract ointments are made	Spain, Italy and Malta for treating scalps, erysipelas and other skin disorders	Guarrera et al., 2005, Benitez et al., 2010. Caruana and Amard, 2016
Amaranthaceae						
Alternanthera caracasana Kunth (2568863)	tlanglus	yes	0.09 rash (62.5), skin bumps (50), varicella (25)	The infusion of the whole plant is used for washing the affected skin area.	Mexico, used for skin scabies	Canales-Martínez et al., 2005.
Chenopodium ambrosioides L. (2568898)	epazote	no	0.02 cuts (100)	The decoction from leaves is used for washing the affected skin area.	Cuba, for cutaneous leishmaniasis. In Guatemala, for burns, skin irritation and wounds.	Cáceres et al., 1987, Monjote et al., 2009
Ibervillea interrupta Benth. (2568621)	hierba de arlomo	yes	0.08 insect bites (100), warts (28.6) psoriasis (14.3)	The aerial part is crushed, and the paste or tincture is applied on skin lesions. The infusion of leaves is used for washing the affected area.	Mexican traditional medicine, for insect bites.	BIOAGRICOOP, 1998; Navarrete-Heredia and Gómez Flores, 2005.

Appendix: Continuation.

Scientific name (voucher specimen number in the herbarium JEB)	Vernacular name	Native plant of Mexico	Use Value Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purepecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Amaryllidaceae						
Allium cepa L. (256837)	cebolla	no	0.69 burns (50), hematomas (37.5), hair fall (12.5)	The bulb is cooked, and the paste is applied on affected skin area	Reported in Mexico for the treatment of cuts, skin bumps and hair fall. Tharu community in India used it for the treatment of ringworms and skin allergy. In the Sannabus region, Italy, used as antiherpetic. In North Pakistan for treating scathe irritation caused by scorpion and hornet stings. In Edo State, Nigeria for rashes and stings. In Central Chaco, Argentina, for bites and warts. In Terengganu, Malaysia, for treating cuts, sores, pimples and abscess. In Morocco for leprosy and skin cancer	Pallitese et al., 2001; Eglarevba and Ebhuoa, 2008; BDMTM, 2009; Martinez and Barboza, 2010; El Mansouri et al., 2011; Ong et al., 2011; Akhtar et al., 2013; Sharma et al., 2014
Allium sativum L.	ajo	no	0.13 fungal infections (54.5), calluses (18.2), psoriasis (18.2), skin bumps (18.2), warts (18.2), superficial infections (18.2), skin inflammation (9.1), varicose veins (9.1)	The bulb is cooked, and the paste is applied on affected skin area. Its alcoholic extract is applied externally on lesions. With the oily bulb extract ointments are prepared.	Sannabus region, Italy, used against insect bites to lessen the pruritus. In North Pakistan for treating scathe irritation caused by scorpion and hornet stings. In Central Chaco, Argentina, for bites and warts. In Terengganu, Malaysia for treating stings, bites, and pins. In Mexico, for warts, ringworm, varicose veins and insect stings.	Pallitese et al., 2001; Andrade-Cetto, 2009; BDMTM, 2009; Martinez and Barboza, 2010; Ong et al., 2011; Akhtar et al., 2013
Anacardiaceae						
Amphipterygium adstringens Schiede ex Standl. (256881)	cuachalalate	yes	0.15 cuts (92.3), skin inflammation (38.5), burns (15.4), rash (15.4), infections with abscesses (7.7), insect bites (7.7), skin bumps (7.7)	The decoction of its bark is taken orally and is also used for washing lesions. The aqueous bark extract is used for ointment preparation.	Mexican traditional medicine for wounds, pimples, scabs, bumps, abscesses, skin inflammation, hair loss and hemorrhoids.	Chávez-Figueroa, 2005; BDMTM, 2009.
Schinus molle L. (256869)	pirul	no	0.02 cuts (50), psoriasis (50)	The alcoholic extract from leaves is used in fermentation form.	Mexican traditional medicine, as cicatrizing agent. In Brazil, as cicatrizing agent	Oliveira et al., 2007; BDMTM, 2009.

Appendix: Continuation

Scientific name (voucher specimen number in the herbarium [EB)	Vernacular name	Native plant of Mexico	Use Value Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Asteraceae						
Jaumea chermoda Mill. (2569109)	churumoyo	no	0.02 skin inflammation (50)	The infusion of leaves is taken orally	Mexico for treatment of bruising, dandruff, skin infections and pimples.	BDMTM, 2009.
Apiaceae						
Eryngium heterophyllum Hook. f. & Arn. (2569000)	hierba del sapo	yes	0.02 skin inflammation (100)	The infusion of the aerial part is taken orally. Additionally, it is used in fomentations form on skin	Not reported	
Apocynaceae						
Asclepias curassavica L. (2569005)	chinguilla	no	0.08 skin bumps (57.1), warts (42.9), calluses (28.6)	The latex of leaves and stems is applied on the skin injury, avoiding contact with healthy skin	Mexico, for treating skin bumps, scabies, erysipelas, edemas, stings of insects and wounds	BDMTM, 2009
Asphodelaceae						
Aloe vera (L.) Burm. f. (2568851)	sábila	no	0.37 cuts (62.5), skin inflammation (28.1), hair fall (21.9), hematomas (21.9), burns (18.8), superficial infections (9.4), infections with abscesses (6.3), insect bites (6.3), skin bumps (6.3), fungal infections (3.1), psoriasis (3.1), skin care (3.1)	The pulp of the fresh or roasted leaves is applied on the affected area. The pulp is also used as shampoo.	broadly used for the treatment of dermatological affections. Used in Mexico, for skin swelling and erysipelas as cicatrizing agent. Additionally, it is used for sunburns, bumps and melasma. In Kelantan, Malaysia, it is used to prevent pimples and to smooth the skin. In district of Tamil Nadu, India, for hair growth. Israelis used it for wound healing. In Wayanad district, India for dandruff treatment.	Ong and Nordiana, 1999; Lev, 2006; Silja et al., 2008; Andrade-Cetto, 2009; BDMTM, 2009; Benítez et al., 2010.
Asteraceae						
Artemisia ludoviciana Nutt. (2568888)	picanancho, pendejosa	yes	0.07 skin inflammation (100)	The aerial part is used as tincture or infusion, using the oral dosage form.	Mexican traditional medicine for treating skin bumps and wounds	BDMTM, 2009.
Artemisia mexicana Willd. ex Spreng. (2569007)	estafiate	yes	0.02 skin inflammation (100)	The infusion of the aerial part is taken orally. The alcoholic extract of the aerial part is used in fumentation form on affected area.	Mexican traditional medicine, to relieve swelling of the feet	Nicholson, 1991.
Bidens pilosa L. (2568381)	acemilla	yes	0.05 cuts (75), skin bumps (50), hair fall (25)	The infusion and decoction of the aerial parts are used for washing the affected skin area.	Mexican traditional medicine, for treating wounds, hair loss, skin irritation, skin inflammations, and burns. In Trinidad and Tobago for baby bath and cuts.	Andrade-Cetto, 2009; BDMTM, 2009; Lans, 2007.

Appendix: Continuation.

Scientific name (voucher specimen number in the herbarium IEB)	Vernacular name	Native plant of Mexico	Use Value Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Calendula officinalis L. (256876)	caléndula, virreina	no	0.17 cuts (54.5), pruritus (26.7), skin inflammation (20), psoriasis (20), burns (13.3), insect bites (13.3), rash (13.3), superficial infections (13.3), blemishes (6.7), skin bumps (6.7)	The infusion of leaves and flowers is taken orally. Additionally, it is used as fomentation form and for washing the skin lesions. The alcoholic extract from leaves and flowers is applied on the affected skin area, being also used in ointment preparation.	Widely used for the treatment of skin diseases. In Eastern Sikkim Himalayan Region, it is used in healing wounds, ulcers and burns. In Turkey, this plant is reported as bulbs for dry skin. In Mexican traditional medicine, for treating wounds as disinfectant and antiinflammatory agent.	HDMTM, 2009; Das et al., 2012; Uguta, 2012.
Eryngium Jelskianum Wilbl. (256846)	arnica blanca	yes	0.08 skin inflammation (85.7), hematomas (14.3)	The infusion from its flowers and leaves is taken orally. Additionally, it is applied in fomentation form on the affected skin area.	Mexico, for treating hematomas.	Bello-González et al., 2015.
Heterotheca inuloides Cass. (256858)	arnica	yes	0.53 skin inflammation (56.5), hematomas (45.7), cuts (28.3), infections with abscesses (4.3), skin bumps (4.3), skin care (4.3), superficial infections (4.3), insect bites (2.2), rash (2.2), varicella (2.2), varicose veins (2.2)	The infusion from its leaves and flowers is taken orally being also used in fomentation form as well as for washing the affected skin area. The alcoholic extract is used on the skin, being a part of preparation of ointments. The infusions of the plant are used as a steam bath for skin health "Temazcal"	Mexico, for the treatment of wounds, internal and external blows, bruises, cutaneous lesions, infected or not, such as pimples, sores, bruises, hives, baby scrapes, erysipelas and skin inflammation	Navarro Pérez and Avendaño Reyes, 2002; Andrade-Cetto, 2009; HDMTM, 2009; Moreno-Ortiz et al. 2014; Bello-González et al., 2015.
Lactuca sativa L.	lechuga	no	0.03 rash (100)	The infusion of its leaves is used as a bath.	Jordanian traditional medicine, it is used as emollients and hair tonic.	Al-Khalil, 1995.
Matricaria recutita L. (256875)	manzanilla	no	0.12 cuts (30), rash (30), blemishes (10), hematomas (10), skin inflammation (20), pruritus (20), skin bumps (10), superficial infections (10)	The infusion of the aerial part of this plant, mainly its flowers, is used as fomentation form and for washing the skin area. The oily flower extract is applied at the injury site.	Its cosmetic value has been established through years of traditional use worldwide. In Mexico, it is used for scorpion stings, wound washing, removal of pimples and to lighten the hair. Among Albanians, Macedonians and Gorani in the Share Mountains, it is used for wound healing. In Kopaonik Mountain (Central Serbia) used externally for skin and mucous complaints (burns, wounds and ulcers). In the Potosí Department, Bolivia, it is used for wounds and acne.	Fernández et al., 2003; Jarić et al., 2007; HDMTM, 2009; Redžepi et al., 2013

Appendix: Continuation.

Scientific name (voucher specimen number in the herbarium IEB)	Vernacular name	Native plant of Mexico	Use Value Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Montanoa tomentosa Cerv. (2568859)	gobernadora	yes	0.83 rash (100), insect bites (66.7)	The infusion of the aerial part is taken orally, being used to wash the affected area	Mexico, to treat a cultural disease called "burnout" in which a woman may have blemishes, hematomas and skin inflammation after childbirth	BDMTM, 2009; Gómez Arpa, 2013; Ávila-Uribe et al., 2016
Piqueria trinervia Cav. (2568616)	cuilcuamachicua	yes	0.81 cuts (100)	The infusion of its aerial parts is taken orally. Moreover, it is used for washing the affected skin area	Mexico, it is used to treat skin bumps and bites, for washing wounds and controlling the measles and rash	BDMTM, 2009
Senecio salignus DC. (2568772)	pata amarilla	no	0.86 hematomas (40), skin bumps (40), cuts (20), skin inflammation (20),	The decoction of flowers and leaves is used to wash the skin and as fomentation foam on the affected area. The leaves are crushed with salt and the paste is applied on the injury site.	Abbottabad City, Pakistan used against snake bite and as antiseptic over wounds. It is also used for treating swelling of joints	Saboor and Ahmad, 2009.
Senecio oliveranus L. (2568791)	ashaxoma	no	0.67 cuts (100), burns (33.3)	The decoction of the aerial part is used in fomentation foam and for washing the affected area. The aerial part is crushed, and the paste is applied at the site of injury.	Mexico, it is used for rash and blemishes treatment. Malayalis tribes in Dharmapuri, India use it externally on wounds. In Italy and Spain, it is used for treating warts.	Guarrera et al., 2005; Ratnya et al., 2008; BDMTM, 2009; Benitez et al., 2010; Bello-Gonzalez et al., 2015.
Sicyos serratus Cav. (2568681)	hierba de burro	yes	0.83 rash (100), skin bumps (33.3)	The infusion of the flowers is taken orally and is used to wash the sites of injury	Mexico used in cuts of feet	Kingsbury, 2001
Tagetes lucida L. (2568471)	cempasúchil, guarecita	yes	0.83 cuts (66.7), rash (33.3)	The infusion of flowers and leaves is used for washing the skin. The flowers are rubbed directly on the affected area	Mexican traditional medicine, for treating erysipelas, wounds, rash, sores, warts and pimples. In Bangladesh, for treating eczema, abscess, ulcer, boils, scabies, itch, infections, dermatitis, rash, sores, scar and warts. In Iran, for treating wounds.	Andrade-Cetto, 2009; BDMTM, 2009; Mollik et al., 2010; Amiri and Joharchi, 2013
Tagetes filifolia Lag. (2568840)	anís de grano	yes	0.67 skin bumps (83.3), cuts (36.7), rash (16.7)	The infusion of the grains is taken orally and is applied as fomentation foam. The aerial parts are crushed, and the paste is applied at the injury site	Mexico, it is used in baths for skin care. In Argentinian traditional medicine, for itch and healing infected wounds.	BDMTM, 2009; Zacchino et al., 2012.

Appendix: Continuation.

Scientific name (voucher specimen number in the herbarium IEB)	Vernacular name	Native plant of Mexico	Use Value Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Tagetes lucida Cav. (256843)	sabia Morita	yes	0.05 cuts (400), skin care (25), superficial infections (25)	The infusion of the aerial part is used for washing the affected area.	Mexican traditional medicine, for various veins and skin inflammation	BDMTM, 2009.
Tagetes remotiflora Kunth (256839)	cinco llagas	no	0.05 cuts (400), superficial infections (50)	The infusion from the aerial part or roots is used for washing the affected area. The flowers and leaves are also used as infusion	Not reported.	
Tanacetum parthenium (L.) Sch. Bip. (256896)	hoja de hilo	no	0.01 insect bites (100)	The infusion from the aerial part is taken orally and used for washing skin lesions	Mexico, for skin inflammation. In Iran for hair care.	Andrade-Cetto, 2009, Amin and Jahanchi, 2013
Taraxacum officinale F.H. Wigg. (256852)	diente de león	no	0.03 infections with abscesses (66.7), rash (33.3)	The infusion from the aerial part is used for washing the affected skin being also taken orally	Mexican traditional medicine, for treating skin bumps, scabies, abscesses, acne and skin infections. In Central Italy for treating warts. In Himachal Pradesh, to cure boils.	Guarrera, 2005; Hurtado-Reco et al. 2016, Lal and Singh, 2008; BDMTM, 2009.
Tithonia diversifolia (Hemsl.) A. Gray (256922)	capitanejo	yes	0.06 cuts (440), infections with abscesses (440), superficial infections (440)	The infusion of flowers and leaves, for washing the skin affected area. It is also used for ointments preparation.	Mexico, it is used to cure bumps, sores and wounds. In addition, it is used for scabies, pimples, blackheads and for removing itching. Among Kalanguya tribe in Philippines for treating cuts and wounds. In Western Uganda, as antifungal and antibacterial agent	Ramatenesi-Mugisha et al., 2008; BDMTM, 2009; Balangcod and Balangcod, 2011.
Boraginaceae						
Borago officinalis L. (256830)	borraja	no	0.07 skin inflammation (500), pruritus (33.3), rash (33.3), psoriasis (16.7), skin bumps (16.7)	The infusion of the aerial part is taken orally and used for washing the affected area. Additionally, it is used in fermentation form	Malta, for skin disorders and minor wounds.	Caruana and Attard, 2016
Tournefortia mutabilis Vent. (256856)	hoja de lija	yes	0.03 cuts (66.7), superficial infections (66.7)	The decoction of the aerial part is used for washing the affected area.	Not reported.	
Brassicaceae						
Lepidium virginicum L. (256908)	pantalillo	yes	0.03 infections with abscesses (100)	The infusion of flowers and leaves is used for washing skin.	Mexican traditional medicine, it is used for rash, burns and scrapes.	BDMTM, 2009.

Appendix: Continuation.

Scientific name (voucher specimen number in the herbarium IEB)	Vernacular name	Native plant of Mexico	Use Value Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Burseraceae						
Bursera bipinnata (DC.) Engl. (2568671)	copal	yes	I:03 superficial infections (100), cuts (66.7)	The resin is applied directly on the injured skin.	Mexican traditional medicine on wounds and skin infections.	BDMTM, 2009.
Commelinaceae						
Commelina diffusa Burm. f. (2568889)	hierba de pollo	yes	I:03 cuts (100), insect bites (33.3)	The decoction of the aerial part is taken orally and used for washing skin. The leaves are crushed, and the paste is applied directly on the lesions.	South Orissa, India, to heal burn injuries, aches, and boils. In Nayagarh District, to stop bleeding of wounds and cuts.	Sarma and Saikia, 2010; Panda and Misra, 2011
Convolvulaceae						
Ipomoea murucoides Roem. & Schult. (2568878)	cazahuate	yes	I:02 infections with abscesses (100), burns (50)	The infusion of flowers and stems is used for washing the affected area.	Mexico, it is used for the treatment of hair loss and rash as well as to remove nails and for managing scorpion stings	Hurtado-Rico et al., 2006; BDMTM, 2009
Equisetaceae						
Equisetum hyemale var. *affine* (Engelm.) A.A. Eaton (2568870)	cola de caballo	yes	I:05 skin inflammation (50), hair fall (50), fungal infections (25)	The infusion of the aerial part is taken orally and used for washing skin. A shampoo is also prepared with the plant.	Not reported	
Euphorbiaceae						
Euphorbia maculata Lag. (2568851)	hierba de la golondrina	yes	I:05 rash (100)	The infusion of the whole plant is used in fomentation form, baths and for washing the affected skin area	Croatia, for treating warts	Jhepons et al., 2004
Euphorbia pulcherrima Willd. ex Klotzsch (2568865)	noche buena, pascua	yes	I:03 skin bumps (100)	Leaf and stem latex are applied directly on the skin lesion.	Mexico, for treating warts, erysipelas, sores, skin infections and wounds. For the management of inflammations and blows in Lawachara National Park, Bangladesh, for cut injury. In Central Nepal to relieve boils, it is also useful for treating skin diseases.	BDMTM, 2009; Malla and Chhetri, 2009; Uddin et al., 2012.
Fabaceae						
Caesalpinia coriaria (Jacq.) Willd. (2569035)	vaquilote	no	I:03 cuts (66.7), skin bumps (33.3)	The decoction of the fruits is used in fomentation form and for washing the affected skin area	Mexican traditional medicine for wounds and blemishes. In Tamilnadu, South India for treatment of acne.	BDMTM, 2009; Vevekraj and Anandgideon, 2015.

Appendix: Continuation.

Scientific name (voucher specimen number at the herbarium IEB)	Vernacular name	Native plant of Mexico	Use Value Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Fagaceae						
Quercus rugosa Née (2568835)	encino	yes	0.05 superficial infections (75), cuts (50), burns (25), infections with abscesses (25), gnorjans (25)	The decoction of its bark is used for washing skin.	Mexico, for treating sores, skin ulcers and bumps	Luna-José et al., 2003; BDMTM, 2009
Geraniaceae						
Geranium seemannii Peyr. (2568887)	pata de león	yes	0.02 cuts (100)	The infusion of the aerial part is used for washing skin.	Mexican traditional medicine for urticaria, scabies, wounds and skin inflammation, sores, pimples, scabs, bruises	BDMTM, 2009; Bello-González et al., 2015; Ávila-Ortiz et al., 2016.
Lamiaceae						
Agastache mexicana (Kunth) Lint & Epling Des. R. Bedolla (2569146)	toronjil	yes	0.05 skin inflammation (75), psoriasis (25), itch (25)	The infusion of the aerial parts is taken orally. Additionally, it is used in fomentation form and for washing the affected skin areas	Mexico, for burns and insect bites. It is also used as antiseptic.	Andrade-Cetto, 2009; BDMTM, 2009.
Dorystaehum microstemum (Moc. & Sessé ex Benth.) Kuntze (2569110)	nurite, te del monte	yes	0.07 skin inflammation (83.3), insect bites (16.7)	The infusion of leaves is taken orally, being also used in fomentation form and baths	Not reported.	
Lavandula officinalis Chaix (2569113)	lavanda	no	0.05 skin care (75), hair fall (25), pimples (25)	The infusions of the aerial parts is used for washing the affected skin area. It is also used for lotion preparation	Southern Italy, in the preparation of anti-varicose mixtures	De Feo et al., 1992
Leonurus inperfolia (L.) R. Br. (2569119)	castilleja	no	0.02 cuts (100), superficial infections (50)	The infusion of the aerial part is used for washing the affected skin area.	Bidar district, India, it is used for treating head sores. In Eastern Himalayan region of India for treating burns. In Middle (Grand) Caicos, Turks and Caicos Islands (West Indies) for treating eczemas, wound and skin disorders.	Halberstein, 1997; Kala, 2005; Prashantkumar and Vidyasagar, 2008.
Lepechinia caulescens (Ortega) Epling (2569229)	salvia	yes	0.08 skin care (60), cuts (40), superficial infections (20)	The infusion of the aerial part of this plant is used to wash the sites of injury and in a steam bath "temazcal". The flower and leaf poultice is applied over the affected area.	Mexico, it is used to treat swelling and blows.	Navarro Perez and Avendaño Reyes, 2002; Bello-González et al., 2015

Appendix: Continuation

Scientific name (voucher specimen number in the herbarium IEB)	Vernacular name	Native plant of Mexico	The Value Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Marrubium vulgare L. (2569111)	manrubio	no	0.05 skin inflammation (100)	The infusion from the aerial part of this plant is used in fomentations form.	Mexico. It is used for blemishes, scabies, pimples, wounds, fungus, measles, dandruff and in cases of hair loss. In Southern Italy, it is used as disinfectant for small wounds. In North of Morocco, used to heal abscesses. In Spain for erysipelas and herpes.	Navarro Pérez and Avendaño Reyes, 2002; Leporini and Guarrera, 2007; BDMTM, 2009; Benítez et al., 2010, Khabbach et al., 2012; Jaimez-Vázquez et al., 2013
Ocimum basilicum L. (2569121)	albahacar	yes	0.02 blemishes (50), rash (50)	The infusion of the aerial part is used for washing skin. The leaves are crushed, and the paste is applied to the affected area.	Mexico, for skin bumps and infections, pimples and hair loss against accents and stings of scorpion. Also, it is used for varicose veins. In Kali Gandaki Watershed Area, Nepal, for the treatment of fungal skin infections. In West Cameroon, it is used against mosquito bites.	Navarro et al., 1996; Joshi and Joshi, 2000; Jirovetz et al., 2008; BDMTM, 2009.
Origanum majorana L. (2569120)	mejorana	no	0.05 rash (100)	The infusion of the aerial part is used as a bath.	Samnium popular medicine for treatment of small abscesses and bruises	Fierro Ipe et al., 2008
Rosmarinus officinalis L. (2569118)	romero	no	0.16 hair fall (35.7), skin care (28.6), insect bites (14.3), hematomas (7.1), skin inflammation (7.1), psoriasis (7.1), rash (7.1).	The infusion of the aerial part is used for washing skin and in fomentation form. It is also used as a foot bath in the "temazcal". The alcoholic extract of the aerial part is used in fomentations form in the sites of skin injury	Mexico, it is used for skin problems, to heal wounds, cancer sores and measles. Additionally, it prevents hair loss, serves to wash hair and against dandruff. It is also used in mens blows. In North Sahara the plant is used for beauty care, hair care, against snake bites and as cicatrizing agent. In Spain as antiinflammatory agent. In Morocco, for treating wounds and skin cancer	Andrade-Cetto, 2009; BDMTM, 2009; Benítez et al., 2010; El Mansouri et al., 2011; Maiza et al., 2011; Khabbach et al., 2012
Salvia amarissima Ortega (2569115)	chan	yes	0.03 skin inflammation (66.7), rash (33.3)	The infusion from the aerial parts of the plant is taken orally. It is also used for washing the affected skin area	Mexico, for hair care.	INEGI, 2014.
Salvia lasiantha Cav. (2569141)	salvia	yes	0.05 skin care (100)	With its flowers an ointment is prepared and applied directly on the skin.	Not reported.	

Appendix: Continuation.

Scientific name (voucher specimen number in the herbarium IEB)	Vernacular name	Native plant of Mexico	Use Value Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Salvia mexicana L. (2569211)	cuaraherapheeeni	yes	0.05 rash (75), insect bites (25)	The infusion of its leaves is taken orally and used in fomentation form and for washing skin. Leaves are directly rubbed on the affected skin area	Mexican traditional medicine for treating skin bumps.	Navarro-Perez and Avendaño Reyes, 2002
Salvia macrophylla Kunth (2569117)	mirto	yes	0.05 rash (50), hemangiomas (25) skin inflammation (25)	The infusion of the aerial part is taken orally, for washing skin and in fomentation form	Mexico, it is used in dermal problems such as pimples, rash, measles and for treating scarlet fever.	BDMTM, 2009.
Lauraceae						
Persea americana Mill. (2568875)	aguacate	yes	0.08 cuts (42.9), hemangiomas (28.6), skin inflammations (28.6) blemishes (14.3), hair fall (14.3).	The infusion and decoction of leaves and seeds are used in fomentation form and for washing skin. The fruit is crushed and directly applied on the skin area.	Mexico, for treating tinea pedis, scabies, rash, bruises, head lice, wounds and sores in Morocco, for hair care	Hurtado-Ríos et al., 2006; Andrade-Cetto, 2009; BDMTM, 2009; El Mansouri et al., 2011; Bello-González et al., 2015.
Malvaceae						
Ceiba aesculifolia (Kunth) Britten & Baker f. (2568681)	pochote	yes	0.02 rash (100)	The infusion of its bark is taken orally.	Mexico, for treating dermatitis and snake bites.	BDMTM, 2009.
Malva parviflora L. (2568481)	malva silvestre	no	0.33 rash (61.8), cuts (36.4), pruritus (18.2), skin inflammations (9.1), skin bumps (9.1)	The infusion of the aerial part of the whole plant is used for baths, washes and in fomentation form.	Mexico, it is used for treating the inflammation caused by blows, wounds or abscesses. Additionally, it is used for deep bumps, cuts or wounds, stings of poisonous animals or spiders, lesions on the lips and purulent abscesses. In Al-Rass province, Saudi Arabia, it is used for promoting hair growth. In Tigray region, Northern Ethiopia, it is used for treating anthrax.	Andrade-Cetto, 2009; BDMTM, 2009; El-Ghazali et al., 2010; Gebrezgabiher et al., 2013
Salvia lavanduloides D. Don (2568491)	humarí	yes	0.33 skin bumps (100), cuts (9.1), hemangiomas (9.1)	The flowers and leaves, mainly the leaves, are crushed with salt and the paste is applied on the affected area	Not reported.	

Appendix: Continuation

Scientific name (voucher specimen number in the herbarium IEB)	Vernacular name	Native plant of Mexico	Use Value Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Meliaceae						
Azadirachta indica A. Juss. (2568931)	neem	no	0.02 cuts (100), skin inflammation (50), psoriasis (50)	The infusion of the leaves is taken orally and used in fomentation form and for washing skin.	this plant appears in classical texts of Ayurveda for its positive effects on the skin. In Trinidad and Tobago, it is used for treating measles. In Morang, Nepal, it is used for scabies. In Coastal Karnataka, for wound healing and for managing herpes, scabies and ringworm. In Manipuri tribal community, Bangladesh, for skin diseases.	Van der Nat et al., 1991; Acharya and Pokharel, 2006; Lans, 2007; Parvez Rana et al., 2010; Bhandary and Chandrashekar 2011; Bhat et al., 2014.
Moraceae						
Ficus carica L. (256883)	higo	no	0.03 skin bumps (100)	The latex from leaves or stems is directly applied on the affected area.	Mexico for calluses, warts and hemorrhages. In North of Morocco, central Italy, and Spain, for treating warts.	Guarrera et al., 2005; BDMTM, 2009. Benitez et al., 2010. Khabbach et al., 2012.
Moringaceae						
Moringa oleifera Lam. (256836)	moringa	no	0.03 skin inflammation (100), infections with abscesses (33.3), psoriasis (33.3)	The infusion of the leaves is taken orally and used in fomentation form. The seeds are eaten	Ugandan rural communities for treating skin diseases. In Karnataka, India for eczema and for treating sores at the tip of penis. In Madhupur forest region of Bangladesh, for treating sores	Harsha et al., 2003; Manzoor-ul-Kadir Mir et al., 2009, Kasolo et al., 2010; Bhat et al., 2014.
Musaceae						
Musa paradisiaca L.	platano	no	0.06 fungal infections (40.0), skin inflammations (40.0), skin bumps (20.0)	The skin of the fruit is rubbed on the affected area. The seed is broken and the infusion of latex is taken orally	Mexican traditional medicine, as cicatrizing agent. In Lawachara National Park, Bangladesh, for cut injury. In Valsad district, Gujarat, it is used for calluses.	BDMTM, 2009. Shuib et al., 2011. Uddin et al., 2012.
Myrtaceae						
Psidium guajava (Kunth) Bonpl. (256872)	arrayan	no	0.03 rash (66.7), skin bumps (33.3)	The infusion of the aerial part is used for washing skin	Chile and Ecuador, for the skin care of women after childbirth	Tera, 2013
Psidium sartorianum (O. Berg) Nied (256892)	bella rosa	yes	0.01 rash (100)	The infusion of its aerial parts is used for washing skin.	Mexican traditional medicine for scabies, hematomas, as cicatrizing and haemostatic agent.	BDMTM, 2009.

Appendix: Continuation.

Scientific name (voucher specimen number in the herbarium IEB)	Vernacular name	Native plant of Mexico	Use Value/Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Onagraceae						
Oenothera rosea L'Hér. ex Aiton (256845)	hierba del golpe	yes	0.21/hematomas (94.4), inflammation (33.3), cuts (5.6), rash (5.6)	The infusion of the aerial part is used for washing skin and in fomentation form on the affected area. The aerial part is crushed, and the paste is applied on the site of injury.	Mexican traditional medicine for treating apparent skin conditions such as infected lesions, bumps, sores, itching, erysipelas and scabies.	Navarro Pérez and Avendaño Reyes, 2002; Hurtado-Rico et al., 2006; Andrade-Cetto, 2009; BDMTM, 2009; Bello-González et al., 2015.
Papaveraceae						
Argemone ochroleuca Sweet (256880)	chicalote	yes	0.02/superficial infections (100), cuts (50)	The decoction of the aerial part is used for washing skin. The alcoholic extract of the aerial part is applied in fomentation form.	Mexico, it is used for the treatment of ringworm, scabies, hair loss, pimples, hives and warts. In Hawassa city, southern Ethiopia, for wound healing.	BDMTM, 2009; Regassa, 2013; Ávila-Uribe et al., 2016.
Bocconia arborea S. Watson (256901)	inguambo	yes	0.13/cuts (72.7), hematomas (36.4), skin inflammation (18.2)	The decoction of the bark is used in fomentation form and for washing skin. A piece of bark is placed in water and taken orally.	Mexican traditional medicine, for the treatment of scabies and warts.	BDMTM, 2009.
Phytolaccaceae						
Phytolacca icosandra L. (256897)	conguera	yes	0.05/superficial infections (50), cuts (25), psoriasis (25), skin care (25)	The infusion of the aerial part is used for washing skin. The leaves and fruits are rubbed directly in the area.	Mexico, it is used to prevent hair loss, against ringworm, measles, and dandruff. Additionally, it is used against the fungi and for treating skin bumps or wounds.	BDMTM, 2009; Bello-González et al., 2015; Ávila-Uribe et al., 2016.

Appendix: Continuation.

Scientific name (voucher specimen number in the herbarium IEB)	Vernacular name	Native plant of Mexico	Use Value (Dermatological conditions (fidelity level))	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Plantaginaceae						
Plantago major L. (2568411)	llantén	no	0.05 cuts (75), infections with abscesses (50), superficial infections (50), skin inflammation (25), psoriasis (25)	The infusion of the aerial part is used in fomentation form and for washing skin.	Mexico, as an anti-inflammatory for bumps, wounds, mumps and abscesses. For pimples, wounds and purulent sores, to suppress hemorrhages. At Arunachal Pradesh, India for treating wound and inflammations. In Kurdish ethnomedicine, it is used topically by its analgesic, antiinflammatory and wound healing properties. Among Croatians living in Northern Istria, it is applied externally as hemostatic and cicatrizing agent. In Zangelan district, Northeast Iran, for treating skin allergies. In Iran, for treating eczema.	Guarrera et al., 2005; Pieroni and Giusti, 2008; BDMTM, 2009; Das et al., 2012; Amiri and Joharchi, 2013; Tabrizian et al., 2014
Poaceae						
Avena sativa L.	avena	no	0.02 skin bumps (98.0), skin care (50.0)	The seed is soaked in water and grinded, the resulting paste is used for preparing a soap that is applied on the skin area.	Mexican traditional medicine, for treating wounds, skin impurities and melasma. In Iran, for treating acne	BDMTM, 2009; Amiri and Joharchi, 2013.
Zea mays L. (2568555)	maíz	yes	0.02 fungal infections (100)	The supernatant of its nixtamalization called "nejayote" is used to wash the affected area.	Mexico, for skin care and as a hot bath in the "temazcal". In Iran, as anti-inflammatory agent	BDMTM, 2009; Amiri and Joharchi, 2013
Polemoniaceae						
Loeselia mexicana (Lam.) Brand (2568644)	espinosilla	yes	0.08 hair fall (42.9), skin inflammation (28.6), insect bites (28.6), varicella (28.6), fungal infections (14.3)	The infusion of the aerial part is taken orally, being also used in baths and washes.	Mexico, for hair loss, dandruff, oily hair, erysipelas, rubella, abscesses, and varicose veins	BDMTM, 2009; Bello-González et al., 2015; Ávila-Uribe et al., 2016
Polygonaceae						
Polygonum aviculare Buch.-Ham. ex D. Don (2568886)	hierba de la golondrina	no	0.05 rash (50), hematomas (25), skin bumps (25)	The infusion of the whole plant is used for washing skin and boils.	Uttar Pradesh, India, for treating boils.	Singh, 1997.

Appendix: Continuation.

Scientific name (voucher specimen number in the herbarium IEJ)	Vernacular name	Native plant of Mexico	Use Value Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Proteaceae						
Macadamia integrifolia Maiden & Betche (256891)	nogal de nuez de Macadamia	no	0.03 hair fall (66.7), cuts (33.3)	The decoction from leaves is used in washing skin.	Hawaiian traditional medicine, it is used against vitiligo.	Ukuma et al., 2011
Rosaceae						
Eriobotrya japonica (Thunb.) Lindl (256853)	níspero	no	0.02 cuts (100), superficial infections (100)	The infusion from its leaves is used for washing skin.	Mexican traditional medicine, for treating varicose veins. In Jammu District, India, for the treatment of skin diseases.	BDMTM, 2009; Priya and Sharma, 2014
Prunus persica (L.) Batsch (256866)	durazno	no	0.03 rash (100), hematomas (33.3)	The infusion of its leaves is taken orally. It is also used for washing skin.	Mexico, for dry skin, rash, scrapes and wounds.	Andrade-Cetto, 2009; BDMTM, 2009.
Rosa gallica L. (256842)	rosa de castilla	no	0.08 skin bumps (57.1), rash (42.9), pruritus (28.6), blemishes (14.3)	The infusion of its leaves is taken orally. Additionally, it is used for washing skin and in fomentation form	Mediterranean coast of Spain, its petals are a part of an ointment used for skin diseases.	Pascual, 1994
Rutaceae						
Citrus aurantiifolia Swingle	limón	no	0.06 blemishes (20), cuts (20), fungal infections (20), infections with abscesses (20), psoriasis (20)	The juice of the fruit is used in infusions and ointments to be applied on the affected area. The juice is placed in a copper bowl and allowed to stand overnight to be applied to the skin area in the morning.	Mexican traditional medicine, for treating skin spots and scabies. In Kelantan, Malaysia, for dandruff treatment. In coastal parts of Karnataka, India, for boils. In Hmar tribe, in India for purulent boils. In Nicaragua Atlantic Coast for itching.	Barnes, 1994; Hug and Nordiana, 1999; BDMTM, 2009; Nath and Choudhury, 2010; Bhat et al., 2014
Ruta graveolens L. (256869)	ruda	no	0.08 hematomas (28.6), rash (28.6), skin inflammation (14.3), psoriasis (14.3) skin bumps (14.3)	The infusion of the aerial part is used for washing skin and in fomentation form. The aerial part is roasted and placed in alcohol for ferments. Fresh leaves are rubbed directly into the affected area.	Mexico, for treating varicose veins and wounds. In Mediterranean traditional medicine, against acne and as a topical analgesic.	Bonilla et al., 2008; BDMTM, 2009
Salicaceae						
Salix humboldtiana Kunth (256902)	eucalipto	yes	0.03 skin acne (66.7), skin inflammation (33.3)	The infusion of the leaves is used as a hot bath in the "temaxcal". The alcoholic extract from the leaves is used in fomentation form	Mexican traditional medicine, for treating gangrene.	BDMTM, 2009.
Scrophulariaceae						
Buddleja sessiliflora Kunth (256904)	tepenja	yes	0.02 hair fall (100), rash (50.0)	The root is crushed and applied on the area. Root decoction is taken orally and used for washing skin	Mexico, it is used in swellings, varicose veins and for healing wounds	BDMTM, 2009.

Appendix: Continuation.

Scientific name (voucher specimen number in the herbarium TEB)	Vernacular name	Native plant of Mexico	Use Value Dermatological conditions (Fidelity level)	Mode of preparation by inhabitants from the Purepecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Schisandraceae						
Illicium verum Hook. f.	anís estrella	no	0.10 skin bumps (300), rash (33.3), pruritus (11.1)	The infusion of its fruits is taken orally and used for washing skin and in fermentation form.	Malaysia, it is used in a mixture for pelting	Ong and Nordiana, 1999
Solanaceae						
Brugmansia suaveolens (Humb. & Bonpl. ex Willd.) Sweet (256884)	floripondio	no	0.03 hematomas (33.3), infections with abscesses (33.3), skin inflammation (33.3)	The infusion of its flowers and leaves is used in fomentation form and for washing skin	Among Zapotecs of the Istmus-Sierra (Oaxaca, Mexico), it is used for dermatological illness. In Sarban Hills, Abbottabad, KP, Pakistan, for treating skin disorders	Frei et al., 1998; Ijaz et al., 2016.
Nicotiana tabacum L.	tabaco	no	0.02 fungal infections (50), psoriasis (50)	The ash from the cigarettes is placed on the affected skin area.	Mexico, for treating wounds, bruises, burns, pimples, rash, erysipelas, dermatitis, scabies, insect bites, herpes and inflammation. In Southern Ethiopia, against skin infections and external parasites. Among the tribals of Mizoram, India, for treating leucoderma, wounds and cuts	Bhandari and Gaikwad, 2005; BDMTM, 2009; Tolossa et al., 2013.
Physalis pubescens L. (256874)	tomate	yes	0.08 rash (100)	The infusion of the fruit, mainly the shell, is used for baths and for washing skin.	Mexican traditional medicine, for treating wounds. In Spain, for burns.	BDMTM, 2009; Benítez et al., 2010.
Solanum nigrescens M. Martens & Galeotti (256837)	hierba mora	yes	0.09 rash (87.5), skin bumps (12.5), varicella (12.5)	The infusion of fruits and leaves is used for baths and for washing skin. The fruits are rubbed directly on the affected area	Mexico, for wounds, eczema, erysipelas, bruises, rash, wounds, burns, abscesses, pimples, inflammation, insect bites, healing and hair care	Navarro Pérez and Avendaño Reyes, 2002; Andrade-Cetto, 2009; BDMTM, 2009; Avila-Uribe et al., 2016.
Solanum tuberosum L.	papa	no	0.05 burns (75), insect bites (25)	The tuber shell is rubbed in the area.	Mexico, to treat dandruff, hair loss, burns, swellings. In North of Morocco, for the treatment of burns. In Bangladesh, for swelling and skin rashes. In Romania, for wounds and swelling.	Pieroni et al., 2003; BDMTM, 2009; Khahbach et al., 2013; Mahbubur Rahman et al., 2015

Appendix: Continuation.

Scientific name (voucher specimen number in the herbarium IEB)	Vernacular name	Native plant of Mexico	Use Value: Dermatological conditions (fidelity level)	Mode of preparation by inhabitants from the Purépecha Plateau	Ethnomedicinal studies worldwide mentioning the use of the species for dermatological conditions	References
Tropaeolaceae						
Tropaeolum majus L. (2568895)	mastuerzo	no	0.03 cuts (33.3), hematomas (33.3), infections with abscesses (33.3), skin inflammation (33.3), superficial infections (33.3)	The infusion of the aerial part is used in fomentations form. The alcoholic extract from the seeds is used for washing skin affected area.	Mexico, for treating melasma and skin bumps. The Angami Tribe, India, used it to cure boils. Used in Uruguay, to prevent hair fall and dandruff, for inflammation, small burns and skin infections.	BDMTM, 2009; Grewal, 2010; Chase and Singh, 2013; Avila-Uribe et al., 2016
Urticaceae						
Urtica subincisa Benth. (2569061)	ortiga	yes	0.17 skin inflammation (60), varicose veins(60), skin bumps (16.7)	The alcoholic extract of the aerial part is applied in the form of fomentations. The infusion of its leaves is taken orally.	Not reported.	
Verbenaceae						
Aloysia citrodora Palau (2568894)	cedrón	yes	0.03 skin inflammation (100)	The infusion of the leaves is taken orally and applied in fomentations form.	Southwest of Morocco. It is used for skin care	Ouhaddou et al., 2015.
Verbena carolina L. (2568854)	verbena	yes	0.06 hair fall (100)	The infusion of the whole plant is used to wash the area.	Mexico, it is used to treat hair loss dandruff, rash, bruising, skin infection, pimples and wounds	Navarro-Pérez and Avendaño-Reyes, 2002. BDMTM, 2009; Bello-González et al., 2015

CAPÍTULO 2. Extracto etanólico, fracciones y bio-aceites de partes aéreas de *Urtica subincisa*: un análisis comparativo de su composición química, toxicidad y actividad anti-IL17 en queratinocitos HaCaT

VI.3. PREFACIO

Este capítulo es el tema del artículo titulado: “Ethanolic extract, solvent fractions and bio-oils from *Urtica subincisa* aerial parts: a comparative analysis of their chemical composition, toxicity and anti-IL-17 activity on HaCaT keratinocytes”. El cual se encuentra en el proceso de revisión de una revista científica.

El presente capítulo se dirige a dar respuesta a los objetivos particulares # 2 y 3. Al contar con el catálogo etnofarmacológico presentado en el capítulo 1, se seleccionó a *Urtica subincisa* como la planta herbácea que sería considerada como candidata terapéutica para estudios subsecuentes. Esta planta, nativa de México, presentó un alto valor de uso (0.17), además de que hasta el presente no cuenta con estudios fitoquímicos, toxicológicos y farmacológicos, lo que garantizó la novedad de la investigación. La extracción convencional de las partes aéreas de la planta se realizó con una mezcla etanol/agua (90:10 v/v), simulando el uso tradicional reportado en la etnomedicina Purépecha. El extracto crudo obtenido fue posteriormente fraccionado por extracción líquido/líquido con vistas a realizar una caracterización más exhaustiva de las moléculas contenidas en el extracto crudo. La biomasa residual, libre de extractivos, fue subsecuentemente sometida a un proceso de pirólisis para obtener aceites pirolíticos. El extracto etanólico, sus fracciones y los aceites pirolíticos se caracterizaron químicamente, se realizaron pruebas para conocer su toxicidad en un cultivo de células HaCaT, y finalmente se probó su capacidad para inhibir la producción de IL-8, a través de la inducción de su síntesis por IL-17.

VI.4. ARTÍCULO

Ethanolic extract, solvent fractions, and bio-oils from *Urtica subincisa* aerial parts: a comparative analysis of their chemical composition, toxicity and anti-IL-17 activity on HaCaT keratinocytes

Roberto Esquivel-García[a,b], Ayca Seker[c], Nehal Abu-Lail[d], Manuel García-Pérez[c], Alejandra Ochoa-Zarzosa[b], Martha-Estrella García-Pérez[a*]

Affilations:

[a]Instituto de Investigaciones Químico-Biológicas. Universidad Michoacana de San Nicolás de Hidalgo. Morelia, Michoacán, Mexico. [b]Centro Multidisciplinario de Estudios en Biotecnología-FMVZ, Universidad Michoacana de San Nicolás de Hidalgo. Tarímbaro, Michoacán, Mexico. [c]Deparment of Biological Systems Engineering, Washington State University, Pullman, WA, USA. [d]Department of Biomedical Engineering, University of Texas at San Antonio, TX, USA.

Address for reprint requests:

Martha Estrella García-Pérez, PhD

[a]Instituto de Investigaciones Químico-Biológicas. Universidad Michoacana de San Nicolás de Hidalgo. Avenida Universidad S/N. Edificio B3 Ciudad Universitaria, Morelia, Michoacán 58030, Mexico. Phone number: (+52) 443 142 9280. E-mail: margar@live.ca

VI.4.1. Abstract

Ethnopharmacological relevance: *Urtica subincisa* Benth. has been used to treat inflammatory skin conditions for centuries by Purépecha traditional medicine, an ancient culture that has left an important legacy to the knowledge of Mexican medicinal plants.

Aim of the study: *This* study compares and chemically characterizes the ethanolic extract, its solvent fractions and bio-oils from *U. subincisa* aerial parts and analyzes their toxicity and anti-IL17 properties on HaCaT keratinocytes in order to propose future applications for treating psoriasis, an incurable, inflammatory, dermatological disorder.

***Methods*:** The aerial parts from *U. subincisa* were extracted by maceration using 90:10 v/v ethanol/water and the crude extract (CE) fractionated with hexane, ethyl acetate and water to obtain phases A, B and C, respectively. The extracted biomass was then pyrolyzed to produce bio-oils 1 and 2. *U. subincisa* raw materials, CE, phases A-C, and the two bio-oils were characterized by Py-GC-MS, proximate, elemental analysis, FTIR, UV-fluorescence and GC-MS to have an overview of their chemical composition. Toxicity of all samples on HaCaT keratinocytes was assessed by the MTT assay and the non-cytotoxic concentrations were used to determine their inhibitory activity on IL-8 production in HaCaT keratinocytes stimulated with IL-17.

Results: FTIR spectra showed a higher diversity of functional groups in CE and its fractions than in bio-oils. On the other hand, UV-fluorescence spectra indicated that solvent extracts are richer in simple phenols, while bio-oils contained a higher number of conjugated and polycondensed molecules. According to GC-MS, CE and phases A and B were rich in esters, whereas in phase C and bio-oil-2, nitrogen-containing compounds prevailed. In contrast, bio-oil-1 showed a preponderance of phenols. CE was characterized by the lowest toxicity on keratinocytes, followed, in an increasing order, by phase C, bio-oil-1, phases B-A, and bio-oil-2, respectively. Bio-oil-2 showed a significant inhibitory effect on IL-8 production comparable to that of dexamethasone at 7.5 μg/mL.

Conclusions: Bio-oil-2 obtained from the extracted biomass, usually considered as a waste, demonstrated to be the most promising anti-inflammatory sample. Further evaluation of its key components and the elucidation of the relevant pathways involved in this activity are needed to confirm its therapeutic potential for psoriasis.

Keywords*:* bio-oils; psoriasis; pyrazole; toxicity; solvent-extracts; *Urtica subincisa*.

VI.4.2. Introduction

In Mexico, the inherited knowledge about the medicinal plants converges with a great floristic wealth that has been exploited for health purposes since the pre-Columbian times. Several ancestral cultures have left important legacies to the current information about Mexican medicinal plants such as the Maya in the southeast, the Mixtec and Zapotec in the south as well as the Teotihuacan and the Purépecha in the center (Chavarría and Espinosa, 2019).

Purépechas live nowadays in the highlands of Central Michoacán, a state located in the center-west of Mexico, where they preserve their language and traditions, including an excellent ancestral inherited knowledge on medicinal plants (Esquivel-García et al., 2018). A recent ethnopharmacological study conducted at the Purépecha Plateau, a zone characterized by an important presence of Purépechas (78.86% of its inhabitants), identified 97 promising plants for treating dermatological conditions (Esquivel-García et al., 2018). Native plants were preferred by Purépechas (56.7%) over the introduced ones. *Urtica subincisa* Benth. (Urticaceae) is one of the most representative native plants which has been widely employed by this population to treat skin inflammation, skin bumps, and varicose veins (Esquivel-García et al., 2018). To the best of our knowledge, this herbaceous Mexican plant has not been analyzed, neither from a phytochemical point of view nor regarding its toxicological and anti-inflammatory properties on the skin cells. Consequently, further phytochemical, toxicological, and pharmacological studies are warranted to support its folk use for dermatological conditions.

Psoriasis is one of the most prevalent inflammatory skin diseases, affecting about 3 million Mexicans (Thorpe and Pandya, 2014). Although its etiology is not fully elucidated, inflammatory mediators crucially involved in its pathogenesis have been recently identified. One of the most critical is the interleukin 17 (IL-17), which exacerbates inflammation, stimulating the hyperproliferation and aberrant differentiation of keratinocytes within the epidermis (Ekman et al., 2019). IL-17 is known to upregulate the release of pro-inflammatory chemokines by keratinocytes such as interleukin-8 (IL-8), leading to the recruitment of macrophages and neutrophils into the epidermis with the consequent flare-up of skin inflammation (Blauvelt and Chiricozzi, 2018). The discovery of biologicals targeting the IL-17 or its receptor such as secukinumab, ixekizumab, and brodalumab offers significant psoriasis improvement; although adverse events, high-costs, and the lack of response in some patients

can limit their use (Bissonnette et al., 2014; Lv et al., 2018). Therefore, the quest for more affordable, effective, and safe therapeutic anti-IL17 agents is ongoing. The development of novel topical natural products showing anti-IL-17 effects offers several advantages compared to biologicals regarding their administration, as biotech agents unlike natural molecules, cannot cross the stratum corneum which prevent their use in topical formulations, preferred by patients having mild-to-moderate psoriasis (Claudia et al., 2020).

The sub-cosmopolitan genus *Urtica* has around 80 species (Steinmann, 2005); some of which have medicinal properties such as *Urtica dioica* L., *Urtica massaica* Mildbr., *Urtica parviflora* Roxb., *Urtica pilulifera* L., and *Urtica urens* L. (Kopyt'ko et al., 2012). Phenolic compounds such as quercetin, isorhamnetin, kaempferol, vicenin-2, isolated from plants of this genus have shown promising results for treating autoimmune diseases due to their immunomodulatory and anti-inflammatory properties (Ibrahim et al., 2018).

Phenols and other bioactive molecules can be classically obtained by conventional solvent extraction but also, they are available as monomers, dimers, and oligomers in bio-oils as a result of lignin degradation during pyrolysis (Esquivel-García et al., 2020). The pyrolysis involves the degradation of biomass by heat in the absence of oxygen and has the advantage that can be performed after conventional extraction, allowing the integral use of the vegetable matrix. In our previous study bio-oils derived from *Amphipterygium adstringens* (Schltdl.) Standl. bark, mainly constituted of phenols, was shown to have an anti-inflammatory potential to be explored for psoriasis (Esquivel-García et al., 2020). Moreover, pyrolytic products, especially the coal tar, a dark, oily material produced by slow pyrolysis of coal, have been used for more than 100 years for this disease (Zeichner, 2010). However, much remains to be known about the pathways involved in the anti-inflammatory properties of these products, the molecules responsible for such effects, and their safety profiles on the skin. Furthermore, the identification of new natural sources of active bio-oils is crucial in the search for new promising pyrolytic products as antipsoriatic agents.

Although the ethanolic macerates of *U. subincisa* have been extensively used by Purépechas for dermatological conditions, to the best of our knowledge no research has been carried out to characterize the chemical, toxicological and anti-inflammatory properties of the solvent extracts or bio-oils from this plant. Therefore, this study aims to compare and chemically characterize

the ethanolic extract, its fractions, and the bio-oils from *U. subincisa* aerial parts and to analyze their toxicity and anti-inflammatory properties on HaCaT keratinocytes. We hypothesized that differences in the chemical compositions of bio-oils and the solvent extracts will significantly affect their toxicity and/or anti-inflammatory properties related to the production of IL-8 in IL-17 stimulated HaCaT keratinocytes.

VI.4.3. Material and methods

Fig. 10 shows the major stages described in this paper. Firstly, the botanical identification and extraction by maceration of the aerial parts of *U. subincisa* were carried out. The residual biomass resulting from the extraction was then pyrolyzed, to obtain bio-oils (oil-1 and 2). Subsequently, the crude extract was fractionated by liquid/liquid extraction to achieve phases A-C. The crude extract, its fractions, and bio-oils were analyzed using a set of analytical techniques (proximate, elemental analysis, FTIR, UV-fluorescence, GC-MS) to have an overview of their chemical composition. Finally, an analysis of their toxicity and inhibitory activity on the IL-8 production in HaCaT keratinocytes stimulated with IL-17 was performed. A detailed explanation of each major step is provided in the following sections.

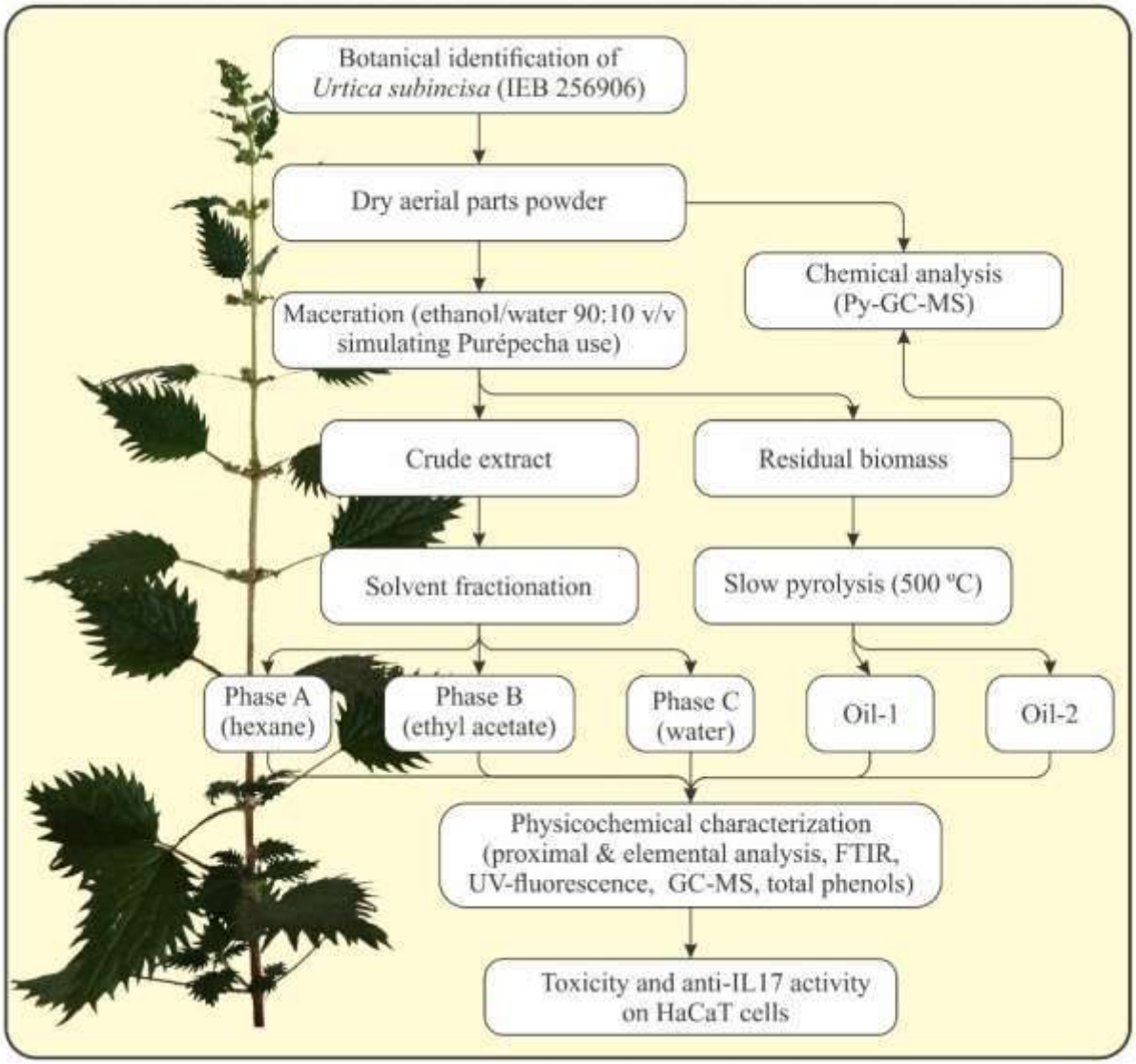

Fig. 10. Major steps depicted in this paper.

VI.4.3.1. Botanical Analysis, extraction, and solvent fractionation

Botanical analysis. The aerial parts of *U. subincisa* (flowers+seeds+leaves+stems) were collected at Michoacán state, Mexico. The plant was botanically identified by Dr. Emmanuel Pérez-Calix and deposited in IEB herbarium at Pátzcuaro, Michoacán (voucher number: 256906). Before used, the plant material was washed and oven-dried (temperature 40±2 °C for 48 h).

Maceration. Aerial parts from *U. subincisa* were extracted by maceration. The choice of the aerial parts and the extraction method was made to simulate the ethnomedicinal use of *U. subincisa* as reported by Purépechas which use the alcoholic extract from the aerial parts as fomentations for skin inflammation (Esquivel-García et al., 2018). Fifteen grams of dry aerial parts powder (DAPP) was grounded to a particle size of ≤800 μm. Then DAPP was extracted by maceration using Lab Companion SI-600 Benchtop Shaker (25 °C) with 90% aqueous ethanol (150 mL) in two 48-hour rounds. The residual biomass (RB) was separated by filtration with a Whatman No. 4 filter paper and washed with an additional 150 mL of 90% aqueous ethanol. The crude extract (CE) was concentrated under vacuum (35 °C, 29 kPa) while the RB) was recovered for pyrolysis experiments.

Solvent fractionation: Thirty grams of CE were resuspended in 300 mL water. Liquid-liquid extraction was performed with hexane, ethyl acetate, and water as previously described (García-Pérez et al., 2018) to obtain the phases A, B and C, respectively. The CE and its fractions were kept under refrigeration (4 °C) until further analysis.

VI.4.3.2. Pyrolysis experiments

Pyrolysis was performed in a quartz-tube furnace reactor of 50 mm outer diameter × 44 mm inner diameter × 1000 mm length. Sixteen grams of the oven-dried RB were introduced into the furnace under nitrogen flow for 5 min at 25 ^{o}C where the slow pyrolysis at 500 °C was performed as previously described (Esquivel-García et al., 2020) to obtain oils 1 and 2. The oils were kept under refrigeration (4 °C) until further analysis.

VI.4.3.3. Chemical analysis

Pyrolysis was performed in a quartz-tube furnace reactor of 50 mm outer diameter × 44 mm inner diameter × 1000 mm length. Sixteen grams of the oven-dried RB were introduced into the furnace under nitrogen flow for 5 min at 25 ^{o}C where the slow pyrolysis at 500 °C was performed as previously described (Esquivel-García et al., 2020) to obtain oils 1 and 2. The oils were kept under refrigeration (4 °C) until further analysis.

2.1 Chemical analyses

Pyrolysis gas chromatography mass spectrometry (Py-GC/MS). Considering that the DAPP and RB were the starting materials for obtaining the solvent extracts and bio-oils respectively, a chemical characterization of both samples was carried out by Py-GC-MS using an Agilent Technologies 6890N gas chromatograph linked to the Agilent 5975B mass selective detector (column: 30 m × 250 µm × 0.25 µm; mass spectral search program NIST 2.0 f). Both samples (≈0.5 mg) were introduced into the probe interface using a CDS Analytical Pyroprobe 5000 following the method described by Ayania et al. (Ayiania et al., 2019). The compounds were identified by combining the data from the Mass Spectra Library and the literature and classified into different chemical groups as previously described (Esquivel-García et al., 2020).

Proximate and elemental analyses. The proximate analysis of the bio-oils, CE, and phases A-C was performed to determine the fixed carbon, volatiles, and ash content with a Mettler Toledo thermogravimetric analyzer (SDTA851e) equipped with the STARe data analysis software to obtain thermogravimetric (TGA)/derivative of thermogravimetric curve (DTG) data curves. The total contents of carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) in the samples were determined using a Leco TruSpec Micro elemental analyzer. The oxygen (O) content was obtained by difference after considering the dry ash content. Both analyses were performed according to the methods previously described (Esquivel-García et al., 2020).

Fourier transform infrared spectroscopy (FTIR) analysis. Small amounts of pyrolytic oils, CE, and phases A-C were deposited in the sample container and FTIR spectra were obtained using a Shimadzu IRPrestige 21 spectrometer. For the analysis of the functional groups, background correction was performed on each spectrum as previously described (Esquivel-García et al., 2020).

Ultraviolet (UV) fluorescence. The UV fluorescence analysis was performed based on a previous methodology (García-Pérez et al., 2018). Briefly, bio-oils, CE, and phases A-C were diluted in high performance liquid chromatography (HPLC) grade methanol at 10 ppm and analyzed on the Shimadzu RF 5301 pc spectrometer with Panorama Fluorescence 2.1 software.

Gas chromatography mass spectrometry (GC-MS). The 1% (w/w) ethanolic solutions of the bio-oils, CE and phases A-C were analyzed by GC-MS using an Agilent Technologies 7890 A

GC set up (Agilent 19091S-433 HP-5MS column: 325 °C: 30 m×250 µm×0.25 µm, Agilent 5975 C MS with NIST 2.0 f Mass Spectral Search Program) and following a method previously described (Esquivel-García et al., 2020).

Determination of total phenols. Total phenol contents of bio-oils, CE, and phases A-C were determined using Folin-Ciocalteu method with some modifications as previously described (García-Pérez et al., 2010). The absorbance was measured at 750 nm on a Spectronic 20 Genesys instrument. Results were expressed as mg gallic acid equivalents (GAE)/g of pyrolytic oils, CE, and phases A-C, respectively.

VI.4.3.4. Toxicity on HaCaT keratinocytes

The toxicity of CE, phases A-C and bio-oils was evaluated by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Human HaCaT keratinocyte cell line was purchased from AddexBio and cultured in DMEM high glucose with sodium pyruvate (Sigma Aldrich) and supplemented with 100 U/mL penicillin (Sigma Aldrich), 100 µg/mL streptomycin (Sigma Aldrich), and 10% fetal bovine serum (Thermo Fisher Scientific) in a humidified incubator containing 5% CO_2 at 37 °C. MTT assay was performed with cells at 80% confluence. Bio-oils, CE and phases A-C (25-1600 µg/mL) were diluted in DMEM medium with 0.2% ethanol and the MTT assay performed as previously described (Esquivel-García et al., 2020; García-Pérez et al., 2010). The toxicity was evaluated through the determination of the initial toxicity value (IC_{90}) which represents the minimal toxic dose of extract causing a 10% reduction of cell viability compared to untreated cells (Esquivel-García et al., 2020).

VI.4.3.5. Anti-inflammatory effects on IL-17-induced IL-8 production

HaCaT cells were seeded into 24-well plates and cultured under the aforementioned conditions until 80% of confluence. Subsequently, the cells were synchronized by serum deprivation during 12 h. After this time, the cells were pre-treated or not for 10 h with dexamethasone (dex, 5 µM, Sigma Aldrich), bio-oils, CE, and phases A-C at concentrations lower than IC_{90} predetermined values. Later, IL-17 (50 ng/mL, Thermo Fisher Scientific) was added to cell cultures for 14 h to induce IL-8 production. IL-8 amounts in IL-17 HaCaT supernatants were measured using

Human IL-8/CXCL8 DuoSet ELISA kit (R&D Systems) according to the manufacturer's protocol. The detection limit for IL-8 production was 30 pg/mL.

VI.4.3.6. Statistical analyses

The Shapiro-Wilk and Levene tests were conducted to compare the analyzed variables. Afterward, variables were analyzed by one-way analysis of variance (ANOVA) followed by a Tukey post-hoc test or Kruskal–Wallis and Holm-Bonferroni tests. The non-parametric Spearman's correlation test was used for establishing the association between the phenol content and the IC_{90} values at $p<0.05$. The statistical analyses were performed employing R and RStudio using the R commander package.

VI.4.4. Results and Discussion

The composition of the raw materials determines the quality, reproducibility, and chemical characteristics of the derived extracts and bio-oils to be used for therapeutic purposes. Considering that the aerial parts of *U. subincisa* constitutes the starting material for obtaining the crude extract and its fractions, while the residual biomass was used for pyrolysis, the chemical characterization of these materials was first performed by Py-GC-MS. This technique is rapid, sensitive, and widely used for simultaneous mass spectrometric detection and identification of pyrolysis products in a vegetable matrix employing small amounts of sample and has been used to evaluate the quality of *U. dioca* raw material for pharmaceutical purposes (Cuinica and Macêdo, 2018).

Fig. 11 shows the obtained pyrograms of the dried aerial parts and residual biomass of *U. subincisa*. The names of the identified compounds are reported in **Table 4**. As expected, a higher diversity of compounds was found in the dried aerial parts powder (DAPP) than in the residual biomass (RB). Predominantly identified compounds from DAPP were aromatics (~34 %), acids (~14 %), and ketones (~14 %), while in the RB, the aromatics (~36 %) and the acids (~18 %) were also the main identified molecules. Among the aromatics, phenols were the major compounds in both samples (62-66 %). Py-GC-MS was used in previous studies to assign the detected compounds to their biochemical origin (Biller and Ross, 2014; Kebelmann et al., 2013).

For example, indole and toluene (compounds 22 and 7 in **Table 4** respectively) have been linked to the proteins, whereas the alkanes (compounds 28 and 33, in **Table 4**) have been related to both fatty acids and triglycerides. Moreover, levoglucosan (compound 27 in **Table 4**) is linked with carbohydrates and phenols with lignin (Biller and Ross, 2014). The hexadecanoic and octadecanoic acids (compounds 32 and 36, **Table 4**) were the main identified molecular fragments from the dried aerial parts and residual biomass of *U. subincisa,* which is consistent with a previous study that used Py-GC-MS to chemically characterize *U. dioica* (Cuinica and Macêdo, 2018).

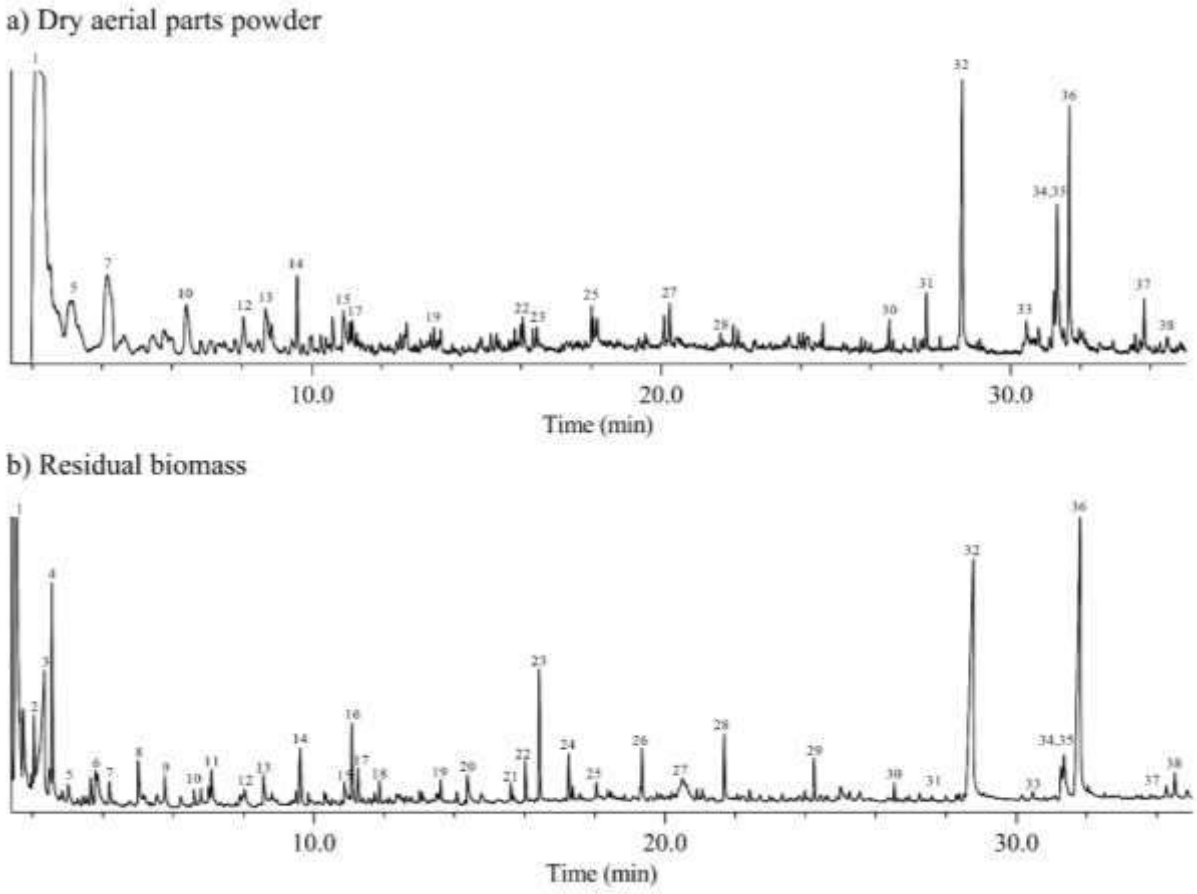

Fig. 11. **Pyrograms of the dried aerial parts and residual biomass of *U. subincisa*.**

List of compounds are in **Table 4.**

Table 4. Compounds identified by Py-GC/MS in *U. subincisa* aerial parts and residual biomass.

No.	Identified compounds	RT (min)	DAPP	RB	Groups
1	Carbon dioxide	1.69	✓	✓	O

No.	Identified compounds	RT (min)	DAPP	RB	Groups
2	2,3-Butanedione	2.04	✓		K
3	Acetic acid	2.34	✓		AC
4	2-Propanone, 1-hydroxy-	2.55	✓		K
5	2-Butenal	3.09	✓	✓	AD
6	Pyridine	3.54	✓		N
7	Toluene	4.11	✓	✓	*
8	3-Furaldehyde	5.01	✓		AD
9	1,2-Ethanediol, diacetate	5.77	✓		A
10	Unknown	6.31	✓	✓	-
11	Cyclohexanone	7.09	✓		K
12	Benzaldehyde	8.05	✓	✓	AD*
13	Phenol	8.57	✓	✓	P*
14	Limonene	9.50	✓	✓	O
15	Phenol, 4-methyl-	10.87	✓	✓	P*
16	Phenol, 2-methoxy-	11.08	✓		P*
17	Mequinol	11.17	✓	✓	P*
18	Maltol	11.74	✓		K
19	Phenol, 2-methoxy-4-methyl-	13.61	✓	✓	P*
20	Catecholborane	14.48	✓		O
21	Phenol, 4-ethyl-2-methoxy-	15.62	✓		P*
22	Indole	16.02	✓	✓	N
23	2-Methoxy-4-vinylphenol	16.42	✓	✓	P*
24	Phenol, 2,6-dimethoxy-	17.26	✓		P*
25	1H-Indole, 3-methyl-	18.05	✓	✓	N
26	Benzoic acid, 4-hydroxy-3-methoxy-	19.30	✓		P*
27	1,6-Anhydro-β-D-glucopyranose	20.49	✓	✓	S
28	Hexadecane	21.68	✓	✓	AK
29	Unknown	24.24	✓		-
30	9-Dodecenol	26.52	✓	✓	A
31	2-Heptadecanone	27.59	✓	✓	K
32	n-Hexadecanoic acid	28.68	✓	✓	AC
33	Cyclopentadecane	30.46	✓	✓	AK
34	Unknown	31.24	✓	✓	-
35	14-Pentadecenoic acid	31.36	✓	✓	AC
36	Octadecanoic acid	31.81	✓	✓	AC
37	Methyl salicylate	33.82	✓	✓	E*
38	Eicosanoic acid	34.51	✓	✓	AC

Dry aerial parts powder (DAPP); residual biomass (RB); retention time (RT). Groups: Alcohol (A); Ketone (K); Alkanes (AK); Phenol (P); Aromatic (*); Sugar (S); Acid (AC); Aldehyde (AD); Ester (E); Nitrogen-containing Compound (N); Others (O).

Table 5 shows the yields of CE, its fractions, and the pyrolytic products (bio-oils and char) with respect to the dried powder from *U. subincisa* aerial parts. The extractive free biomass, which represents an important component of the initial vegetable matrix (73.5±3.3 wt.%), was used to obtain the pyrolytic products (chars and bio-oils). As observed, the char, the crude extract, and oil-2 exhibited higher yields compared to other samples. The recovery of char was higher than that reported for nine other herbaceous plants such as *Bromus marginatus* Nees and *Trifolium pratense* L., whereas lower yields of pyrolytic bio-oils (oil-1 + oil-2) were obtained compared to the plants mentioned above (Hlavsová et al., 2016) which can be explained both, by the lack of filters and electrostatic precipitators to collect aerosols in our pyrolytic system, but also by differences in the chemical composition among plants. It is known that cellulose and hemicellulose are the primary components responsible to produce bio-oils, whereas lignin is associated with the char yield (Stefanidis et al., 2014). Therefore, differences in the chemical composition of the feedstock and secondary reactions of bio-oils and char account for the recovery of pyrolytic products (Hlavsová et al., 2016). Regarding the yield of the crude extract, it was similar to that reported for the ethanolic extract of *Urtica circularis* (Hicken) Sorarú (Gorzalczany et al., 2011).

Table 5. Yields of the crude extract, its fractions, and pyrolytic products from aerial parts from *U. subincisa.*

Sample	Recovery (%) *
Crude extract	13.4 ± 0.6^{b}
Phase A	5.5 ± 0.1^{d}
Phase B	1.6 ± 0.2^{e}
Phase C	6.2 ± 0.5^{d}
Char	31.1 ± 2.1^{a}
Oil-1	5.0 ± 0.5^{d}
Oil-2	10.0 ± 0.6^{c}

*Percentages determined from the dry powder of aerial parts (wt.%). Data represent the Mean ± SD of three experiments. Statistical: one-way ANOVA followed by Tukey`s post hoc test. Means without a common letter differ ($p<0.05$).

Table 6 illustrates the proximate and elemental analyses of all samples. Bio-oils showed the highest water content (17-25 wt.%) compared to the crude extract and its fractions (0.5-3.4 wt.%). This finding can be explained by the dehydration of carbohydrate fractions during

pyrolysis (Mohan et al., 2006). On the contrary, the content of volatiles was similar for all samples, but not the fixed carbon, which was higher for phase B and bio-oils than for the reminder of samples. A previous study also showed a high content of fixed carbon for the ethyl acetate fraction obtained from *Petiveria alliacea* L. aerial parts (García-Pérez et al., 2018). On the other hand, the ash content was higher in phase C than that in the other samples.

The elemental analysis revealed higher contents of carbon, nitrogen, and sulfur in bio-oils than in the crude extract and its fractions. Conversely, a higher content of hydrogen was constated in phase A, while the oxygen was significantly higher in the crude extract and its fractions than in the bio-oils. The oxygen content is related to the contribution of oxygen to the total mass of the sample and indicates an important presence of oxygenated metabolites likely polyphenols, oxygenated fatty acids, carotenoids. In bio-oils, oxygen has been linked to the presence of water, phenols, carboxylic acids, hydroxy ketones, and hydroxy aldehydes (Han et al., 2017).

Table 6. Proximate and elemental analysis of the crude extract, its fractions, and pyrolytic products from *U. subincisa.*

Proximate analysis (wt. %)					
Sample	Moisture*	Volatiles**	Fixed carbon**		Ash**
Crude extract	3.4±1.2 ^{b,c}	94.8±1.1 ^{a}	1.3±0.6 ^{b}		3.9±1.7 ^{b}
Phase A	0.5±0.3 ^{c}	95.6±0.9 ^{a}	2.8±0.0 ^{a,b}		1.6±0.8 ^{b}
Phase B	2.4±0.6 ^{c}	86.6±5.5 ^{a}	8.7±3.2 ^{a}		4.7±2.3 ^{b}
Phase C	4.2±1.1 ^{b,c}	85.9±2.1 ^{a}	0.2±0.1 ^{b}		13.9±2.1 ^{a}
Oil 1	17.6±5.2 ^{a,b}	96.5±2.9 ^{a}	4.9±1.2 ^{a,b}		0.9±0.8 ^{b}
Oil 2	25.5±6.9 ^{a}	94.4±0.7 ^{a}	4.9±1.2 ^{a,b}		0.7±0.5 ^{b}
Elemental analysis (wt. %)					
Sample	C**	H**	N**	S**	O***
Crude extract	34.4±0.2 ^{c}	6.9±0.07 ^{c}	4.7±0.00 ^{d}	0.06±0.01 ^{c}	50.04±1.5 ^{a}
Phase A	68.0±0.3 ^{a,b}	10.4±0.3 ^{a}	1.0±0.09 ^{f}	0.06±0.00 ^{c}	18.94±0.8 ^{b}
Phase B	60.7±2.9 ^{b}	8.6±0.53 ^{b}	2.4±0.05 ^{e}	0.00±0.00 ^{c}	23.6±1.2 ^{b}
Phase C	18.4±0.1 ^{d}	3.8±0.15 ^{d}	7.7±0.21 ^{c}	0.03±0.04 ^{c}	56.17±1.9 ^{a}
Oil 1	77.4±3.2 ^{a}	7.5±0.15 ^{b,c}	10.4±0.23 ^{a}	0.34±0.03 ^{a}	3.46±2.8 ^{c}
Oil 2	76.8±3.9 ^{a}	8.5±0.18 ^{b}	9.4±0.54 ^{b}	0.18±0.01 ^{b}	4.42±3.5 ^{c}

* As-received basis. ** Dry basis. *** By difference. Data represent Mean ± SD of two experiments. Statistical: one-way ANOVA followed by Tukey`s post hoc test. Means without a common letter differ ($p<0.05$).

The thermogravimetric analyses of all samples are presented in **Fig. 12**. TGA and DTG provide information about the volatility and thermal stability of a sample. As observed, bio-oils had lower thermal stability than solvent extracts, as the evaporation and thermal degradation of their components occurred faster at lower temperatures. It is worth noting that the crude extract and phase C showed the highest thermal stability, which can be observed from the presence of three main decomposition steps. The first step at <200 ◦C corresponds to dehydration. In the second step (200-450 ^{o}C) the devolatilization and decomposition of organic matter occurs, whereas at temperatures higher than 450 ^{o}C, the decomposition of residues resulting from the previous steps takes place (Marcilla et al., 2009).

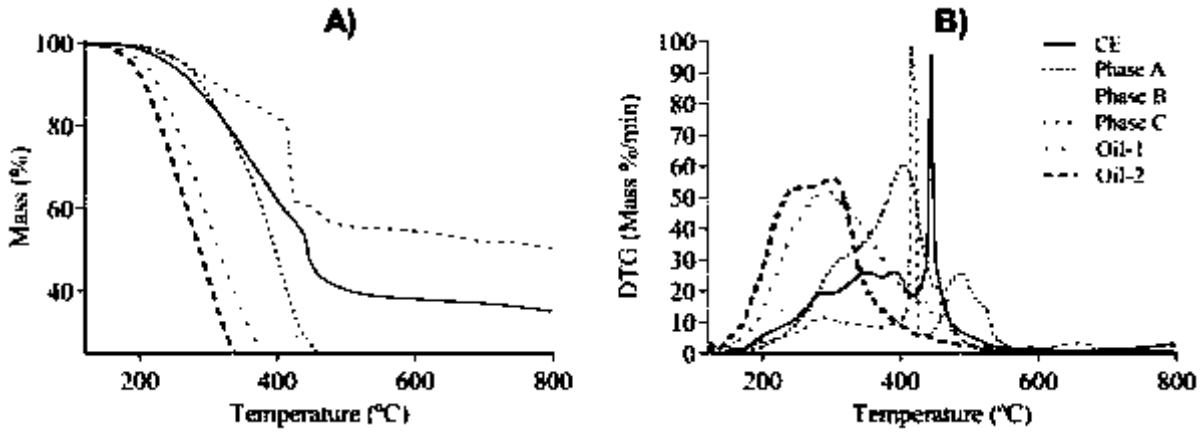

Fig. 12. **TGA (A) and DTG (B) curves of the crude extract, its fractions, and pyrolytic products from *U. subincisa.***

FTIR has been used to analyze the presence of functional groups in natural extracts and its findings complement data obtained using other analytical assessments (dos Santos Grasel et al., 2016; Esquivel-García et al., 2020; Nunes Oliveira et al., 2016). **Fig. 13** shows the infrared spectrum of the crude extract, its fractions, and pyrolytic oils from *U. subincisa*. Stretching vibrations of the O-H bond (3600-3100 cm^{-1}), usually assigned to water, carboxylic acids, alcohols, and phenols (Stankovikj and Garcia-Perez, 2017) were more intense for oil-1 followed by phase A than the reminder of samples. GC/MS, UV-fluorescence, and the total phenol content results showed that oil-1 is abundant in phenols. As such, vibrations observed in the spectrum are likely related to the presence of phenols. Stretching signals for the C-H bond

(3000-2800 cm^{-1}) could be linked to saturated and unsaturated aliphatic and aromatic compounds. These records were more intense for the ethyl acetate fraction (phase B). Similarly, the carbonyl stretching signal (C=O, 1780-1680 cm^{-1}) typically related to aldehydes, ketones, carboxylic acids, and esters was also more intense for phase B, which agrees with the analysis of this fraction by GC-MS. The stretch signal for C=C (1680-1530 cm^{-1}), is linked to conjugated aromatic systems (Esquivel-García et al., 2020). This signal was more dominant in phases A and C likely due to the presence of flavonoids or even proanthocyanidins which are widely distributed in the plant kingdom and characterized by their aromatic conjugated structures (Kumar and Pandey, 2013). It is worth noting that the intense signal between 1520-1250 cm^{-1} associated with the C-O bond, observed for both bio-oils and the crude extract, could indicate the presence of terpenes and acetates, while the signal at 1050 cm^{-1} could be related to the occurrence of anhydrides (Stankovikj and Garcia-Perez, 2017). The vibrations observed between 900-800 cm^{-1} could be considered complementary to those of C-O and C-N (Esquivel-García et al., 2020). Overall, FTIR spectra showed a higher diversity of functional groups in the crude extract and its fractions than that of the bio-oils, although the O-H and C-O signals suggest that the latter are rich in phenols, terpenes, and acetates.

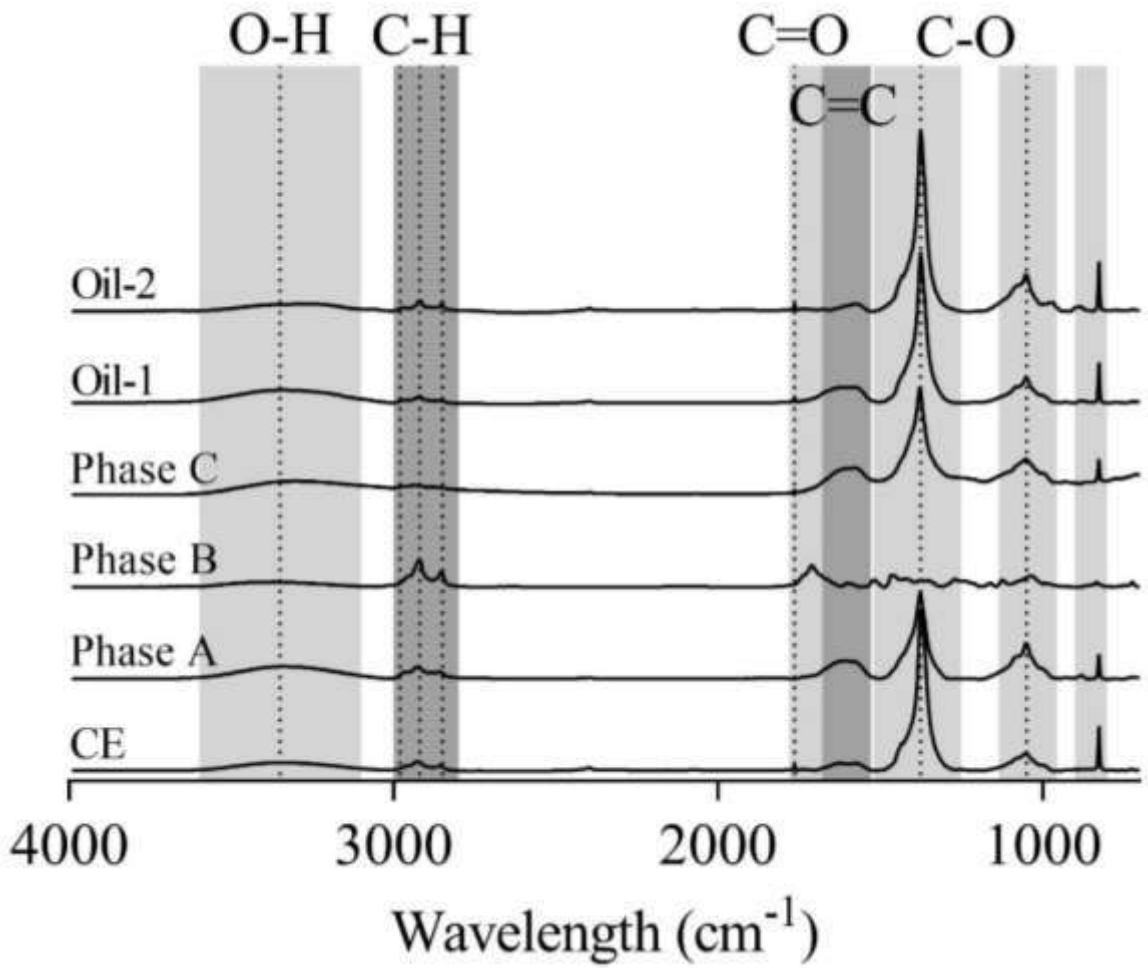

Fig. 13. **FTIR spectra of the crude extract, its fractions, and bio-oils from *U. subincisa.***

UV-fluorescence has been used to characterize natural extracts and bio-oils (Bolea et al., 2016; García-Pérez et al., 2018; Mourant et al., 2013) as molecules such as phenols that are widely distributed in the plant kingdom are fluorescent. Phenols occur in nature as simple phenols, short conjugated systems, and polycondensed structures. In a previous study, it was demonstrated that conformers having different dihedral angles between aromatic rings related to the presence of simple structures, dimers, oligomers, and polymeric molecules have their own contribution to the absorption in the fluorescence spectra (Barsotti et al., 2016). Thus, as the number of aromatic rings in the molecule increases, the wavelength of fluorescence emission also raises (Dieguez-Alonso et al., 2015). **Fig. 14** shows the UV-fluorescence spectra of all samples. In the range from 260-300 nm, the crude extract showed a more intense peak, followed by phase C, and then oils 1 and 2. The intensity of the peak reveals that crude extract is rich in monophenols. At an

excitation wavelength of around 310 nm, dimers and short conjugated structures can be observed, whereas around 323 nm, large conjugated systems occur (Stankovikj et al., 2017). According to **Fig. 14**, the presence of these compounds is higher in oil-1 than in the other samples. In summary, these results indicate that solvent extracts are generally richer in simple phenols, while bio-oils contain a higher number of conjugated molecules and polycondensed structures, likely related to lignin decomposition during pyrolysis. Further studies using *nuclear magnetic resonance* (NMR) spectroscopy should be performed to determine the chemical structure of these molecules in the studied samples.

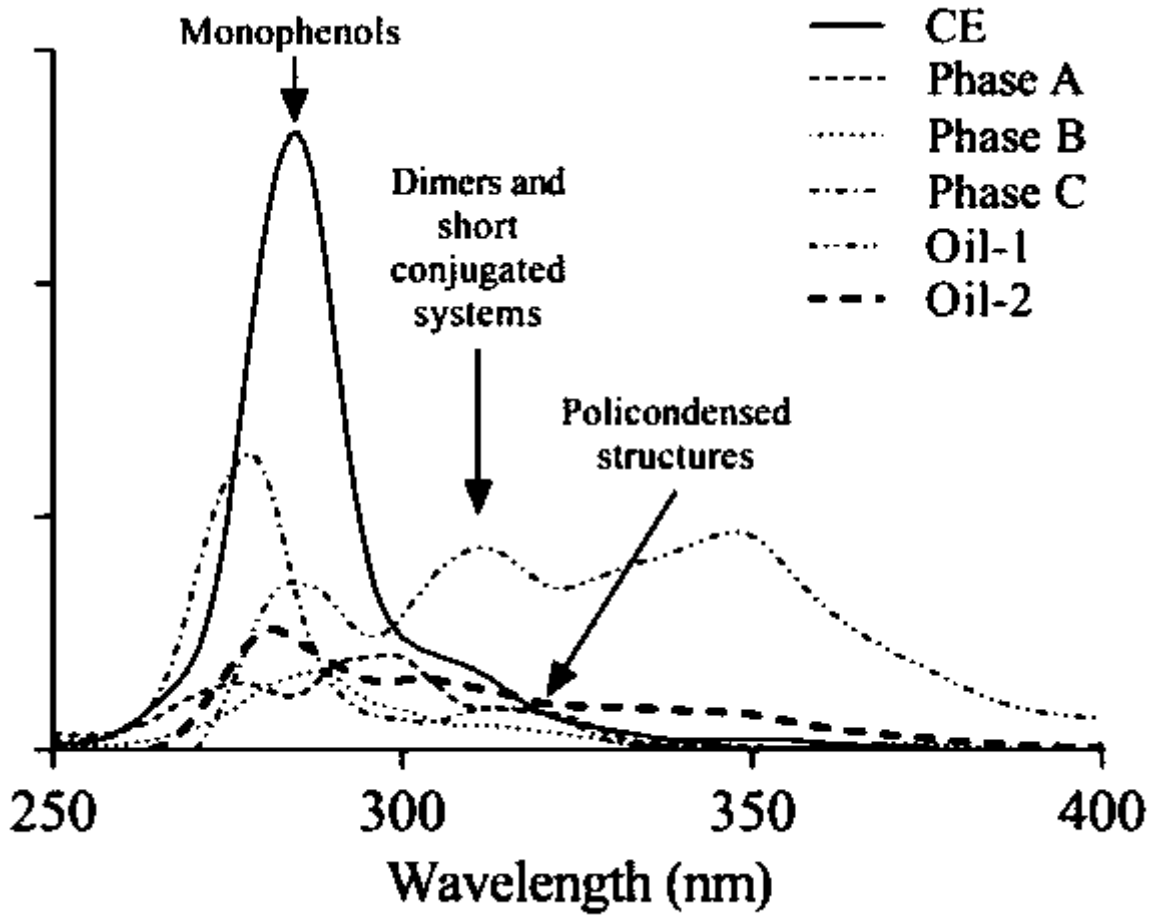

Fig. 14. UV-Fluorescence spectra of the crude extract, its fractions, and bio-oils from *U. subincisa.*

GC-MS has been used to perform the phytochemical analysis of volatile compounds from solvent extracts and bio-oils (Ezhilan and Neelamegam, 2012; Mourant et al., 2013). GC-MS chromatograms of all samples are presented in **Fig. 15**, whereas the list of the numbered compounds is shown in **Table 7**. The number of identified molecules was higher in CE, followed by phase B and then the bio-oils. As observed, depending on the solvent extraction or pyrolysis, the relative compositions of samples changed. The crude extract and phases A and B showed a higher concentration of esters (25, 56, and 20% of the identified molecules, respectively) whereas, in phase C and oil-2, nitrogen-containing compounds prevailed (26 and 40%, respectively). In contrast, oil-1 showed a preponderance of phenols (33%) and nitrogenous compounds (28%). These results agree with the elemental analysis which demonstrated that oxygen was significantly higher in the crude extract and its fractions than in the bio-oils, whereas the nitrogen was higher in bio-oils than in solvent extracts. These findings are in good agreement with the previously reported FTIR results.

As expected, the nature of the main compounds in each sample changed. Linoleic acid ethyl ester was the dominant compound in CE and phase A, while 2-cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-3-oxo-1-butenyl- was the major molecule within phase B. Acetamide N-(3-methyl-2-buten-1-yl)- on the other hand, predominated in phase C. Indole and phenol were prevalent within oil-1, while oil-2 proved to be rich in 3,4,5-trimethylpyrazole and phenol. The presence of fatty acids in the form of ethyl esters, as linoleic acid ethyl ester has been documented in *U. dioica* (Lapinskaya and Kopyt'ko, 2008).

It is worth noting the significant occurrence of nitrogenous compounds in both solvent extracts and pyrolytic oils. Although to the best of our knowledge this study is the first to analyse the chemical composition of *U. subincisa*, it is known that other species of this genus such as *U. dioica* and *U. urens* are nitrophilic weeds (Garmendia et al., 2018; Rosnitschek-Schimmel, 1985a) as they compete with most other herbal plants for nitrogen. It has been documented that the excess of nitrogen supply led to the accumulation of nitrogenous compounds such as nitrate and free amino acid in those plants. Proteins can function as nitrogen stores too, particularly in seeds (Rosnitschek-Schimmel, 1985b). Our results suggest that *U. subincisa* could also be considered a nitrophilic species, but further studies should be accomplished to determine its capacity to store this compound and the impact of seasonal variations on this process.

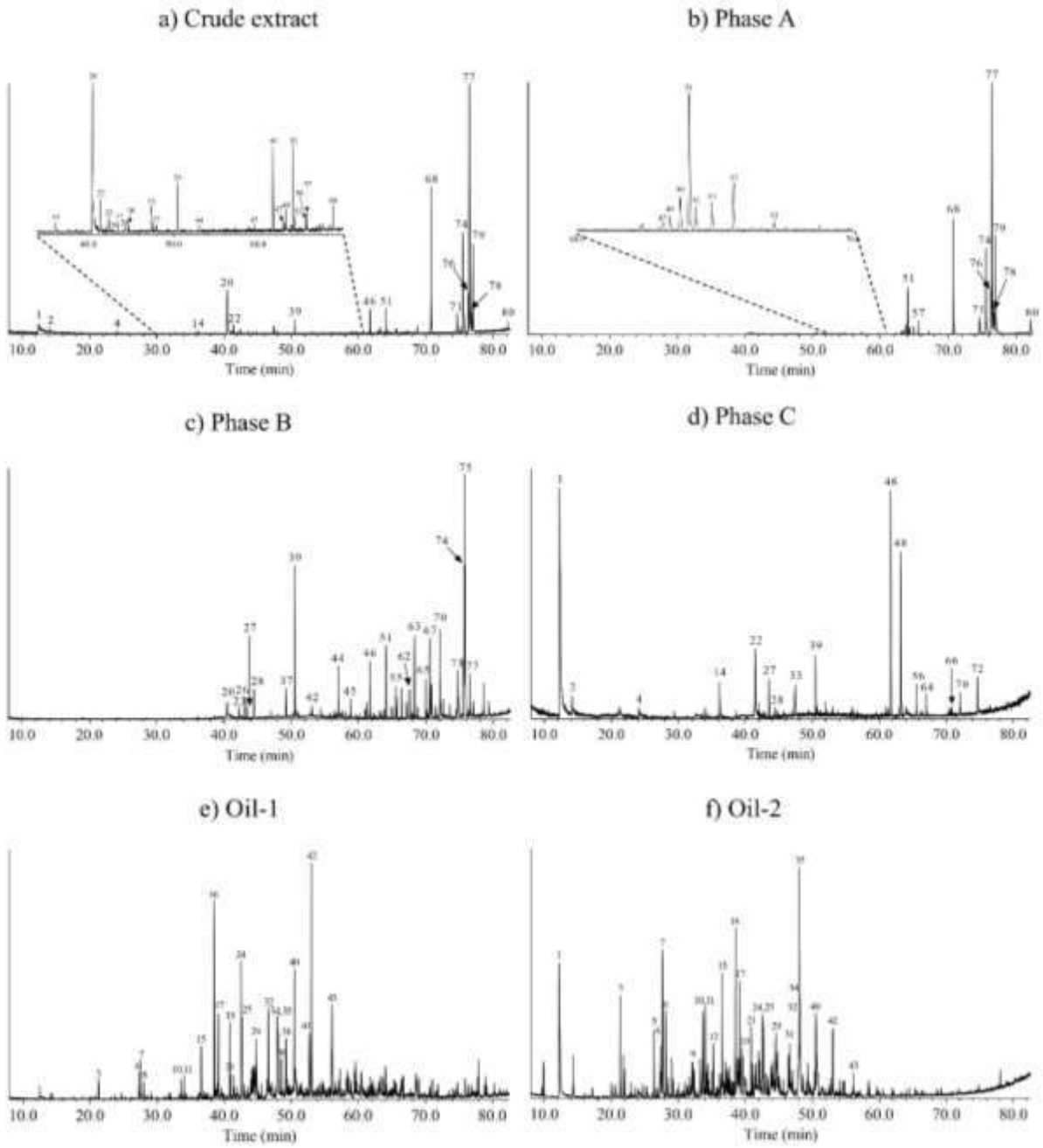

Fig. 15. GC-MS chromatograms of the crude extract, its fractions, and bio-oils from *Urtica subincisa*. Phase A (hexane); B (ethyl acetate) and C (aqueous fraction) (the list of numbered compounds is provided in **Table 7**).

Table 7. Compounds identified by GC-MS in the crude extract, phases A-C and bio-oils obtained from aerial parts of *U. subincisa*.

No.	Identified compounds	RT (min)	Crude extract	Phase A	Phase B	Phase C	Oil 1	Oil 2	Groups
1	Acetic acid	12.30	✓			✓	✓	✓	AC
2	Propanoic acid, 2-oxo-	14.12	✓			✓			AC
3	Pyrrole	21.32					✓	✓	N
4	Pyruvic acid	24.11	✓			✓			AC

No.	Identified compounds	RT (min)	Crude extract	Phase A	Phase B	Phase C	Oil 1	Oil 2	Groups
5	4-Methyl-2-hexene	26.37					✓	✓	HD
6	Acetamide	27.32					✓	✓	N
7	2-Furanmethanol	27.65					✓	✓	F
8	2-Cyclopenten-1-one, 2-methyl-	28.12					✓		K
9	Propanenitrile, 2-hydroxy-	32.18						✓	N
10	2-Cyclopenten-1-one, 3-methyl-	33.65					✓	✓	K
11	Butyrolactone	34.02					✓	✓	E
12	1-Methyl-2-piperidinemethanol	35.21						✓	N
13	1H-1,2,4-Triazol-3-amine, 5-methyl	35.24					✓		N
14	Glycolaldehyde dimer	36.14	✓			✓			AD
15	2-Cyclopenten-1-one, 2,3-dimethyl-	36.62					✓	✓	K
16	Phenol	38.54					✓	✓	P*
17	Phenol, 2-methoxy-	39.17					✓	✓	P*
18	2-Cyclohexen-1-one, 4-(2-oxopropyl)-	39.25						✓	K
19	Cyclohexanone, 3-methyl-	39.50					✓	✓	K
20	dl-Glycerldehyde	40.52	✓		✓	✓			AD
21	Phenol, 2-methyl-	40.87					✓	✓	P*
22	2-Hydroxy-gamma-butyrolactone	41.45	✓			✓			E
23	Benzeneacetic acid, methyl ester	42.29	✓		✓				AC*
24	Phenol, 4-methyl-	42.60					✓	✓	P*
25	Phenol, 3-methyl-	42.70					✓	✓	P*
26	Ethanol, 2-(2-butoxyethoxy)-	43.10	✓		✓				A
27	Vinyl butyrate	43.53	✓		✓	✓			E
28	Cyclobutanol	44.33	✓			✓			A
29	1H-Imidazole, 2,4-dimethyl-	44.54					✓	✓	N
30	L-Prolinamide	44.73	✓			✓			N
31	Tetradecane	46.45						✓	HD
32	Phenol, 4-ethyl-	46.62					✓	✓	P*
33	Unknown	47.42	✓			✓			-
34	Phenol, 4-ethyl-2-methoxy-	47.99					✓		P*
35	3,4,5-Trimethylpyrazole	48.06					✓	✓	N
36	1,3-Benzenediamine	48.62						✓	N*
37	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	49.20	✓		✓				N
38	Benzenepropanenitrile	49.27						✓	N*
39	2,3,5-Trimethylanisole	50.50	✓		✓	✓			*
40	Unknown	50.54					✓	✓	-
41	Pyrazine, 2,5-dimethyl-	51.56						✓	N
42	Indole	53.04	✓		✓		✓	✓	N
43	Unknown	56.23						✓	-
44	Vanillin	56.95	✓		✓				P*
45	Benzeneacetamide	58.84	✓		✓				N*
46	Acetamide, N-(3-methyl-2-buten-1-yl)-	61.72	✓		✓	✓			N
47	Unknown	63.11	✓	✓	✓				-
48	Mannitol, 1,4-anhydro-	63.20	✓			✓			A
49	2-Hexadecene, 2,6,10,14-teramethyl-	63.37	✓	✓					HD
50	2-Hexadecene, 3,7,11,15-tetramethyl	63.74	✓	✓	✓				HD
51	3-Eicosyne	64.07	✓	✓	✓				HD
52	Tetradecanoic acid, ethyl ester	64.31	✓	✓					E
53	7-Octadecyne, 2-methyl-	64.89	✓	✓	✓				HD

No.	Identified compounds	RT (min)	Crude extract	Phase A	Phase B	Phase C	Oil 1	Oil 2	Groups
54	Propanoic acid, 2-methyl-, 2-phenylethyl ester	64.99	✓		✓				E*
55	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-	65.48	✓		✓				K
56	β-D-Glucopyranose, 1,6-anhydro-	65.55	✓			✓			S
57	1,4-Eicosadiene	65.67	✓	✓	✓				HD
58	Unknown	66.33	✓		✓				-
59	Unknown	66.99	✓			✓			-
60	3-(1-Methylhept-1-enyl)-5-methyl-2,5-dihydrofuran-2-one	67.06	✓		✓				F
61	2-Pentadecanone, 6,10,14-trimethyl-	67.14	✓	✓					K
62	3-Buten-2-one, 4-(5-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-	67.95	✓		✓				K
63	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	68.30	✓		✓				K
64	Hexadecanoic acid, methyl ester	68.77	✓	✓	✓				E
65	3,7-Cyclodecadiene-1-methanol, 4,8-tetramethyl-	69.90	✓		✓				A
66	Unknown	70.43	✓			✓			-
67	4-3-Hydroxy-1-propenyl)-2-methoxyphenol	70.58	✓		✓				P*
68	Hexadecanoic acid, ethyl ester	70.80	✓	✓	✓				E
69	2-Propanone, 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-	71.38	✓		✓				P*
70	Benzamide, N-(2-hydroxyethyl)-	72.05	✓		✓	✓			N*
71	11,14-Eicosadienoic acid, methyl ester	74.65	✓	✓					E
72	Adenine	74.69	✓			✓			N
73	Phenethyl alcohol, 2,5-dihydroxy-α-methyl, (-)-	74.70	✓		✓				P*
74	Phytol	75.56	✓	✓	✓				A
75	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-3-oxo-1-butenyl)-	75.77	✓		✓				K
76	Ethyl oleate	76.32	✓	✓					E
77	Linoleic acid ethyl ester	76.49	✓	✓	✓				E
78	Methyl 17-methyl-octanoate	76.73	✓	✓					E
79	9,12,15-Octadecatrienoic acid, ethyl ester	76.99	✓	✓	✓				E
80	Eicosanoic acid, ethyl ester	82.18	✓	✓					E

Retention time (RT). Groups: Alcohol (A); Furans (F); Ketone (K); Phenol (P), Aromatic (*); Sugar (S); Hydrocarbon (HD); Acid (AC); Aldehyde (AD); Ester (E); Nitrogen-containing Compound (N).

The preponderance of nitrogenous compounds in bio-oils, determined by elemental analysis and GC-MS, among which indole and 3,4,5-trimethylpyrazole distinguish, could be related to protein pyrolysis (Biller and Ross, 2014). Phenols, also identified as major compounds in these oils, could result from lignin pyrolysis (Fu et al., 2014). Indeed, bio-oils were found to be significantly richer in total phenols than solvent extracts as determined by the Folin-Ciocalteu method (see **Table 8**).

Table 8. Total phenols and initial toxicity values (IC_{90}) of different fractions of *U. subincisa*.

Sample	Total phenols (mg GAE/g)	IC_{90} (µg/mL)
Crude extract	13.5 ± 0.1^{d}	1614.8 ± 67.0^{a}
Phase A	13.9 ± 0.1^{d}	116.7 ± 3.5^{e}
Phase B	18.5 ± 0.5^{c}	194.9 ± 5.8^{d}
Phase C	13.4 ± 0.2^{d}	443.9 ± 5.0^{b}
Oil-1	30.6 ± 1.2^{a}	284.2 ± 5.6^{c}
Oil-2	25.4 ± 1.3^{b}	33.8 ± 3.0^{f}

Statistical: one-way ANOVA followed by Tukey's post hoc test. Means without a common letter in the same column differ ($p<0.05$).

Although the GC-MS provides valuable information on the chemical composition of the studied samples, it could be considered as a preliminary phytochemical study as it only determines the nature of volatile molecules. Further in-depth chemical analyses (HPLC, NMR) should be performed to provide more information about other non-volatile compounds present in *U. subincisa* extracts and bio-oils.

To evaluate the potential of the samples as anti-inflammatory agents, it is not only indispensable to determine their capacity to inhibit the production of crucial mediators involved in psoriasis, but it is also imperative to assess their toxicity on keratinocytes in advance as they should demonstrate low toxicity at pharmacological concentrations. In this study, the toxicity of all samples was evaluated through the determination of the initial toxicity value (IC_{90}) which represents the minimum toxic dose causing a 10% reduction of the cell viability compared with untreated keratinocytes (García-Pérez et al., 2010; Teepe et al., 1993). MTT assay has been considered as a sensitive test to evaluate the toxicity of natural extracts, drugs, and excipients used for pharmaceutical goals (García-Pérez et al., 2010; Hundie et al., 2016; Scherließ, 2011). **Table 8** shows the IC_{90} values obtained for all samples. The crude extract had the lowest toxicity, followed in increasing toxicity order by phase C, oil-1, phases B-A, and oil-2, respectively. In rationalizing the initial toxicity of the samples in terms of the total phenol content, it became clear from the Spearman's correlation data that higher content of phenols in samples, the lower IC_{90} values and higher toxicity on keratinocytes ($r=-0.5209$; $p=0.0266$). It is well known that phenols used in household and pharmaceutical formulations can produce a rash, dermal inflammation, and contact dermatitis (Murray et al., 2007). The toxicity of phenol on keratinocytes depends not only on its concentration and hydrophobicity (Newby et al., 2000) but also on the generation of organic radicals and reactive oxygen species (Gami, 2014). One of

the main compounds identified by GC-MS in oil-2 (the most toxic sample) was phenol (compound 16, **Table 7**) The metabolism of phenol in the skin results in the formation of the phenoxyl radicals which can modify proteins, DNA, and lipids. A potential mechanism for phenol' cutaneous toxicity is also linked to the glutathione and protein thiols oxidation. Moreover, this compound decreases vitamin E, thereby reducing the antioxidant capacity of the skin (Murray et al., 2007).

Although the presence of phenols could impact the toxicity, their sole occurrence cannot explain the overall observed toxic effects of samples. Indeed, oil-1 which shows a preponderance of these compounds according to the total phenol content assessment, GC-MS, FTIR, and UV-fluorescence analyses, was less toxic on keratinocytes than the phases A-B and oil-2. Thus, we hypothesized that the toxicity on keratinocytes is also related to the relative proportion of the chemical compounds within the sample, which could lead to interactions between molecules that would ultimately translate into harmful effects on cells. Our hypothesis was tested though exploration of the GC-MS data (**Fig. 15**, **Table 7**). GC-MS analysis showed the important occurrence of nitrogenous compounds in the samples. The CE (the sample with the least toxicity) showed a relatively low proportion of nitrogenous compounds (14%), while oil-2 (the most toxic sample) showed the highest proportion of these compounds (36%). On the contrary, the CE presented a high proportion of esters (25%), while oil-2 showed the lowest proportion (4%) of these molecules. The major ester in CE was linoleic acid ethyl ester (compound 77, Table 3), which is a neutral, lipid-soluble form of linoleic acid. In the skin epidermis, mainly composed of keratinocytes, linoleic acid is transformed in 13-hydroxyoctadecadienoic acid which exerts antiproliferative and anti-inflammatory activities (Ziboh et al., 2000) thereby protecting skin against toxicity. The dominant nitrogenous compound in oil-2 was 3,4,5-trimethylpyrazole (compound 35, **Table 7**). Although the toxicity of this compound on keratinocytes is not documented, the pyrazole derivative 3,4-dimethyl-1-H-pyrazole has been demonstrated to be irritant for the skin (Jírová et al., 2010) and other pyrazole-based compounds have been shown to have slight toxicity to dermal fibroblasts (Nada et al., 2018). More *in vitro* and *in vivo* toxicological studies should be carried out to accurately determine the nature of the molecules impacting the toxicity on keratinocytes and their threshold doses.

The critical role of IL-17 is now recognized in psoriasis and the impact of keratinocytes as crucial targets for its inflammatory effects on the skin is also documented (Moos et al., 2019).

The production of IL-8, a potent neutrophil chemoattractant, was found to be higher in the serum and skin of psoriasis patients compared to healthy ones (Bai et al., 2017) and its levels were correlated with psoriasis severity (Arican et al., 2005). Consequently, in this work, it was considered that the inhibition of IL-8 production by IL-17 stimulated HaCaT keratinocytes would be a good *in vitro* model for screening the anti-inflammatory potential of the solvent extracts and bio-oils and their possible application for psoriasis. For this, all samples were tested at non-cytotoxic concentrations, under their respective IC_{90} values. **Fig. 16** shows their effect on IL-8 production.

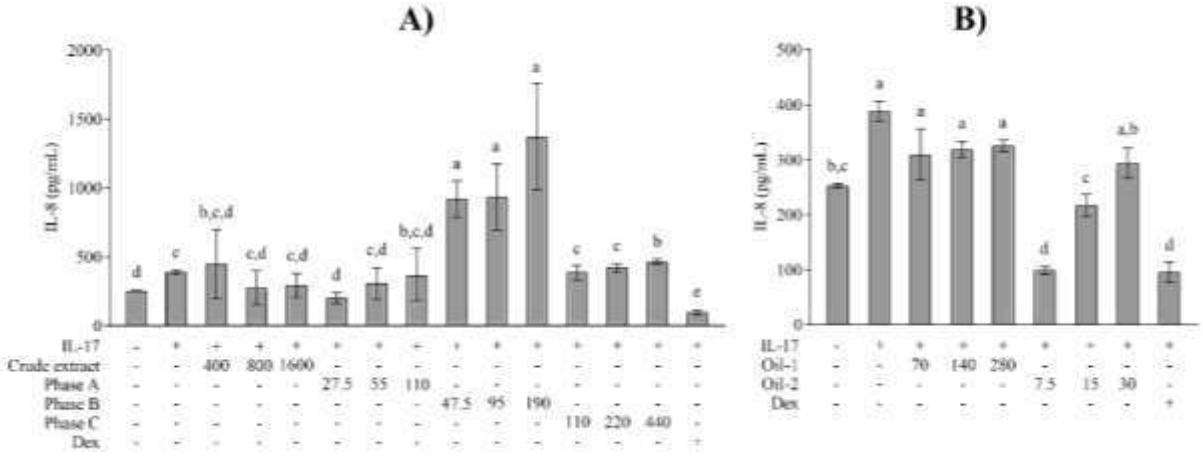

Fig. 16. Effect of the crude extract, phases A-C, and bio-oils on the IL-8 production in keratinocytes HaCaT stimulated with IL-17.

A) Solvent extracts, B) Bio-oils. The confluent HaCaT cells were pre-treated or not with the crude extract (400-1600 µg/mL), phase A (27.5-110 µg/mL), phase B (47.5-190 µg/mL), phase C (110-440 µg/mL), oil-1 (70-280 µg/mL), oil-2 (7.5-30 µg/mL) or Dex (5 µM) for 10h, then cells were stimulated with IL-17 (50 ng/mL) for 14 h. IL-8 production in HaCaT supernatants was assessed by ELISA. Results are expressed in pg/mL and correspond to 10^6 cells. Histograms represent the mean ± SD. A) statistical: Kruskal-Wallis followed by Holm's post hoc test. B) statistical: one-way ANOVA followed by Tukey's post hoc test. In all cases, means without a common letter differ ($p < 0.05$).

As observed, the solvent extracts did not inhibit the IL-8 production, except when phase A was used at the lowest concentration, which decreased IL-8 to basal concentrations. Conversely, a significant and unexpected proinflammatory effect of phase B was noted, whereas dexamethasone, a corticoid used for psoriasis (Le and Howard, 2013) significantly decreased the IL-8 under basal levels. In the case of bio-oils, oil-1 failed to induce an anti-inflammatory effect, while oil-2 at 15 and 7.5 µg/mL significantly decreased the IL-8 production. It should be

noted, that, unlike solvent extracts, this effect at 7.5 μg/mL was comparable to that of dexamethasone. Interestingly, oil-2 at intermediate and higher concentrations (15 and 30 μg/mL, respectively) showed lesser anti-IL17 activity than did the lowest tested concentration (7.5 μg/mL). This opposite effect was also observed for the anti-inflammatory activity of pyrolytic oils from *A. adstringens* bark (Esquivel-García et al., 2020). A possible explanation could be related to the fact that at higher concentrations close to the minimum toxic concentration, the bio-oils trigger cellular defense mechanisms, which counteract the anti-inflammatory effect, but further research is needed to prove it.

Our results demonstrate that differences in chemical composition, as observed between phase B and oil 2 (the samples with highest pro-inflammatory and anti-inflammatory effects, respectively) could be associated with changes in the anti-IL17 activity. The most important differences in the relative composition of phase B and oil-2 according to GC-MS data are related to the presence of esters (20 vs. 4%); nitrogenous compounds (16 vs. 36%), hydrocarbons (13 vs. 8%) and phenols (13 vs. 24%), respectively. One of the major identified compounds in phase B is phytol (compound 74, **Table 7**) which has been shown to have pro-inflammatory and tumor-promoting activities on mouse skin (Kagoura et al., 1999). Phytol derivatives are strong inducers of cytokines such as IL-17 (Roy Chowdhury et al., 2013) and can stimulate the NF-kappaB transcription factor (Ghosh and Chowdhury, 2013) linked to the IL-8 production. Phytol-based immunostimulants can boost the expression of $CXCL_{1,}$ a murine equivalent of IL-8 (Chowdhury and Ghosh, 2012) so we think that the important presence of phytol in Phase B could explain, at least partially, the unexpected pro-inflammatory activity. Furthermore, the significant occurrence of hydrocarbons in this phase compared to oil-2 is striking. Hydrocarbons have been associated with immune activation and development of autoimmune disease (Yau et al., 2017), so it is likely that these compounds act alone or in synergism with phytol to stimulate the IL-8 production, but further research is warranted to confirm it.

Currently, the main utilization of bio-oils is related to fuel's development, while their use as valuable sources of bioactive molecules has been largely neglected. However, the tar's use as a treatment for dermatological conditions dates back to over 2000 years, whereas coal tar has been used precisely for psoriasis for more than 100 years (Zeichner, 2010). The Goeckerman regimen, presently used for this illness, which consists of the ultraviolet B exposure combined with the coal tar application is an example of the importance of pyrolytic products as effective

antipsoriatic therapies. Nevertheless, the mechanism of action of coal tar in psoriasis remains to be elucidated and it is unknown whether other pyrolytic products derived from new natural sources, as *U. subincisa*, could have an anti-inflammatory potential for this cutaneous condition. Results here presented demonstrate that oil-2 has an inhibitory effect on the IL-8 production triggered by IL-17 comparable to that of dexamethasone. The major compound detected by GC-MS in oil-2 was 3,4,5-trimethylpyrazole. The pyrazole scaffold provides varied stereochemical complexity and functionality due to the presence of two nitrogen atoms and aromaticity. This complexity explains its wide range of drug-like properties including anti-inflammatory activity (Ansari et al., 2017). Pyrazole derivatives can ameliorate psoriasis-like dermatitis in mice by inhibiting IL-17 mRNA expression (Seki et al., 2014). Moreover, these compounds can inhibit NF-kappaB (Fu et al., 2017) linked to IL-8 production. Another bioactive nitrogenous compound present in oil-2 is pyrrole (compound 3, **Table 7**). Pyrrole derivatives have been proposed for the prophylaxis and treatment of inflammatory diseases involving the over-production of cytokines such as IL-8 (Zablocki et al., 2003). Moreover, the significant proportion of phenols in oil-2 could also be linked to its anti-inflammatory potential as these molecules are recognized by their anti-psoriatic activity (Działo et al., 2016).

Even though our results show the potential of oil-2 as an anti-IL-17 agent for psoriasis, additional research should be performed to elucidate the mechanisms involved in this activity. Also, since this oil at concentrations higher than those showing anti-inflammatory activity proved to be toxic on keratinocytes, further studies should be carried out to comprehensively evaluate its pharmacodynamic and toxicity-related compositions to ensure its efficacy and safety on skin.

VI.4.5. Conclusions

Overall, results here presented allowed for the first time to compare the chemical composition, toxicity, and anti-inflammatory character of the crude extract, its fractions, and bio-oils from *U. subincisa*. The use of aerial parts of the plant and the extracted residual biomass to obtain solvent extracts and bio-oils allowed the integral use of this medicinal plant employed by Purépechas for dermatological conditions. The relative chemical compositions of the samples were assessed using a set of analytical techniques. FTIR spectra showed a higher diversity of functional groups

in the crude extract and its fractions than in bio-oils. UV-fluorescence indicated that solvent extracts were generally richer in simple phenols, while bio-oils contained a higher number of conjugated molecules and polycondensed structures. According to GC-MS, the crude extract and phases A and B showed a higher concentration of esters (25, 56, and 20% of the identified molecules, respectively) whereas, in phase C and oil-2, nitrogen-containing compounds prevailed (26 and 40%, respectively). In contrast, oil-1 showed a preponderance of phenols (33%). These results agree with the elemental analysis which demonstrated that the oxygen was significantly higher in the crude extract and its fractions than in bio-oils, whereas the nitrogen was higher in bio-oils than in solvent extracts. As to the toxicity, the crude extract had the lowest toxicity on HaCaT keratinocytes, followed in increasing toxicity order by phase C, oil-1, phases B-A, and oil-2, respectively. Regarding the anti-IL-17 activity, only oil-2 showed a significant inhibitory effect on IL-8 production and this effect, at 7.5 μg/mL, was comparable to that of dexamethasone.

The evidence presented in this work does not support the traditional topical use of the ethanolic extract from aerial parts of *U. subincisa* by Purépechas as an anti-inflammatory agent for treating psoriasis. However, this folk preparation could possess other anti-inflammatory properties not linked to IL-17's inhibition useful for other cutaneous conditions, but further investigations should be performed to prove it. Our results demonstrate for the first time that oil-2 obtained from the extracted biomass, usually considered as a waste, has anti-IL-17 properties that deserve to be studied in detail in the future to elucidate the involved mechanisms. The significant proportion of nitrogenous compounds in this bio-oil, particularly 3,4,5-trimethylpyrazole, opens the door for a more exhaustive analysis of the anti-IL17 effects of this molecule and its possible application in psoriasis. As the toxic effects linked to the use of oil-2 on the skin could be a concern, it is expected that preclinical dermal toxicological evaluations can be performed to asses any complication arising during its topical exposition to anticipate adverse events in humans.

VI.4.6. Author contributions

REG: investigation, formal analysis, writing original draft. **AS**: investigation, formal analysis. **NAL**: investigation, visualization, writing, review & editing. **MGP**: investigation,

methodology, resources, writing, review, and editing. **AOZ**: investigation, formal analysis, writing, review, and editing. **MEGP**: conceptualization, methodology, formal analysis, resources, supervision, writing, review, and editing.

VI.4.7. Conflict of interest

The authors declare that they have no competing interests.

VI.4.8. Acknowledgements

The "Consejo Nacional de Ciencia y Tecnología" (CONACYT, Mexico) for the financial support of Roberto Esquivel-García (Doctoral Fellowship No.285764).

VI.4.9. References

Ansari, A., Ali, A., Asif, M., Shamsuzzaman, 2017. Review: biologically active pyrazole derivatives. New. J. Chem. 41, 16–41. https://doi.org/10.1039/C6NJ03181A

Arican, O., Aral, M., Sasmaz, S., Ciragil, P., 2005. Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. Mediat. Inflamm. 2005, 273–279. https://doi.org/10.1155/MI.2005.273

Ayiania, M., Terrell, E., Dunsmoor, A., Carbajal-Gamarra, F.M., Garcia-Perez, M., 2019. Characterization of solid and vapor products from thermochemical conversion of municipal solid waste woody fractions. Waste Manag. 84, 277–285. https://doi.org/10.1016/j.wasman.2018.11.042

Bai, F., Zheng, W., Dong, Y., Wang, J., Garstka, M.A., Li, R., An, J., Ma, H., 2017. Serum levels of adipokines and cytokines in psoriasis patients: a systematic review and meta-analysis. Oncotarget 9, 1266–1278. https://doi.org/10.18632/oncotarget.22260

Barsotti, F., Ghigo, G., Vione, D., 2016. Computational assessment of the fluorescence emission of phenol oligomers: a possible insight into the fluorescence properties of humic-like substances (HULIS). J Photochem. Photobiol. A. 315, 87–93. https://doi.org/10.1016/j.jphotochem.2015.09.012

Biller, P., Ross, A.B., 2014. Pyrolysis GC–MS as a novel analysis technique to determine the biochemical composition of microalgae. Algal Res. 6, 91–97. https://doi.org/10.1016/j.algal.2014.09.009

Bissonnette, R., Nigen, S., Langley, R.G., Lynde, C.W., Tan, J., Fuentes-Duculan, J., Krueger, J.G., 2014. Increased expression of IL-17A and limited involvement of IL-23 in patients with palmo-plantar (PP) pustular psoriasis or PP pustulosis; results from a randomised controlled trial. J. Eur. Acad. Dermatol. Venereol. 28, 1298–1305. https://doi.org/10.1111/jdv.12272

Blauvelt, A., Chiricozzi, A., 2018. The immunologic role of IL-17 in psoriasis and psoriatic arthritis pathogenesis. Clin. Rev. Allergy Immunol. 55, 379–390. https://doi.org/10.1007/s12016-018-8702-3

Bolea, C., Turturică, M., Stănciuc, N., Vizireanu, C., 2016. Thermal degradation kinetics of bioactive compounds from black rice flour (*Oryza sativa* L.) extracts. J. Cereal Sci. 71, 160–166. https://doi.org/10.1016/j.jcs.2016.08.010

Chavarría, A., Espinosa, G., 2019. Cruz-Badiano Codex and the importance of the Mexican medicinal plants. J. Pharm. Technol. Res. Manag. 7, 15–22.

Chowdhury, R.R., Ghosh, S.K., 2012. Phytol-derived novel isoprenoid immunostimulants. Front. Immunol. 3. https://doi.org/10.3389/fimmu.2012.00049

Claudia, C.-D., María-Elena, V.-H., Josué, V.-E., María-Carmen, B.-C., Alain-Raimundo, R.-O., Martha-Estrella, G.-P., 2020. Small molecules under development for psoriasis: on the road to the individualized therapies. Arch. Dermatol. Res. https://doi.org/10.1007/s00403-020-02056-3

Cuinica, L.G., Macêdo, R.O., 2018. Thermoanalytical characterization of plant drug and extract of *Urtica dioica* L. and kinetic parameters analysis. J. Therm. Anal. Calorim. 133, 591–602. https://doi.org/10.1007/s10973-018-6986-4

Dieguez-Alonso, A., Anca-Couce, A., Zobel, N., Behrendt, F., 2015. Understanding the primary and secondary slow pyrolysis mechanisms of holocellulose, lignin and wood with laser-induced fluorescence. Fuel 153, 102–109. https://doi.org/10.1016/j.fuel.2015.02.097

dos Santos Grasel, F., Flôres Ferrão, M., Wolf, C.R., 2016. Development of methodology for identification the nature of the polyphenolic extracts by FTIR associated with multivariate analysis. Spectrochim. Acta A. Mol. Biomol. Spectrosc. 153, 94–101. https://doi.org/10.1016/j.saa.2015.08.020

Działo, M., Mierziak, J., Korzun, U., Preisner, M., Szopa, J., Kulma, A., 2016. The potential of plant phenolics in prevention and therapy of skin disorders. Int. J. Mol. Sci. 17. https://doi.org/10.3390/ijms17020160

Ekman, A.-K., Bivik Eding, C., Rundquist, I., Enerbäck, C., 2019. IL-17 and IL-22 Promote Keratinocyte Stemness in the Germinative Compartment in Psoriasis. J. Invest. Dermatol. 139, 1564-1573.e8. https://doi.org/10.1016/j.jid.2019.01.014

Esquivel-García, R., Ayiania, M., Abu-Lail, N., López-Meza, J.E., del Río, R.E., García-Pérez, M., Ochoa-Zarzosa, A., García-Pérez, M.-E., 2020. Pyrolytic oils from *Amphipterygium adstringens* bark inhibit IL-8 production of IL-17-stimulated HaCaT keratinocytes. J. Anal. Appl. Pyrol. 145, 104749. https://doi.org/10.1016/j.jaap.2019.104749

Esquivel-García, R., Pérez-Calix, E., Ochoa-Zarzosa, A., García-Pérez, M.-E., 2018. Ethnomedicinal plants used for the treatment of dermatological affections on the Purépecha Plateau, Michoacán, Mexico. Acta Bot Mex 125, 95–132. https://doi.org/10.21829/abm125.2018.1339

Ezhilan, B.P., Neelamegam, R., 2012. GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L. Pharmacognosy Res 4, 11–14. https://doi.org/10.4103/0974-8490.91028

Fu, D., Farag, S., Chaouki, J., Jessop, P.G., 2014. Extraction of phenols from lignin microwave-pyrolysis oil using a switchable hydrophilicity solvent. Bioresour. Technol. 154, 101–108. https://doi.org/10.1016/j.biortech.2013.11.091

Fu, Y., Ma, J., Shi, X., Song, X.-Y., Yang, Y., Xiao, S., Li, J., Gu, W.-J., Huang, Z., Zhang, J., Chen, J., 2017. A novel pyrazole-containing indolizine derivative suppresses NF-κB activation and protects against TNBS-induced colitis via a PPAR-γ-dependent pathway. Biochem. Pharmacol. 135, 126–138. https://doi.org/10.1016/j.bcp.2017.03.013

Gami, A.A., 2014. Phenol and its toxicity. Journal of Environmental Microbiology and Toxicology 2.

García-Pérez, M.-E., Alfonso-Castillo, A., Fong Lores, O., Batista-Duharte, A., Lemus-Rodríguez, Z., 2018. Toxicological evaluation of an aqueous suspension from leaves and stems of *Petiveria alliacea* L. (Phytolaccaceae). J. Ethnopharmacol. 211, 29–37. https://doi.org/10.1016/j.jep.2017.09.022

García-Pérez, M.-E., Royer, M., Duque-Fernandez, A., Diouf, P.N., Stevanovic, T., Pouliot, R., 2010. Antioxidant, toxicological and antiproliferative properties of Canadian polyphenolic extracts on normal and psoriatic keratinocytes. J. Ethnopharmacol. 132, 251–258. https://doi.org/10.1016/j.jep.2010.08.030

Garmendia, A., Raigón, M.D., Marques, O., Ferriol, M., Royo, J., Merle, H., 2018. Effects of nettle slurry (*Urtica dioica* L.) used as foliar fertilizer on potato (*Solanum tuberosum* L.) yield and plant growth. PeerJ 6, e4729. https://doi.org/10.7717/peerj.4729

Ghosh, S.K., Chowdhury, R.R., 2013. Synthetic adjuvants for vaccine formulations: phytol derivatives. Expert Opin. Drug Deliv. 10, 437–450. https://doi.org/10.1517/17425247.2013.757591

Gorzalczany, S., Marrassini, C., Miño, J., Acevedo, C., Ferraro, G., 2011. Antinociceptive activity of ethanolic extract and isolated compounds of *Urtica circularis*. J. Ethnopharmacol. 134, 733–738. https://doi.org/10.1016/j.jep.2011.01.025

Han, Y., Stankovikj, F., Garcia-Perez, M., 2017. Co-hydrotreatment of tire pyrolysis oil and vegetable oil for the production of transportation fuels. Fuel Process Technol. 159, 328–339. https://doi.org/10.1016/j.fuproc.2017.01.048

Hlavsová, A., Corsaro, A., Raclavská, H., Vallová, S., Juchelková, D., 2016. The effect of feedstock composition and taxonomy on the products distribution from pyrolysis of nine herbaceous plants. Fuel Process Technol. 144, 27–36. https://doi.org/10.1016/j.fuproc.2015.11.022

Hundie, G.B., Woldemeskel, D., Gessesse, A., 2016. Evaluation of direct colorimetric MTT assay for rapid detection of rifampicin and isoniazid resistance in *Mycobacterium tuberculosis*. PLoS ONE 11, e0169188. https://doi.org/10.1371/journal.pone.0169188

Ibrahim, M., Rehman, K., Razzaq, A., Hussain, I., Farooq, T., Hussain, A., Akash, M.S.H., 2018. Investigations of phytochemical constituents and their pharmacological properties isolated from the genus *Urtica*: critical review and analysis. Crit. Rev. Eukaryot. Gene Expr. 28, 25–66. https://doi.org/10.1615/CritRevEukaryotGeneExpr.2018020389

Jírová, D., Basketter, D., Liebsch, M., Bendová, H., Kejlová, K., Marriott, M., Kandárová, H., 2010. Comparison of human skin irritation patch test data with in vitro skin irritation assays and animal data. Contact Dermatitis 62, 109–116. https://doi.org/10.1111/j.1600-0536.2009.01640.x

Kagoura, M., Matsui, C., Morohashi, M., 1999. Phytol is a novel tumor promoter on ICR mouse skin. Jpn. J. Cancer Res. 90, 377–384. https://doi.org/10.1111/j.1349-7006.1999.tb00758.x

Kebelmann, K., Hornung, A., Karsten, U., Griffiths, G., 2013. Intermediate pyrolysis and product identification by TGA and Py-GC/MS of green microalgae and their extracted protein and lipid components. Biomass Bioenergy 49, 38–48. https://doi.org/10.1016/j.biombioe.2012.12.006

Kopyt'ko, Ya.F., Lapinskaya, E.S., Sokol'skaya, T.A., 2012. Application, chemical composition, and standardization of nettle raw material and related drugs (review). Pharm. Chem. J. 45, 622–631. https://doi.org/10.1007/s11094-012-0690-7

Kumar, S., Pandey, A.K., 2013. Chemistry and biological activities of flavonoids: an overview. Sci. World J. 2013, e162750. https://doi.org/10.1155/2013/162750

Lapinskaya, E.S., Kopyt'ko, Ya.F., 2008. Composition of the lipophilic fraction of stinging nettle (*Urtica dioica* L. and *U. urens* L.) homeopathic matrix tinctures. Pharm. Chem. J. 42, 699–702. https://doi.org/10.1007/s11094-009-0204-4

Le, Q.V., Howard, A., 2013. Dexamethasone iontophoresis for the treatment of nail psoriasis. Australas. J. Dermatol. 54, 115–119. https://doi.org/10.1111/ajd.12029

Lv, J., Zhou, D., Wang, Y., Zhao, J., Chen, Z., Zhang, J., Di, T., Hu, J., Li, B., Li, P., Huang, F., 2018. Quantitative evaluation to efficacy and safety of therapies for psoriasis: a network meta-analysis. Mol. Pain 14, 1744806918762205. https://doi.org/10.1177/1744806918762205

Marcilla, A., Gómez-Siurana, A., Gomis, C., Chápuli, E., Catalá, M.C., Valdés, F.J., 2009. Characterization of microalgal species through TGA/FTIR analysis: application to *Nannochloropsis sp.* Thermochim. Acta 484, 41–47. https://doi.org/10.1016/j.tca.2008.12.005

Mohan, D., Pittman, Charles U., Steele, P.H., 2006. Pyrolysis of wood/biomass for bio-oil: a critical review. Energy Fuels 20, 848–889. https://doi.org/10.1021/ef0502397

Moos, S., Mohebiany, A.N., Waisman, A., Kurschus, F.C., 2019. Imiquimod-induced psoriasis in mice depends on the IL-17 signaling of keratinocytes. J. Invest. Dermatol. 139, 1110–1117. https://doi.org/10.1016/j.jid.2019.01.006

Mourant, D., Lievens, C., Gunawan, R., Wang, Y., Hu, X., Wu, L., Syed-Hassan, S.S.A., Li, C.-Z., 2013. Effects of temperature on the yields and properties of bio-oil from the fast pyrolysis of mallee bark. Fuel 108, 400–408. https://doi.org/10.1016/j.fuel.2012.12.018

Murray, A.R., Kisin, E., Castranova, V., Kommineni, C., Gunther, M.R., Shvedova, A.A., 2007. Phenol-induced *in vivo* oxidative stress in skin: evidence for enhanced free radical generation, thiol oxidation, and antioxidant depletion. Chem. Res. Toxicol. 20, 1769–1777. https://doi.org/10.1021/tx700201z

Nada, A., Al-Moghazy, M., Soliman, A.A.F., Rashwan, G.M.T., Eldawy, T.H.A., Hassan, A.A.E., Sayed, G.H., 2018. Pyrazole-based compounds in chitosan liposomal emulsion for antimicrobial cotton fabrics. Int. J. Biol. Macromol. 107, 585–594. https://doi.org/10.1016/j.ijbiomac.2017.09.031

Newby, C.S., Barr, R.M., Greaves, M.W., Mallet, A.I., 2000. Cytokine release and cytotoxicity in human keratinocytes and fibroblasts induced by phenols and sodium dodecyl sulfate. J. Invest. Dermatol. 115, 292–298. https://doi.org/10.1046/j.1523-1747.2000.00056.x

Nunes Oliveira, R., Cordeiro Mancini, M., Cabral Salles de Oliveira, F., Passos, T., Quilty, B., da Silva Moreira Thiré, R.M., McGuinness, G.B., 2016. FTIR analysis and quantification of phenols and flavonoids of five commercially available plants extracts used in wound healing. Matéria (Rio J.) 21, 767–779. https://doi.org/10.1590/S1517-707620160003.0072

Rosnitschek-Schimmel, I., 1985a. Seasonal dynamics of nitrogenous compounds in a nitrophilic weed I. Changes in inorganic and organic nitrogen fractions of the different plant parts of *Urtica dioica*. Plant Cell Physiol. 26, 169–176. https://doi.org/10.1093/oxfordjournals.pcp.a076885

Rosnitschek-Schimmel, I., 1985b. Seasonal dynamics of nitrogenous compounds in a nitrophilic weed II. The role of free amino acids and proteins as nitrogen store in *Urtica dioica*. Plant Cell Physiol. 26, 177–183. https://doi.org/10.1093/oxfordjournals.pcp.a076886

Roy Chowdhury, R., Fitch, R.W., Ghosh, S.K., 2013. Efficacy of phytol-derived diterpenoid immunoadjuvants over alum in shaping the murine host's immune response to *Staphylococcus aureus*. Vaccine 31, 1178–1186. https://doi.org/10.1016/j.vaccine.2012.12.069

Scherließ, R., 2011. The MTT assay as tool to evaluate and compare excipient toxicity *in vitro* on respiratory epithelial cells. Int. J. Pharm. 411, 98–105. https://doi.org/10.1016/j.ijpharm.2011.03.053

Seki, N., Shimano, K., Kondou, T., Murase, M., Mitamura, M., Kataoka, H., Sugahara, K., Nakamura, H., Yamada, M., 2014. OP0105 Pyrazole-anilide derivatives, a new class of immunomodulator which inhibits IL-17 production, ameliorate CIA and psoriasis-like dermatitis in mice. Ann. Rheum. Dis. 73, 100–101. https://doi.org/10.1136/annrheumdis-2014-eular.3054

Stankovikj, F., Garcia-Perez, M., 2017. TG-FTIR method for the characterization of bio-oils in chemical families. Energy Fuels 31, 1689–1701. https://doi.org/10.1021/acs.energyfuels.6b03132

Stankovikj, F., McDonald, A.G., Helms, G.L., Olarte, M.V., Garcia-Perez, M., 2017. Characterization of the water-soluble fraction of woody biomass pyrolysis oils. Energy Fuels 31, 1650–1664. https://doi.org/htpps://doi.org/10.1021/acs.energyfuels.6b02950

Stefanidis, S.D., Kalogiannis, K.G., Iliopoulou, E.F., Michailof, C.M., Pilavachi, P.A., Lappas, A.A., 2014. A study of lignocellulosic biomass pyrolysis via the pyrolysis of cellulose, hemicellulose and lignin. J. Anal. Appl. Pyrol. 105, 143–150. https://doi.org/10.1016/j.jaap.2013.10.013

Steinmann, V.W., 2005. Urticaceae. Flora del Bajío y de Regiones Adyacentes 134, 1–74.

Teepe, R.G., Koebrugge, E.J., Löwik, C.W., Petit, P.L., Bosboom, R.W., Twiss, I.M., Boxma, H., Vermeer, B.J., Ponec, M., 1993. Cytotoxic effects of topical antimicrobial and antiseptic agents on human keratinocytes *in vitro*. J. Trauma 35, 8–19. https://doi.org/10.1097/00005373-199307000-00002

Thorpe, R., Pandya, A.G., 2014. Psoriasis, in: Jackson-Richards, D., Pandya, A.G. (Eds.), Dermatology Atlas for Skin of Color. Springer, Berlin, Heidelberg, pp. 131–138. https://doi.org/10.1007/978-3-642-54446-0_25

Yau, A.C.Y., Lönnblom, E., Zhong, J., Holmdahl, R., 2017. Influence of hydrocarbon oil structure on adjuvanticity and autoimmunity. Sci. Rep. 7, 14998. https://doi.org/10.1038/s41598-017-15096-z

Zablocki, J.A., Tarlton, E., Rizzi, J.P., Mantlo, N.B., 2003. Aryl and heteroaryl substituted fused pyrrole anti-inflammatory agents. US6605634B2.

Zeichner, J.A., 2010. Use of topical coal tar foam for the treatment of psoriasis in difficult-to-treat areas. J. Clin. Aesthet. Dermatol. 3, 37–40.

Ziboh, V.A., Miller, C.C., Cho, Y., 2000. Metabolism of polyunsaturated fatty acids by skin epidermal enzymes: generation of antiinflammatory and antiproliferative metabolites. Am J. Clin. Nutr. 71, 361s–366s. https://doi.org/10.1093/ajcn/71.1.361s

CAPÍTULO 3. Los aceites pirolíticos de la corteza de *Amphipterygium adstringens* inhiben la producción de IL-8 en queratinocitos HaCaT estimulados con IL-17.

VI.5. PREFACIO

Este capítulo es el tema de un artículo titulado: “Pyrolytic oils from *Amphipterygium adstringens* bark inhibit IL-8 production of IL-17-stimulated HaCaT keratinocytes”. El cual fue publicado en *Journal of Analytical and Applied Pyrolysis*, 145: 104749. Su identificador de objeto digital es: https://doi.org/10.1016/j.jaap.2019.104749

El presente capítulo se dirige a dar respuesta a los objetivos particulares # 2 y 3. La especie, nativa de México, *Amphipterygium adstringens* fue escogida como un árbol prometedor, dado su valor alto valor de uso por la etnomedicina Purépecha (0.15), que se demostró durante el estudio etnofarmacológico. La especie fue sometida a una extracción con agua caliente, siguiendo el uso tradicional reportado y posteriormente la biomasa residual fue sometida a un proceso de pirólisis para obtener aceites pirolíticos. Estos aceites fueron caracterizados químicamente, se hizo un análisis de su toxicidad en un cultivo de queratinocitos HaCaT, y finalmente se probó su capacidad para inhibir la producción de IL-8 en queratinocitos estimulados con IL-17.

Journal of Analytical and Applied Pyrolysis 145 (2020) 104749

Contents lists available at ScienceDirect

Journal of Analytical and Applied Pyrolysis

journal homepage: www.elsevier.com/locate/jaap

Pyrolytic oils from *Amphipterygium adstringens* bark inhibit IL-8 production of IL-17-stimulated HaCaT keratinocytes

Roberto Esquivel-García[a,b], Michael Ayiania[c], Nehal Abu-Lail[d], Joel E. López-Meza[b], Rosa E. del Río[a], Manuel García-Pérez[c], Alejandra Ochoa-Zarzosa[b], Martha-Estrella García-Pérez[a,*]

[a] Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, Mexico
[b] Centro Multidisciplinario de Estudios en Biotecnología-FMVZ, Universidad Michoacana de San Nicolás de Hidalgo, Tarímbaro, Michoacán, Mexico
[c] Biological Systems Engineering Department, Washington State University, Pullman, WA, USA
[d] Department of Biomedical Engineering, University of Texas at San Antonio, TX, USA

ARTICLE INFO

Keywords:
Amphipterygium adstringens
Slow pyrolysis
IL-8
Phenol
HaCaT
IL-17

ABSTRACT

Pyrolytic products have been historically used as a therapeutic treatment for psoriasis, a dermatological inflammatory disease. However, little is known about the mechanisms involved in their pharmacological activity. The main goals of this work were to characterize pyrolytic fractions derived from *Amphipterygium adstringens* bark, a plant used in Mexican traditional medicine, and to study the effects of pyrolytic oils as anti-IL-17 agents on HaCaT keratinocytes. *A. adstringens* biomass and pyrolytic fractions were first characterized by proximate, elemental analysis, FTIR, Py-GC–MS, GC–MS, UV-fluorescence and HPLC-DAD to determine its physicochemical composition. Toxicity of pyrolytic oils was further assessed on HaCaT keratinocytes by the MTT method and the non-cytotoxic concentrations were used to determine their effects on IL-8 production of IL-17-stimulated HaCaT keratinocytes. The result obtained show that pyrolytic oils are very rich in phenols, some of them recognized by their anti-inflammatory properties. Pyrolytic oils showed significant inhibitory effects on IL-8 production, suggesting that they could be potentially explored for treating IL-17 driven dermatological diseases such as psoriasis.

1. Introduction

Psoriasis is a cutaneous disorder characterized by the presence of desquamative erythematous plaques [1]. The prevalence of this disease in adults worldwide ranges from 0.51 to 11.43 % [2]. The estimated prevalence in Mexico is 2.9 %, so more than 3 million Mexicans are affected [3], 12.4–25.9 % of them having moderate to severe forms of the disease [4]. The incidence of death directly caused by this disease is low, but its physical and psychological manifestations are comparable to those observed in cancer, diabetes, and depression [5]. Although psoriasis' pathogenesis has not been fully elucidated; it is recognized that the disturbances in keratinocytes and the hyperproliferation of these cells are distinctive features of this disorder [6]. In recent years, it has been shown that the pathogenesis of this disease involves a complex interaction between neutrophils, dendritic cells (CD), T lymphocytes (mainly Th1 and Th17) and keratinocytes [7].

The crucial role of cytokines, such as interleukin (IL)-17 in the pathophysiology of psoriasis is now widely recognized by the scientific community. Indeed, it has been postulated that psoriasis is an IL-17-driven disease [8]. In psoriasis, IL-17 is mainly produced by T cells, mast cells and neutrophils [9,10]. Once released, IL-17 cytokines bind their respective transmembrane IL-17 receptor (IL-17R) which is expressed, among others, on keratinocyte surface, making these cutaneous cells important targets for IL-17 promoting effects [11]. IL-17 induces the keratinocyte proliferation with a negative impact on their differentiation [12], contributing to the skin barrier disruption. Additionally, IL-17 cytokines amplify the psoriasis inflammatory network as they can promote the keratinocyte release of other proinflammatory chemokines such as IL-8 [11] (a potent neutrophil chemoattractant), overexpressed in psoriatic lesions [13]. In psoriasis context, keratinocytes have been suggested to be a major IL-8 source [13,14] and psoriasis improvement correlates with the diminution of this chemokine in the skin [15].

Biological agents that neutralize IL-17 (secukinumab, ixekizumab)

* Corresponding author at: Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Avenida Universidad S/N. Edificio, B1 Ciudad Universitaria, Morelia, Michoacán, 58030, Mexico.
E-mail address: margar@live.ca (M.-E. García-Pérez).

https://doi.org/10.1016/j.jaap.2019.104749
Received 21 June 2019; Received in revised form 29 November 2019; Accepted 4 December 2019
Available online 05 December 2019
0165-2370/ © 2019 Elsevier B.V. All rights reserved.

or antagonize its receptor (brodalumab) have been demonstrated to be highly efficacious in clinical studies for psoriasis [16]. However, some adverse events have been described with these drugs such as nasopharyngitis, headache, upper respiratory tract infection, arthralgia, hypertension, back pain and cough [17,18].

Global surveys have clearly shown that some psoriasis patients are dissatisfied with biological treatments (10–25 %), mainly due to adverse events (12–28 %), lack of effectiveness and high costs [19,20]. As their quality of life is affected, those patients usually use alternative therapies [21] such as coal tar and medicinal plants to treat their disease [22]. The use of distillation and pyrolysis products from coal, such as tar and pitch, became popular after 1925 when W.H. Goeckerman established a method to use them as a therapeutic option for psoriasis treatment [23]. Nowadays, research on the use of coal tar as an antipsoriatic drug continues, due to its efficacy and safety [24,25]. Despite its wide use, little is known about its exact mechanism of action [26]. Some compounds isolated from the coal tar such as carbazole and its derivatives have shown antipsoriatic activity in human cell lines [27]. Tar from other sources such as pinewood has also been used as part of the therapy to treat this skin illness because of its antipruritic, antimicrobial and anti-inflammatory properties [28].

Amphipterygium adstringens (Schltdl.) Standl. is a species endemic to Mexico belonging to the Anacardiaceae family that inhabits the tropical deciduous forest region [29]. The bark from this tree is widely used in Mexican traditional medicine for the treatment of various diseases, including dermatological disorders [29,30]. The chemical characterization of *A. adstringens* bark has been performed using conventional natural extracts obtained by ebullition or maceration [31–33]. Some compounds have been isolated from bark extracts, such as anacardic acid, masticadienonic acid, and β-sitosterol [32], which have shown anti-inflammatory and gastroprotective properties [31,33]. During the filtration process of these conventional extracts, the recovered residual biomass has been considered waste and nothing is known about its chemical characterization and/or biological properties.

Considering that it has not been described if pyrolytic fractions of the bark from this tree have biological actions, the aims of this study were to chemically characterize the pyrolytic fractions and residual biomass from *A. adstringens* bark and to evaluate the capacity of pyrolytic oils to inhibit IL-8 production of IL-17-stimulated HaCaT keratinocytes in order to propose future applications of pyrolytic oils of this species for psoriasis treatment.

2. Material and methods

This work is divided into four major parts (see Fig. 1). In the first one *A. adstringens* bark was subjected to hot water extraction and the residual biomass was retained to be pyrolyzed. In the second part, residual biomass and pyrolytic fractions were thoroughly characterized by a tandem of analytical techniques. The third part consisted of toxicological evaluation of the effects of pyrolytic oils on HaCaT keratinocytes, whereas in the fourth part, the anti-inflammatory activities of these oils on HaCaT cells were analyzed. Each of these major parts will be explained in more detail in the sections to follow.

2.1. Materials

Barks from *A. adstringens* trees were collected from San Francisco, Municipality of San Lucas, Michoacán state, Mexico. The bark samples were taken in a single strip approximately 50 cm long by 8 cm wide and less than 3 cm thick from 20 trees that had not been previously barked with an age between 20–25 years (20 − 25 cm diameter) avoiding affecting its viability. The trees were identified botanically, and a sample deposited in the IEB herbarium located in Pátzcuaro, Michoacán, Mexico (voucher number 256881). Before used, the bark was washed with water at 25 °C, oven-dried (temperature 40 ± 2 °C) and grounded at a particle size ≤800 μm. The bark powder was then subjected to an

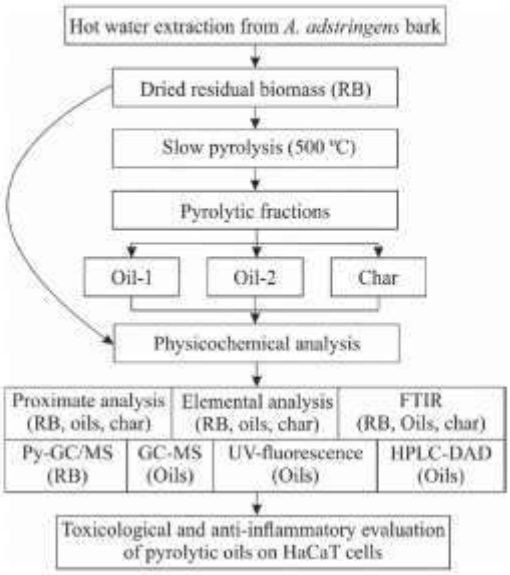

Fig. 1. Schematic representation of mayor tasks described in this paper.

aqueous extraction by boiling under reflux for 1 h, the extract filtered under vacuum and the residual biomass recovered for pyrolysis studies. The residual biomass recovered after hot-water extraction of the dry bark powder from *A. adstringens* was 66.2 ± 2.9 wt.%. Valorization of this residue, which represents more than half of the original biomass, is very important to improve the economic viability of *A. adstringens* commercialization [34,35]. The moisture content of the residual biomass was 4.4 ± 0.9 %, whereas the volatile content determined by proximate analysis was 76.0 ± 0.8 wt.%. The contents of fixed carbon and ash were 17.2 ± 0.3 and 6.8 ± 1.1 wt.% respectively. Its elemental composition was the following: carbon (C): 46.9 ± 0.3 wt.%; hydrogen (H): 5.2 ± 0.1 wt.%; nitrogen (N): 0.8 ± 0.02 wt.%; sulfur (S): 0.02 ± 0.0 wt.%; oxygen (O): 40.3 ± 0. 9 wt.%.

2.2. Pyrolysis experiments

Pyrolytic samples were obtained by slow pyrolysis at a temperature of 500 °C from 16 g of residual biomass in a quartz-tube furnace reactor of 50 mm outer diameter (OD) x 44 mm inner diameter (ID) x1000 mm length (L) (0.05 m D x1 mL) (see Fig. 2). The sample containers were introduced into the furnace under nitrogen flow for 5 min at 25 °C. The temperature was increased from 25 °C to 500 °C at a heating rate of 10 °C per min. After the final temperature had been reached, the reactor was then cooled to room temperature under a nitrogen gas before exposure to air. Biochar was obtained in sample containers. After recording the weight differences of the quartz tube, oil-1 was recovered from inside the tube with a spatula. Oil-2 samples were collected in two vacuum traps (Sigma-Aldrich SYNV164034). The traps were connected in series and placed in an ice bath to condense the volatile phase. Each trap was washed using ultrasonic agitation for three 2-min periods using a total of 500 mL of methanol. The solvent extract was concentrated under vacuum (30 °C, 26 kPa). Oil samples were weight and kept under refrigeration (4 °C) until further analysis.

2.3. Chemical characterization of residual biomass and pyrolytic fractions

2.3.1. Proximate and elemental analyses

The characterization of the biomass pyrolyzed, its pyrolytic oils and char was carried out according to methodologies described elsewhere

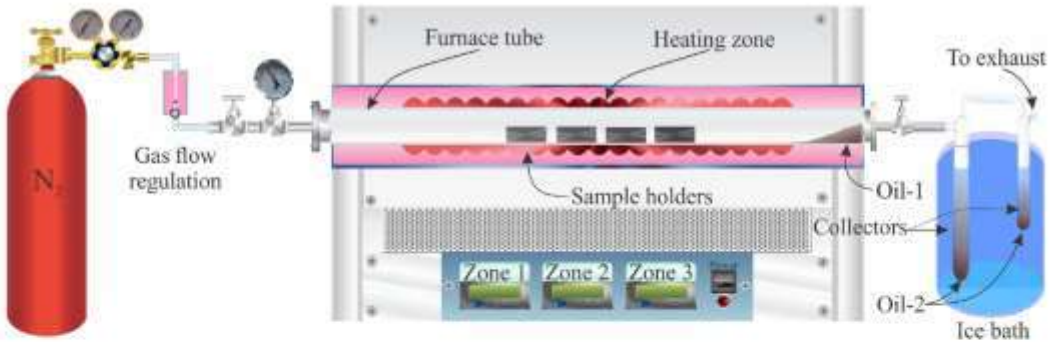

Fig. 2. System used for pyrolytic collection of oil-1 and oil-2.

Table 1
Pyrolytic products yield of residual biomass from *A. adstringens*.

Sample	Recovery (%)
Char	39.9 ± 2.7
Oil-1	10.1 ± 0.5
Oil-2	13.8 ± 0.4

Data represent mean ± SD of four experiments.

Table 2
Proximate and elemental analyses of *A. adstringens* pyrolytic products.

Parameter	Sample		
	Char	Oil-1	Oil-2
Proximate analysis (wt.%)			
Moisture[a]	3.5 ± 0.6	16.1 ± 1.5	26.9 ± 2.2
Volatiles[b]	26.1 ± 0.9	95.6 ± 0.8	92.8 ± 2.8
Fixed carbon[b]	56.6 ± 0.0	4.2 ± 0.5	6.9 ± 2.8
Ash[b]	17.3 ± 0.9	0.2 ± 0.3	0.4 ± 0.0
Elemental analysis (wt.%)			
C[b]	63.6 ± 0.8	71.7 ± 1.2	87.7 ± 0.9
H[b]	2.7 ± 0.05	7.5 ± 0.3	8.0 ± 0.4
N[b]	1.1 ± 0.06	1.1 ± 0.03	1.4 ± 0.03
S[b]	0.00 ± 0.0	0.03 ± 0.0	0.01 ± 0.0
O[c]	15.3 ± 0.1	19.4 ± 1.8	2.5 ± 0.5

[a] As-received basis.
[b] Dry basis.
[c] By difference. Carbon (C), Hydrogen (H), Nitrogen (N), Sulfur (S), Oxygen (O). Data represent mean ± SD of two experiments.

[36]. Fixed carbon, volatiles, and the ash content of samples were determined with a Mettler Toledo thermogravimetric analyzer (SDTA851e) equipped with STARe data analysis software for obtaining thermogravimetric/differential thermal data curves (TGA/DTG). The elemental composition (contents C, H, N and S) of pyrolytic oils, char and residual biomass was determined using a Leco TruSpec Micro elemental analyzer 628 series instrument. The oxygen content was obtained by difference after considering the ash content on a dry basis.

2.3.2. FTIR of residual biomass and pyrolytic fractions

FTIR spectra were obtained using a Shimadzu IRPrestige 21 spectrometer. A small liquid or solid sample from pyrolytic oils, char, and residual biomass was applied to cover the crystal window, and spectra of 32 scans with a resolution of 4 cm^{-1} within the range 4000 to 600 cm^{-1} were acquired. The spectra were baseline corrected with a multi-point interactive method using version 1.30 of IRsolution software and absorbance wavenumbers that can be correlated to certain characteristic functional groups were selected [37].

2.3.3. Py-GC–MS of residual biomass

The method was conducted using Agilent Technologies 6890 N Gas Chromatograph linked to Agilent 5975B Mass Selective Detector with NIST 2.0 f Mass Spectral Search Program. GC was equipped with Agilent 19091S-433 column with dimensions of 30 m x 250 µm x 0.25 µm [38]. Approximately 0.5 mg of residual biomass was loaded into a clean quart tube. The sample was introduced in the probe interface using a CDS Analytical Pyroprobe 5000 Series. The initial and final temperatures of pyroprobe were adjusted to 500 °C, with a probe run time of 1 min. The sample was injected using a split ratio 50:1. Front inlet parameters were set as: 250 °C, 7.06 psi, total flow He

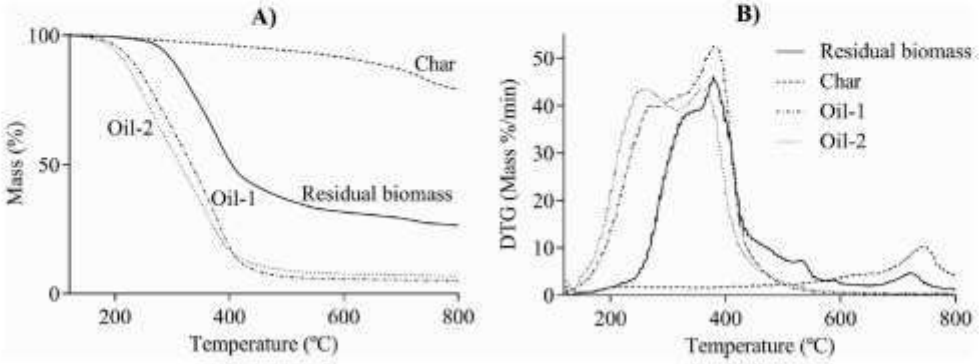

Fig. 3. TGA (A) and DTG (B) curves for the residual biomass and pyrolytic products from *A. adstringens* bark.

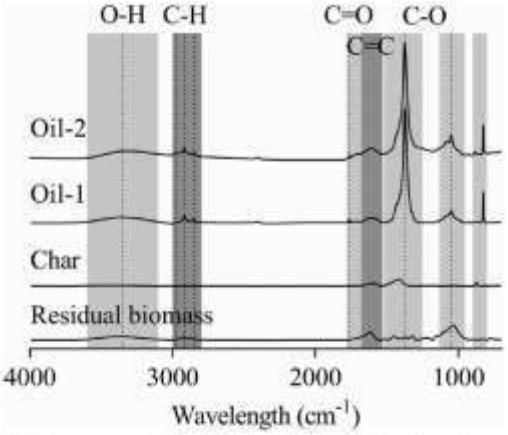

Fig. 4. FTIR spectra of residual biomass and pyrolytic fractions from *A. adstringens* bark.

53.8 mL/min, septum purge flow and 1.0 mL/min, 7.07 psi, 36 cm/s of column flow. Oven temperature was set at 40 °C for 1 min and then increased to 280 °C at the rate of 6 °C/min and the final hold time was 58 min. Compounds were identified by a combination with literature data, and the data provided by the Mass Spectral Library. Additionally, the chemical nature of the *A. adstringens* biomass and the previous chemical characterization performed in Anacardiaceae family were considered. The assignment of chemical groups to each compound was performed as previously described [39,40].

2.3.4. GC–MS of pyrolytic oils

Oil samples were analyzed to identify compounds by GC–MS using an Agilent Technologies 7890 A GC set up (Agilent 19091S-433 HP-5MS column: 325 °C: 30 m × 250 µm × 0.25 µm, Agilent 5975 C MS with NIST 2.0 f Mass Spectral Search Program). Small samples (1.8 mL) were collected with a filter unit for syringe (Miller® GP Filter Unit 0.22 µm) from the stock solutions (1 % w/w in methanol) for the GC–MS analysis. The GC–MS analytical method used was that described by Han et al. [41]. The structural assignments were performed as previously described for Py-GC–MS analysis.

2.3.5. UV-fluorescence of pyrolytic oils

Oil samples were diluted in HPLC grade methanol (Fisher Scientific), at a 10 ppm and analyzed on Shimadzu RF 5301 pc spectrometer with the software Panorama Fluorescence 2.1. The analysis was based on the methodology of García-Pérez and collaborators [36]. Briefly, the excitation wavelength was constant and scanned from 250 to 500 nm, and emission wavelengths were recorded with a 5 nm difference (from 260 to 510 nm; excitation and emission slit widths of 5 nm).

2.3.6. HPLC-DAD analysis of pyrolytic oils

Compounds' separation was performed via Agilent Technologies HPLC 1200 series using an Agilent Technologies Poroshell C18 column (150 × 4.6 mm I.D., 2.7 µm particle size), operated at 30 °C. The multi-wavelength detector was set to 280 and 325 nm. The flow-rate was 0.3 mL/min and the mobile phases consisted of 0.1 % formic acid in water (Solvent A) and 0.1 % formic acid in acetonitrile (Solvent B) [42]. The elution program included a gradient as previously detailed by Berry and Henderson [42]. Compounds were identified by comparing the retention times and UV spectra with those of pure standards: gallic acid, epigallocatechin, chlorogenic acid, catechin hydrate, caffeic acid, syringic acid, ellagic acid, *p*-coumaric acid, (-)-epicatechin gallate, scopoletin, ferulic acid, naringin, salicylic acid, resveratrol, quercetin and naringenin (all standards were purchased from Sigma Aldrich, St. Louis, MO, USA). Calibration curves served to quantify the identified compounds ($r > 0.99$).

2.4. Toxicity of pyrolytic oils on HaCaT cells and effects on IL-17-induced IL-8 production

Considering that a successful drug candidate for psoriasis treatment should demonstrate low toxicity at pharmacological concentrations [43], the toxicological properties of the pyrolytic oils obtained here were evaluated on HaCaT keratinocytes through the determination of the initial toxicity value (IC_{90}) by the MTT method [44,45]. The IC_{90} value represents the minimal toxic dose of the extract causing a 10 % reduction of cell viability compared with untreated cells [44]. MTT test is considered to be a measure of toxicity effects on mitochondria [46].

2.4.1. Cell culture

Human HaCaT keratinocyte cell line was purchased from AddexBio San Diego, CA, USA. HaCaT cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) high glucose with sodium pyruvate (Sigma Aldrich, St. Louis, MO, USA) and supplemented with 100 U/mL penicillin (Sigma Aldrich, St. Louis, MO, USA), 100 µg/mL streptomycin (Sigma Aldrich, St. Louis, MO, USA), and 10 % fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) in a humidified incubator containing 5 % CO_2 at 37 °C. Only cells at 80 % confluence were used in experiments as previously described [44].

2.4.2. MTT assay

Approximately, 2.0×10^4 cells were seeded in 96-well microplate and maintained in a supplemented medium for 24 h. Fresh medium without supplements was added to wells and was left 12 h to allow for cell synchronization. Oil samples obtained from pyrolysis of *A.*

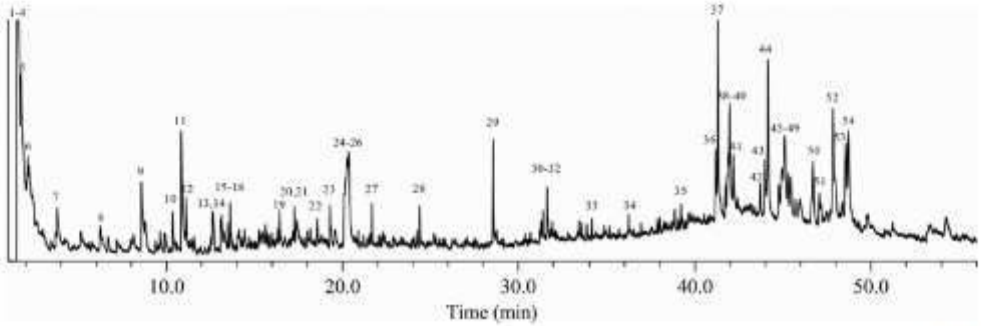

Fig. 5. Py-GC–MS analysis chromatogram of residual biomass from *A. adstringens* (list of the numbered compounds is shown in Table 3).

Table 3
Py-GC–MS characterization of the main compounds found in the residual biomass from *A. adstringens*.

No.	Identified compounds	RT (min)	Groups
1	Carbon dioxide	1.49	O
2	sec-Butylamine	1.72	AM
3	Cyclopropyl carbinol	1.88	A
4	Cyclobutanol	1.96	A
5	Butanal	2.10	AD
6	Pentanal	2.16	AD
7	Toluene	3.78	*
8	C8H8 alkane	6.23	C
9	Phenol	8.56	P*
10	Phenol, 2-methyl-	10.33	P*
11	Phenol, 4-methyl-	10.83	P*
12	Mequinol	11.10	P*
13	Phenol, 2,4-dimethyl-	12.59	P*
14	Phenol, 2,5-dimethyl-	12.64	P*
15	Phenol, 4-ethyl-	13.07	P*
16	Phenol, 3-ethyl-	13.12	P*
17	2-Dodecene, (Z)-	13.48	O
18	Phenol, 2-methoxy-4-methyl-	13.61	P*
19	2-Methoxy-4-vinylphenol	16.42	P*
20	Phenol, 2,6-dimethoxy-	17.26	P*
21	3-Phenyl-2-propyn-1-ol	17.43	A*
22	2-Methyl-5-hydroxybenzofuran	18.56	F
23	Benzenamine, 2-methoxy-4-nitro-	19.28	*
24	Unknown anhydrosugar	20.11	S
25	Ethyl α-D-glucopyranoside	20.24	S
26	1,6-Anhydro-β-D-glucopyranose	20.35	S
27	Unknown	21.67	–
28	Unknown	24.38	–
29	n-Hexadecanoic acid	28.57	AC
30	Oxacycloheptadecan-2-one	31.29	K
31	Retinol	31.39	A
32	Octadecanoic acid	31.64	AC
33	Cyclohexane, decyl-	34.14	C
34	Phenol, 3-pentadecyl-	36.22	P*
35	Phenol, 4,4'-methylenebis[2,6-dimethoxy-	39.21	P*
36	Carbamic acid, methyl-, 3-methylphenyl ester	41.16	E*
37	1,7,7-trimethylbicyclo[2.2.1]heptan-2-one	41.22	K
38	1-(3,5-Dimethyl-2-pyrazinyl)-3-methyl-1-butanone	41.29	K
39	Phenol, 3-methyl-	41.73	P*
40	dl-Erythro-1-phenyl-1,2-propanediol	41.88	A*
41	2-[5-(2-Hydroxy-propyl)-furan-2-yl]-propan-1-ol	42.18	F
42	2-Amino-4,4,6,6-tetramethyl-4,6-dihydro-thieno[2,3-c]furan-3-carbonitrile	43.71	F
43	Unknown	43.98	–
44	Bicyclo[6.1.0]nonane-9-carboxylic acid	44.15	C
45	Carbamic acid, 3-methylphenyl-, butyl ester	44.76	E*
46	3-Cyclopentene-1-acetaldehyde, 2,2,3-trimethyl-	44.95	AD
47	1,2-Ethanediol, 1,2-diphenyl-	45.09	A*
48	1H-Indole, 1-methyl-2-phenyl-	45.27	PAC
49	3,5,9-Trimethyl-deca-2,4,8-trien-1-ol	45.44	A
50	2-Ethylacridine	46.71	PAC
51	Benzo[h]quinoline, 2,4-dimethyl-	47.06	PAC
52	Carbamic acid, 3-methylphenyl-, isobutyl ester	47.85	E*
53	Unknown	48.56	–
54	Propanal, 2-(4-ethoxyphenyl)-2-methyl-	48.71	AD*

Groups: Alcohol (A); Furans (F); Ketone (K); Phenol (P), Aromatic (*); Amine (AM); Sugar (S); Acid (AC); Polycyclic aromatic compound (PAC); Aldehyde (AD); Cyclic (C); Ester (E); Others (O).

adstringens bark (25 – 1600 μg/mL) diluted in DMEM medium with 0.2 % ethanol were added to HaCaT cells and incubated for 24 h. The cells were washed with phosphate-buffered saline (PBS) and a solution of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; R&D Systems, Minneapolis, MN, USA) (1 mg/mL in PBS) was added to each well with fresh medium and incubated for 4 h at 37 °C. Formazan crystals were dissolved with 0.2 mL of acidic isopropanol and absorbances were read at 570 nm with an Accuris SmartReader 96. The cytotoxicity IC_{90} values on HaCaT cells were estimated from the dose-response curve, using a non-linear regression algorithm (GraphPad Software, Inc., San Diego, CA, USA) considering triplicate independent experiments and the mean of three wells for each oil concentration tested. Results were presented as a Mean ± SD.

2.4.3. Effects of pyrolytic oils on IL-17-induced IL-8 production of HaCaT cell

IL-8 amounts in Il-17 HaCaT supernatants were measured using Human IL-8/CXCL8 DuoSet ELISA kit (R&D System, Minneapolis, MN, USA) according to the manufacturer's protocol. Approximately, 8.0×10^4 HaCaT cells were seeded into 24-well plates until 80 % of confluence and subsequently synchronized by serum deprivation during 12 h. After this time, the cells were pre-treated for 10 h with dexamethasone (Dex, 5 μM, Sigma Aldrich, St. Louis, MO, USA) as an anti-inflammatory positive control or oil samples from *A. adstringens* bark at concentrations lower than IC_{90} values (17.5, 35, 70 μg/mL oil-1; 15, 30, 60 μg/mL oil-2). Later, IL-17 (50 ng/mL, Thermo Fisher Scientific, Waltham, MA, USA) was added to cell cultures for 14 h to induce IL-8 production. IL-8 amount is calculated by interpolation using a four-parameter logistic (4-PL) curve-fit as specified by the manufacturer. The detection limit for IL-8 production was 30 pg/mL.

3. Results and discussion

3.1. Recovered pyrolytic fractions

Table 1 shows the yields of the pyrolytic fractions of extractive free biomass (63.8 ± 3.6 wt.%). The increasing order of recovery was oil-1, oil-2 and char. The pyrolytic gases formed, and non-collected aerosols accounted for close to 36.2 wt.%. The recovery of char was higher than that reported for eucalyptus bark [47], but lower yield of the pyrolytic fractions (oil-1 + oil-2) was obtained likely due to the lack of filters and electrostatic precipitators to collect the aerosols [47]. Still, high yields of charcoal and comparable low yield of oil are reported in slow pyrolysis systems processing feedstock with high lignin content (such as bark) [48].

3.2. Chemical characterization of the pyrolytic fractions and the residual biomass

3.2.1. Proximate and elemental analyses

The moisture content in the residual biomass less than 10 % makes this suitable for a thermochemical conversion process [49]. Its volatile content is comparable with values reported in the literature for other lignocellulosic materials [34]. Regarding the ash content of the residual biomass, the obtained value to that reported for eucalyptus bark [47], and higher to those determined for pine and oak barks (2.9 and 5.3 wt. %, respectively) [50]. Contents of O, H, and S of *A. adstringens* residual biomass were similar to those reported for pine and oak bark [50]. However, the C content was lower than those reported for eucalyptus, pine and oak barks (52.5, 54.9 and 52.6 wt.%, respectively) [47,50], while the N content was greater than those of the aforementioned barks [47,50].

Table 2 shows the proximate and elemental analyses of *A. adstringens* pyrolytic products. The oil-1 and oil-2 have 16.1 and 26.9 wt.% of water, respectively. This water is mostly formed from the dehydration of carbohydrate fractions [35,51]. In both cases, the content of volatiles was greater than 90 wt.%. The fixed carbon content was in both cases close to 5 wt.% [38]. Carbon content was higher in pyrolytic fractions than to residual biomass. C and N contents were higher in oil-2 than those obtained for oil-1, while oxygen content was greater for the oil-1. The O content is related to the presence of water and oxygenated compounds in the oil such as hydroxyaldehydes, hydroxyketones and carboxylic acids [35,41,51].

The small moisture content observed in the char is likely to be re-adsorbed water from the environment. Its volatile content (26.1 wt.%), fixed carbon (56.6 wt.%) and ash (17.3 wt.%) are comparable to values reported for other chars [38,49,52]. The C content in the char was

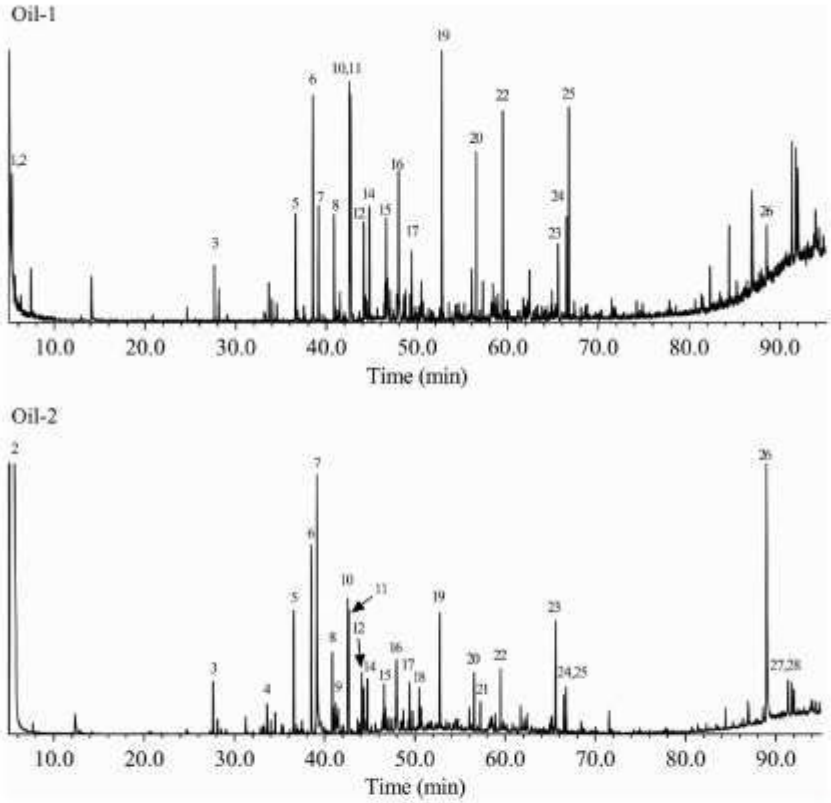

Fig. 6. GC–MS analysis chromatograms of pyrolytic oils from *A. adstringens* (list of the numbered compounds is shown in Table 4).

lower than those reported for chars derived from waste woody fractions [38].

The thermogravimetric analyses of residual biomass and pyrolytic fractions from *A. adstringens* are presented in Fig. 3. TGA curve represents the weight loss depending on the volatility and thermal stability of the chemical compounds present in the sample [37]. As expected, the mass loss is greater in the oils than in the char as the evaporation and thermal degradation of their components occurs faster at lower temperatures, which has a direct relationship with the content of the volatile compounds previously discussed.

Unlike the char and the residual biomass, the oils present a large peak in the range of 200–300 °C, a region detectable for the GC–MS analysis [53]. In this region, volatile compounds such as monophenols, monosugars, and furans can be identified [37,53]. Around 400 °C, the DTG analysis shows the presence of another large peak for oils and residual biomass. The compounds found in this region have a greater mass, lower volatility and may correspond to lignin oligomeric fractions that cannot be analyzed by conventional GC–MS [37,53]. For the analysis of these heavy compounds other techniques can be used, such as Py-GC–MS, LC–MS, among others.

3.2.2. FTIR

The FTIR analysis provides information on certain functional groups of chemical families and complements the data of other analytical techniques [35,37]. Fig. 4 shows the infrared spectrum of oils, char and residual biomass from *A. adstringens*. Stretching vibrations of the O−H bond ($3600-3100\ cm^{-1}$) are typically assigned to phenols, alcohols, water, and carboxylic acids [37]. Based on our Py-GC/MS, GC/MS and UV-fluorescence we can confirm that these oils are rich in phenols. The vibrations between 3600 and 3100 are likely to be associated with phenols. Stretching signals for the C–H bond ($3000-2800\ cm^{-1}$) could correspond to several saturated and unsaturated aliphatic and aromatic compounds. These records were more intense for oils and were similar to those reported for other pyrolytic oils [37]. The carbonyl stretching signal (C=O) between 1780-1680 cm^{-1}, characteristic for aldehydes, ketones, carboxylic acids, and esters was not visible in the char spectrum. The stretch signal for C=C at 1680-1530 cm^{-1} indicates the presence of conjugated aromatic systems. The most intense stretching region for the oil spectra between $1520-1250\ cm^{-1}$ associated with C−O bond could indicate the presence of terpenes and acetates, whereas the associated signal at $1050\ cm^{-1}$ could indicate the presence of other oxygenated compounds such as anhydrides [37]. Vibrations between $900-800\ cm^{-1}$ can be considered complementary to some stretches such as C−O and C−N.

The functional groups estimated in the FTIR spectrum of the residual biomass coincide with those reported for other barks and indicate the presence of hemicellulose, cellulose and lignin components [54], while the thermal conversion of these macromolecules can be seen at higher wavelengths [37]. Oils showed a strong signal of C−O and C−H bonds, these may correspond to the abundance of aromatic and cyclical compounds such as alkylphenols or terpenes recognized by their antioxidant, antiproliferative and anti-inflammatory properties [32,33,55]. As observed, FTIR analysis for the char shows a lower diversity of functional groups than the oils [34,52].

3.2.3. Py-GC–MS

The Py-GC–MS analysis of residual biomass is useful to identify compounds that cannot be identified by GC–MS in pyrolytic oils. Fig. 5

Table 4
GC–MS characterization of the main compounds found in the pyrolytic oils from *A. adstringens*.

No.	Identified compounds	RT (min)	Oil-1	Oil-2	Groups
1	Methanol	5.0	✓		A
2	Ethanol	5.8	✓	✓	A
3	2-Furanmethanol	27.6	✓	✓	F
4	2-Cyclopenten-1-one, 3-methyl-	33.6		✓	K
5	1,2-Cyclopentanedione, 3-methyl-	36.6	✓	✓	K
6	Phenol	38.5	✓	✓	P*
7	Phenol, 2-methoxy-	39.2	✓	✓	P*
8	Phenol, 2-methyl-	40.2	✓	✓	P*
9	Maltol	40.8		✓	K
10	Phenol, 4-methyl-	42.6	✓	✓	P*
11	Phenol, 3-methyl-	42.7	✓	✓	P*
12	2-Methoxy-5-methylphenol	44.1	✓	✓	P*
13	2-Heptanamine, 5-methyl-	44.3	✓	✓	AM
14	Phenol, 2,3-dimethyl-	44.8	✓	✓	P*
15	Phenol, 2-ethyl-	46.6	✓	✓	P*
16	Phenol, 4-ethyl-2-methoxy-	48.0	✓	✓	P*
17	1,4:3,6-Dianhydro-α-D-glucopyranose	49.4	✓	✓	S
18	2-Methoxy-4-vinylphenol	50.5		✓	P*
19	Phenol, 2,6-dimethoxy-	52.8	✓	✓	P*
20	1,2,4-Trimethoxybenzene	56.9	✓	✓	*
21	Hydroquinone	57.3		✓	P*
22	5-tert-Butylpyrogallol	59.5	✓	✓	P*
23	Unknown anhydrosugar	65.6	✓	✓	S
24	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	66.5	✓	✓	P*
25	7H-Furo[3,2-g][1]benzopyran-7-one	66.7	✓	✓	F
26	1,2-Benzenedicarboxylic acid, diisooctyl ester	89.0	✓	✓	E*
27	Docosanoic acid	91.4		✓	AC
28	12,13-Dihydro-12-methyl-13,14-dioxo-14H-naphtho(1',2':5,6)pyrano(3,2-c) quinoline	91.8		✓	PAC

Groups: Alcohol (A); Furans (F); Ketone (K); Phenol (P), Aromatic (*); Amine (AM); Sugar (S); Ester (E); Acid (AC); Polycyclic aromatic compound (PAC).

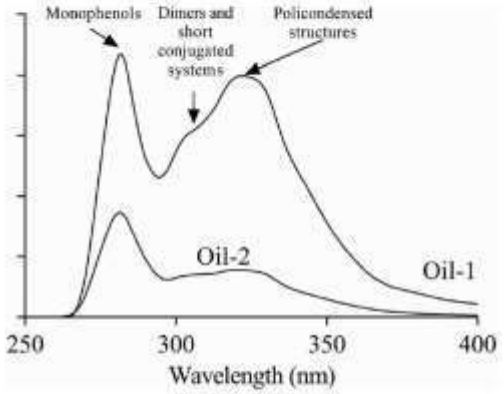

Fig. 7. UV-fluorescence spectra of the oil samples from *A. adstringens*.

shows the chromatogram from Py-GC–MS and the list of major identified peaks is given in Table 3. Of the total identified compounds belonging to various chemical families, twenty-six were aromatic compounds. Of these approximately 53 % corresponds to phenols which can be associated with lignin degradation. Indeed, their presence was greater than that of the sugars (6 %) derived from the pyrolysis of cellulose-hemicellulose. This result coincides with what was observed in the GC–MS analysis for bio-oils.

The identification of alkylphenols such as phenol, 3-pentadecyl- has been described in species of the Anacardiaceae family [31,55,56]. These compounds are related to the presence of cardanols (cardol and cardanol), which have shown cytotoxic activity in cancer cell lines [56,57]. Octadecanoic acid (stearic acid) and hexadecanoic acid (palmitic acid) identified in this analysis were also reported in an ethanolic extract from *A. adstringens* bark and have been related to its anti-inflammatory activity [31].

3.2.4. GC–MS analysis

The pyrolytic oils from *A. adstringens* were analyzed by GC-MS. The chromatograms corresponding to each fraction are shown in Fig. 6, these chromatograms are very useful in the identification of compounds associated with the DTG curves and the chemical groups described in the FTIR and UV-fluorescence analyzes [37,53,58]. As shown in Table 4, there is a great similarity between the compounds present in oil-1 and oil-2, although the areas of the peaks differ when both oils are compared. The number of identified GC-MS peaks was higher in oil-2 regarding oil-1. Additionally, the relative proportion of compounds changes between oils. For example, phenol, 2-methoxy- (guaiacol) a product of lignin [35] is present in greater proportion in oil-2 regarding oil-1, while the opposite occurs with phenol, 2,6-dimethoxy- (syringol) which is present in a higher concentration in oil-1. Cresols such as phenol, 2-methyl- (o-cresol), phenol, 3-methyl- (m-cresol) and phenol, 4-methyl- (p-cresol) are among the phenols identified in oils. These compounds have antiseptic activity but have also shown moderate toxicity [59] and can be found in the coal tar [26]. Hydroquinone present in oil-2 has been used in the treatment of various hyperpigmentation skin disorders [60].

In both oils, phenols (51–54 %) represent the dominant group of molecules, as compared with other groups such as sugars (7–9 %) or ketones (4–11 %). It could be explained by the chemical composition of structural components of barks [35].

3.2.5. UV-fluorescence

UV-fluorescence has been used for the chemical characterization of pyrolytic oils and herbal drugs in order to identify compounds capable of emitting fluorescence [58]. At wavelengths that range from 260 to 290 nm, molecules could be compounds with carbonyl groups and one-ring aromatic compounds, mainly phenolics [61]. The wavelengths of fluorescence emission increase with the increase of the number of aromatic rings, being able to observe short conjugated systems first and then polycondensed structures in the range of 290–360 nm [58,61].

As demonstrated by the GC–MS analysis, phenols are the dominant molecules identified in pyrolytic oils. In nature, these compounds can be present in plants as simple phenols, short conjugated ring systems with different dihedral angles in their aromatic structures and consequently, different fluorescence spectra [62]. As shown in Fig. 7, the peak intensity at 281 nm was greater in oil-1 than in oil-2. This indicates that oil-1 is very rich in monophenols of small structure, which is in accord with results of proximate analysis here presented which demonstrated a higher content of volatile compounds in this sample. With an excitation wavelength of ≈305 nm, dimers and short conjugated systems such as polyphenols and large conjugated ring systems can be observed [58,61]. The presence of these compounds was also lower in oil-2. Around 321 nm, a greater presence of large conjugated systems was observed in oil-1 compared to the oil-2 [58]. Further studies using NRM are needed to determine the nature of the multi-aromatic systems present in pyrolytic oils from *A. adstringens* bark.

3.2.6. HPLC-DAD analysis

In order to obtain a comprehensive characterization of the non-volatile and thermal unstable analytes present in pyrolytic oils, an HPLC-DAD analysis was performed considering molecules previously described in Anacardiaceae family [63]. HPLC chromatograms of oils are shown in Fig. 8. Nine phenolic compounds were identified by comparing the retention times and UV spectra with those of the standards (Table 5). Pyrolytic oils were very abundant in epigallocatechin, catechin, and quercetin. A previous study had also identified the presence of catechin and naringin in an ethanolic extract of *A. adstringens* bark [31]. Most of the molecules identified in *A. adstringens* pyrolytic

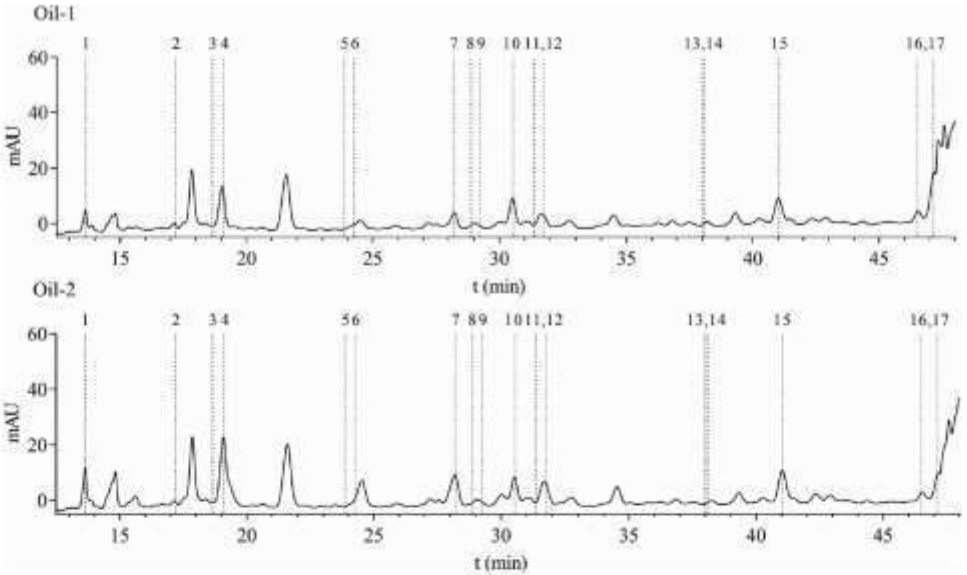

Fig. 8. HPLC-DAD profiles of liquid pyrolysis samples from *A. adstringens*. Dotted lines represent the standards used and the list of numbered compounds is shown in Table 5.

Table 5
HPLC-DAD quali-quantitative analysis of phenolic compounds in *A. adstringens* oils.

No.	Phenolic compound	RT (min)	Oil-1 (mg/L)	Oil-2 (mg/L)
1	Gallic acid	13.81	0.005	0.013
2	Epigallocatechin	17.29	0.191	0.150
3	Chlorogenic acid	18.65	ND	ND
4	Catechin hydrate	19.13	0.126	0.249
5	Caffeic acid	23.86	ND	ND
6	Syringic acid	24.25	ND	ND
7	Ellagic acid	28.58	0.012	0.035
8	*p*-Coumaric acid	28.88	ND	ND
9	(-)-Epicatechin gallate	29.25	ND	ND
10	Scopoletin	30.83	0.048	0.045
11	Ferulic acid	31.37	ND	ND
12	Naringin	31.89	0.011	0.033
13	Salicylic acid	37.99	0.004	0.001
14	Resveratrol	38.09	ND	ND
15	Quercetin	41.47	0.061	0.077
16	Naringenin	46.32	ND	ND
17	Kaempferol	47.14	0.013	0.003

ND = not detected.

oils have demonstrated positive effects on skin diseases such as melanoma and psoriasis. The catechins (epigallocatechin, catechin or epigallocatechin-3-gallate) have shown anti-melanogenic activity on B16 murine melanoma cells stimulated with α-melanocyte-stimulating hormone in a dose-dependent manner (5 – 20 μM) [64]. In HaCaT cells, quercetin showed antiproliferative activity (62.42 ± 10.2 μg/mL), whereas in a murine psoriatic model quercetin inhibited leukocyte migration and exhibited an anti-inflammatory effect (50 mg/kg) [65]. Quercetin decreases reactive oxygen species (ROS) generation and inflammation caused by UV-B radiation in human keratinocytes by suppressing NF-κB pathway [66] and the expression of proinflammatory cytokines such as TNF-α, IL-1β, IL-6 and IL-8 [66]. Because the HPLC-DAD is a quali-quantitative technique, NMR (^{1}H and ^{13}C) and LC–MS-MS analysis are necessary to confirm the presence of these compounds in pyrolytic oils from *A. adstringens* bark.

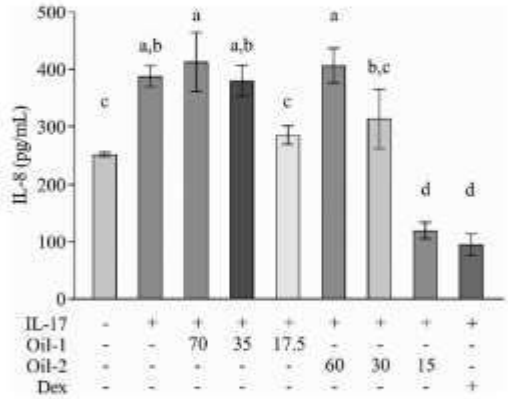

Fig. 9. Effect of pyrolytic oils on the IL-17-induced production of IL-8 on HaCaT keratinocytes. The confluent HaCaT cells were pretreated or not with pyrolytic fractions (17.5, 35, 70 μg/mL oil-1; 15, 30, 60 μg/mL oil-2) or Dex (5 μM) for 10 h, then cells were stimulated with IL-17 (50 ng/mL) for 14 h. IL-8 production in HaCaT supernatants was measured using ELISA kit. Results are expressed in pg/mL and correspond to 10^{6} cells. The histogram represents the mean ± standard deviation. Statistical: one-way ANOVA followed by Tukey's post hoc test. Means without a common letter differ ($p < 0.05$).

3.3. Toxicity of oils on HaCaT cells and effects on IL-17-induced IL-8 production

Cell viability was determined by treating three wells of cells with different concentrations of oil samples (25 – 1600 μg/mL) during 24 h of culture [67]. IC_{50} values were (60 ± 4 μg/mL) and (78 ± 3 μg/mL) for oil-2 and oil-1 respectively. As observed, oil-2 was more toxic on HaCaT cells than oil-1, likely due to a significantly higher proportion of compounds such as hydroquinone and guaiacol. In HaCaT keratinocytes, quinones have been demonstrated to induce reactive oxygen species formation and depletion of cellular glutathione, leading to the accumulation of DNA strand breaks [68]. Repeated exposure to

guaiacol produces irritation, suggesting that keratinocytes could be toxicological targets for these compounds [69].

Fig. 9 shows that the basal production of IL-8 in HaCaT cells was 252 ± 4 pg/mL whereas IL-17 stimulated 1.5 times the production of IL-8 after 14 h of treatment ($p < 0.05$). Interestingly, at higher and intermediate concentrations tested for both pyrolytic oils, IL-8 production did not significantly change regarding the control, but it significantly decreased at lower concentrations of both oils. This opposite effect could be explained considering that at the higher concentrations close to the minimum toxicity value (IC_{90}), pyrolytic oils will not be able to promote an anti-inflammatory anti-IL17 response since other cellular defense mechanisms are taking place. Interestingly, oil-2 at a 15 μg/mL had an inhibitory effect on IL-8 production similar to dexamethasone, a drug used for psoriasis treatment [70], suggesting that pyrolytic oils have a potential to be explored for treating this disease.

The antipsoriatic effect of pyrolytic fractions for psoriasis is complex and mostly unknown [26]. It has been proposed that most bioactive compounds from tar are oxygenated polycyclic aromatic hydrocarbons [26,71] and that its antipsoriatic action is mediated by the activation of aryl hydrocarbon receptor resulting in induction of the epidermal differentiation [26,72]. Carbazole has been hypothesized to be the responsible for antipsoriatic effect of tar as it can reduce the IL-17 levels by inhibiting the signal transducer and activator of transcription 3 (STAT3) [27] but further research is necessary to elucidate other possible mechanisms mediated by relevant cytokines present in psoriasis by which pyrolytic fractions can exhibit anti-inflammatory effects.

As described in the HPLC analysis section, some of the phenolic compounds identified in this study such as epigallocatechin, catechin, and quercetin have anti-inflammatory properties [65]. Moreover, other compounds here identified such as docosahexaenoic acid can inhibit the secretion of the IL-8 in TNF-stimulated HaCaT cells [73]. Future studies are necessary to elucidate the mechanism by which pyrolytic oils inhibit the IL-8 production by blocking IL-17 pathways and the molecules responsible for these effects.

Though the results here presented demonstrated the anti-IL17 activity of the pyrolytic oils *in vitro*, these results cannot be extrapolated to their use in humans. One aspect that should be evaluated on animal models in the future is the toxicity, mainly considering the presence of hydroquinone and guaiacol in pyrolytic oils with a recognized toxic potential on the skin. Therefore, further investigations are needed to determine the acute, subchronic and chronic toxicity of these pyrolytic oils on the skin in order to prevent side effects in humans. Additionally, the stability of pyrolytic oils overtime should be verified to ensure the reproducibility of the obtained results.

4. Conclusions

The present work allowed for the first time to perform the physicochemical characterization of pyrolytic fractions from extracted *A. adstringens* bark. This is a plant used in Mexican traditional medicine to treat dermatological affections. The obtained results from a tandem of analytical techniques were combined in order to give an overview of the molecules present in pyrolytic oils and residual biomass. Pyrolytic oils showed significant inhibitory effects on IL-8 production, suggesting that they have the potential to be explored for treating IL-17 driven dermatological diseases such as psoriasis. It is worth mentioning that oil-2 at 15 μg/mL had a similar inhibitory effect on IL-8 as dexamethasone, a drug used for managing this disease. Results of this work, also demonstrated that the residual biomass from *A. adstringens* bark obtained after conventional extraction, commonly considered as a waste, is a valuable source of bioactive molecules. Pyrolysis of biomass after extraction can thus be an interesting alternative that allows the rational use of this waste for developing new therapeutic candidates with high added value. Although these findings are encouraging, further research is still necessary before establishing the therapeutic use of pyrolytic oils from *A. adstringens* bark for psoriasis. Notably, it would be important to identify and quantify the molecules responsible for the anti-inflammatory activity and to study their impact on IL-17 related pathways.

When compared with other groups of compounds such as sugars or heterocyclic compounds, phenols represented the dominant group of molecules in pyrolytic oils from *A. adstringens* bark. Although polyphenols present in solvent extracts are recognized by their pharmacological properties, little is known about the action of pyrolytic phenols as therapeutic candidates. Therefore, this work opens the door for a more exhaustive study of their mechanism of action.

CRediT authorship contribution statement

Roberto Esquivel-García: Investigation, Formal analysis, Writing - original draft. **Michael Ayiania:** Investigation, Formal analysis. **Nehal Abu-Lail:** Investigation, Visualization, Writing - review & editing. **Joel E. López-Meza:** Formal analysis, Visualization, Writing - review & editing. **Rosa E. del Río:** Investigation, Formal analysis, Visualization. **Manuel García-Pérez:** Investigation, Formal analysis, Resources, Writing - review & editing. **Alejandra Ochoa-Zarzosa:** Investigation, Formal analysis, Writing - review & editing. **Martha-Estrella García-Pérez:** Conceptualization, Methodology, Formal analysis, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We thank the "Consejo Nacional de Ciencia y Tecnología" (CONACYT, Mexico) for the financial support of Roberto Esquivel-García (Doctoral Fellowship No.285764).

References

[1] W.-H. Boehncke, M.P. Schön, Psoriasis, Lancet 386 (2015) 983–994, https://doi.org/10.1016/S0140-6736(14)61909-7.
[2] I.M. Michalek, B. Loring, S.M. John, A systematic review of worldwide epidemiology of psoriasis, J. Eur. Acad. Dermatol. Venereol. 31 (2017) 205–212, https://doi.org/10.1111/jdv.13854.
[3] International Psoriasis Council, IPC Psoriasis Review, IPC, Dallas, 2009, pp. 1–12 (accessed 18 June 2019), https://www.psoriasiscouncil.org/docs/ipcpsoriasisreview_dec_2009_english.pdf.
[4] C.G. Helmick, H. Lee-Han, S.C. Hirsch, T.L. Baird, C.L. Bartlett, Prevalence of psoriasis among adults in the U.S.: 2003-2006 and 2009-2010 National Health and Nutrition Examination Surveys, Am. J. Prev. Med. 47 (2014) 37–45, https://doi.org/10.1016/j.amepre.2014.02.012.
[5] S.K. Kurd, A.B. Troxel, P. Crits-Christoph, J.M. Gelfand, The risk of depression, anxiety, and suicidality in patients with psoriasis: a population-based cohort study, Arch. Dermatol. 146 (2010) 891–895, https://doi.org/10.1001/archdermatol.2010.186.
[6] H. Zhang, W. Hou, L. Henrot, S. Schnebert, M. Dumas, C. Heusèle, J. Yang, Modelling epidermis homoeostasis and psoriasis pathogenesis, J. R. Soc. Interface 12 (2015) 20141071, , https://doi.org/10.1098/rsif.2014.1071.
[7] E. Ogawa, Y. Sato, A. Minagawa, R. Okuyama, Pathogenesis of psoriasis and development of treatment, J. Dermatol. 45 (2018) 264–272, https://doi.org/10.1111/1346-8138.14139.
[8] N.C. Brembilla, L. Senra, W.-H. Boehncke, The IL-17 family of cytokines in psoriasis: IL-17A and beyond, Front. Immunol. 9 (2018) 1682, https://doi.org/10.3389/fimmu.2018.01682.
[9] A.M. Lin, C.J. Rubin, R. Khandpur, J.Y. Wang, M. Riblett, S. Yalavarthi, E.C. Villanueva, P. Shah, M.J. Kaplan, A.T. Bruce, Mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis, J. Immunol. 187 (2011) 490–500, https://doi.org/10.4049/jimmunol.1100123.
[10] P.C.M. Res, G. Piskin, O.J. de Boer, C.M. van der Loos, P. Teeling, J.D. Bos, M.B.M. Teunissen, Overrepresentation of IL-17A and IL-22 producing CD8 T cells in lesional skin suggests their involvement in the pathogenesis of psoriasis, PLoS One 5 (2010) e14108, , https://doi.org/10.1371/journal.pone.0014108.
[11] K.E. Nograles, L.C. Zaba, E. Guttman-Yassky, J. Fuentes-Duculan, M. Suárez-Fariñas, I. Cardinale, A. Khatcherian, J. Gonzalez, K.C. Pierson, T.R. White, C. Pensabene, I. Coats, I. Novitskaya, M.A. Lowes, J.G. Krueger, Th17

cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways, Br. J. Dermatol. 159 (2008) 1092–1102, https://doi.org/10.1111/j.1365-2133.2008.08769.x.
[12] Y. Lai, D. Li, C. Li, B. Muehleisen, R.A. Radek, H.J. Park, Z. Jiang, Z. Li, H. Lei, Y. Quan, T. Zhang, Y. Wu, P. Kotol, S. Morizane, T.R. Hata, K. Iwatsuki, C. Tang, R.L. Gallo, The antimicrobial protein REG3A regulates keratinocyte proliferation and differentiation after skin injury, Immunity 37 (2012) 74–84, https://doi.org/10.1016/j.immuni.2012.04.010.
[13] M. Ozawa, T. Terui, H. Tagami, Localization of IL-8 and complement components in lesional skin of psoriasis vulgaris and pustulosis palmaris et plantaris, Dermatology (Basel) 211 (2005) 249–255, https://doi.org/10.1159/000087019.
[14] H.S. Anttila, S. Reitamo, P. Erkko, M. Ceska, B. Moser, M. Baggiolini, Interleukin-8 immunoreactivity in the skin of healthy subjects and patients with palmoplantar pustulosis and psoriasis, J. Invest. Dermatol. 98 (1992) 96–101, https://doi.org/10.1111/1523-1747.ep12495817.
[15] K. Ghoreschi, P. Thomas, S. Breit, M. Dugas, R. Mailhammer, W. van Eden, R. van der Zee, T. Biedermann, J. Prinz, M. Mack, U. Mrowietz, E. Christophers, D. Schlöndorff, G. Plewig, C.A. Sander, M. Röcken, Interleukin-4 therapy of psoriasis induces Th2 responses and improves human autoimmune disease, Nat. Med. 9 (2003) 40–46, https://doi.org/10.1038/nm804.
[16] M. Hohenberger, L.A. Cardwell, E. Oussedik, S.R. Feldman, Interleukin-17 inhibition: role in psoriasis and inflammatory bowel disease, J. Dermatol. Treat. 29 (2018) 13–18, https://doi.org/10.1080/09546634.2017.1329511.
[17] P.C.M. van de Kerkhof, C.E.M. Griffiths, K. Reich, C.L. Leonardi, A. Blauvelt, T.-F. Tsai, Y. Gong, J. Huang, C. Papavassilis, T. Fox, Secukinumab long-term safety experience: a pooled analysis of 10 phase II and III clinical studies in patients with moderate to severe plaque psoriasis, J. Am. Acad. Dermatol. 75 (2016) 83–98, https://doi.org/10.1016/j.jaad.2016.03.024 e4.
[18] S. Rusta-Sallehy, M. Gooderham, K. Papp, Brodalumab: a review of safety, Skin Therapy Lett. 23 (2018) 1–3,
[19] A.W. Armstrong, A.D. Robertson, J. Wu, C. Schupp, M.G. Lebwohl, Undertreatment, treatment trends, and treatment dissatisfaction among patients with psoriasis and psoriatic arthritis in the United States: findings from the National Psoriasis Foundation Surveys, 2003-2011, JAMA Dermatol. 149 (2013) 1180–1185, https://doi.org/10.1001/jamadermatol.2013.5264.
[20] O.D. van Cranenburgh, J. de Korte, M.A.G. Sprangers, M.A. de Rie, E.M.A. Smets, Satisfaction with treatment among patients with psoriasis: a web-based survey study, Br. J. Dermatol. 169 (2013) 398–405, https://doi.org/10.1111/bjd.12372.
[21] W. Talbott, N. Duffy, Complementary and alternative medicine for psoriasis: what the dermatologist needs to know, Am. J. Clin. Dermatol. 16 (2015) 147–165, https://doi.org/10.1007/s40257-015-0128-6.
[22] M.T. Svendsen, J. Jeyabalan, K.E. Andersen, F. Andersen, H. Johannessen, Worldwide utilization of topical remedies in treatment of psoriasis: a systematic review, J. Dermatolog. Treat. 28 (2017) 374–383, https://doi.org/10.1080/09546634.2016.1254331.
[23] W.H. Goeckerman, Treatment of psoriasis: continued observations on the use of crude coal tar and ultraviolet light, Arch. Derm. Syphilol. 24 (1931) 446–450, https://doi.org/10.1001/archderm.1931.01450010455012.
[24] M.B. Alora-Palli, A.C. Perkins, A. van Cott, A.B. Kimball, Efficacy and tolerability of a cosmetically acceptable coal tar solution in the treatment of moderate plaque psoriasis, Am. J. Clin. Dermatol. 11 (2010) 275–283, https://doi.org/10.2165/11530380-000000000-00000.
[25] M.L. Moscaliuc, M.M. Heller, E.S. Lee, J. Kro, Goeckerman therapy: a very effective, yet often forgotten treatment for severe generalized psoriasis, J Dermatol Treat. 24 (2013) 34–37, https://doi.org/10.3109/09546634.2012.658014.
[26] S. Sekhon, C. Jeon, M. Nakamura, L. Afifi, D. Yan, J.J. Wu, W. Liao, T. Bhutani, Review of the mechanism of action of coal tar in psoriasis, J. Dermatol. Treat. 29 (2018) 230–232, https://doi.org/10.1080/09546634.2017.1369494.
[27] J.L. Arbiser, B. Govindarajan, T.E. Battle, R. Lynch, D.A. Frank, M. Ushio-Fukai, B.N. Perry, D.F. Stern, G.T. Bowden, A. Liu, E. Klein, P.J. Kolodziejski, N.T. Eissa, C.F. Hossain, D.G. Nagle, Carbazole is a naturally occurring inhibitor of angiogenesis and inflammation isolated from antipsoriatic coal tar, J. Invest. Dermatol. 126 (2006) 1396–1402, https://doi.org/10.1038/sj.jid.5700276.
[28] T.M. Barnes, K.A. Greive, Topical pine tar: history, properties and use as a treatment for common skin conditions, Australas. J. Dermatol. 58 (2017) 80–85, https://doi.org/10.1111/ajd.12427.
[29] R. Mata, M. Figueroa, A. Navarrete, I. Rivero-Cruz, Chemistry and biology of selected Mexican medicinal plants, in: A.D. Kinghorn, H. Falk, S. Gibbons, J. Kobayashi, Y. Asakawa, J.-K. Liu (Eds.), Progress in the Chemistry of Organic Natural Products, Springer, Cham, 2019, pp. 1–142, , https://doi.org/10.1007/978-3-030-01099-7_1.
[30] R. Esquivel-García, E. Pérez-Calix, A. Ochoa-Zarzosa, M.-E. García-Pérez, Ethnomedicinal plants used for the treatment of dermatological affections on the Purépecha Plateau, Michoacán, Mexico, Acta Bot. Mex. 125 (2018) 95–132, https://doi.org/10.21829/abm125.2018.1339.
[31] M. Rodríguez-Canales, R. Jiménez-Rivas, M.M. Canales-Martínez, A.J. García-Lopez, N. Rivera-Yañez, O. Nieto-Yañez, Y. Ledesma-Soto, L.E. Sánchez-Torres, M. Rodríguez-Sosa, L.I. Terrazas, M.A. Rodríguez-Monroy, Protective effect of *Amphipterygium adstringens* extract on dextran sulphate sodium-induced ulcerative colitis in mice, Mediators Inflamm. 2016 (2016) 8543561, , https://doi.org/10.1155/2016/8543561.
[32] M. Makino, T. Motegi, Y. Fujimoto, Tirucallane-type triterpenes from Juliania adstringens, Phytochemistry 65 (2004) 891–896, https://doi.org/10.1016/j.phytochem.2003.12.012.
[33] I. Oviedo-Chávez, T. Ramírez-Apan, M. Soto-Hernández, M. Martínez-Vázquez, Principles of the bark of *Amphipterygium adstringens* (Julianaceae) with anti-inflammatory activity, Phytomedicine 11 (2004) 436–445, https://doi.org/10.1016/j.phymed.2003.05.003.
[34] J.A. Libra, K.S. Ro, C. Kammann, A. Funke, N.D. Berge, Y. Neubauer, M.-M. Titirici, C. Fühner, O. Bens, J. Kern, K.-H. Emmerich, Hydrothermal carbonization of biomass residuals: a comparative review of the chemistry, processes and applications of wet and dry pyrolysis, Biofuels 2 (2011) 71–106, https://doi.org/10.4155/bfs.10.81.
[35] D. Mohan, U. Pittman Charles, P.H. Steele, Pyrolysis of wood/biomass for bio-oil: a critical review, Energy Fuels 20 (2006) 848–889, https://doi.org/10.1021/ef0502397.
[36] M.-E. García-Pérez, A. Alfonso-Castillo, O. Fong Lores, A. Batista-Duharte, Z. Lemus-Rodríguez, Toxicological evaluation of an aqueous suspension from leaves and stems of *Petiveria alliacea* L. (Phytolaccaceae), J. Ethnopharmacol. 211 (2018) 29–37, https://doi.org/10.1016/j.jep.2017.09.022.
[37] F. Stankovikj, M. García-Pérez, TG-FTIR method for the characterization of bio-oils in chemical families, Energy Fuels 31 (2017) 1689–1701, https://doi.org/10.1021/acs.energyfuels.6b03132.
[38] M. Ayianta, E. Terrell, A. Dunnmoor, F.M. Carbajal-Gamarra, M. García-Pérez, Characterization of solid and vapor products from thermochemical conversion of municipal solid waste woody fractions, Waste Manag. 84 (2019) 277–285, https://doi.org/10.1016/j.wasman.2018.11.042.
[39] G. Lv, S. Wu, Analytical pyrolysis studies of corn stalk and its three main components by TG-MS and Py-GC/MS, J. Anal. Appl. Pyrol. 97 (2012) 11–18, https://doi.org/10.1016/j.jaap.2012.04.010.
[40] A. Aho, N. Kumar, K. Eränen, T. Salmi, M. Hupa, D.Y. Murzin, Catalytic pyrolysis of woody biomass in a fluidized bed reactor: influence of the zeolite structure, Fuel 87 (2008) 2493–2501, https://doi.org/10.1016/j.fuel.2008.02.015.
[41] Y. Han, F. Stanković, M. García-Pérez, Co-hydrotreatment of tire pyrolysis oil and vegetable oil for the production of transportation fuels, Fuel Process Technol. 159 (2017) 328–339, https://doi.org/10.1016/j.fuproc.2017.01.048.
[42] J. Berry, J.W. Henderson, UHPLC Of Polyphenols in Red Wine, Agilent Technologies, Inc, 2010 (accessed 17 June 2019), https://www.agilent.com/cs/library/applications/5990-6659EN.pdf.
[43] A. Pol, M. Bergers, J. Schalkwijk, Comparison of antiproliferative effects of experimental and established antipsoriatic drugs on human keratinocytes, using a simple 96-well-plate assay, In Vitro Cell. Dev. Biol. Anim. 39 (2003) 36–42.
[44] M.-E. García-Pérez, M. Royer, A. Duque-Fernandez, P.N. Diouf, T. Stevanovic, R. Pouliot, Antioxidant, toxicological and antiproliferative properties of Canadian polyphenolic extracts on normal and psoriatic keratinocytes, J. Ethnopharmacol. 132 (2010) 251–258, https://doi.org/10.1016/j.jep.2010.08.030.
[45] R.G. Teepe, E.J. Koebrugge, C.W. Lowik, P.L. Petit, R.W. Bosboom, I.M. Twiss, H. Bouma, B.J. Vermeer, M. Ponec, Cytotoxic effects of topical antimicrobial and antiseptic agents on human keratinocytes in vitro, J. Trauma 35 (1993) 8–19.
[46] J. van Meerloo, G.J.L. Kaspers, J. Cloos, Cell sensitivity assays: the MTT assay, in: I. Cree (Ed.), Cancer Cell Culture. Methods in Molecular Biology (Methods and Protocols), 2nd ed., Humana Press, New York, 2011, pp. 237–245, , https://doi.org/10.1007/978-1-61779-080-5_20.
[47] D. Mourant, C. Lievens, R. Gunawan, Y. Wang, X. Hu, L. Wu, S.S.A. Syed-Hassan, C.-Z. Li, Effects of temperature on the yields and properties of bio-oil from the fast pyrolysis of mallee bark, Fuel 108 (2013) 400–408, https://doi.org/10.1016/j.fuel.2012.12.018.
[48] J.A. García-Nunez, M.R. Pelaez-Samaniego, M.-E. García-Perez, I. Fonts, J. Abrego, R.J.M. Westerhof, M. García-Perez, Historical developments of pyrolysis reactors: a review, Energy Fuels 31 (2017) 5751–5775, https://doi.org/10.1021/acs.energyfuels.7b00641.
[49] C.L. Williams, T.L. Westover, R.M. Emerson, J.S. Tumuluru, C. Li, Sources of biomass feedstock variability and the potential impact on biofuels production, Bioenergy Res. 9 (2016) 1–14, https://doi.org/10.1007/s12155-015-9694-y.
[50] K.W. Ragland, D.J. Aerts, A.J. Baker, Properties of wood for combustion analysis, Bioresour. Technol. 37 (1991) 161–168, https://doi.org/10.1016/0960-8524(91)90205-X.
[51] S. Kersten, M. García-Pérez, Recent developments in fast pyrolysis of ligno-cellulosic materials, Curr. Opin. Biotechnol. 24 (2013) 414–420, https://doi.org/10.1016/j.copbio.2013.04.003.
[52] S.V. Vassilev, C.G. Vassileva, Y.-C. Song, W.-Y. Li, J. Feng, Ash contents and ash-forming elements of biomass and their significance for solid biofuel combustion, Fuel 208 (2017) 377–409, https://doi.org/10.1016/j.fuel.2017.07.036.
[53] M. García-Pérez, A. Chaala, H. Pakdel, D. Kretschmer, C. Roy, Characterization of bio-oils in chemical families, Biomass Bioenergy 31 (2007) 222–242, https://doi.org/10.1016/j.biombioe.2006.02.006.
[54] A. Ahmed, S. Hidayat, M.S. Abu Bakar, A.K. Azad, R.S. Sukri, N. Phusunti, Thermochemical characterisation of *Acacia auriculiformis* tree parts via proximate, ultimate, TGA, DTG, calorific value and FTIR spectroscopy analyses to evaluate their potential as a biofuel resource, Biofuels (2018), https://doi.org/10.1080/17597269.2018.1442663.
[55] M. Saitta, D. Giuffrida, G.L. La Torre, A.G. Potorti, G. Dugo, Characterisation of alkylphenols in pistachio (*Pistacia vera* L.) kernels, Food Chem. 117 (2009) 451–455, https://doi.org/10.1016/j.foodchem.2009.04.043.
[56] I. Kubo, T. Nitoda, F.E. Tocoli, I.R. Green, Multifunctional cytotoxic agents from Anacardium occidentale, Phytother. Res. 25 (2011) 38–45, https://doi.org/10.1002/ptr.3109.
[57] D. Teerasripreecha, P. Phuwapraisirisan, S. Puthong, K. Kimura, M. Okuyama, H. Mori, A. Kimura, C. Chanchao, *In vitro* antiproliferative/cytotoxic activity on cancer cell lines of a cardanol and a cardol enriched from Thai *Apis mellifera* propolis, BMC Complement. Altern. Med. 12 (2012) 27, https://doi.org/10.1186/1472-6882-12-27.

CAPÍTULO 4. Caracterización química, toxicología y actividad antiinflamatoria del extracto acuoso de la corteza de *Amphipterygium adstringens* y sus fracciones en queratinocitos HaCaT.

VI.7. PREFACIO

Este capítulo es el tema del artículo titulado: “Chemical characterization, toxicology and anti-inflammatory activity of the *Amphipterygium adstringens* aqueous bark extract and its solvent fractions on HaCaT keratinocytes”. El cual se enviará próximamente a una revista científica.

El presente capítulo se dirige a dar respuesta a los objetivos particulares # 2 y 3. Como se explicó en el capítulo anterior, la especie nativa de México *Amphipterygium adstringens* fue escogida como un árbol prometedor. Las cortezas del árbol fueron sometidas a una extracción con agua caliente, siguiendo el uso tradicional reportado, y el extracto acuoso fue fraccionado por extracción líquido/líquido para una caracterización más exhaustiva de su composición química. El extracto y sus fracciones fueron químicamente caracterizados, se determinó su toxicidad en queratinocitos HaCaT y se evaluó su capacidad para inhibir la producción de IL-8 en un modelo de inducción por IL-17. Los resultados mostraron un alto rendimiento de extracción, potente efecto anti-IL17 y una baja toxicidad del extracto crudo, por lo que fue considerado el candidato más prometedor.

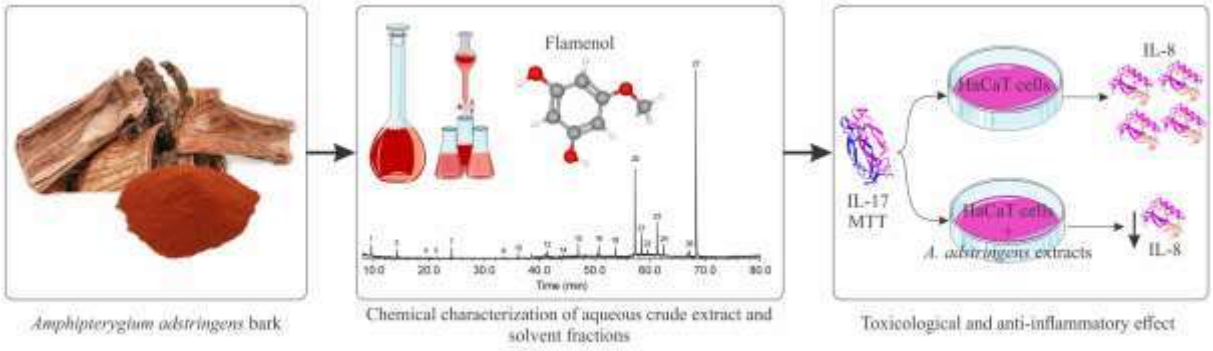

Fig. 17. Graphical abstract.

VI.8. ARTÍCULO

Chemical characterization, toxicology and anti-inflammatory activity of the *Amphipterygium adstringens* aqueous bark extract and its solvent fractions on HaCaT keratinocytes

Roberto Esquivel-García [1,2], José Salomón Martínez-Fernández [3], Nehal Abu-Lail [4], Manuel García-Pérez [3], Alejandra Ochoa-Zarzosa [2], Martha-Estrella García-Pérez [1]

Affilations:

[1] Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo. Morelia, Michoacán, Mexico. [2] Centro Multidisciplinario de Estudios en Biotecnología-FMVZ, Universidad Michoacana de San Nicolás de Hidalgo. Tarímbaro, Michoacán, Mexico. [3] Department of Biological Systems Engineering, Washington State University, Pullman, WA, USA. [4] Department of Biomedical Engineering, University of Texas at San Antonio, TX, USA.

Address for reprint requests:

Martha Estrella García-Pérez, PhD

[a]Instituto de Investigaciones Químico-Biológicas. Universidad Michoacana de San Nicolás de Hidalgo. Avenida Universidad S/N. Edificio B3 Ciudad Universitaria, Morelia, Michoacán 58030, Mexico. Phone number: (+52) 443 142 9280

E-mail: margar@live.ca

VI.8.1. Abstract

Previous ethnopharmacological research on the Purépecha Plateau, Michoacán, Mexico demonstrated that its indigenous population used topically the aqueous extract from *Amphipterygium adstringens* bark to treat dermatological disorders. However, no exhaustive investigations exist about the chemical characterization of the aqueous extract from the bark of this tree, its toxicology and anti-inflammatory properties on skin cells. In this work, this extract and its solvent fractions were chemically characterized by a tandem of analytical techniques (proximate, elemental analysis, FTIR, GC-MS and UV-fluorescence). Its toxicity and potential anti-inflammatory activity on IL-8 production of IL-17-stimulated HaCaT keratinocytes was studied by MTT and ELISA methods respectively. Acids and aromatics were the predominant compounds identified in all samples whereas the flamenol was the major molecule present in the crude extract, the ethyl acetate, and the aqueous fractions. The crude extract and the aqueous fraction were less toxic on keratinocytes and exhibited a significantly higher inhibitory activity on IL-8 production than dexamethasone ($p<0.01$), a drug used for treating inflammatory skin conditions such as psoriasis. These results support the traditional topical use of *A. adstringens* bark' decoctions by Purépechas for treating dermatological inflammatory diseases and open the door for a more exhaustive study of its anti-IL17 mechanism.

Keywords: *Amphipterygium adstringens*, Anacardiaceae, aqueous extract, IL-17, psoriasis.

VI.8.2. Introduction

Amphipterygium adstringens (Schltdl.) Standl. is an endemic tree from Mexico commonly known as “cuachalate” and belongs to the Anacardiaceae family [1]. This medicinal plant is widely prescribed by Mexican traditional healers since, in the pre-Hispanic period, its bark was used to treat gastrointestinal disorders, skin affections, and respiratory problems [2].

Our recently conducted ethnopharmacological survey at the Purépecha Plateau, Michoacán, Mexico demonstrated its ancestral population, locally recognized by its inherited knowledge on medicinal plants, has used the aqueous extract from the bark of this specie orally and topically to treat dermatological disorders [3]. This pharmacological potential warrant further chemical characterization, toxicology and anti-inflammatory evaluation on skin cells.

Although previous studies report the medicinal properties of bark extracts from this tree, most of them use organic solvent extraction methodologies with ethanol, hexane or methanol [4–9]. These extraction protocols do not reflect the traditional decoction usage form of this plant by the Mexican population [3,10]. The anti-inflammatory effect of the aqueous extract has been reported in two models of acute inflammation: the 12-O- tetradecanoylphorbol-13-acetate (TPA)-induced ear edema and the carrageenan-induced paw edema (CIPE) [11]. The CIPE represents a biphasic inflammation reaction where multiple mediators participate, whereas the TPA-induced ear edema can be used to study the skin inflammation. The aqueous extract was inactive to inhibit the cutaneous inflammation in TPA-model [11] which is contradictory with the extensive use of the decoctions from the *A. adstringens* bark by the Mexican population to treat dermatological conditions. The lack of data about the effects of the extracts from this specie on the skin prompted us to evaluate the anti-inflammatory potential of the aqueous extract on HaCaT keratinocytes.

Psoriasis is a common inflammatory skin disease that affects 2.9 % of the Mexican population [12]. In a psoriasis context, an intricate interaction occurs between hyperproliferative keratinocytes and the innate and adaptive immune cells [13]. Although the exact cause of this condition is not yet known, the significant role of pro-inflammatory cytokines such as interleukin (IL)-17 is now recognized and psoriasis is currently considered to be an IL-17 driven disease [14]. When IL-17 binds to its receptor on the keratinocyte surface (IL-17RA), it can induce an accelerated division of these cells, their aberrant differentiation [15], and the release

of other pro-inflammatory mediators such as the neutrophil-attracting chemokine IL-8 which contributes to the exacerbation of the cutaneous inflammation [15].

Biological agents that neutralize IL-17 (secukinumab and ixekizumab) or antagonize its receptor (brodalumab) have been demonstrated to be highly efficacious in clinical studies for psoriasis [16]. However, some adverse events have been described with these drugs [17,18], which has lead patients to search for complementary and alternative therapies [19]. Nowadays, the pharmaceutical industry continues the pursuit of new therapeutic antipsoriatic candidates effective and safe for patients [20].

Some phytochemical studies performed with *A. adstringens* bark have shown that the dichloromethane fraction contains gastroprotective triterpenoids such as 3α-hydroxymasticadienonic acid and β-sitosterol and 3-epi-oleanolic acid [6]. Masticadienonic, α-hydroxymasticadienonic and masticadienonic/isomasticadienonic acid mixtures were also identified in the hexanoic bark extract from these species [21]. The presence of masticadienonic acid and 3-hydroxymasticadienonic acid has also been reported in the methanolic bark extract from this tree [22]; whereas anacardic acids, recognized by their anti-inflammatory properties have been identified in the ether fraction [23].

Up to date, most of the phytochemical investigations to characterize the bioactive compounds present in *A. adstringens* bark have been made in the nonpolar extracts and little is known about the identity of the major compounds present in the aqueous extract. This constitutes an important limitation since Mexican traditional medicine mostly employs aqueous preparations from this plant [3,10].

Regarding the toxicology studies, the methanol-chloroform extract (1:1) from *A. adstringens* bark showed no toxic effect against *Artemia salina* (LC_{50}>1000 μg/mL) or against a mice model (LD_{50}>5000 mg/kg) [24]. The toxicity of the aqueous extract from this plant on the skin or cutaneous-related cells is unknown.

Taking into account the traditional dermatological use of *A. adstringens* by Purépechas and the Mexican population and the fact that little is known about the phytochemistry, toxicology and anti-inflammatory character of the aqueous extract on the skin cells, this study aimed to the chemical characterization of the *A. adstringens* aqueous bark extract and its solvent fractions and to analyze their toxicology on HaCaT keratinocytes. Moreover, their capacity to inhibit the

IL-8 production on IL-17-stimulated HaCaT keratinocytes was also studied to propose future applications for treating inflammatory skin conditions such as psoriasis.

VI.8.3. Material and methods

The experiment procedure of the present work is divided into four major steps. In the first step, *A. adstringens* bark was subject to a hot water extraction and afterward fractionated with hexane, ethyl acetate and water to obtain the phases A, B, and C respectively. In the second step, the CE and the fractions were chemically characterized by a tandem of analytical techniques to have an overview of the molecules present in each sample. As the DBP is the starting material, and its composition can affect the chemical composition of the extract and its fractions, it was also characterized in this step. The third step consisted of the analysis of the toxicology of the CE and its fractions on HaCaT keratinocytes. Finally, the anti-inflammatory character of the CE and the solvent fractions on the IL-8 production on HaCaT keratinocytes stimulated with IL-17 was determined and compared to that of dexamethasone. Each of these major parts will be more exhaustively explained in the sections that follow.

VI.8.3.1. Botanical identification and solvent extraction

Botanical identification: The bark from *A. adstringens* was collected from Michoacán state, Mexico. Bark samples were taken in a single strip approximately 50 cm long by 8 cm wide and less than 3 cm thick from 20 trees that had not been previously barked with an age between 20–25 years (20−25 cm diameter) avoiding affecting its viability. Botanical characterization was performed by Dr. Emmanuel Pérez-Calix and a sample deposited in the IEB herbarium located in Pátzcuaro, Michoacán, Mexico (voucher number: 256881) (collection date 07/01/2017). Before extraction, the bark was washed and oven-dried (40±2 °C) by 48 h, then it was grounded at a particle size of ≤ 800 μm.

Solvent extraction: Considering that the decoction was reported to be used to treat dermatological conditions by Purépechas [3], in this work the DBP (40 g) was subjected to hot water extraction (400 mL) for 1 h to simulate this traditional use. The CE was then concentrated under vacuum (60 °C, 29 kPa) and solvent fractionated. Thirty grams of CE were resuspended

in 300 mL water, decanted through a 100-mL Gooch crucible (Pyrex®, 40–60 µm, coarse porosity) and the filtrate collected in a 500 mL Erlenmeyer flask. In a separation funnel, the aqueous solution was first extracted with hexane (3 × 100 mL, phase A) and ethyl acetate (3 × 100 mL, phase B) whereas the remained aqueous solution was called phase C. The three phases were evaporated under vacuum to remove hexane (40 °C, 23 kPa), ethyl acetate (60 °C, 21 kPa) and water (60 °C, 29 kPa). The CE and fractions were kept under refrigeration (4 °C) until further analyses.

VI.8.3.2. Chemical analysis of the starting material, the CE and its fractions

Py-GC-MS of the starting material: The Py-GC-MS analysis was conducted on the DBP using an Agilent Technologies 6890N Gas Chromatograph linked to Agilent 5975B Mass Selective Detector (column: 30 m×250 µm×0.25 µm) with NIST 2.0 f Mass Spectral Search Program. The DBP sample (≈0.5 mg) was introduced in the probe interface using a CDS Analytical Pyroprobe 5000 Series and the analytical method used was described by Ayania et al. [43]. The compounds were identified by combining the data from the Mass Spectral Library and literature data, and these were classified into the different chemical groups as previously described [30].

Proximate and elemental analyses: Fixed carbon, volatiles, and the ash content of DBP, CE and phases A-C were determined with a Mettler Toledo thermogravimetric analyzer (SDTA851e) equipped with STARe data analysis software for obtaining TGA/derivative of thermogravimetric curve (DTG) data curves. The carbon, hydrogen, nitrogen and sulfur contents of the aforementioned samples were determined using a Leco TruSpec Micro elemental analyzer. The oxygen content was obtained by difference after considering the ash content on a dry basis. Both analyses were performed according to the methods previously described [30].

FTIR analysis: FTIR spectra of DBP, CE, and phases A-C were obtained using a Shimadzu IRPrestige 21 spectrometer. For the analysis of functional groups, a background correction was done on each spectrum [30].

GC–MS: The CE and phases A-C were analyzed by GC–MS using an Agilent Technologies 7890 A GC set up (Agilent 19091S-433 HP-5MS column: 325 °C: 30 m×250 µm×0.25 µm,

Agilent 5975 C MS with NIST 2.0 f Mass Spectral Search Program) using a method previously described [29].

UV-fluorescence: The analysis was based on the methodology described by García-Pérez and collaborators [29]. CE and phases A-C were diluted in HPLC grade methanol at 10 ppm and analyzed on Shimadzu RF 5301 pc spectrometer with the software Panorama Fluorescence 2.1.

VI.8.3.3. Toxicological evaluation of the CE and its fractions on HaCaT cells

CE and phases A-C were evaluated on HaCaT keratinocytes by the MTT method (R&D Systems) through the determination of the initial toxicity value (IC_{90}) [38]. The IC_{90} represents the minimal toxic dose of extract causing 10% reduction of cell viability compared with untreated cells. MTT test is a measure of toxicity on the mitochondria.

Cell culture and MTT assay: Human HaCaT keratinocyte cell line was purchased from AddexBio. HaCaT cells were cultured in DMEM high glucose with sodium pyruvate (Sigma Aldrich) and supplemented with 100 U/mL penicillin (Sigma Aldrich), 100 µg/mL streptomycin (Sigma Aldrich), and 10% fetal bovine serum (Thermo Fisher Scientific) in a humidified incubator containing 5% CO_2 at 37 ºC. Only cells at 80% confluence were used in experiments as previously described. MTT assay was performed as previously described [30]. CE and phases A-C from *A. adstringens* (25-1600 µg/mL) diluted in DMEM medium with 0.2% ethanol was added to HaCaT cells and incubated for 24 h. The cells were washed with PBS and a solution of MTT (1 mg/ml in PBS) was added to each well with fresh medium and incubated for 4 h at 37 ºC. Formazan crystals were dissolved with 0.2 mL of acidic isopropanol and absorbance was read at 570 nm with an Accuris SmartReader 96. The IC_{90} values were estimated from the dose-response curve, using a non-linear regression algorithm.

VI.8.3.4. Anti-inflammatory activity on HaCaT cells

HaCaT cells were seeded into 24-well plates until 80% of confluence and subsequently synchronized by serum deprivation during 12 h. After this time, the cells were pre-treated or not for 10 h with dex (5 µM, Sigma Aldrich), CE and phases A-C from *A. adstringens* bark at

concentrations lower than IC_{90} values. Later, IL-17 (50 ng/mL, Thermo Fisher Scientific) was added to cell cultures for 14 h to induce IL-8 production. IL-8 amounts in Il-17 HaCaT supernatants were measured using Human IL-8/CXCL8 DuoSet ELISA kit (R&D Systems) according to the manufacturer's protocol. The detection limit for IL-8 production was 30 pg/mL.

VI.8.4. Results and Discussion

This study presents novel results regarding the chemical characterization, the toxicological and anti-inflammatory analyses of the aqueous extract from the *A. adstringens* bark and its solvent fractions on HaCaT keratinocytes.

The yield of crude extract (CE) was 28.6±2.1% (w/w respect to the dry bark powder (DBP)). This recovery was higher than that previously reported (14.4%) [11]. As to the liquid-liquid fractionation, the phase C (aqueous fraction) exhibited a higher recovery (10.2±1.9%) than the phase A (hexanoic fraction, 5.3±0.1%) followed by the phase B (ethyl acetate fraction, 4.9±0.1%). In a previous study in which the methanolic extract from *A. adstringens* bark was fractionated with hexane, ethyl acetate and water, higher yields were also observed in the aqueous phase [6], probably due to the presence of polar molecules with high molecular weight such as tannins.

Considering that the *A. adstringens* bark was the starting material and that biomass composition may change according to the collection site with the consequent variation of the chemical composition of the extract and its fractions, a characterization of the DBP by pyrolysis–gas chromatography mass spectrometry (py-GC/MS) was firstly performed. **Fig. 18** shows the pyrogram whereas the list of major identified peaks is presented in **Table 9**.

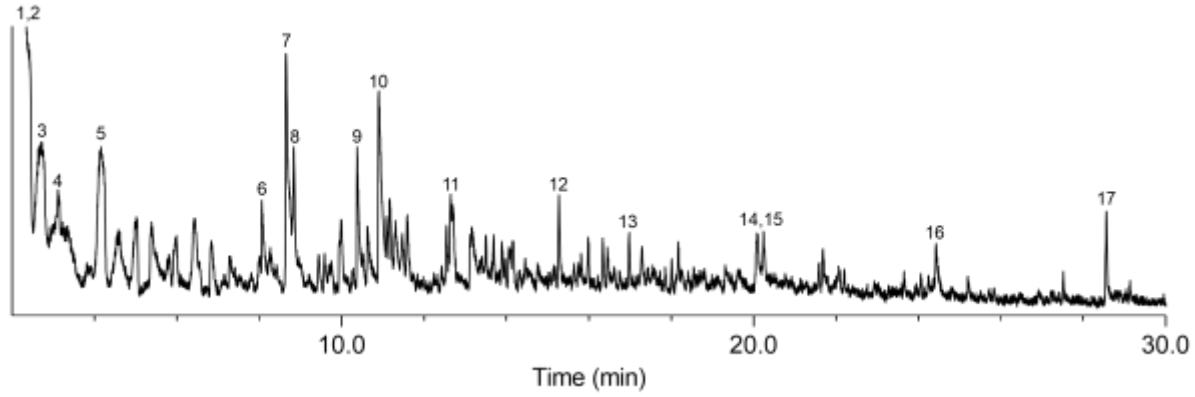

Fig. 18. Py-GC/MS analysis chromatogram of dry bark powder from *A. adstringens.* List of the numbered compounds is shown in Table 9.

Table 9. Py-GC/MS characterization of dry bark from *A. adstringens*.

No.	Identified compounds	RT (min)	Groups
1	Carbon dioxide	2.10	O
2	Nitrous oxide	2.12	O
3	n-Hexylmethylamine	2.24	AM
4	Butanal	3.11	AD
5	Toluene	4.12	*
6	Benzaldehyde	8.06	AD*
7	Phenol	8.65	P*
8	Cyclotetrasiloxane, octamethyl-	8.84	O
9	Phenol, 2-methyl-	10.38	P*
10	Phenol, 4-methyl-	10.90	P*
11	Phenol, 2,4-dimethyl-	12.64	P*
12	Naphthalene, 2,6-bis(1,1-dimethylethyl)-	15.28	*
13	Phenol, 2,6-dimethoxy-	16.97	P*
14	1,6-Anhydro-β-D-glucopyranose	20.07	S
15	Ethyl-α-d-glucopyranoside	20.08	S
16	Unknown	24.42	-
17	n-Hexadecanoic acid	28.56	AC

Groups: Phenol (P), Aromatic (*); Amine (AM); Sugar (S); Acid (AC); Aldehyde (AD); Others (O)

Aromatic compounds related to lignin degradation reached 43% from the total identified compounds with 71% of them being phenols. In contrast, sugars derived from cellulose/hemicellulose degradation represented only 12.5% from the total compounds. This

correlates with previous studies that highlighted the importance of lignin content in barks [25]. Indeed, the predominant peaks (7 and 10) were phenols.

The pyrogram also showed the presence of the fatty acid, hexadecanoic acid, in the *A. adstringens* bark. The anti-inflammatory activity of an ethanolic extract from *A. adstringens* bark has been related to the content of fatty acids [5]. The ethyl-α-d-glucopyranoside, here identified has been previously reported in rice wine and showed photoprotective activity in rat skin exposed to UVB-radiation [26]. Similarly, when it was isolated from *Stichopus japonicus* Sel., it inhibited the activity of tyrosinase, an enzyme that participates in cutaneous hyperpigmentation [27].

Table 10 presents the proximate and elemental analysis of the *A. adstringens* bark, CE, and fractions. The volatiles' content of this bark was similar to those reported for pine (68.21%) and eucalyptus (68.73) barks, whereas the fixed carbon content was comparable to that of pine park (25.27%) [28]. In contrast, *A. adstringens* bark had a higher ash content than those of the pine and eucalyptus bark (1.52 and 4.22%, respectively). In terms of the elemental analysis, the content of oxygen was greater in *A. adstringens* bark than in the aforementioned barks, which could be related to the presence of oxygenated compounds such as phenols previously described in this species [10]. In the *A. adstringens* bark, the sulfur content was not detectable while in the pine and eucalyptus barks a minimal presence of it (0.03 and 0.04, respectively) has been reported [28]. However, as shown in **Table 10** when fractionating the CE with hexane, ethyl acetate and water, the sulfur concentration increased compared to the starting extract and the bark. This noted surge in sulfur content after fractionation is in agreement with results previously described for other species such as *Petiveria alliacea* L. [29].

Thermogravimetric analysis (TGA) of the dry bark, the crude aqueous extract and its fractions are presented in **Fig. 19**. TGA curve represents the weight loss depending on the volatility and thermal stability of the chemical compounds present in the samples [30]. The greater weight loss from the DBP could be related to a higher concentration of volatiles as compared with the CE and the fractions (**Fig. 19-A**). All these samples showed a faster change in the range of 200 to 300 ºC (**Fig. 19-B**), which can be related to the presence compounds such as monophenols, monosugars and furans [31], some of them identified in the GC-MS analysis.

Table 10. Proximate and elemental analysis of *A. adstringens* bark, crude extract and fractions.

Parameter	Sample				
	Dry bark powder	Crude extract	Phase A	Phase B	Phase C
Proximate analysis (wt. %)					
Moisture[a]	6.7±0.1	3.9±0.5	6.1±0.2	5.5±0.1	6.4±0.3
Volatiles[b]	69.4±0.9	54.5±0.4	56.9±0.4	55.2±0.8	53.3±0.8
Fixed carbon[b]	22.1±0.1	36.5±1.0	31.4±3.4	35.5±0.0	33.7±0.5
Ash[b]	8.5±1.0	9.0±1.4	11.7±3.0	9.3±0.9	13.0±0.3
Elemental analysis (wt. %)					
C[b]	45.2±0.03	18.6±0.21	42.6±0.08	45.1±0.11	43.3±0.74
H[b]	5.1±0.01	8.4±0.05	4.7±0.00	4.2±0.03	3.9±0.14
N[b]	0.6±0.01	0.1±0.02	0.2±0.00	0.2±0.00	0.2±0.01
S[b]	0.00±0.00	0.01±0.00	0.32±0.02	0.26±0.00	0.24±0.02
O[c]	40.55±1.0	63.85±1.5	40.47±2.8	40.94±1.0	39.28±1.17

[a]As-received basis. [b]Dry basis. [c]By difference. Carbon (C), Hydrogen (H), Nitrogen (N), Sulfur (S), Oxygen (O). Data represent mean ± SD of two experiments.

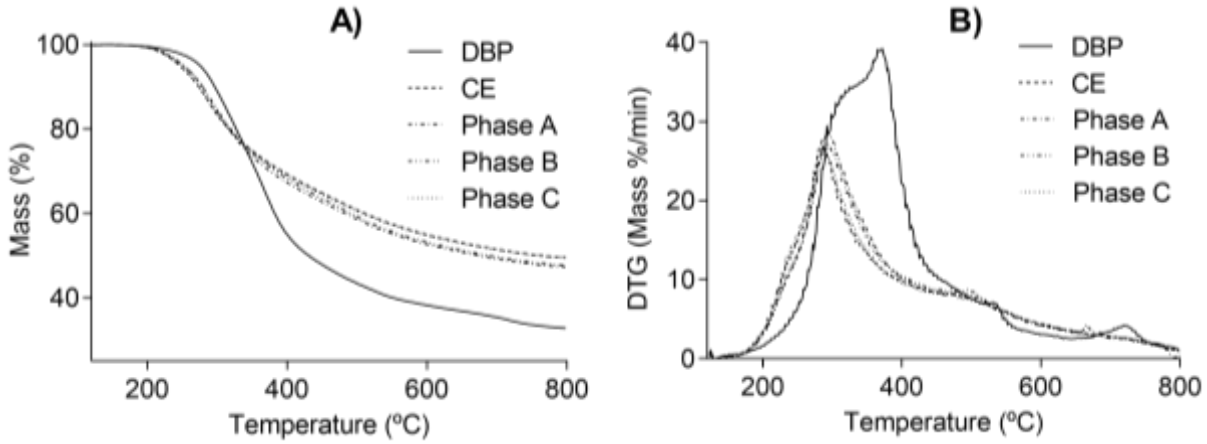

Fig. 19. TGA (A) and DTG (B) curves for the dry bark, crude aqueous extract and its fractions from *A. adstringens*.

DBP (dry bark powder), CE (crude aqueous extract), phase A (hexane), phase B (ethyl acetate), and phase C (aqueous fraction).

The FTIR analysis provides information on certain functional groups of chemical families in the studied natural samples [31]. **Fig. 20** (supporting information) shows the infrared spectrum of the DBP, CE and phases A-C. O-H and N-H bonds generated stretching vibrations between 3600-3100 cm^{-1} which correspond to water, alcohols or some amines [31]. Aliphatic and aromatic compounds presented stretching signals for the C-H bond in the range of 3000 to 2800

cm^{-1}. These peaks were more intense in phase B, which agrees with the GC-MS analysis that showed a higher proportion of aromatic compounds in this phase (44.4% of the identified compounds from phase B). The C=O signal characteristic for aldehydes, ketones, carboxylic acids and esters was less visible in the CE and phases A and B between 1780-1680 cm^{-1} regarding DBP and phase C. In all samples, the stretch signal for C=C at 1680-1530 cm^{-1} was intense and was associated with conjugated aromatic systems. The C-O bond had the most intense signals in the spectra between 1520-1250 cm^{-1} associated with another signal at 1050 cm^{-1} which could indicate the presence of oxygenated compounds such as terpenes, acetates or anhydrides [31]. Overall, the FTIR spectrum revealed an abundance of functional groups in the DBP, CE and fractions linked with the presence of bioactive aromatic or cyclical compounds such as phenols or terpenes previously described for this species [5].

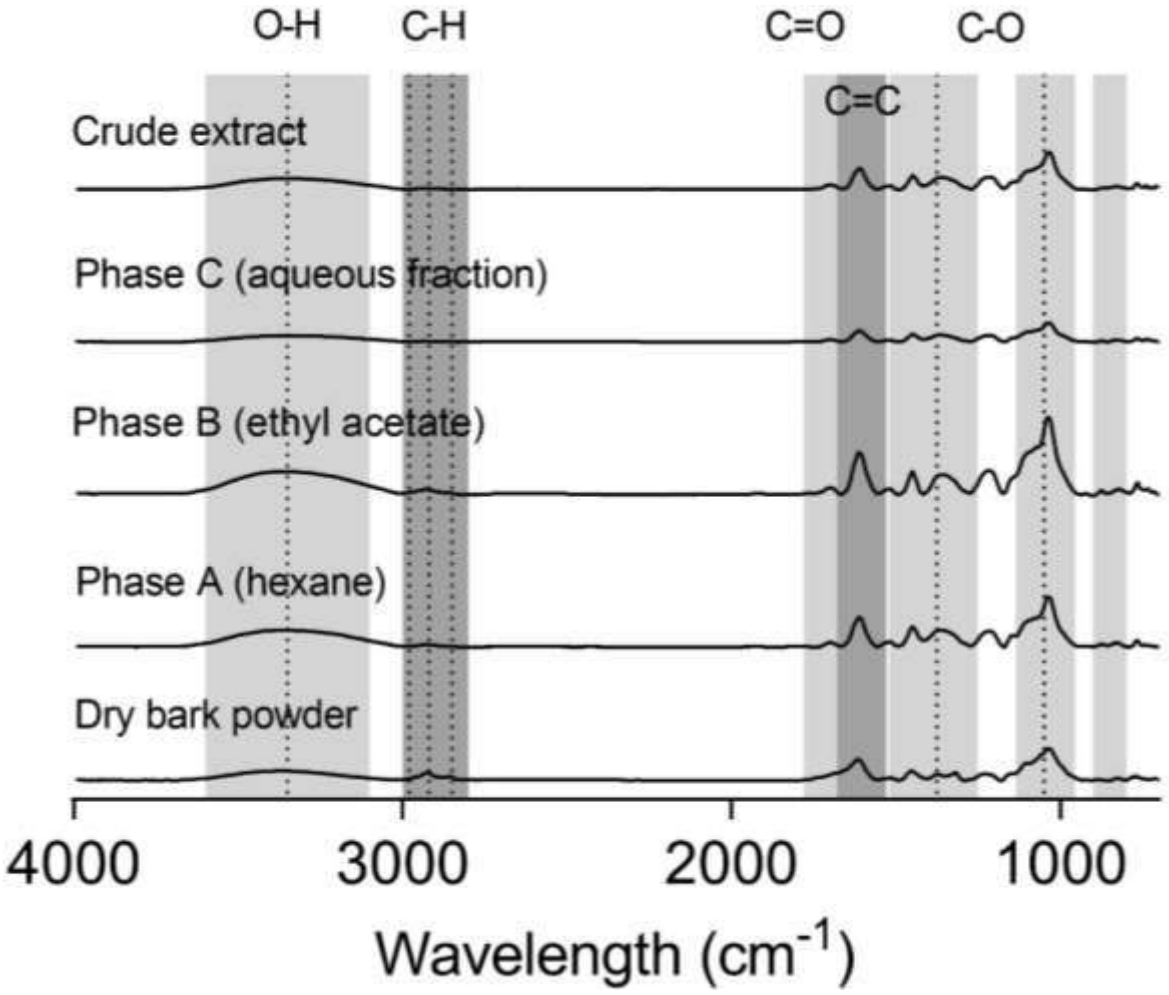

Fig. 20. FTIR spectra of the dry bark, aqueous crude extract and fractions from *A. adstringens*.

The CE and the A-C phases from *A. adstringens* bark were analyzed by GC-MS. **Fig. 21** shows the chromatograms of each sample and **Table 11** reports the name of the numbered compounds. Acids and aromatics were the predominant identified compounds in all samples. Within the aromatics, phenols represented the most extracted compounds, mainly in CE and phase B (≈ 62%). Flamenol, one of the major compounds was concentrated in a greater proportion in phases B and C. This is the first report for this phenolic compound in the *Amphipterygium* genus, but it has also been described in seeds of *Terminalia chebula* Retz. [32] and in *Sinapis alba* L., a plant recognized by its antibacterial activity [33]. Hydroquinone, identified in the CE and phases B and C, has been used for the treatment of various skin hyperpigmentation disorders [34] whereas pyrogallol, present in the CE and phase A has been topically used for psoriasis treatment [35].

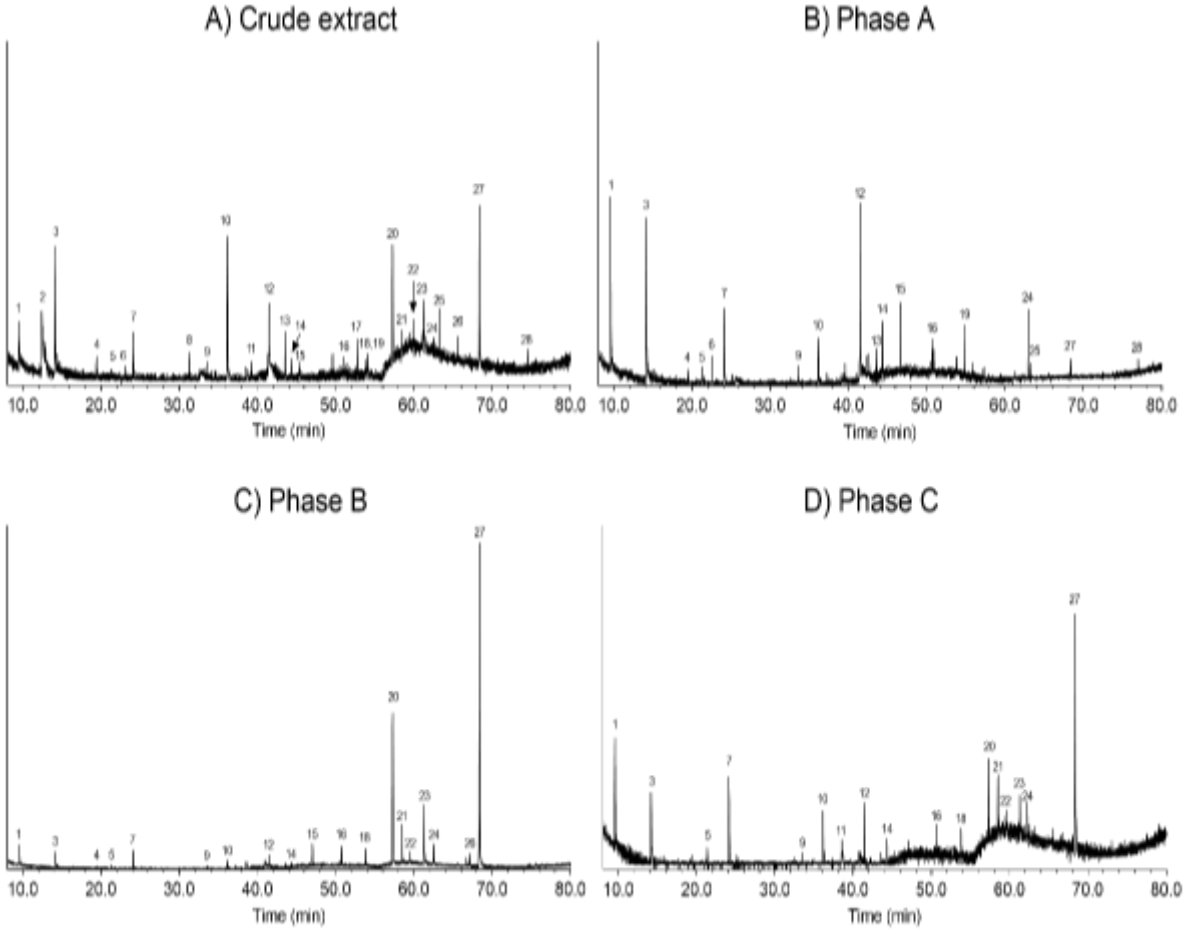

Fig. 21. Chromatograms obtained by GC-MS in aqueous bark extract from *A. adstringens* and its solvent fractions.

Phase A (hexane); B (ethyl acetate) and C (aqueous fraction) (list of the numbered compounds is shown in **Table 11**).

Table 11. Compounds identified by GC-MS in the crude aqueous extract from *A. adstringens* bark and its fractions.

No.	Identified compounds	RT (min)	Crude extract	Phase A	Phase B	Phase C	Groups
1	Metil formate	9.56	✓	✓	✓	✓	E
2	Hydrazine, ethyl-	12.41	✓				O
3	Acetic acid, methyl ester	14.15	✓	✓	✓	✓	AC
4	3,3-Dimethylacrylic acid	19.48	✓	✓	✓		AC
5	Pyruvic acid	21.32	✓	✓	✓	✓	AC
6	Glycoaldehyde dimethyl acetal	23.11	✓	✓			AD
7	Propanoic acid, 2-oxo-, methyl ester	24.11	✓	✓	✓	✓	E
8	Proline, 2-methyl-5-oxo-, methyl ester	31.23	✓				E
9	Acetic acid, hydrazide	33.53	✓	✓	✓	✓	AC
10	2-Propanone, 1,3-dihydroxy-	36.13	✓	✓	✓	✓	K
11	Picolinic acid N-oxide	38.51	✓			✓	AC
12	(S)-(+)-3-Hydroxytetrahydrofuran	41.43	✓	✓	✓	✓	F
13	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	43.51	✓	✓			K
14	Thiophene-3-ol, tetrahydro-, 1,1-dioxide	44.31	✓	✓		✓	A
15	7-Octen-2,6-diol, 2,6-dimethyl-	47.02	✓	✓			A
16	Butanedioic acid, methoxy-, dimethyl ester	50.68		✓	✓	✓	AC
17	Unknown	50.88	✓		✓		-
18	3,5-Dimethyl-1-dimethylphenylsilyloxybenzene	53.79	✓		✓	✓	O*
19	11-Tricosene	54.41	✓	✓			O
20	Carbonic acid, ethyl 3-(1-methylethoxy)phenyl ester	57.26	✓		✓	✓	E*
21	Hydroquinone	58.48	✓		✓	✓	P*
22	2-(Methylmercapto)-benzothiazol	59.63	✓		✓	✓	O*
23	Benzeneethanol, 4-hydroxy-	61.28	✓	✓	✓	✓	P*
24	Homovanillyl alcohol	62.55		✓	✓	✓	P*
25	Pyridine, 4-tert-butyl-2-(tert-butylthio)-	63.21	✓	✓			O
26	Unknown	66.12	✓		✓		-
27	Flamenol	68.34	✓	✓	✓	✓	P*
28	Pyrogallol	75.74	✓	✓			P*

Retention time (RT, min). Groups: Alcohol (A); Furans (F); Ketone (K); Phenol (P), Aromatic (*); Acid (AC); Aldehyde (AD); Ester (E); Others (O)

UV-fluorescence has been used in the analysis of natural extracts to identify compounds capable of emitting fluorescence, mainly phenolics [29]. Phenolic compounds can be present in plants as monomers, short conjugated ring systems and as polycondensed structures [36]. **Fig. 22** shows that the spectra of all samples had the highest intensity peak in the range from 260 to 290 nm, which could be related to a greater amount of monophenols of a small structure. The higher proportion of monophenols in the CE could be explained by the presence of a larger diversity of molecules in this CE compared to its fractions. It is worth noting the significantly more intense signal related to the monophenols of phase B as compared with phases A and C (**Fig. 22**). Ethyl acetate has been described as a good solvent to extract phenols of low molecular

weight [37]. Interestingly, phase C presents signals from short conjugated systems and polycondensed structures in the range of 290 to 340 nm. It could indicate a higher diversity of polyphenolic structures as tannins in this phase as compared with phases A and B.

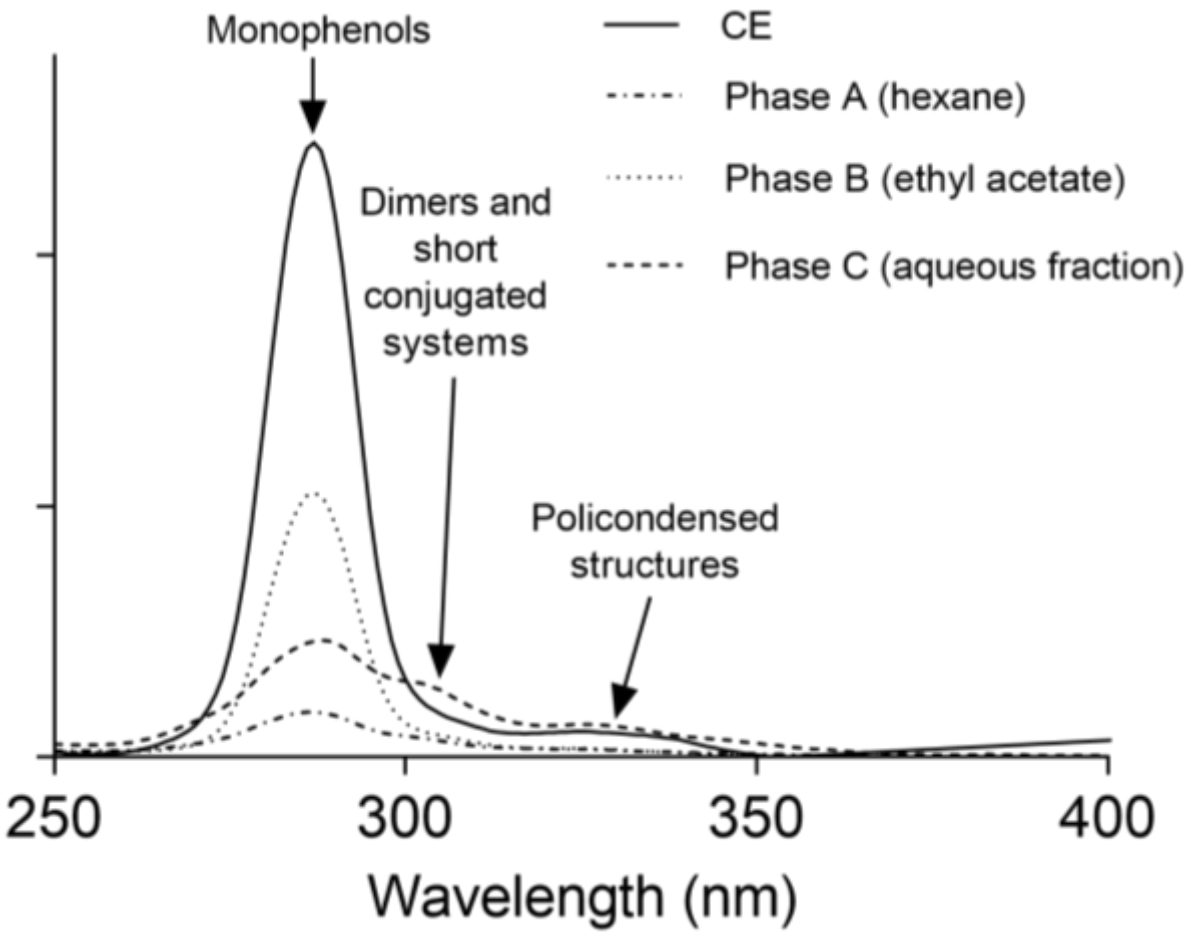

Fig. 22. UV-fluorescence spectra of the crude extract and its fractions from *A. adstringens* bark.

The toxicological MTT test performed on HaCaT cells through the determination of the minimum toxic dose (IC_{90}) showed that phase C had the lowest toxicity with an IC_{90}=911.3±10.8 μg/mL followed by phase A (IC_{90}= 283.2±7.2 μg/mL), the CE (IC_{90}=223±6 μg/mL) and then phase B (IC_{90}=74.0±5.5 μg/mL), respectively. According to the UV-fluorescence analysis, phase C presents a higher abundance of dimers, short conjugated molecules, and polycondensed structures when compared to phases A and B. Proanthocyanidins are a group of biologically active oligomeric and polymeric bioflavonoids which can be obtained

in the aqueous fraction [38]. These compounds have protective effects on keratinocyte's UV-induced oxidative stress and could be related to the lower toxicity observed in phase C compared to phases A and B [39].

Fig. 23 shows the anti-inflammatory activity of CE and its fractions at concentrations lower than IC_{90} on IL-17-induced IL-8 production on HaCaT keratinocytes. Results were compared with dexamethasone (dex), a drug used for psoriasis treatment. Dexamethasone has demonstrated to decrease the deleterious Th17 immune response in a model of arthritis [40] showing anti-IL17 activities on articular chondrocytes [41]. As shown in **Fig. 23**, the CE and all fractions significantly decreased ($p < 0.05$) the IL-8 release from keratinocytes. Interestingly, the CE and phase C, at all tested concentrations, demonstrated to be more potent than dexamethasone by significantly decreasing the IL-8 production under basal concentrations.

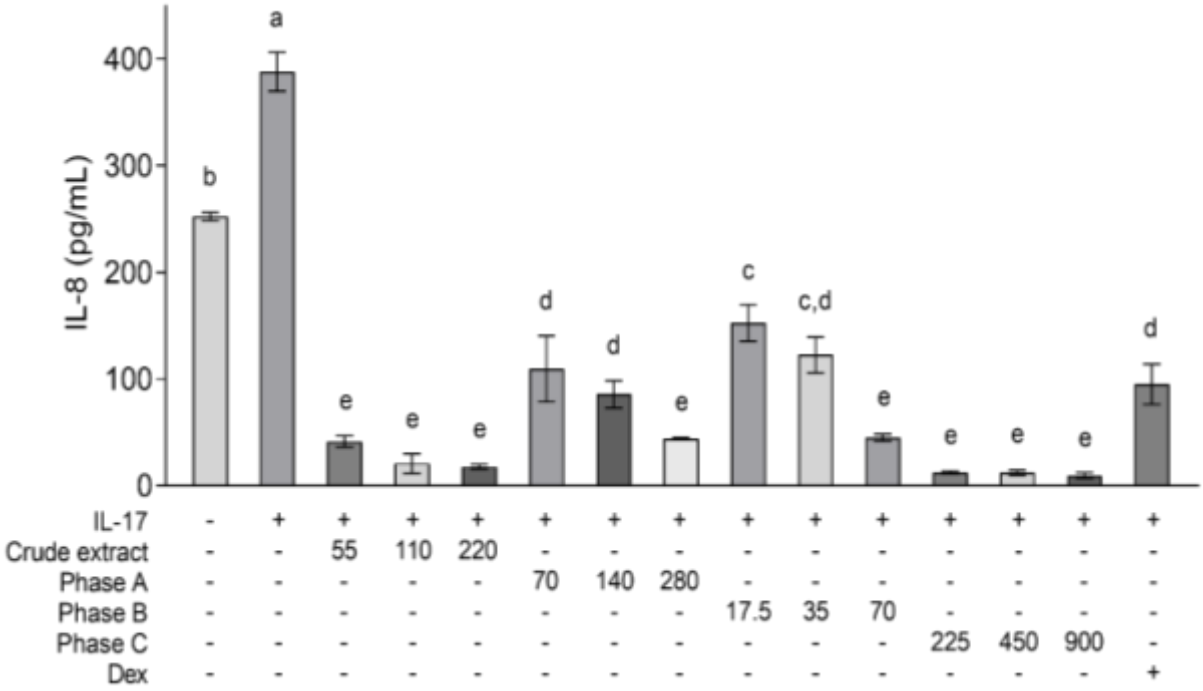

Fig. 23. Effect of crude extract and phases A-C from *A. adstringens* bark on the IL-17-induced production of IL-8 on HaCaT keratinocytes.

The confluent HaCaT cells were pretreated or not with crude extract and phases A-C (μg/mL) or dexamethasone (dex, 5 μM) for 10 h, then cells were stimulated with IL-17 (50 ng/mL) for 14 h. IL-8 production in HaCaT supernatants was measured by ELISA. Results are expressed in pg/mL and correspond to 10^6 cells. The histogram represents the mean ± standard deviation. Statistical: one-way ANOVA followed by Tukey's post hoc test. Means without a common letter differ ($p<0.05$).

In a previous study, it was demonstrated that a pyrolytic oil from the bark of this tree had a comparable anti-inflammatory effect as dexamethasone [30]. However, the results here presented show a more important anti-inflammatory effect of CE and fraction C on the IL-8 production ($p<0.01$). This can be explained by the higher diversity of chemical structures present in both samples. Most of the identified compounds with variable structures are present in CE, while phase C is rich in dimers, small conjugated structures and polycondensed molecules which could significantly contribute to the IL-17 inhibition. It has been reported that proanthocyanidins inhibit the IL-17 in human pulmonary epithelial cells by blocking mitogen-activated protein kinases and nuclear factor-κB-mediated signaling pathway [42]. Further research is needed to elucidate the involved anti-inflammatory mechanism from CE and phase C and the molecules responsible for this activity.

Overall, our results demonstrate for the first time the significant anti-inflammatory effect and low toxicity of the CE and aqueous fraction on HaCaT keratinocytes, which supports the traditional topical use of *A. adstringens* bark' decoctions by Purépechas for the treatment of dermatological diseases and opens the door for an exhaustive study of their anti-IL17 mechanism. Acids and aromatics were the predominant compounds identified in all samples. Moreover, flamenol, a phenol identified for the first time in the *Amphipterygium* genus, was the major compound in CE and phases B and C. Other investigations should be performed to determine the anti-inflammatory properties of flamenol and quantify this molecule into the analyzed samples.

VI.8.5. Conflict of interest statement

The authors declare no conflict of interest

VI.8.6. Authors' contributions

REG: investigation, formal analysis, writing-original draft. JSMF: Investigation, formal analysis. NAI: Investigation, reviewing and editing. MGP: investigation, formal analysis, methodology, resources, reviewing and editing. AOZ: investigation, formal analysis,

methodology, reviewing and editing. MEGP: conceptualization, methodology, formal analysis, resources, supervision, reviewing and editing.

VI.8.7. References

1 Villaseñor JL. Checklist of the native vascular plants of Mexico. Rev Mex Biodivers 2016; 87: 559–902

2 Alonso-Castro AJ, Domínguez F, Maldonado-Miranda JJ, Castillo-Pérez LJ, Carranza-Álvarez C, Solano E, Isiordia-Espinoza MA, Juárez-Vázquez M del C, Zapata-Morales JR, Argueta-Fuertes MA, Ruiz-Padilla AJ, Solorio-Alvarado CR, Rangel-Velázquez JE, Ortiz-Andrade R, González-Sánchez I, Cruz-Jiménez G, Orozco-Castellanos LM. Use of medicinal plants by health professionals in Mexico. J Ethnopharmacol 2017; 198: 81–86

3 Esquivel-García R, Pérez-Calix E, Ochoa-Zarzosa A, García-Pérez M-E. Ethnomedicinal plants used for the treatment of dermatological affections on the Purépecha Plateau, Michoacán, Mexico. Acta Bot Mex 2018; 125: 95–132

4 Rodriguez-Garcia A, Peixoto ITA, Verde-Star MJ, De la Torre-Zavala S, Aviles-Arnaut H, Ruiz ALTG. *In vitro* antimicrobial and antiproliferative activity of *Amphipterygium adstringens*. Evid Based Complement Alternat Med 2015; 2015: 175497

5 Rodriguez-Canales M, Jimenez-Rivas R, Canales-Martinez MM, Garcia-Lopez AJ, Rivera-Yañez N, Nieto-Yañez O, Ledesma-Soto Y, Sanchez-Torres LE, Rodriguez-Sosa M, Terrazas LI, Rodriguez-Monroy MA. Protective effect of *Amphipterygium adstringens* extract on dextran sulphate sodium-induced ulcerative colitis in mice. Mediators Inflamm 2016; 2016: 8543561

6 Arrieta J, Benitez J, Flores E, Castillo C, Navarrete A. Purification of gastroprotective triterpenoids from the stem bark of *Amphipterygium adstringens*; role of prostaglandins, sulfhydryls, nitric oxide and capsaicin-sensitive neurons. Planta Med 2003; 69: 905–909

7 Knauth P, Acevedo-Hernández GJ, Cano ME, Gutiérrez-Lomelí M, López Z. *In vitro* bioactivity of methanolic extracts from *Amphipterygium adstringens* (Schltdl.) Schiede ex Standl., *Chenopodium ambrosioides* L., *Cirsium mexicanum* DC., *Eryngium carlinae* F. Delaroche, and *Pithecellobium dulce* (Roxb.) Benth. used in traditional medicine in Mexico. Evid Based Complement Alternat Med 2018; 2018: 3610364

8 Navarrete A, Sánchez-Mendoza ME, Arrieta J, Cruz-Antonio L, Oliva I, Castañeda-Hernández G. Gastroprotection and effect of the simultaneous administration of cuachalalate (*Amphipterygium adstringens*) on the pharmacokinetics and anti-inflammatory activity of diclofenac in rats. J Pharm Pharmacol 2005; 57: 1629–1636

9 Rosas-Piñón Y, Mejía A, Díaz-Ruiz G, Aguilar MI, Sánchez-Nieto S, Rivero-Cruz JF. Ethnobotanical survey and antibacterial activity of plants used in the Altiplane region of Mexico for the treatment of oral cavity infections. J Ethnopharmacol 2012; 141: 860–865

10 Mata R, Figueroa M, Navarrete A, Rivero-Cruz I. Chemistry and biology of selected Mexican medicinal plants. In: Kinghorn AD, Falk H, Gibbons S, Kobayashi J, Asakawa Y, Liu J-K (Hrsg.). Progress in the Chemistry of Organic Natural Products. Cham: Springer, 2019: 1–142 Im Internet: https://doi.org/10.1007/978-3-030-01099-7_1

11 Oviedo-Chávez I, Ramírez-Apan T, Soto-Hernández M, Martínez-Vázquez M. Principles of the bark of *Amphipterygium adstringens* (Julianaceae) with anti-inflammatory activity. Phytomedicine 2004; 11: 436–445

12 International Psoriasis Council. IPC Psoriasis Review. Dallas, Texas: IPC, 2009: 1–12 Im Internet: http://www.psoriasiscouncil.org/docs/ipcpsoriasisreview_dec_2009_english.pdf?LanguageID=EN-US

13 Boehncke W-H, Schön MP. Psoriasis. Lancet 2015; 386: 983–994

14 Blauvelt A, Chiricozzi A. The immunologic role of IL-17 in psoriasis and psoriatic arthritis pathogenesis. Clin Rev Allergy Immunol 2018; 55: 379–390

15 Nograles KE, Zaba LC, Guttman-Yassky E, Fuentes-Duculan J, Suárez-Fariñas M, Cardinale I, Khatcherian A, Gonzalez J, Pierson KC, White TR, Pensabene C, Coats I, Novitskaya I, Lowes MA, Krueger JG. Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. Br J Dermatol 2008; 159: 1092–1102

16 Hohenberger M, Cardwell LA, Oussedik E, Feldman SR. Interleukin-17 inhibition: role in psoriasis and inflammatory bowel disease. J Dermatol Treat 2018; 29: 13–18

[17] Rusta-Sallehy S, Gooderham M, Papp K. Brodalumab: a review of safety. Skin Therapy Lett 2018; 23: 1–3
[18] van de Kerkhof PCM, Griffiths CEM, Reich K, Leonardi CL, Blauvelt A, Tsai T-F, Gong Y, Huang J, Papavassilis C, Fox T. Secukinumab long-term safety experience: a pooled analysis of 10 phase II and III clinical studies in patients with moderate to severe plaque psoriasis. J Am Acad Dermatol 2016; 75: 83-98.e4
[19] Murphy EC, Nussbaum D, Prussick R, Friedman AJ. Use of complementary and alternative medicine by patients with psoriasis. J Am Acad Dermatol 2019; 81: 280–283
[20] Esquivel-García R, Estévez-Delgado G, Rodríguez-Orozco A-R, Ochoa-Zarzosa A, García-Pérez ME. La psoriasis: de la investigación básica y clínica al desarrollo de nuevos tratamientos. Gaceta Médica de México 2018; 154: 502–508
[21] Olivera Ortega AG, Soto Hernández M, Martínez Vázquez M, Terrazas Salgado T, Solares Arenas F. Phytochemical study of cuachalalate (*Amphiptherygium adstringens*, Schiede ex Schlecht). J Ethnopharmacol 1999; 68: 109–113
[22] Navarrete A, Avula B, Joshi VC, Ji X, Hersh P, Khan IA. Quantitative determination of triterpenes from *Amphiptherygium adstringens* by liquid chromatography and thin-layer chromatography and morphological analysis of cuachalalate preparations. J AOAC Int 2006; 89: 1–7
[23] Castillo-Juárez I, Rivero-Cruz F, Celis H, Romero I. Anti-*Helicobacter pylori* activity of anacardic acids from *Amphipterygium adstringens*. J Ethnopharmacol 2007; 114: 72–77
[24] Déciga-Campos M, Rivero-Cruz I, Arriaga-Alba M, Castañeda-Corral G, Angeles-López GE, Navarrete A, Mata R. Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. J Ethnopharmacol 2007; 110: 334–342
[25] Kersten S, Garcia-Perez M. Recent developments in fast pyrolysis of ligno-cellulosic materials. Curr Opin Biotechnol 2013; 24: 414–420
[26] Hirotsune M, Haratake A, Komiya A, Sugita J, Tachihara T, Komai T, Hizume K, Ozeki K, Ikemoto T. Effect of ingested concentrate and components of sake on epidermal permeability barrier disruption by UVB irradiation. J Agric Food Chem 2005; 53: 948–952
[27] Husni A, Jeon J-S, Um B-H, Han NS, Chung D. Tyrosinase inhibition by water and ethanol extracts of a Far Eastern sea cucumber, *Stichopus japonicus*. J Sci Food Agric 2011; 91: 1541–1547
[28] ECN, TNO. Database for the physico-chemical composition of (treated) lignocellulosic biomass, micro- and macroalgae, various feedstocks for biogas production and biochar. Phyllis2 2020; Im Internet: https://phyllis.nl/Browse/Standard/ECN-Phyllis#bark
[29] García-Pérez M-E, Alfonso-Castillo A, Fong Lores O, Batista-Duharte A, Lemus-Rodríguez Z. Toxicological evaluation of an aqueous suspension from leaves and stems of *Petiveria alliacea* L. (Phytolaccaceae). J Ethnopharmacol 2018; 211: 29–37
[30] Esquivel-García R, Ayiania M, Abu-Lail N, López-Meza JE, del Río RE, García-Pérez M, Ochoa-Zarzosa A, García-Pérez M-E. Pyrolytic oils from *Amphipterygium adstringens* bark inhibit IL-8 production of IL-17-stimulated HaCaT keratinocytes. J Anal Appl Pyrol 2020; 145: 104749
[31] Stankovikj F, Garcia-Perez M. TG-FTIR method for the characterization of bio-oils in chemical families. Energy Fuels 2017; 31: 1689–1701
[32] Nandagopal S, Ganesh Kumar A, Dhanalakshmi DP, Prakash P. Bio-prospecting the antibacterial and anticancer activities of silver nanoparticles synthesized using *Terminalia chebula* seed extract. Int J Pharm Pharm Sci 2014; 6: 368–373
[33] Al-Qudah MA, Al-Jaber HI, Muhaidat R, Hussein EI, Hamid AAA, Al-Samadi ML, Abaza IF, Afifi FU, Abu-Orabi ST. Chemical composition and antimicrobial activity of the essential oil from *Sinapis alba* L. and *Sinapis arvensis* L. (Brassicaceae) growing wild in Jordan. Res J Pharm Biol Chem Sci 2011; 2: 1136–1144
[34] Nordlund JJ, Grimes PE, Ortonne JP. The safety of hydroquinone. J Eur Acad Dermatol Venereol 2006; 20: 781–787
[35] Willsteed E, Regan W. Psoriasis, pyrogallol and skin cancer. Australas J Dermatol 1985; 26: 144–145
[36] Barsotti F, Ghigo G, Vione D. Computational assessment of the fluorescence emission of phenol oligomers: a possible insight into the fluorescence properties of humic-like substances (HULIS). J Photochem Photobiol A 2016; 315: 87–93
[37] García-Pérez M-E, Royer M, Herbette G, Desjardins Y, Pouliot R, Stevanovic T. *Picea mariana* bark: a new source of *trans*-resveratrol and other bioactive polyphenols. Food Chem 2012; 135: 1173–1182
[38] García-Pérez M-E, Royer M, Duque-Fernandez A, Diouf PN, Stevanovic T, Pouliot R. Antioxidant, toxicological and antiproliferative properties of Canadian polyphenolic extracts on normal and psoriatic keratinocytes. J Ethnopharmacol 2010; 132: 251–258

[39] Mantena SK, Katiyar SK. Grape seed proanthocyanidins inhibit UV-radiation-induced oxidative stress and activation of MAPK and NF-kappaB signaling in human epidermal keratinocytes. Free Radic Biol Med 2006; 40: 1603–1614

[40] Peña C, Gárate D, Contreras-Levicoy J, Aravena O, Catalán D, Aguillón JC. Dexamethasone preconditioning improves the response of collagen-induced arthritis to treatment with short-term lipopolysaccharide-stimulated collagen-loaded dendritic cells. Clin Dev Immunol 2013; 2013: 296031

[41] Shalom-Barak T, Quach J, Lotz M. Interleukin-17-induced Gene Expression in Articular Chondrocytes Is Associated with Activation of Mitogen-activated Protein Kinases and NF-κB. J Biol Chem 1998; 273: 27467–27473

[42] Kim H, Kim JY, Song HS, Park KU, Mun K-C, Ha E. Grape seed proanthocyanidin extract inhibits interleukin-17-induced interleukin-6 production via MAPK pathway in human pulmonary epithelial cells. Naunyn Schmiedebergs Arch Pharmacol 2011; 383: 555–562

[43] Ayiania M, Terrell E, Dunsmoor A, Carbajal-Gamarra FM, Garcia-Perez M. Characterization of solid and vapor products from thermochemical conversion of municipal solid waste woody fractions. Waste Manag 2019; 84: 277–285

VII.DISCUSIÓN GENERAL

El presente trabajo se enmarca en una visión de desarrollo farmacéutico, donde se siguió un enfoque etnofarmacológico para la búsqueda de candidatos terapéuticos de origen natural con posible actividad antipsoriásica. La región de la Meseta Purépecha se seleccionó como área de estudio para la realización del estudio etnofarmacológico de partida (**Fig. 1-Capítulo 1**), ya que las características culturales de su población y la diversidad biológica de su territorio favorecen que la práctica de la medicina tradicional continúe vigente. Además, ningún estudio etnofarmacológico había sido reportado con relación al tratamiento de enfermedades dermatológicas por esta población, lo que garantizó la novedad de la investigación presentada.

Se identificaron 97 plantas medicinales para el tratamiento de afecciones dermatológicas utilizadas por los habitantes de esta región (Esquivel-García et al., 2018b) y 19 condiciones dermatológicas entre las que destacaron el tratamiento de heridas (40.20%), inflamación de la piel (37.11%) y erupciones cutáneas (37.11%) (**Fig. 4-Capítulo 1**, **Apéndice-Capítulo 1**). Además, se constató que las partes aéreas, las hojas y las flores son los tejidos más empleados, los que se usan preferentemente por vía oral en forma de infusiones y cocimientos (**Fig. 5-Capítulo 1**). Una observación interesante es que a diferencia de otros estudios etnofarmacológicos centrados en enfermedades de la piel (Afolayan et al., 2014; Njoroge & Bussmann, 2007; Sharma et al., 2014) los Purépechas prefieren el uso de preparaciones por vía oral en lugar de la administración tópica, esto debido a que poseen la creencia de que las enfermedades dermatológicas se deben a una contaminación sanguínea interna.

La comparación con la literatura etnomedicinal permitió registrar por primera vez 8 nuevas plantas utilizadas para tratar afecciones de la piel (*Eryngium beecheyanum, Tagetes remotiflora, Tournefortia mutabilis, Equisetum hyemale* var. *affine, Clinopodium macrostemum, Salvia leucantha, Sida haenkeana* y *Urtica subincisa*). Desde el punto de vista etnofarmacológico, se trata de una nueva información que podría servir para el desarrollo de nuevos candidatos orientados a tratar este tipo de patologías. Además, permite la difusión del conocimiento heredado de generación en generación por los Purépecha sobre las preparaciones herbolarias para enfermedades cutáneas. Lo anterior sirvió para confeccionar un catálogo

etnofarmacológico de la zona con características similares a los ya publicados en otras regiones del mundo (Prashantkumar & Vidyasagar, 2008; Sharma et al., 2014).

A partir de la información etnofarmacológica, se determinaron las 10 plantas con mayor valor de uso (**Fig. 6-Capítulo 1**). De estas plantas se seleccionaron dos especies mexicanas (una herbácea y un árbol) que se emplearon en etapas sucesivas de la investigación para la búsqueda de compuestos prometedores como tratamientos antipsoriásicos. Tres criterios fundamentales se consideraron en la elección de ambas plantas: 1) ser altamente reportadas por los Purépechas; 2) ser nativas de México; 3) no haber sido farmacológicamente estudiadas para el tratamiento de enfermedades dermatológicas. La lógica tras la elección de una planta herbácea y un árbol estuvo motivada por las diferencias en cuanto a la composición química de ambas especies, respecto al tipo de compuestos extraíbles y lignina, con el consiguiente impacto en la diversidad de moléculas obtenidas en los extractos convencionales y aceites pirolíticos, así como en su actividad biológica. En consecuencia, la especie herbácea conocida comúnmente como ortiga (*Urtica subincisa*), identificada como la quinta planta con mayor valor de uso, y el árbol de cuachalalate (*Amphipterygium adstringens*), séptimo reportado con mayor valor de uso, fueron seleccionados para etapas subsecuentes de la investigación.

El cuachalalate (**Fig. 17**) se utiliza desde tiempos prehispánicos en el tratamiento de úlceras gástricas y hay registro en la literatura científica de que algunos de sus compuestos tienen acción antimicrobiana y antiinflamatoria (Mata et al., 2019). En la región de la Meseta Purépecha su corteza es preparada como cocimiento, tomada oralmente y/o usada para lavar las áreas de piel afectada por cortaduras, inflamación, quemaduras, sarpullido, infecciones con abscesos, picaduras de insectos (**Apéndice-Capítulo 1**). Con respecto a la ortiga (**Fig. 10**) en la literatura solamente se menciona el uso de las partes aéreas en la medicina tradicional sin una aplicación en particular (Steinmann, 2005), y no hay estudios de su composición química, acciones farmacológicas o toxicológicas. En la etnomedicina Purépecha se describe que el extracto etanólico de sus partes aéreas es utilizado por vía tópica en forma de fomentos, mientras que la infusión se administra oralmente para el tratamiento de inflamaciones cutáneas, várices y erupciones (**Apéndice-Capítulo 1**).

Posterior a la recolección, preparación de las muestras y depósito en el herbario del Instituto de Ecología del Bajío (IEB) de ambas plantas se obtuvieron los extractos crudos convencionales

apegándose a la forma de uso tradicional, los que fueron fraccionados utilizando tres solventes de menor a mayor polaridad (hexano, acetato de etilo y agua) con vistas a caracterizarlos más exhaustivamente desde el punto de vista químico. Las fracciones resultantes fueron denominadas fases A, B y C, respectivamente. La biomasa residual libre de extractivos (más del 60% de la muestra inicial) fue sometida a un proceso de pirólisis lenta (**Fig. 2-Capítulo 3**). Este procedimiento permitió el aprovechamiento integral del material biológico y se amplió la cantidad de compuestos bioactivos obtenidos, ya que los aceites pirolíticos presentaron en su composición compuestos provenientes de la degradación de la celulosa, hemicelulosa y lignina, imposibles de obtener con métodos convencionales de extracción (Mohan et al., 2006). Los extractos crudos, sus fracciones y los aceites pirolíticos de ambas plantas fueron caracterizados químicamente, además de que se determinó su toxicidad y efecto sobre la producción de IL-8 en queratinocitos HaCaT estimulados con IL-17.

Con respecto al rendimiento de extracción, el extracto crudo de *A. adstringens* presentó el mayor rendimiento de extracción (28.6 %) comparativamente a 13.4 % del extracto etanólico de *U. subincisa*. Con relación a la extracción líquido/líquido en ambos casos, la fracción acuosa generó los mayores rendimientos, mientras que el aceite pirolítico 2 comparativamente al aceite pirolítico 1 fue el que se obtuvo en mayores cantidades (13.8 y 10.0 % para *A. adstringens* y *U. subincisa* respectivamente). El análisis realizado por GC-MS permitió constatar que la composición química de los extractos convencionales de ambas plantas fue variable tal y como se esperaba dada su naturaleza (Stevanovic, 2005).

El extracto etanólico de *U. subincisa* presentó una alta concentración de esteres (25% de las moléculas identificadas), mientras que los compuestos fenólicos y ácidos prevalecieron en el extracto acuoso de *A. adstringens* (19 % de las moléculas reportadas para ambos grupos). Además de las variaciones en las proporciones relativas de los compuestos identificados, los compuestos mayoritarios fueron diferentes en ambos extractos convencionales. El extracto etanólico de *U. subincisa* demostró ser rico en linoleato de etilo, mientras que el extracto acuoso derivado de cortezas de *A. adstringens* mostró altas concentraciones del compuesto fenólico flamenol. La presencia de esta molécula es documentada por vez primera para el género *Amphipterygium.*

Las moléculas mayoritarias de los aceites pirolíticos también mostraron variaciones relacionadas con la especie analizada. En el caso de *U. subincisa*, el indol y el fenol mostraron una prevalencia en el aceite pirolítico-1, mientras que el aceite pirolítico-2 demostró ser rico en 3,4,5 trimetilpirazol y fenol. En el caso de *A. adstringens* existe una gran similitud entre los compuestos presentes en el aceite pirolítico-1 y el aceite pirolítico-2, aunque las áreas de los picos difirieron cuando se compararon ambos aceites. Por ejemplo, el guaiacol, un producto de degradación de la lignina (Mohan et al., 2006) estuvo presente en mayor proporción en el aceite pirolítico-2, mientras que ocurre lo contrario con el siringol que estuvo presente en una concentración más alta en el aceite pirolítico-1.

Las diferencias observadas en cuanto a la composición química de los extractos convencionales y aceites pirolíticos de *U. subincisa* y *A. adstringens* podrán explicarse por la propia naturaleza de las plantas estudiadas. En general se considera que las plantas leñosas tienen un contenido elevado de compuestos fenólicos como taninos, lignanos y estilbenos, mientras que las herbáceas suelen ser ricas en flavonoides y ácidos fenólicos (Gómez-Fuentes-Galindo et al., 2017). Además, los aceites pirolíticos pueden contener compuestos diferentes dadas las diferencias en cuanto a la composición de la lignina, el tipo de planta, la etapa de desarrollo, lugar de crecimiento y anatomía propia de la especie analizada (Sykes et al., 2009).

El análisis de la toxicidad de las muestras analizadas sobre los queratinocitos HaCaT se realizó siguiendo el método de MTT mediante la determinación de la concentración toxica mínima (IC_{90}). Tanto para *U. subincisa*, como para *A. adstringens* la muestra con mayor toxicidad estuvo representada por el aceite pirolítico-2.

Comparando la toxicidad de las muestras con el contenido de compuestos fenólicos identificados por las distintas técnicas analíticas se puede apreciar que a mayor concentración de fenoles se constató una mayor toxicidad sobre los queratinocitos. De hecho, en el caso de *U subincisa* se encontró una correlación indirecta entre el contenido de fenoles totales y el valor de la IC_{90}, representativo de una mayor toxicidad ($r=-0.5209$; $p=0.0266$). De igual manera, para *A. adstringens*, pudo apreciarse que las muestras con mayor toxicidad correspondían a aquellas con altos contenidos de fenoles, según el análisis por GC-MS y FTIR. Es conocido que fenoles utilizados en formulaciones tradicionales y farmacéuticas pueden producir erupciones cutáneas, inflamación dérmica y dermatitis de contacto (Murray et al., 2007). La toxicidad de estos

compuestos fenólicos dependerá no solo de su concentración e hidrofobicidad (Newby et al., 2000), sino también de la generación de radicales libres y especies reactivas de oxígeno (Gami, 2014).

Aunque la presencia de fenoles podría afectar la toxicidad, su sola aparición no es suficiente para explicar en su totalidad los efectos tóxicos observados en las muestras analizadas. Si bien en el caso de *A. adstringens* la mayor toxicidad estuvo en la fase B y los aceites 1-2, que mostraron el mayor contenido fenólico, el aceite pirolítico-1 de *U. subincisa* que mostró una preponderancia de estos compuestos de acuerdo con la evaluación del contenido de fenoles totales, GC-MS, FTIR y análisis de UV fluorescencia, fue menos tóxico que las fases A-B y el aceite pirolítico-2 de esta misma planta. Por lo tanto, se propuso la hipótesis de que la toxicidad en los queratinocitos pudiera también estar relacionada con la proporción relativa de los compuestos químicos dentro de la muestra, lo que podría conducir a interacciones entre las moléculas que finalmente se traducirían en efectos nocivos para las células. Otros estudios toxicológicos *in vitro* e *in vivo* deben ser realizados para determinar con precisión la naturaleza de las moléculas que impactan sobre la toxicidad en los queratinocitos y sus dosis umbral.

Los extractos crudos de ambas plantas, sus respectivas fracciones (fases A, B y C), así como los aceites pirolíticos 1 y 2 de cada una de las especies analizadas fueron evaluados a concentraciones no citotóxicas con relación a su efecto inhibitorio sobre la producción de IL-8 en un modelo *in vitro* de inducción por IL-17 en queratinocitos HaCaT. Se escogió este modelo por varias razones: a) el reconocimiento del papel significativo de la IL-17 en la patogénesis de la psoriasis; b) el papel de los queratinocitos como células cruciales que promueven los efectos proinflamatorios de esta citocina a nivel epidérmico (Moos et al., 2019); c) la correlación de los niveles de IL-8 con la gravedad de la psoriasis (Arican et al., 2005). En consecuencia, para el cribado farmacológico, se consideró que la inhibición de la producción de IL-8 por los queratinocitos HaCaT estimulados por IL-17 sería un buen modelo *in vitro* para detectar el potencial antiinflamatorio de los extractos solventes y bio-aceites y su posible aplicación para la psoriasis. Lo anterior con el objetivo de seleccionar la especie más prometedora.

La producción basal de IL-8 en células HaCaT fue de 252±4 pg/ml, mientras que la IL-17 estimuló 1.54 veces la producción de IL-8 después de 14 horas de tratamiento ($p < 0.05$). En el caso de *U. subincisa* (**Fig. 16**), los extraíbles mostraron un efecto proinflamatorio y no

inhibieron la producción de IL-8, excepto cuando la fase C fue probada a la concentración más baja de 27.5 μg/mL, generando una disminución en los niveles de IL-8 hasta las concentraciones basales; mientras que el aceite-2 a una concentración de 7.5 μg/mL provocó una disminución significativa de IL-8 inferior a las concentraciones basales en forma comparable a la dexametasona. Uno de los resultados más inesperados fue el significativo efecto proinflamatorio observado en la fase B de *U. subincisa*. Uno de los principales compuestos identificados en esta fase es el fitol, al que se le han demostrado actividades proinflamatorias y promotoras de tumores en piel de ratón (Kagoura et al., 1999). Los derivados de fitol son inductores potentes de citocinas como la IL-17 (Roy Chowdhury et al., 2013) y pueden estimular al factor de transcripción NF-κB (Ghosh & Chowdhury, 2013) vinculado a la producción de IL-8. Los inmunoestimulantes basados en fitol pueden aumentar la expresión de CXCL1, un equivalente murino de IL-8 (Chowdhury & Ghosh, 2012), por lo que creemos que la importante presencia de fitol en la Fase B podría explicar, al menos parcialmente, la actividad proinflamatoria encontrada. Además, la presencia significativa de hidrocarburos en esta fase pueden generar activación inmunitaria (Yau et al., 2017), que podría estar relacionada con los efectos observados ya que estos compuestos podrían actuar solos o en sinergia con el fitol para estimular la producción de IL-8, pero nuevas investigaciones deben aún ser realizadas para confirmar tales efectos. El efecto antinflamatorio mostrado por el aceite pirolítico-2 de *U. subincisa* sugiere que este bioaceite posee un potencial farmacológio a ser explorado en el tratamiento de la psoriasis. El compuesto mayoritario detectado por GC-MS en el mismo fue el 3,4,5-trimetilpirazol. Se ha demostrado que derivados pirazólicos pueden mejorar la psoriasis inducida en ratones al inhibir la expresión del ARNm de la IL-17 (Seki et al., 2014). Además, estos compuestos pueden inhibir la activación de NF-κB (Fu et al., 2017), factor de transcripción vinculado a la producción de IL-8. Otro compuesto nitrogenado bioactivo presente en el aceite-2 es el pirrol, quien se ha sugerido para la profilaxis y el tratamiento de enfermedades inflamatorias que implican la sobreproducción de citocinas como IL-8 (Zablocki et al., 2003).

A diferencia de los resultados presentados para *U. subincisa*, las muestras más antiinflamatorias en el caso de *A. adstringens* resultaron provenientes de la extracción convencional, aunque los aceites pirolíticos mostraron también efectos anti-IL17. De hecho, el extracto crudo y las fases A-C disminuyeron significativamente la liberación de IL-8 ($p<0.05$). Un resultado notable fue el constatar que el extracto crudo y la fase C, a todas las concentraciones probadas, demostraron

ser significativamente más potentes que la dexametasona ($p < 0.05$). El análisis realizado por fluorescencia UV mostró que la fase C presentaba señales de sistemas conjugados y estructuras policondensadas en el rango de 290 a 340 nm, lo que podría indicar una mayor diversidad de estructuras polifenólicas como las proantocianidinas en esta fase en comparación con las fases A y B. Se ha reportado que las proantocianidinas tienen actividad anti-IL17 en el epitelio pulmonar humano (H. Kim et al., 2011), por lo que podrían estar relacionadas con esta actividad. El aceite pirolítico-1 de *A. adstringens* a una concentración de 17.5 μg/mL disminuyó la producción de IL-8 hasta el valor basal ($p < 0.05$), mientras que el efecto antiinflamatorio fue mayor en el aceite pirolítico 2, pues a una concentración de 15 μg/mL provocó la disminución de la producción de IL-8 por debajo de los valores iniciales y en forma comparable a la dexametasona ($p < 0.05$). Algunos de los compuestos fenólicos identificados por HPLC en los aceites pirólicos de *A. adstringens*, como la epigalocatequina, la catequina y la quercetina, poseen propiedades antiinflamatorias (Vijayalakshmi et al., 2012). Además, otras moléculas también identificadas, como el ácido docosahexaenoico, pueden inhibir la secreción de la IL-8 en las células HaCaT estimuladas por TNF (Storey et al., 2005). Nuevas investigaciones son necesarias para dilucidar el mecanismo por el cual los aceites pirolíticos inhiben la producción de IL-8 al bloquear las vías de IL-17 y las moléculas responsables de estos efectos.

Los resultados anteriormente presentados permitieron seleccionar la muestra más prometedora. Dicha selección se realizó considerando tres elementos principales: a) baja toxicidad sobre los queratinocitos HaCaT; b) alto rendimiento de extracción y 3) efecto anti-IL17 (**Fig. 24**). Estos tres elementos llevaron a considerar como el candidato más prometedor para continuar con estudios farmacodinámicos, toxicológicos y farmacocinéticos al extracto acuoso de *A. adstringens*.

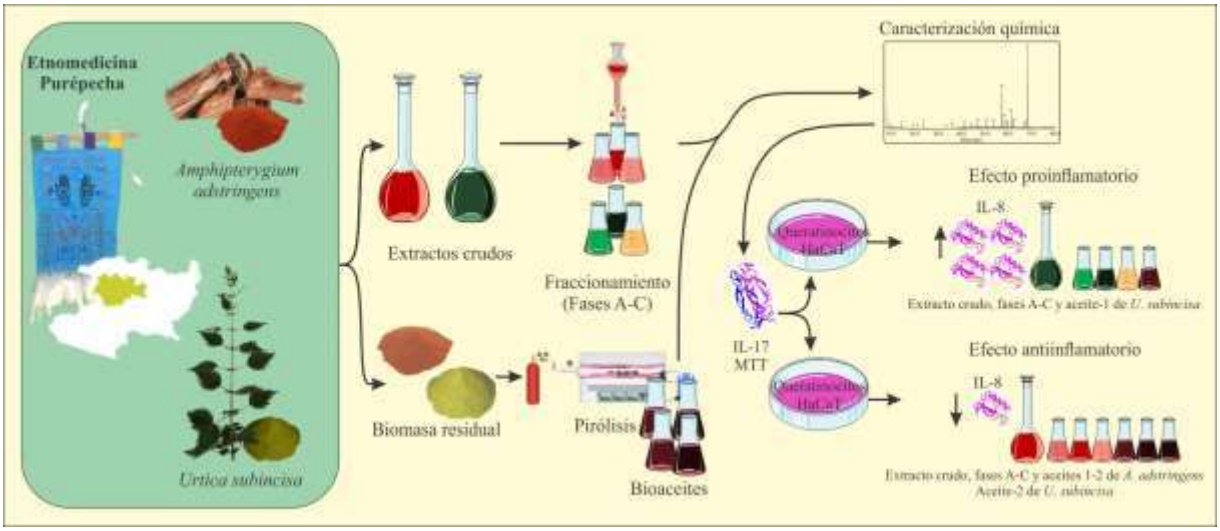

Fig. 24. Esquema representativo de los resultados obtenidos en este trabajo.

Los extractos crudos de *A. adstringens* y *U. subincisa* se obtuvieron simulando su uso tradicional por la etnomedicina Purépecha y fueron fraccionados por extracción líquido/líquido en las fases A-C. La biomasa residual libre de extractivos de ambas plantas fue pirolizada, permitiendo la obtención de los aceites pirolíticos 1 y 2. Después de conocer la composición química y la concentración mínima tóxica (IC_{90}) de cada una de las muestras estudiadas, se evaluó el potencial antiinflamatorio de las muestras en un modelo de inducción de inflamación por IL-17 en queratinocitos HaCaT. El aceite pirolítico-2 de *U.subincisa*, así como el extracto crudo, las fases A-C y los aceites pirolíticos 1 y 2 de *A. adstringens* mostraron un efecto antiinflamatorio. El alto rendimiento de extracción, la baja toxicidad y el efecto antiinflamatorio mostrado por el extracto crudo de *A. adstringens* permitieron seleccionarlo como la muestra más prometedora.

VIII. CONCLUSIÓN GENERAL

El extracto acuoso, las fracciones y el aceite pirolítico-2 derivados de la corteza de *A. adstringens*, así como el aceite pirolítico-2 de *U. subincisa* mostraron efectos anti-IL17 en el modelo de inducción en queratinocitos humanos HaCaT. El extracto acuoso de *A. adstringens* fue considerado el candidato más prometedor dado su alto rendimiento de extracción, baja toxicidad y acción anti-IL17; lo que respalda su uso tradicional por vía tópica reportado por la población Purépecha, al menos en el modelo *in vitro* empleado.

IX. PERSPECTIVAS

La investigación presentada podrá probablemente contribuir al desarrollo de nuevas terapias utilizando extractos convencionales y aceites pirolíticos de especies utilizadas en la etnomedicina Purépecha para tratar la psoriasis, lo que podría tener repercusiones significativas en la calidad de vida y la salud de los pacientes afectados con esta enfermedad.

X. BIBLIOGRAFÍA

Abreu Guirado, O. A., & Cuéllar Cuéllar, A. (2008). Estrategias en la selección de las plantas medicinales a investigar. *Revista Cubana de Plantas Medicinales*, *13*(3), 0-0.

Adami, S., Cavani, A., Rossi, F., & Girolomoni, G. (2014). The role of interleukin-17A in psoriatic disease. *BioDrugs*, *28*(6), 487-497. https://doi.org/10.1007/s40259-014-0098-x

Afolayan, A. J., Grierson, D. S., & Mbeng, W. O. (2014). Ethnobotanical survey of medicinal plants used in the management of skin disorders among the Xhosa communities of the Amathole District, Eastern Cape, South Africa. *Journal of Ethnopharmacology*, *153*(1), 220-232. https://doi.org/10.1016/j.jep.2014.02.023

Alonso-Castro, A. J., Domínguez, F., Maldonado-Miranda, J. J., Castillo-Pérez, L. J., Carranza-Álvarez, C., Solano, E., Isiordia-Espinoza, M. A., del Carmen Juárez-Vázquez, M., Zapata-Morales, J. R., Argueta-Fuertes, M. A., Ruiz-Padilla, A. J., Solorio-Alvarado, C. R., Rangel-Velázquez, J. E., Ortiz-Andrade, R., González-Sánchez, I., Cruz-Jiménez, G., & Orozco-Castellanos, L. M. (2017). Use of medicinal plants by health professionals in Mexico. *Journal of Ethnopharmacology*, *198*, 81-86. https://doi.org/10.1016/j.jep.2016.12.038

Amatya, N., Garg A. V., Gaffen S. L. (2017). IL-17 signaling: the yin and the yang. *Trends in immunology*, *38*(5), 310-322. https://doi.org/10.1016/j.it.2017.01.006

Arican, O., Aral, M., Sasmaz, S., & Ciragil, P. (2005). Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. *Mediators of Inflammation*, *2005*(5), 273-279. https://doi.org/10.1155/MI.2005.273

Armstrong, A. W., Robertson, A. D., Wu, J., Schupp, C., & Lebwohl, M. G. (2013). Undertreatment, treatment trends, and treatment dissatisfaction among patients with psoriasis and psoriatic arthritis in the United States: Findings from the National Psoriasis Foundation surveys, 2003-2011. *JAMA Dermatology*, *149*(10), 1180-1185. https://doi.org/10.1001/jamadermatol.2013.5264

Arora, N., Shah, K., & Pandey-Rai, S. (2016). Inhibition of imiquimod-induced psoriasis-like dermatitis in mice by herbal extracts from some Indian medicinal plants. *Protoplasma*, *253*(2), 503-515. https://doi.org/10.1007/s00709-015-0829-y

Bai, F., Zheng, W., Dong, Y., Wang, J., Garstka, M. A., Li, R., An, J., & Ma, H. (2017). Serum levels of adipokines and cytokines in psoriasis patients: A systematic review and meta-analysis. *Oncotarget*, *9*(1), 1266-1278. https://doi.org/10.18632/oncotarget.22260

BDMTM. (2009). *Biblioteca Digital de la Medicina Tradicional Mexicana* [Base de datos]. Biblioteca Digital de la Medicina Tradicional Mexicana - Universidad Nacional Autónoma de México. http://www.medicinatradicionalmexicana.unam.mx/index.php

Bello-González, M. Á., Hernández-Muñoz, S., Lara-Chávez, M. B. N., & Salgado-Garciglia, R. (2015). Plantas útiles de la comunidad indígena Nuevo San Juan Parangaricutiro, Michoacán, México. *Polibotánica*, *39*, 175-215. https://doi.org/10.18387/polibotanica.39.10

Bissonnette, R., Nigen, S., Langley, R. G., Lynde, C. W., Tan, J., Fuentes-Duculan, J., & Krueger, J. G. (2014). Increased expression of IL-17A and limited involvement of IL-23 in patients with palmo-plantar (PP) pustular psoriasis or PP pustulosis; results from a randomised controlled trial. *Journal of the European Academy of Dermatology and Venereology*, *28*(10), 1298-1305. https://doi.org/10.1111/jdv.12272

Boehncke, W.-H., & Schön, M. P. (2015). Psoriasis. *The Lancet*, *386*(9997), 983-994. https://doi.org/10.1016/S0140-6736(14)61909-7

Bogaard, E. H. van den, Bergboer, J. G. M., Vonk-Bergers, M., Vlijmen-Willems, I. M. J. J. van, Hato, S. V., Valk, P. G. M. van der, Schröder, J. M., Joosten, I., Zeeuwen, P. L. J. M., & Schalkwijk, J. (2013). Coal tar induces AHR-dependent skin barrier repair in atopic dermatitis. *The Journal of Clinical Investigation*, *123*(2), 917-927. https://doi.org/10.1172/JCI65642

Bruhn, J. G., & Helmstedt, B. (1981). Ethnopharmacology: Objectives, principles and perspectives. *Natural products as medicinal agents*.

Cai, Y., Fleming, C., & Yan, J. (2012). New insights of T cells in the pathogenesis of psoriasis. *Cellular and Molecular Immunology*, *9*(4), 302-309. https://doi.org/10.1038/cmi.2012.15

Calvo, M. I., Akerreta, S., & Cavero, R. Y. (2011). Pharmaceutical ethnobotany in the Riverside of Navarra (Iberian Peninsula). *Journal of Ethnopharmacology*, *135*(1), 22-33. https://doi.org/10.1016/j.jep.2011.02.016

Cervantes-Durán, C., Velázquez-Hernández, M.-E., Valentín-Escalera, J., Bartolomé-Camacho, M., Rodríguez-Orozco, A.-R., & García-Pérez, M.-E. (2020). Small molecules under development for psoriasis: On the

road to the individualized therapies. *Archives of Dermatological Research*. https://doi.org/10.1007/s00403-020-02056-3

Chávez-Sifontes, M., & Domine, M. E. (2013). Lignina, estructura y aplicaciones: Métodos de despolimerización para la obtención de derivados aromáticos de interés industrial. *Avances en Ciencias e Ingeniería*, *4*(4), 15-46.

Chern, E., Yau, D., Ho, J.-C., Wu, W.-M., Wang, C.-Y., Chang, H.-W., & Cheng, Y.-W. (2011). Positive effect of modified Goeckerman regimen on quality of life and psychosocial distress in moderate and severe psoriasis. *Acta Dermato-Venereologica*, *91*(4), 447-451. https://doi.org/10.2340/00015555-1096

Chiricozzi, A., Guttman-Yassky, E., Suárez-Fariñas, M., Nograles, K. E., Tian, S., Cardinale, I., Chimenti, S., & Krueger, J. G. (2011). Integrative responses to IL-17 and TNF-α in human keratinocytes account for key inflammatory pathogenic circuits in psoriasis. *The Journal of Investigative Dermatology*, *131*(3), 677-687. https://doi.org/10.1038/jid.2010.340

Chowdhury, R. R., & Ghosh, S. K. (2012). Phytol-derived novel isoprenoid immunostimulants. *Frontiers in Immunology*, *3*. https://doi.org/10.3389/fimmu.2012.00049

Czernik, S., & Bridgwater, A. V. (2004). Overview of applications of biomass fast pyrolysis oil. *Energy & Fuels*, *18*(2), 590-598. https://doi.org/10.1021/ef034067u

Dhanabal, S. P., Dwarampudi, L. P., Muruganantham, N., & Vadivelan, R. (2012). Evaluation of the antipsoriatic activity of *Aloe vera* leaf extract using a mouse tail model of psoriasis. *Phytotherapy Research*, *26*(4), 617-619. https://doi.org/10.1002/ptr.3589

Di Meglio, P., Duarte, J. H., Ahlfors, H., Owens, N. D. L., Li, Y., Villanova, F., Tosi, I., Hirota, K., Nestle, F. O., Mrowietz, U., Gilchrist, M. J., & Stockinger, B. (2014). Activation of the Aryl Hydrocarbon Receptor Dampens the Severity of Inflammatory Skin Conditions. *Immunity*, *40*(6), 989-1001. https://doi.org/10.1016/j.immuni.2014.04.019

Dimitris, D., Ekaterina-Michaela, T., Christina, K., Ioannis, S., Ioanna, S. K., Aggeliki, L., Sophia, H., Michael, R., & Helen, S. (2020). *Melissa officinalis* ssp. Altissima extracts: A therapeutic approach targeting psoriasis in mice. *Journal of Ethnopharmacology*, *246*, 112208. https://doi.org/10.1016/j.jep.2019.112208

Duan, H., Koga, T., Kohda, F., Hara, H., Urabe, K., & Furue, M. (2001). Interleukin-8-positive neutrophils in psoriasis. *Journal of Dermatological Science*, *26*(2), 119-124. https://doi.org/10.1016/s0923-1811(00)00167-5

Edeas, M. (2008). Les polyphénols et les polyphénols de thé. *Phytothérapie*, *5*(5), 264-270. https://doi.org/10.1007/s10298-007-0268-9

Ekman, A.-K., Bivik Eding, C., Rundquist, I., & Enerbäck, C. (2019). IL-17 and IL-22 Promote Keratinocyte Stemness in the Germinative Compartment in Psoriasis. *Journal of Investigative Dermatology*, *139*(7), 1564-1573.e8. https://doi.org/10.1016/j.jid.2019.01.014

Esquivel-García, R., Estévez-Delgado, G., Rodríguez-Orozco, A.-R., Ochoa-Zarzosa, A., & García-Pérez, M. E. (2018a). La psoriasis: De la investigación básica y clínica al desarrollo de nuevos tratamientos. *Gaceta Médica de México*, *154*(4), 502-508. https://doi.org/10.24875/GMM.17003182

Esquivel-García, R., Pérez-Calix, E., Ochoa-Zarzosa, A., & García-Pérez, M.-E. (2018b). Ethnomedicinal plants used for the treatment of dermatological affections on the Purépecha Plateau, Michoacán, Mexico. *Acta Botanica Mexicana*, *125*, 95-132. https://doi.org/10.21829/abm125.2018.1339

Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*, *109*(Suppl 1), 69-75.

Fleischer, A. B., Feldman, S. R., Rapp, S. R., Reboussin, D. M., Exum, M. L., & Clark, A. R. (1996). Alternative therapies commonly used within a population of patients with psoriasis. *Cutis*, *58*(3), 216-220.

Fu, Y., Ma, J., Shi, X., Song, X.-Y., Yang, Y., Xiao, S., Li, J., Gu, W.-J., Huang, Z., Zhang, J., & Chen, J. (2017). A novel pyrazole-containing indolizine derivative suppresses NF-κB activation and protects against TNBS-induced colitis via a PPAR-γ-dependent pathway. *Biochemical Pharmacology*, *135*, 126-138. https://doi.org/10.1016/j.bcp.2017.03.013

Gallardo Ruiz, J. (2002). *Medicina tradicional P'urhépecha*. El Colegio de Michoacán A.C.

Gami, A. A. (2014). Phenol and its toxicity. *Journal of Environmental Microbiology and Toxicology*, *2*(1), Article 1. https://journal.hibiscuspublisher.com/index.php/JEMAT/article/view/89

García, A. Á., & Carril, E. P.-U. (2011). Metabolismo secundario de plantas. *REDUCA (Biología)*, *2*(3), Article 3. http://revistareduca.es/index.php/biologia/article/view/798

García-Pérez, M. E., Allaeys, I., Rusu, D., Pouliot, R., Janezic, T. S., & Poubelle, P. E. (2014). Picea mariana polyphenolic extract inhibits phlogogenic mediators produced by TNF-α-activated psoriatic keratinocytes:

Impact on NF-κB pathway. *Journal of Ethnopharmacology*, *151*(1), 265-278. https://doi.org/10.1016/j.jep.2013.10.034

García-Pérez, M. E., Jean, J., & Pouliot, R. (2012). Antipsoriatic drug development: Challenges and new emerging therapies. *Recent Patents on Inflammation & Allergy Drug Discovery*, *6*(1), 3-21. https://doi.org/10.2174/187221312798889248

Gellerstedt, G., & Henriksson, G. (2008). Chapter 9 - Lignins: Major sources, structure and properties. En M. N. Belgacem & A. Gandini (Eds.), *Monomers, Polymers and Composites from Renewable Resources* (pp. 201-224). Elsevier. https://doi.org/10.1016/B978-0-08-045316-3.00009-0

Gendrisch, F., Nováčková, A., Sochorová, M., Haarhaus, B., Vávrová, K., Schempp, C. M., & Wölfle, U. (2020). *Gentiana lutea* extract modulates ceramide synthesis in primary and psoriasis-Like keratinocytes. *Molecules*, *25*(8). https://doi.org/10.3390/molecules25081832

Gertsch, J. (2009). How scientific is the science in ethnopharmacology? Historical perspectives and epistemological problems. *Journal of Ethnopharmacology*, *122*(2), 177-183. https://doi.org/10.1016/j.jep.2009.01.010

Ghosh, S. K., & Chowdhury, R. R. (2013). Synthetic adjuvants for vaccine formulations: Phytol derivatives. *Expert Opinion on Drug Delivery*, *10*(4), 437-450. https://doi.org/10.1517/17425247.2013.757591

Gómez-Fuentes-Galindo, T., González-Rebeles, C., López-Ortiz, S., Ku-Vera, J. C., Albor-Pinto, C. de J., Sangines-García, J. R., Gómez-Fuentes-Galindo, T., González-Rebeles, C., López-Ortiz, S., Ku-Vera, J. C., Albor-Pinto, C. de J., & Sangines-García, J. R. (2017). Dominancia, composición química-nutritiva de especies forrajearas y fitomasa potencial en una selva secundaria. *Agricultura, sociedad y desarrollo*, *14*(4), 617-634.

Griffiths, C. E. M., Strober, B. E., van de Kerkhof, P., Ho, V., Fidelus-Gort, R., Yeilding, N., Guzzo, C., Xia, Y., Zhou, B., Li, S., Dooley, L. T., Goldstein, N. H., Menter, A., & ACCEPT Study Group. (2010). Comparison of ustekinumab and etanercept for moderate-to-severe psoriasis. *The New England Journal of Medicine*, *362*(2), 118-128. https://doi.org/10.1056/NEJMoa0810652

Gulliver, W. P., & Donsky, H. J. (2005). A report on three recent clinical trials using *Mahonia aquifolium* 10% topical cream and a review of the worldwide clinical experience with *Mahonia aquifolium* for the treatment of plaque psoriasis. *American Journal of Therapeutics*, *12*(5), 398-406. https://doi.org/10.1097/01.mjt.0000174350.82270.da

Gupta, A., Nagariya, A. K., Mishra, A. K., Bansal, P., Kumar, S., Gupta, V., & Singh, A. K. (2010). Ethno-potential of medicinal herbs in skin diseases: An overview. *Journal of Pharmacy Research*, *3*(3), 435-441.

Guttman-Yassky, E., & Krueger, J. G. (2007). Psoriasis: Evolution of pathogenic concepts and new therapies through phases of translational research. *The British Journal of Dermatology*, *157*(6), 1103-1115. https://doi.org/10.1111/j.1365-2133.2007.08135.x

Harvey, A. L., Edrada-Ebel, R., & Quinn, R. J. (2015). The re-emergence of natural products for drug discovery in the genomics era. *Nature Reviews Drug Discovery*, *14*(2), 111-129. https://doi.org/10.1038/nrd4510

Heinrich, M., Edwards, S., Moerman, D. E., & Leonti, M. (2009). Ethnopharmacological field studies: A critical assessment of their conceptual basis and methods. *Journal of Ethnopharmacology*, *124*(1), 1-17. https://doi.org/10.1016/j.jep.2009.03.043

Heinrich, M., Frei Haller, B., & Leonti, M. (2014). A perspective on natural products research and ethnopharmacology in Mexico: The eagle and the serpent on the prickly pear cactus. *Journal of Natural Products*, *77*(3), 678-689. https://doi.org/10.1021/np4009927

Heinrich, M., & Gibbons, S. (2001). Ethnopharmacology in drug discovery: An analysis of its role and potential contribution. *Journal of Pharmacy and Pharmacology*, *53*(4), 425. https://doi.org/10.1211/0022357011775712

Hert, J., Irwin, J. J., Laggner, C., Keiser, M. J., & Shoichet, B. K. (2009). Quantifying biogenic bias in screening libraries. *Nature chemical biology*, *5*(7), 479-483. https://doi.org/10.1038/nchembio.180

Hsu, S., Dickinson, D., Borke, J., Walsh, D. S., Wood, J., Qin, H., Winger, J., Pearl, H., Schuster, G., & Bollag, W. B. (2007). Green tea polyphenol induces caspase 14 in epidermal keratinocytes via MAPK pathways and reduces psoriasiform lesions in the flaky skin mouse model. *Experimental Dermatology*, *16*(8), 678-684. https://doi.org/10.1111/j.1600-0625.2007.00585.x

International Psoriasis Council. (2009). *IPC Psoriasis Review*. *5*, 1-12. http://www.psoriasiscouncil.org/docs/ipcpsoriasisreview_dec_2009_english.pdf?LanguageID=EN-US

Jírová, D., Basketter, D., Liebsch, M., Bendová, H., Kejlová, K., Marriott, M., & Kandárová, H. (2010). Comparison of human skin irritation patch test data with in vitro skin irritation assays and animal data. *Contact Dermatitis*, *62*(2), 109-116. https://doi.org/10.1111/j.1600-0536.2009.01640.x

Johansen, C., Usher, P. A., Kjellerup, R. B., Lundsgaard, D., Iversen, L., & Kragballe, K. (2009). Characterization of the interleukin-17 isoforms and receptors in lesional psoriatic skin. *The British Journal of Dermatology*, *160*(2), 319-324. https://doi.org/10.1111/j.1365-2133.2008.08902.x

Kagoura, M., Matsui, C., & Morohashi, M. (1999). Phytol is a novel tumor promoter on ICR mouse skin. *Japanese Journal of Cancer Research: Gann*, *90*(4), 377-384. https://doi.org/10.1111/j.1349-7006.1999.tb00758.x

Kim, H., Kim, J. Y., Song, H. S., Park, K. U., Mun, K.-C., & Ha, E. (2011). Grape seed proanthocyanidin extract inhibits interleukin-17-induced interleukin-6 production via MAPK pathway in human pulmonary epithelial cells. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *383*(6), 555-562. https://doi.org/10.1007/s00210-011-0633-y

Kim, W. B., Jerome, D., & Yeung, J. (2017). Diagnosis and management of psoriasis. *Canadian Family Physician*, *63*(4), 278-285.

King, L., Jurima-Romet, M., & Ichhpurani, N. (2010). Biomarkers in pharmaceutical development: The essential role of project management and teamwork. En *Pharmaceutical Sciences Encyclopedia* (pp. 1-22). American Cancer Society. https://doi.org/10.1002/9780470571224.pse278

Koo, J., & Lebwohl, M. (1999). Duration of remission of psoriasis therapies. *Journal of the American Academy of Dermatology*, *41*(1), 51-59. https://doi.org/10.1016/s0190-9622(99)70406-8

Kviecinski, M. R., David, I. M. B., Fernandes, F. de S., Correa, M. dos R., Clarinda, M. M., Freitas, A. F., da Silva, J., Gava, M., Müller, S. D., Florentino, D., Petronilho, F., Moterle, D., Kanis, L. A., & Pedrosa, R. C. (2016). Healing effect of *Dillenia indica* fruit extracts standardized to betulinic acid on ultraviolet radiation-induced psoriasis-like wounds in rats. *Pharmaceutical Biology*, *55*(1), 641-648. https://doi.org/10.1080/13880209.2016.1266672

Lapolla, W., Yentzer, B. A., Bagel, J., Halvorson, C. R., & Feldman, S. R. (2011). A review of phototherapy protocols for psoriasis treatment. *Journal of the American Academy of Dermatology*, *64*(5), 936-949. https://doi.org/10.1016/j.jaad.2009.12.054

Lebwohl, M. (2016). Psoriasis therapy: Breakthroughs in pharmacogenomics or in pharmacology? *Journal of Investigative Dermatology*, *136*(12), 2339-2340. https://doi.org/10.1016/j.jid.2016.07.019

Lee, D. H., & Lee, C. S. (2016). Flavonoid myricetin inhibits TNF-α-stimulated production of inflammatory mediators by suppressing the Akt, mTOR and NF-κB pathways in human keratinocytes. *European Journal of Pharmacology*, *784*, 164-172. https://doi.org/10.1016/j.ejphar.2016.05.025

Lee, E., & Koo, J. (2005). Modern modified 'ultra' Goeckerman therapy: A PASI assessment of a very effective therapy for psoriasis resistant to both prebiologic and biologic therapies. *Journal of Dermatological Treatment*, *16*(2), 102-107. https://doi.org/10.1080/09546630510033140

Lee, S. Y., Nam, S., Hong, I. K., Kim, H., Yang, H., & Cho, H.-J. (2018). Antiproliferation of keratinocytes and alleviation of psoriasis by the ethanol extract of *Artemisia capillaris*. *Phytotherapy Research*, *32*(5), 923-932. https://doi.org/10.1002/ptr.6032

Liaw, S.-S., Zhou, S., Wu, H., & Garcia-Perez, M. (2013). Effect of pretreatment temperature on the yield and properties of bio-oils obtained from the auger pyrolysis of Douglas fir wood. *Fuel*, *103*, 672-682. https://doi.org/10.1016/j.fuel.2012.08.016

Lowes, M. A., Bowcock, A. M., & Krueger, J. G. (2007). Pathogenesis and therapy of psoriasis. *Nature*, *445*(7130), 866-873. https://doi.org/10.1038/nature05663

Lowes, M. A., Suárez-Fariñas, M., & Krueger, J. G. (2014). Immunology of psoriasis. *Annual Review of Immunology*, *32*, 227-255. https://doi.org/10.1146/annurev-immunol-032713-120225

Lv, J., Zhou, D., Wang, Y., Zhao, J., Chen, Z., Zhang, J., Di, T., Hu, J., Li, B., Li, P., & Huang, F. (2018). Quantitative evaluation to efficacy and safety of therapies for psoriasis: A network meta-analysis. *Molecular Pain*, *14*, 1744806918762205. https://doi.org/10.1177/1744806918762205

Mata, R., Figueroa, M., Navarrete, A., & Rivero-Cruz, I. (2019). Chemistry and Biology of Selected Mexican Medicinal Plants. En A. D. Kinghorn, H. Falk, S. Gibbons, J. Kobayashi, Y. Asakawa, & J.-K. Liu (Eds.), *Progress in the Chemistry of Organic Natural Products 108* (pp. 1-142). Springer International Publishing. https://doi.org/10.1007/978-3-030-01099-7_1

Medina, C. (2003). La flora: Riqueza, diversidad y sus relaciones fitogeográficas. En *Las enseñanzas de San Juan* (1a ed., pp. 165–200). INE-SEMARNAT. http://www2.inecc.gob.mx/publicaciones2/libros/420/ocho.html

Michalek, I. M., Loring, B., & John, S. M. (2017). A systematic review of worldwide epidemiology of psoriasis. *Journal of the European Academy of Dermatology and Venereology*, *31*(2), 205-212. https://doi.org/10.1111/jdv.13854

Miquel, J., Bernd, A., Sempere, J. M., Díaz-Alperi, J., & Ramírez, A. (2002). The curcuma antioxidants: Pharmacological effects and prospects for future clinical use. A review. *Archives of Gerontology and Geriatrics*, *34*(1), 37-46.

Mohan, D., Pittman, Charles U., & Steele, P. H. (2006). Pyrolysis of wood/biomass for bio-oil: A critical review. *Energy & Fuels*, *20*(3), 848-889. https://doi.org/10.1021/ef0502397

Montoya Arbeláez, J. I., Chejne Janna, F., Castillo Monroy, E. F., Acero Reyes, J. R., Gómez Gutiérrez, C. A., Sarmiento Chaparro, J. A., Valdés Rentería, C. F., Garzón Fuentes, L. L., Osorio Velasco, J., Fernando Tirado, D., Blanco, L. A., Moreno Safra, N., Marrugo, G., & Ospina Aguirre, E. Y. (2013). *Pirólisis rápida de biomasa* (1.ª ed.). Universidad Nacional de Colombia.

Moos, S., Mohebiany, A. N., Waisman, A., & Kurschus, F. C. (2019). Imiquimod-induced psoriasis in mice depends on the IL-17 signaling of keratinocytes. *Journal of Investigative Dermatology*, *139*(5), 1110-1117. https://doi.org/10.1016/j.jid.2019.01.006

Moscaliuc, M. L., Heller, M. M., Lee, E. S., & Koo, J. (2013). Goeckerman therapy: A very effective, yet often forgotten treatment for severe generalized psoriasis. *Journal of Dermatological Treatment*, *24*(1), 34-37. https://doi.org/10.3109/09546634.2012.658014

Moss, G. P. (2000). Nomenclature of lignans and neolignans (IUPAC recommendations 2000). *Pure and Applied Chemistry*, *72*(8), 1493-1523. https://doi.org/10.1351/pac200072081493

Müller, K., Ziereis, K., & Gawlik, I. (1995). The antipsoriatic *Mahonia aquifolium* and its active constituents; II. Antiproliferative activity against cell growth of human keratinocytes. *Planta Medica*, *61*(1), 74-75. https://doi.org/10.1055/s-2006-958005

Murray, A. R., Kisin, E., Castranova, V., Kommineni, C., Gunther, M. R., & Shvedova, A. A. (2007). Phenol-induced *in vivo* oxidative stress in skin: Evidence for enhanced free radical generation, thiol oxidation, and antioxidant depletion. *Chemical Research in Toxicology*, *20*(12), 1769-1777. https://doi.org/10.1021/tx700201z

Nada, A., Al-Moghazy, M., Soliman, A. A. F., Rashwan, G. M. T., Eldawy, T. H. A., Hassan, A. A. E., & Sayed, G. H. (2018). Pyrazole-based compounds in chitosan liposomal emulsion for antimicrobial cotton fabrics. *International Journal of Biological Macromolecules*, *107*(Pt A), 585-594. https://doi.org/10.1016/j.ijbiomac.2017.09.031

Newby, C. S., Barr, R. M., Greaves, M. W., & Mallet, A. I. (2000). Cytokine release and cytotoxicity in human keratinocytes and fibroblasts induced by phenols and sodium dodecyl sulfate. *Journal of Investigative Dermatology*, *115*(2), 292-298. https://doi.org/10.1046/j.1523-1747.2000.00056.x

Njoroge, G. N., & Bussmann, R. W. (2007). Ethnotherapeautic management of skin diseases among the Kikuyus of Central Kenya. *Journal of Ethnopharmacology*, *111*(2), 303-307. https://doi.org/10.1016/j.jep.2006.11.025

Novaes, E., Kirst, M., Chiang, V., Winter-Sederoff, H., & Sederoff, R. (2010). Lignin and Biomass: A Negative Correlation for Wood Formation and Lignin Content in Trees. *Plant Physiology*, *154*(2), 555-561. https://doi.org/10.1104/pp.110.161281

N-S Hon, D., & Shiraishi, N. (2000). *Wood and cellulosic chemistry*. Marcel Dekker Inc. https://www.nhbs.com/wood-and-cellulosic-chemistry-book

Oasmaa, A., Fonts, I., Pelaez-Samaniego, M. R., Garcia-Perez, M. E., & Garcia-Perez, M. (2016). Pyrolysis Oil Multiphase Behavior and Phase Stability: A Review. *Energy & Fuels*, *30*(8), 6179-6200. https://doi.org/10.1021/acs.energyfuels.6b01287

Patwardhan, B. (2005). Ethnopharmacology and drug discovery. *Journal of Ethnopharmacology*, *100*(1-2), 50-52. https://doi.org/10.1016/j.jep.2005.06.006

Prashantkumar, P., & Vidyasagar, G. M. (2008). Traditional knowledge on medicinal plants used for the treatment of skin diseases in Bidar district, Karnataka. *Indian Journal of Traditional Knowledge*, *7*(2), 273-276.

Rapp, S. R., Feldman, S. R., Exum, M. L., Fleischer, A. B., & Reboussin, D. M. (1999). Psoriasis causes as much disability as other major medical diseases. *Journal of the American Academy of Dermatology*, *41*(3), 401-407. https://doi.org/10.1016/S0190-9622(99)70112-X

Roy Chowdhury, R., Fitch, R. W., & Ghosh, S. K. (2013). Efficacy of phytol-derived diterpenoid immunoadjuvants over alum in shaping the murine host's immune response to *Staphylococcus aureus*. *Vaccine*, *31*(8), 1178-1186. https://doi.org/10.1016/j.vaccine.2012.12.069

Sakkas, L. I., & Bogdanos, D. P. (2017). Are psoriasis and psoriatic arthritis the same disease? The IL-23/IL-17 axis data. *Autoimmunity Reviews*, *16*(1), 10-15. https://doi.org/10.1016/j.autrev.2016.09.015

Scholze, B., & Meier, D. (2001). Characterization of the water-insoluble fraction from pyrolysis oil (pyrolytic lignin). Part I. PY–GC/MS, FTIR, and functional groups. *Journal of Analytical and Applied Pyrolysis*, *60*(1), 41-54. https://doi.org/10.1016/S0165-2370(00)00110-8

Schultz, J. C., Hunter, M. D., & Appel, H. M. (1992). Antimicrobial activity of polyphenols mediates plant-herbivore interactions. En R. W. Hemingway & P. E. Laks (Eds.), *Plant Polyphenols: Synthesis, Properties, Significance* (pp. 621-637). Springer US. https://doi.org/10.1007/978-1-4615-3476-1_35

Sekhon, S., Jeon, C., Nakamura, M., Afifi, L., Yan, D., Wu, J. J., Liao, W., & Bhutani, T. (2018). Review of the mechanism of action of coal tar in psoriasis. *Journal of Dermatological Treatment*, *29*(3), 230-232. https://doi.org/10.1080/09546634.2017.1369494

Seki, N., Shimano, K., Kondou, T., Murase, M., Mitamura, M., Kataoka, H., Sugahara, K., Nakamura, H., & Yamada, M. (2014). OP0105 Pyrazole-anilide derivatives, a new class of immunomodulator which inhibits IL-17 production, ameliorate CIA and psoriasis-like dermatitis in mice. *Annals of the Rheumatic Diseases*, *73*(Suppl 2), 100-101. https://doi.org/10.1136/annrheumdis-2014-eular.3054

Sharma, J., Gairola, S., Sharma, Y. P., & Gaur, R. D. (2014). Ethnomedicinal plants used to treat skin diseases by Tharu community of district Udham Singh Nagar, Uttarakhand, India. *Journal of Ethnopharmacology*, *158*(Part A), 140-206. https://doi.org/10.1016/j.jep.2014.10.004

Shen, T., Wang, X.-N., & Lou, H.-X. (2009). Natural stilbenes: An overview. *Natural Product Reports*, *26*(7), 916-935. https://doi.org/10.1039/b905960a

Steinmann, V. W. (2005). Urticaceae. *Flora del Bajío y de Regiones Adyacentes*, *134*, 1-74.

Stevanovic, T. (2005). *Chimie du bois*. U. Laval.

Stevanovic, T., Diouf, P. N., & Garcia-Perez, M. E. (2009). Bioactive polyphenols from healthy diets and forest biomass. *Current Nutrition & Food Science*, *5*(4), 264-295.

Stevanovic, T., & Perrin, D. (2009). Extractives. En *Chimie du bois*. EPFL PRESS. https://www.epflpress.org/produit/448/9782880747992/chimie-du-bois

Storey, A., McArdle, F., Friedmann, P. S., Jackson, M. J., & Rhodes, L. E. (2005). Eicosapentaenoic acid and docosahexaenoic acid reduce UVB- and TNF-α-induced IL-8 secretion in keratinocytes and UVB-induced IL-8 in fibroblasts. *Journal of Investigative Dermatology*, *124*(1), 248-255. https://doi.org/10.1111/j.0022-202X.2004.23543.x

Svendsen, M. T., Jeyabalan, J., Andersen, K. E., Andersen, F., & Johannessen, H. (2017). Worldwide utilization of topical remedies in treatment of psoriasis: A systematic review. *The Journal of Dermatological Treatment*, *28*(5), 374-383. https://doi.org/10.1080/09546634.2016.1254331

Sykes, R., Yung, M., Novaes, E., Kirst, M., Peter, G., & Davis, M. (2009). High-throughput screening of plant cell-wall composition using pyrolysis molecular beam mass spectroscopy. *Methods in Molecular Biology (Clifton, N.J.)*, *581*, 169-183. https://doi.org/10.1007/978-1-60761-214-8_12

Talbott, W., & Duffy, N. (2015). Complementary and alternative medicine for psoriasis: What the dermatologist needs to know. *American Journal of Clinical Dermatology*, *16*(3), 147-165. https://doi.org/10.1007/s40257-015-0128-6

Terui, T., Ozawa, M., & Tagami, H. (2000). Role of neutrophils in induction of acute inflammation in T-cell-mediated immune dermatosis, psoriasis: A neutrophil-associated inflammation-boosting loop. *Experimental Dermatology*, *9*(1), 1-10. https://doi.org/10.1034/j.1600-0625.2000.009001001.x

Turner, J. R. (2010). *New Drug Development—An Introduction to Clinical Trials*. http://www.springer.com/la/book/9781441964175

van de Kerkhof, P. C. M. (2008). Options for the treatment of psoriasis: A multifactorial approach. *Clinics in Dermatology*, *26*(5), 419-423. https://doi.org/10.1016/j.clindermatol.2007.10.029

Vijayalakshmi, A., Priyanka, M., Priyadharshini, S., Kumar, S. S., & Jayakumari, S. (2019). Antipsoriatic activity of *Cassia auriculata* L. flowers in Freund's adjuvant-formaldehyde induced animal model. *IJNPR Vol.10(4) [December 2019]*. http://nopr.niscair.res.in/handle/123456789/54201

Vijayalakshmi, A., Ravichandiran, V., Malarkodi, V., Nirmala, S., & Jayakumari, S. (2012). Screening of flavonoid "quercetin" from the rhizome of *Smilax china* Linn. For anti-psoriatic activity. *Asian Pacific Journal of Tropical Biomedicine*, *2*(4), 269-275. https://doi.org/10.1016/S2221-1691(12)60021-5

Woolf, R. T., & Smith, C. H. (2010). How genetic variation affects patient response and outcome to therapy for psoriasis. *Expert Review of Clinical Immunology*, *6*(6), 957-966. https://doi.org/10.1586/eci.10.74

Yau, A. C. Y., Lönnblom, E., Zhong, J., & Holmdahl, R. (2017). Influence of hydrocarbon oil structure on adjuvanticity and autoimmunity. *Scientific Reports*, *7*(1), 14998. https://doi.org/10.1038/s41598-017-15096-z

Zablocki, J. A., Tarlton, E., Rizzi, J. P., & Mantlo, N. B. (2003). *Aryl and heteroaryl substituted fused pyrrole anti-inflammatory agents* (United States Patent N.º US6605634B2). https://patents.google.com/patent/US6605634B2/en

Zeichner, J. A. (2010). Use of topical coal tar foam for the treatment of psoriasis in difficult-to-treat areas. *The Journal of Clinical and Aesthetic Dermatology*, *3*(9), 37-40.

Zhang, S., Liu, X., Mei, L., Wang, H., & Fang, F. (2016). Epigallocatechin-3-gallate (EGCG) inhibits imiquimod-induced psoriasis-like inflammation of BALB/c mice. *BMC Complementary and Alternative Medicine*, *16*(1), 334. https://doi.org/10.1186/s12906-016-1325-4

Zhang, X., Rakesh, K. P., Shantharam, C. S., Manukumar, H. M., Asiri, A. M., Marwani, H. M., & Qin, H.-L. (2018). Podophyllotoxin derivatives as an excellent anticancer aspirant for future chemotherapy: A key current imminent needs. *Bioorganic & Medicinal Chemistry*, *26*(2), 340-355. https://doi.org/10.1016/j.bmc.2017.11.026

Zhu, T. H., Nakamura, M., Farahnik, B., Abrouk, M., Singh, R. K., Lee, K. M., Hulse, S., Koo, J., Bhutani, T., & Liao, W. (2016). The patient's guide to psoriasis treatment. Part 4: Goeckerman therapy. *Dermatology and Therapy*, *6*(3), 333-339. https://doi.org/10.1007/s13555-016-0132-7

Ziboh, V. A., Miller, C. C., & Cho, Y. (2000). Metabolism of polyunsaturated fatty acids by skin epidermal enzymes: Generation of antiinflammatory and antiproliferative metabolites. *The American Journal of Clinical Nutrition*, *71*(1), 361s-366s. https://doi.org/10.1093/ajcn/71.1.361s

XI. ANEXOS

XI.1. ANEXO 1 - La psoriasis: de la investigación básica y clínica al desarrollo de nuevos tratamientos

XI.1.1. PREFACIO

Este anexo corresponde a un artículo de revisión titulado “La psoriasis: de la investigación básica y clínica al desarrollo de nuevos tratamientos”. El cual fue publicado en *Gaceta Médica de México*. 154: 502-508. Su identificador de objeto digital es: https://doi.org/10.24875/GMM.17003182

El artículo consiste en una revisión de la información científica para analizar el impacto de la medicina traslacional en el desarrollo de medicamentos antipsoriásicos asociados a nuevas dianas farmacológicas.

La psoriasis: de la investigación básica y clínica al desarrollo de nuevos tratamientos

Roberto Esquivel-García,[1] Gabino Estévez-Delgado,[1] Alain Raimundo Rodríguez-Orozco,[2] Alejandra Ochoa-Zarzosa[3] y Martha Estrella García-Pérez[1]

[1]Facultad de Químico-Farmacobiología, [2]Facultad de Ciencias Médicas y Biológicas, [3]Facultad de Medicina Veterinaria y Zootecnia, Centro Multidisciplinario de Estudios en Biotecnología. Universidad Michoacana de San Nicolás de Hidalgo. Michoacán, México

Resumen

La psoriasis es una enfermedad cutánea incurable que afecta a 2.9 % de la población mexicana, por lo que es trascendente analizar el impacto de la medicina traslacional en el desarrollo de medicamentos antipsoriásicos. En esta revisión se discuten conceptos etiopatogénicos de la enfermedad y se analizan artículos publicados entre 2005 y 2017 en torno a medicamentos en desarrollo, además, se presenta un análisis crítico sobre las perspectivas futuras en el desarrollo de nuevos tratamientos. El uso de estrategias bidireccionales de la medicina traslacional ha permitido incrementar significativamente el número de tratamientos antipsoriásicos disponibles. Se encontraron 18 nuevos fármacos en exploración. La caracterización de antígenos responsables de la activación inmunológica, la identificación de biomarcadores predictivos de eficacia farmacológica, el desarrollo de modelos más representativos de la enfermedad, así como la integración de aspectos farmacogenómicos a estrategias de medicina traslacional fueron identificados como elementos relevantes que deben ser incorporados en el desarrollo de nuevas opciones terapéuticas.

PALABRAS CLAVE: *Psoriasis. Objetivos farmacológicos. Medicamentos. Medicina traslacional.*

Abstract

Psoriasis is an incurable cutaneous disease that affects 2.9% of the Mexican population, and it is therefore important for the impact of translational medicine on the development of anti-psoriatic drugs to be analyzed. In this review, current etiopathogenic concepts of the disease are discussed, and articles on drugs under development published between 2005 and 2017 are reviewed; in addition, a critical analysis on future perspectives for the development new treatments is presented. The use of translational medicine bi-directional strategies of has allowed to significantly increase the number of available anti-psoriatic therapies. Eighteen new investigational drugs were found. Characterization of antigens responsible for immune activation, identification of predictive biomarkers with pharmacologic efficacy, and the development of more representative disease models, as well as the integration of pharmacogenomic aspects to translational medicine strategies were identified as relevant aspects that should be incorporated in the development of new therapeutic options.

KEY WORDS: *Psoriasis Pharmacological targets. Drugs. Translational medicine*

Correspondencia:
Martha Estrella García-Pérez
E-mail: margarc@live.ca

Fecha de recepción 13-01-2017
Fecha de aceptación 27-09-2017
DOI: http://dx.doi.org/10.24875/GMM.17003182

Gac Med Mex. 2018;154:502-508
Disponible en PubMed
www.gacetamedicademexico.com

Introducción

La psoriasis es una enfermedad cutánea incurable de naturaleza inflamatoria caracterizada por placas eritematosas. Se estima que afecta de 2 a 3 % de la población mundial; es menos frecuente en las regiones ecuatoriales que en las nórdicas.[1] En México hay pocos estudios epidemiológicos sobre la enfermedad, por lo que la magnitud real del problema sigue siendo desconocida. En un simposio celebrado en 2009 en Dallas, Texas, se estimó que la prevalencia en México sería de 2.9 %,[2] lo que equivaldría a más de 3 millones de mexicanos afectados. La incidencia de muerte debida directamente a la psoriasis es baja, pero sus manifestaciones físicas y psicológicas la hacen incapacitante, comparable al cáncer, la diabetes y la depresión.[3]

Los tratamientos antipsoriásicos actuales se dirigen fundamentalmente al manejo de los síntomas e incluyen fototerapia, así como medicamentos tópicos y sistémicos orientados a:[4]

- Inhibir la hiperproliferación de los queratinocitos (análogos de la vitamina D).
- Normalizar el proceso de diferenciación de los queratinocitos (análogos de la vitamina D, retinoides).
- Disminuir el reclutamiento y activación de los inmunocitos (ciclosporina A, medicamentos biotecnológicos).
- Neutralizar las citocinas proinflamatorias (medicamentos biotecnológicos).

Sin embargo, su utilización a largo plazo está limitada por la pérdida de eficacia, la toxicidad y los elevados costos. Como consecuencia, de 31 a 70 % de los pacientes con psoriasis se encuentran altamente insatisfechos.[5] La industria farmacéutica está cada vez más interesada en el desarrollo de nuevos tratamientos.

En los últimos años ha nacido el término "medicina traslacional", que se refiere a un nuevo paradigma basado en la necesidad de integrar los conocimientos científicos que se generan durante la investigación básica al desarrollo de tratamientos con impacto clínico (*bench-to-bedside*). Se trata de un concepto bidireccional, donde los nuevos hallazgos derivados de la investigación clínica sirven de base para la generación de hipótesis científicas utilizadas en ciencia fundamental (*bedside-to-bench*). Bajo este modelo se utilizan saberes derivados de la genómica, proteómica, medicina y farmacología en aras de conocer con mayor profundidad la fisiopatología de las enfermedades, definir los posibles objetivos farmacológicos y obtener moléculas prometedoras, aportando una visión realista orientada a la transferencia de conocimientos entre diferentes áreas del conocimiento, para generar fármacos cada vez más eficaces.

La psoriasis constituye un excelente ejemplo de la importancia de la medicina traslacional para el desarrollo de nuevos tratamientos. De hecho, los primeros tratamientos antipsoriásicos, como la fototerapia o la ciclosporina, se desarrollaron a partir de una aproximación empírica que partió de la evidencia clínica. Esto conllevó a la generación de hipótesis etiopatogénicas elucidadas posteriormente en investigación básica.[6]

En la actualidad, el desarrollo de tratamientos para la psoriasis ha experimentado un cambio de paradigma, promovido por un mayor conocimiento sobre las causas de la enfermedad, lo que se ha evidenciado con la salida al mercado de medicamentos biotecnológicos dirigidos a neutralizar quimiocinas y citocinas específicas.

La presente revisión tiene como objetivo analizar el impacto de la medicina traslacional en el desarrollo de medicamentos antipsoriásicos. La fisiopatología de la psoriasis se examina con base en los conceptos actuales. Además, se explora la literatura científica sobre nuevos medicamentos en desarrollo y sus objetivos farmacológicos para ofrecer una visión actualizada del desarrollo farmacéutico en esta patología. Finalmente, se presenta un análisis crítico sobre las perspectivas futuras en el desarrollo de nuevos tratamientos para esta enfermedad.

Conceptos etiopatogénicos actuales de la psoriasis

La etiología de la psoriasis es compleja y multifactorial, ya que implica la interacción compleja entre las células constitutivas de la piel y las respuestas innata y adaptativa del sistema inmunológico (Figura 1).[7] Las primeras hipótesis consideraban que el defecto primario causante de la enfermedad consistía en los queratinocitos. De hecho, hasta hoy día se considera que la hiperproliferación de estas células es una característica distintiva de la patología. La división rápida de los queratinocitos psoriásicos comparativamente a los normales (siete a 10 días *versus* 28 a 50 días) conlleva aumento del grosor de la epidermis y cambios en la expresión de los marcadores de diferenciación.[8] La capa granulosa de la piel, donde comienza la

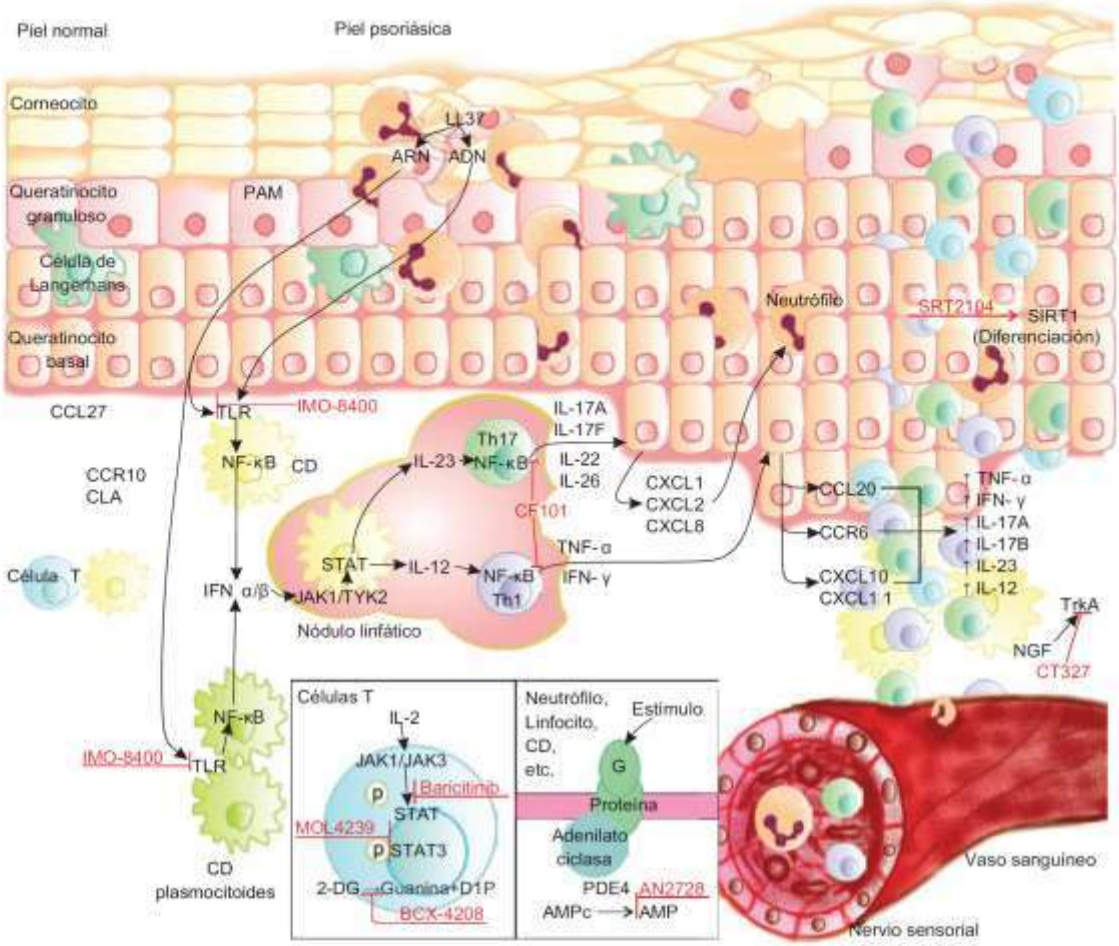

Figura 1. *Patogénesis de la psoriasis e implicación de los nuevos fármacos en desarrollo. La psoriasis implica la interacción compleja entre ne trófilos, células dendríticas (CD), linfocitos T (Th1 y Th17) y queratinocitos. La activación de los queratinocitos por una lesión inicial induce la pr ducción de péptidos antimicrobianos (PAM), propiciando que las CD liberen citocinas (IFN-α y β, IL-12 e IL-23) implicadas en la diferenciaci de linfocitos Th1 y Th17, que producen mediadores (IL-17A, IL-17F, IL-22, TNF-α e IFN-β) que estimulan la generación de quimiocinas (CXCL CCL) en los queratinocitos, con el consecuente reclutamiento de más inmunocitos en la piel. Algunos de los nuevos fármacos antipsoriásicos investigación actúan en: a) el bloqueo de la vía de señalización JAK/STAT (baricitinib, MOL4239, BCX-4208); b) la inhibición de enzimas con la PDE4 (AN2728) y la PNP (BCX-4208); c) el bloqueo de los receptores TLR (IMO-8400); d) la inhibición del receptor de la TrkA (CT327); e) activación de la SIRT1 (SRT2104) y del A3AR (CF101). 2-DG = 2-dexosiguanosina, A3AR = receptor de adenosina A3, AMPc = monofosfa de adenosina cíclico, D1P = dexosiribosa-1-fosfato, JAK = Janus cinasa, NF-κB, factor nuclear potenciador de las cadenas ligeras kappa de l células B activadas, NGF = factor de crecimiento nervioso, PNP = fosforilasa de nucleósidos de purina, STAT = transductor de señal y activad de la transcripción, SIRT1 = sirtuina-1, TLR = receptores tipo Toll, TrkA = tropomiosina cinasa A.*

diferenciación terminal de los queratinocitos, está disminuida o ausente en la piel psoriásica, con una capa córnea formada por células no diferenciadas.[8]

En los últimos años se ha puesto en evidencia el papel del sistema inmune en la psoriasis, lo que ha facilitado la comprensión de las bases fisiopatológicas de la enfermedad. Las lesiones psoriásicas se caracterizan por aumento de la infiltración de linfocitos T. Se estima que un paciente con 20 % de la superficie corporal afectada con psoriasis tendrá unos 8 billones de células T en la sangre, mientra que 20 millones se localizarán en la dermis y la ep dermis.[7] Los estudios inmunohistoquímicos confirma que la dermis alberga predominantemente linfocit T CD4+, macrófagos y células dendríticas (CD), mie tras que los linfocitos T CD8 predominan en la ep dermis.[7] A pesar de la indudable importancia d sistema inmunitario en la psoriasis, el debate sob su causa primaria continúa y numerosas dudas debe resolverse, en particular respecto a la identificació

de antígenos (exógenos o endógenos) y su interacción con las células epidérmicas e inmunológicas en el contexto de las lesiones cutáneas.[9]

Por estudios genéticos se han identificado nueve locus de susceptibilidad para la psoriasis (*PSORS1* a *PSORS9*). Uno de los principales determinantes genéticos es *PSORS1*, una región de 220 kb en el complejo mayor de histocompatibilidad localizado en el cromosoma 6p21, con el cual se explica de 35 a 50 % de heredabilidad de la enfermedad.[10] Otros genes específicos también han sido identificados en distintas poblaciones; sus polimorfismos se han asociado con numerosos procesos inmunológicos, incluyendo la diferenciación de queratinocitos, la proliferación de células T, la modulación de vías de señalización, así como la regulación y adhesión de leucocitos por células T cooperadoras (Th) 1 y 17.[11] En fechas recientes también se ha destacado la participación de divide the word as: micro-RNA en la regulación inflamatoria de la enfermedad.[12]

De la investigación básica y clínica al desarrollo de tratamientos antipsoriásicos

El desarrollo de nuevos tratamientos desde una visión convencional involucra las siguientes etapas:

- Establecimiento de hipótesis etiopatogénicas de la enfermedad.
- Identificación de objetivos farmacológicos.
- Selección de candidatos terapéuticos.
- Optimización química de los compuestos seleccionados.
- Pruebas preclínicas en animales de experimentación.
- Evaluación de la seguridad en seres humanos y establecimiento de las propiedades farmacocinéticas (fase I).
- Pruebas de eficacia (prueba de concepto, fase II).
- Ensayos clínicos multicéntricos a mayor escala para evaluar la seguridad y eficacia (fase III).

En este esquema clásico, el traslado de conocimientos se realiza desde la investigación básica hacia la clínica en un sentido unidireccional.

El desarrollo de medicamentos antipsoriásicos a lo largo de su historia rompe con ese modelo, ya que el traslado de conocimientos no siempre ha partido desde la ciencia básica, sino que muchas hipótesis han surgido a partir de la experiencia clínica pudiendo ser verificadas en investigación fundamental para luego trasladarse nuevamente hacia escenarios clínicos. Así, aunque el concepto de medicina traslacional es relativamente reciente, su implementación en el desarrollo de tratamientos antipsoriásicos no es novedosa.

De hecho, uno de los primeros cambios en cuanto a los paradigmas sobre la patogénesis de la enfermedad se produjo cuando una observación accidental demostró que pacientes trasplantados que recibían ciclosporina evidenciaban mejoría de su psoriasis.[13] Esta experiencia clínica propició el inicio de nuevas investigaciones que demostraron que la acción de este medicamento reducía el número de linfocitos T, células presentadoras de antígenos, monocitos y macrófagos.[14] En consecuencia, los linfocitos T se consideraron blancos terapéuticos, lo cual propició el desarrollo de una proteína de fusión que selectivamente bloqueaba su activación sin afectar a los queratinocitos.[15] Curiosamente se utilizaron estrategias de la medicina traslacional que conllevaron a la generación de nuevas hipótesis a partir de las experiencias clínicas (*bedside-to-bench*).

La hipótesis que consideraba la activación de linfocitos T como un evento central en la etiología de la enfermedad fue posteriormente confirmada con la realización de experimentos en los que el traslado de conocimientos se realizó bidireccionalmente (*bench-to-bedside/bedside-to-bench*). En uno de ellos se utilizaron ratones inmunosuprimidos en los que se injertó piel no lesionada proveniente de pacientes con psoriasis. Esta piel adquirió características de placas psoriásicas después de la inyección de leucocitos.[16] Para probar la misma hipótesis se usaron antagonistas específicos (anticuerpos antiCD60 y proteína de fusión CTLA4-Ig) de moléculas de superficie implicadas en la activación de los linfocitos T.[17] Estos fueron experimentos clínicos acoplados con investigaciones básicas que condujeron a la elucidación de vías de señalización vinculadas con la patogénesis de la enfermedad.

En esta época se extendió la idea de que la presencia de una red de citocinas generadas por los linfocitos en individuos genéticamente predispuestos tenía influencia en la hiperproliferación de los queratinocitos. Como consecuencia se diseñaron productos biotecnológicos para bloquear la acción de dichas citocinas. Un ejemplo de ello fue el daclizumab, un anticuerpo monoclonal dirigido contra el receptor de la IL-2 y la salida al mercado de dos nuevos agentes dirigidos a inactivar a los linfocitos T: alefacept y efalizumab. Aunque efalizumab fue retirado más tarde por generar efectos adversos graves, su uso constituyó otra prueba de la importancia de los linfocitos T en esta patología.

En la década de 1990 ya se había demostrado que el factor de necrosis tumoral (TNF) se encontraba en altas concentraciones en las placas psoriásicas.[18] La remisión de las lesiones cutáneas en pacientes con artritis psoriásica luego del uso de un agente anti-TNF (etanercept)[19] puso en evidencia el papel relevante de esta citocina y de la respuesta inmunológica innata en la psoriasis.[20] Estudios clínicos posteriores, complementados con herramientas de biología celular y molecular, confirmaron que los agentes anti-TNF también tenían efectos sobre la proliferación de otros linfocitos, los Th17.[21] Estos abordajes bidireccionales de medicina traslacional contribuyeron a la identificación de mediadores clave en la enfermedad, permitiendo la salida al mercado de fármacos con mayor selectividad sobre citocinas específicas.

Aunque los primeros medicamentos anti-TNF surgieron a principios del siglo XXI (etanercept, adalimumab, infliximab), nuevas estrategias farmacológicas han permitido el desarrollo de otros agentes anti-TNF novedosos. Es el caso del certolizumab pegol, que difiere de otros fármacos anti-TNF por su estructura. Este medicamento está compuesto por un fragmento Fab de un anticuerpo monoclonal murino humanizado anti-TNF combinado con dos moléculas de polietilenglicol,[22] lo que incrementa su tiempo de vida media a aproximadamente 14 días con mayor distribución tisular.[22] Al estar libre de la porción Fc, el certolizumab pegol no forma complejos inmunes ni induce citotoxicidad mediada por anticuerpos ni por complemento. Además, posee una afinidad por el TNF superior a otros medicamentos como infliximab y adalimumab,[22] sin inducir apoptosis en linfocitos T y monocitos de individuos sanos.

La importancia de las células Th17 en la psoriasis fue también establecida utilizando enfoques de medicina traslacional. Primeramente, la existencia de este grupo de células productoras de IL-17, su impacto en enfermedades inflamatorias autoinmunes y su importante presencia en piel y sangre periférica de individuos con psoriasis se demostró en modelos animales y pacientes.[23,24] Otras investigaciones evidenciaron que la activación de las células Th17 estaba influida por la IL-23[25] y que esta compartía la subunidad p40 con la IL-12.[26] Estos estudios de ciencia básica combinados con comprobaciones clínicas permitieron el establecimiento de un nuevo paradigma en la fisiopatología de la enfermedad relacionado con el eje IL-23/IL-17.[27] La consecuencia lógica de estas investigaciones fue el desarrollo de dos anticuerpos contra la subunidad p40: el ustekinumab y briakinumab, así como de inhibidores de la IL-17 o de su receptor (secukinumab, ixekizumab, brodalumab).

Un análisis exhaustivo de lo sucedido hace dos décadas con la generación de nuevos medicamentos para la psoriasis permite comprender el impacto de la medicina traslacional en este proceso. De hecho, el éxito de los biotecnológicos se debió a la realización de investigaciones en las que se establecieron hipótesis en modelos biológicos con posibilidad de trasladarse rápidamente al campo clínico, al mismo tiempo que esta experiencia clínica servía de base para refinar los conceptos etiopatogénicos.

Este proceso bidireccional ha garantizado el éxito en la generación de tratamientos y, aunque muchos de ellos han quedado en el camino (anticuerpo anti-IL8, anticuerpo anti-CD5, anticuerpo anti-L selectina), no cabe duda de que esta estrategia ha permitido la salida al mercado de nuevos fármacos, contribuyendo a la mejoría en la calidad de vida de los pacientes.

Nuevos fármacos antipsoriásicos en desarrollo

La comprensión más profunda de los eventos celulares y moleculares implicados en la etiología de la psoriasis ha permitido que se haya producido un salto considerable en cuanto al número de fármacos en desarrollo asociados con nuevos objetivos terapéuticos (Figura 1). Destacan los que activan o bloquean la fosfodiesterasa 4, el receptor de la tropomiosina cinasa A, la fosforilasa de nucleósidos de purina, el receptor de adenosina A3, el receptor de esfingosina-1-fosfato y la familia de las Janus cinasas, entre otros (Tabla 1).

Perspectivas futuras en el desarrollo de nuevos medicamentos antipsoriásicos

El uso de estrategias de medicina traslacional en el desarrollo de medicamentos antipsoriásicos ha permitido en los últimos 20 años incrementar significativamente el número de los disponibles para esta patología. La salida al mercado de los productos biotecnológicos constituyó un hito en los esquemas de desarrollo de este tipo de fármacos, ya que combinó conscientemente una estrategia bidireccional donde los conocimientos derivados de la ciencia básica se incorporaron rápidamente a la investigación clínica, al mismo tiempo que la experiencia clínica sirvió de base para aceptar o refutar hipótesis de la ciencia básica. Por ello, no resulta sorprendente que existan 18

Tabla 1. Nuevos medicamentos en desarrollo para el tratamiento de la psoriasis

Fármaco	Mecanismo de acción/Objetivo terapéutico (Compañía farmacéutica)	Fase del desarrollo
AN2728	Inhibidor selectivo de la fosfodiesterasa 4 (Pfizer)	Fase II[a]
CT327	Inhibidor selectivo del receptor de la tropomiosina cinasa A (TrkA) (Creabilis, S.A)	Fase II[b]
IMO-8400	Bloqueo de la activación de los receptores de tipo Toll (TLR) 7,8 y 9 (Idera Pharmaceuticals)	Fase II[c]
SRT2104	Activa a la sirtuina-1 (SIRT1) modulando la función de NF-κB y p53, inhibe la proliferación de queratinocitos (GlaxoSmithKline)	Fase II[d]
AZD0284	Agonista inverso del receptor de ácido retinoide asociado con el receptor nuclear huérfano (AstraZeneca)	Fase II[e]
CF101	Agonista del receptor de adenosina A3 (A3AR) (Can-Fite BioPharma Ltd)	Fase II[f]
BCX-4208	Inhibidor de la fosforilasa de nucleósidos de purina (PNP) (BioCryst Pharmaceuticals, Inc/Hoffman-La Roche)	Fase II[g]
Namilumab (MT-203)	Anticuerpo monoclonal que neutraliza de forma potente y específica al factor estimulante de colonias de granulocitos y macrófagos (GM-CSF) (Takeda Pharmaceutical)	Fase II[h]
Ponesimod (ACT-128800)	Agonista selectivo del receptor de esfingosina-1-fosfato (S1P) reduce la disponibilidad de células efectoras B/T circulantes. (Actelion Pharmaceuticals)	Fase II[i]
RWJ-445380	Inhibidor de la calpaína 5 (Alza Corporation, DE)	Fase II[j]
Ruxolitinib	Inhibidor de la familia de las cinasas Janus JAK1/JAK2 (Incyte Corporation)	Fase II[k]
Baricitinib	Inhibidor de la familia de las cinasas Janus JAK1/JAK2 (Eli Lilly)	Fase II[l]
GSK2586184	Bloqueador selectivo de JAK1 (GlaxoSmithKline)	Fase II[m]
INCB039110	Bloqueador selectivo de JAK1 (Incyte Corporation)	Fase II[n]
Lestaurtinib	Inhibidor del receptor de tirosina cinasa FLT3 y de JAK2 (Teva Pharmaceutical)	Fase II[o]
ASP015K	Inhibidor selectivo de JAK3 (Astellas Pharma)	Fase II[p]
MCL4239	Inhibidor de la fosforilación del transductor de señal y activador de la transcripción 3 (STAT3) (Moleculin, LLC)	Fase II[q]
KD025	Inhibidor de la quinasa 2 asociada con Rho (ROCK2) bloqueando la fosforilación de STAT3 y la producción de IL-17 e IL-21(Kadmon Holdings, Inc)	Fase II[r]

fármacos antipsoriásicos asociados con nuevos objetivos, los cuales que están siendo desarrollados por múltiples compañías farmacéuticas en el mundo.

A pesar de estas alentadoras noticias, la psoriasis sigue siendo una enfermedad incurable y existen todavía varios aspectos que pueden dificultar el desarrollo futuro de fármacos, que deben ser profundizados en su integración con esquemas de medicina traslacional. Como ejemplo podrían enumerarse los siguientes:

- La caracterización de antígenos específicos responsables de la activación de la respuesta inmunológica.
- La identificación de biomarcadores predictivos de toxicidad, farmacocinética y eficacia farmacológica.
- El desarrollo de modelos *in vivo* e *in vitro* más representativos de la enfermedad.
- La integración de aspectos genómicos al proceso del descubrimiento de fármacos.

Mucho queda por comprender las causas de esta enfermedad, así como la influencia de marcadores genéticos en la respuesta a nuevos tratamientos, pero seguramente el traslado bidireccional de conocimientos permitirá la introducción futura de nuevas opciones individualizadas en las que la cooperación entre todos los actores (científicos, médicos, pacientes) será necesaria para el cambio de paradigmas.

Bibliografía

1 Parisi R, Symmons DPM, Griffiths CEM, Ashcroft DM. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. J Invest Dermatol. 2013;133:377–385.

2 International Psoriasis Council [Sitio web]. IPC psoriasis review. Dallas, Texas: IPC; 2009. p. 1-12. [Consultado 2017 Sep 10]. Disponible en: http://www.psoriasiscouncil.org/docs/ipcpsoriasisreview_dec_2009_english.pdf?LanguageID=EN-US

3. Kurd SK1, Troxel AB, Crits-Christoph P, Gelfand JM. The risk of depression, anxiety, and suicidality in patients with psoriasis: a population-based cohort study. Arch Dermatol. 2010;146:891–5.
4. Nast A, Boehncke WH, Mrowietz U, Ockenfels HM, Philipp S, Reich K, et al. S3-Guidelines on the treatment of psoriasis vulgaris (English version). Update. J Dtsch Dermatol Ges. 2012;10:S1-S95.
5. Dubertret L, Mrowietz U, Ranki A, Van-De-Kerkhof PC, Chimenti S, Lotti T, et al. European patient perspectives on the impact of psoriasis: the EUROPSO patient membership survey. Br J Dermatol. 2006; 155:729-736.
6. Lowe NJ, Wieder JM, Rosenbach A, Johnson K, Kunkel R, Bainbridge C, et al. Long-term low-dose cyclosporine therapy for severe psoriasis: effects on renal function and structure. J Am Acad Dermatol. 1996; 35:710-719.
7. Cai Y, Fleming C, Yan J. New insights of T cells in the pathogenesis of psoriasis. Cell Mol Immunol. 2012;9:302-309.
8. Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis. Nature. 2007;445:866-873.
9. Guttman-Yassky E, Krueger JG. Psoriasis: evolution of pathogenic concepts and new therapies through phases of translational research. Br J Dermatol. 2007;157:1103-1115.
10. Hébert HL, Ali FR, Bowes J, Griffiths CE, Barton A, Warren RB. Genetic susceptibility to psoriasis and psoriatic arthritis: implications for therapy. Br J Dermatol. 2012;166:474-482.
11. Das S, Stuart PE, Ding J, Tejasvi T, Li Y, Tsoi LC, et al. Fine mapping of eight psoriasis susceptibility loci. Eur J Hum Genet. 2015;23:844-853.
12. Hawkes JE, Nguyen GH, Fujita M, Fiorell SR, Callis Duffin K, Krueger GG, et al. microRNAs in psoriasis. J Invest Dermatol. 2016;136:365-371.
13. Picascia DD, Garden JM, Freinkel RK, Roenigk HH. Resistant severe psoriasis controlled with systemic cyclosporine therapy. Transplant Proc. 1988;20:58-62.
14. Griffiths CE, Voorhees JJ. Cyclosporine A in the treatment of psoriasis: a clinical and mechanistic perspective. J Invest Dermatol. 1990;95:S53-S55.
15. Gottlieb SL, Gilleaudeau P, Johnson R, Estes L, Woodworth TG, Gottlieb AB, et al. Response of psoriasis to a lymphocyte-selective toxin (DAB389IL-2) suggests a primary immune, but not keratinocyte, pathogenic basis. Nat Med. 1995;1:442-447.
16. Wrone-Smith T, Nickoloff BJ. Dermal injection of immunocytes induces psoriasis. J Clin Invest. 1996;98:1878-1887.
17. Gottlieb AB, Lebwohl M, Totoritis MC, Abdulghani AA, Shuey SR, Romano P, et al. Clinical and histologic response to single-dose treatment of moderate to severe psoriasis with an anti-CD80 monoclonal antibody. J Am Acad Dermatol. 2002;47:692-700.
18. Nickoloff BJ, Karabin GD, Barker JN, Griffiths CEM, Sarma V, Mitra RS, et al. Cellular localization of interleukin-8 and its inducer, tumor necrosis factor-alpha in psoriasis. Am J Pathol. 1991;138:129-140.
19. Mease PJ, Goffe BS, Metz J, VanderStoep A, Finck B, Burge DJ. Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. Lancet. 2000;356:385-390.
20. Victor FC, Gottlieb AB. TNF-alpha and apoptosis: implications for the pathogenesis and treatment of psoriasis. J Drugs Dermatol. 2002; 1:264-275.
21. Zaba LC, Cardinale I, Gilleaudeau P, Sullivan-Whalen M, Suárez-Fariñas M, Fuentes-Duculan J, et al. Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. J Exp Med. 2007;204:3183-3194.
22. Pasut G. Pegylation of biological molecules and potential benefits: pharmacological properties of certolizumab pegol. BioDrugs. 2014;28:S15-S23.
23. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005;6:1123-1132.
24. Lowes MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, Haider AS, et al. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. J Invest Dermatol. 2008;128:1207-1211.
25. McKenzie BS, Kastelein RA, Cua DJ. Understanding the IL-23-IL-17 immune pathway. Trends Immunol. 2006;27:17-23.
26. Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity. 2000; 13:715-725.
27. Di-Cesare A, Di-Meglio P, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. J Invest Dermatol. 2009;129:1339-1350.
28. Nazarian R, Weinberg JM. AN-2728, a PDE4 inhibitor for the potential topical treatment of psoriasis and atopic dermatitis. Curr Opin Investig Drugs. 2009;10:1236-1242.
29. Roblin D, Yosipovitch G, Boyce B, Robinson J, Sandy J, Mainero V, et al. Topical TrkA kinase inhibitor CT327 is an effective, novel therapy for the treatment of pruritus due to psoriasis: results from experimental studies, and efficacy and safety of CT327 in a phase 2b clinical trial in patients with psoriasis. Acta Derm Venereol. 2015; 95:542-548.
30. Balak DMW, Van-Doorn MBA, Rissmann R, Sullivan T, Burggraaf J, Arbeit RD. Results from a randomized, double-blind, placebo-controlled, monotherapy trial of IMO-8400 demonstrate clinical proof-of-concept for Toll-like receptor 7, 8, and 9 antagonism in psoriasis. J Am Acad Dermatol. 2015;72:AB247.
31. Krueger JG, Suárez-Fariñas M, Cueto I, Khacherian A, Matheson R, Parish LC, et al. A randomized, placebo-controlled study of SRT2104, a SIRT1 activator, in patients with moderate to severe psoriasis. PLoS ONE. 2015;10:e0142061.
32. ClinicalTrials.gov. [Sitio web]. Study to assess the safety, tolerability and pharmacokinetics and pharmacodynamics of AZD0284 in healthy subjects. [Consultado 2017 Sep 10]. Disponible en: https://clinicaltrials.gov/ct2/show/NCT02976831?term=AZD0284&rank=2
33. David M, Gospodinov DK, Gheorghe N, Mateev GS, Rusinova MV, Hristakieva E, et al. Treatment of plaque-type psoriasis with oral CF101: data from a phase II/III multicenter, randomized, controlled trial. J Drugs Dermatol. 2016;15:931-938.
34. Bantia S, Parker C, Upshaw R, Cunningham A, Kotian P, Kilpatrick JM, et al. Potent orally bioavailable purine nucleoside phosphorylase inhibitor BCX-4208 induces apoptosis in B- and T-lymphocytes. A novel treatment approach for autoimmune diseases, organ transplantation and hematologic malignancies. Int Immunopharmacol. 2010;10:784-790.
35. ClinicalTrials.gov. [Sitio web]. Efficacy and safety of namilumab (MT203) for plaque psoriasis. [Consultao 2017 Sep 10]. Disponible en: https://clinicaltrials.gov/ct2/show/NCT02129777?term=Namilumab&rank=1
36. Vaclavkova A, Chimenti S, Arenberger P, Holló P, Sator PG, Burcklen M, et al. Oral ponesimod in patients with chronic plaque psoriasis: a randomized, double-blind, placebo-controlled phase 2 trial. Lancet. 2014; 384:2036-2045.
37. ClinicalTrials.gov. [Sitio web]. Study to investigate the safety, tolerability, absorption, distribution, metabolism, and elimination of RWJ-445380 administered to patients with plaque psoriasis. [Consultado 2017 Sep 10]. Disponible en: https://clinicaltrials.gov/ct2/show/NCT00396422?term=RWJ-445380&rank=1
38. Punwani N, Scherle P, Flores R, Shi J, Liang J, Yeleswaram S, et al. Preliminary clinical activity of a topical JAK1/2 inhibitor in the treatment of psoriasis. J Am Acad Dermatol. 2012;67:658-664.
39. Papp KA, Menter MA, Raman M, Disch D, Schlichting DE, Gaich C, et al. A randomized phase 2b trial of baricitinib, an oral Janus kinase (JAK) 1/JAK2 inhibitor, in patients with moderate-to-severe psoriasis. Br J Dermatol. 2016;174:1266-1276.
40. Ludbrook VJ, Hicks KJ, Hanrott KE, Patel JS, Binks MH, Wyres MR, et al. Investigation of selective JAK1 inhibitor GSK2586184 for the treatment of psoriasis in a randomized placebo-controlled phase IIa study. Br J Dermatol. 2016;174:985-995.
41. Bissonnette R, Luchi M, Fidelus-Gort R, Jackson S, Zhang H, Flores R, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of the safety and efficacy of INCB039110, an oral janus kinase 1 inhibitor, in patients with stable, chronic plaque psoriasis. J Dermatol Treat. 2016;27:332-338.
42. ClinicalTrials.gov. [Sitio web]. Study of the efficacy, safety and tolerability of oral CEP-701 in patients with severe psoriasis. [Consultado 2017 Sep 10]. Disponible en: https://clinicaltrials.gov/ct2/show/NCT00236119?term=Lestaurtinib&cond=psoriasis&rank=1
43. Papp KA, Pariser D, Catlin M, Wierz G, Ball G, Akinlade B, et al. A phase 2a randomized, double-blind, placebo-controlled, sequential dose-escalation study to evaluate the efficacy and safety of ASP015K, a novel Janus kinase inhibitor, in patients with moderate-to-severe psoriasis. Br J Dermatol. 2015;173:767-776.
44. ClinicalTrials.gov. [Sitio web]. Paired psoriasis lesion, comparative, study to evaluate MOL4239 in psoriasis. [Consultado 2017 Sep 10]. Disponible en: https://clinicaltrials.gov/ct2/show/NCT01826201?term=MOL4239&rank=1
45. Zanin-Zhorov A, Weiss JM, Nyuydzefe MS, Chen W, Scher JU, Mo R, et al. Selective oral ROCK2 inhibitor down-regulates IL-21 and IL-17 secretion in human T cells via STAT3-dependent mechanism. Proc Natl Acad Sci U S A. 2014;111:16814-16819.