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Loeselia mexicana: Antioxidant and antimicrobial properties by Soxhlet differential extraction

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Abstract

Loeselia mexicana or "Espinosilla" commonly known in Mexico is a plant with a great diversity of traditional medicinal uses, especially as an aid in the treatment of superficial wounds, however, said use lacks a scientific basis beyond ethnobotany, so studies of differential extraction that allowed to separate the secondary metabolites according to their polarity to carry out antioxidant tests, associated with the healing process and antimicrobial, associated with avoiding wound infection. The antioxidant potential is divided into two polarities with better results for those extracted by Ethanol (59.38% DPPH and 456.7 mg/kg Folin) and ethyl acetate (63.83% DPPH and 340.1 mg/kg Folin), indicating that both oily and alcoholic has metabolites that prevent skin oxidation; coinciding with the best inhibition halos in Gram negative bacteria. The use of medicinal plants in a traditional way cannot be stopped without affecting the traditions of the Mexican peoples and the world, however, with studies of differential extraction and evaluation of biological activity, it allows to establish scientific bases for its use and to continue to standardize it.

Keywords: *Loeselia Mexicana*; Differential extraction; DPPH; Antioxidant; Gram-negative; Antimicrobial activity

1. Introduction

The use of medicinal plants in world culture widely distributed, given the accessibility of these and the traditions that each culture (1). Mexico as a megadiverse country in species of the plant kingdom has used this diversity for medicinal purposes, from imported species as well as endemic species; this last group is of particular interest since they have been little studied and although they have reported traditional uses, they lack investigations in scientific reports (2,3). *Loeselia mexicana* (Figure 1) is a clear example of this, it is a plant belonging to the *Polemoniaceae* family, which grows as small shrubs with erect stems that can be simple or branched (4). Its geographical distribution ranges from southwestern Texas to the state of Chiapas. *L. mexicana* has been attributed properties for shamanic rituals as well as medicinal and cosmetic properties (5,6), with respect to the latter they have been focused on the treatment of diarrhea, fever, dandruff and in turn as an aid in hair care, being consumed both orally (infusions and tinctures) and topical (creams and plasters) (7).

The association with respiratory, gastrointestinal, and topical pathologies associates the antimicrobial use of *L. mexicana*, since these pathologies are mainly responses to a bacterial infection such as *A. baumanni* (8), *S. aureus* (9–12), *E. coli* (13,14), *E. fecalis* (9,15–17), *S. saprophyticus* (18–20), *S. typhimurium* (21), *E. Coli BLEE* (14,22), *M. morganii* (23), among others, being necessary to establish if there is an antimicrobial effect. While topical use as a healing agent is associated with antioxidant effects to reduce the oxidative process (24), the other interesting study approach is to

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establish a correlation between the traditional uses of *L. mexicana* and a scientific basis, for which differential extraction studies allow establishing what type of metabolites are associated with biological activity and choose the appropriate extraction method.

Figure 1 *Loeselia mexicana* and traditional uses

2. Material and methods

2.1. Material

Fresh samples of *L. mexicana* were collected in the botanical garden of the Benemérita Universidad Autónoma de Puebla, to perform the extraction of its active compounds by means of a Soxhlet extractor.

2.2. Methods

2.2.1. Differential extraction by Soxhlet.

30.0 g of dry plant were put in a Soxhlet extraction equipment, with 150 mL of solvent (hexane, ethyl ether, ethyl acetate, dichloromethane, acetone, ethanol, methanol and water) in sequence for 3 h each one, then all the solvent was removed, and a stock solution is prepared at 1 mg/ml.

2.2.2. Total phenol content

150 μL of each extract diluted in 2250 μL of distilled water were used. Once the solution was homogenized, 150 μL of Folin-Ciocalteau reagent was added and 450 μL of 20% Na_2CO_2 was added and placed in incubation in the absence of light for 2 h. At the end of the time, its absorbance at 760 nm is determined and by linear regression the result is expressed in mg of gallic acid per g of plant material (25). Gallic acid was used as controls at a defined concentration and in accordance with reports in the literature.

2.2.3. DPPH radical inhibition assay

1 mL of the stock solution diluted in 1 mL of methanol was used, then 1 mL of the DPPH free radical solution was added, it was homogenized and left to incubate isolated from light for 60 minutes (26). Express the result as a percentage of inhibition as reported and gallic acid (0.4836 mg/mL) were used as control.

2.2.4. Antimicrobial activity

The inhibition halo technique was used, culturing each of the bacteria in LB agar medium at 37 °C, with a filter paper disc with 8 μL of the extract in mother solution for 24 h to proceed to determine the inhibition halos (27,28). The result is expressed in mm and standard penicillin 10 mg antibiotic sensidiscs were used as a control.

2.3. Statistical Analysis

The extraction data, DPPH radical scavening, total phenol content and inhibition halos were statistically analyzed by ANOVA post-Tuckey test in Minitab 18.0 software and the results are expressed in means with letter assignment to identify statistically different groups for $p<0.05$ (29).

3. Results and discussion

3.1. Differential extraction

The use of medicinal plants such as *L. Mexicana* culture is very diverse, however, many times the use of this is incorrect, even generating even serious poisoning, or in the best of cases the beneficial effect is not observed, the latter mainly associated with a bad extraction of the secondary metabolites with the biological activity, for which it is necessary to carry out a differential extraction increasing the polarity of the solvent to separate these metabolites and associate if the activity is independent of the extraction technique or a particular extract must be used to obtain a health benefit. Table 1 shows the results of the differential extraction in Soxhlet equipment, obtaining a low percentage in general, this indicates that the content of secondary metabolites in the plant matrix is low, however, there is a trend towards a higher content in polar than non-polar solvents.

Table 1 Solvent extraction percentage used by differential extraction in Soxhlet

Solvent	Dielectric constant (D)	Extract[1]
Hexane	1.9	1.5088±0.6889
Ethyl ether	4.3	16.1958±8.1801
Ethyl acetate	6.0	23.7221±6.3116
DCM	9.1	4.1688±1.2083
Acetone	20.6	20.9160±6.2301
Ethanol	22.4	22.0329±8.6376
Methanol	32.6	14.7662± 8.7449
Water	79.7	-

[1]Results expressed as mg of extract/g of plant.

The most suitable method for extraction is through aqueous or alcoholic extractions in traditional use, although this is only in terms of quantity, given that in terms of biological activity this does not always have a direct relationship, therefore the effect on the antioxidant property, related to the healing process, and the antibacterial activity, was studied to relate the use of this medicinal plant to the treatment of superficial wounds to reduce the risk of infection and improve the healing process.

3.2. Antioxidant properties

Table 2 Antioxidant activity by differential extract at 1.0 mg/mL

Solvent	Dielectric constant (D)	DPPH[1]	Folin-Ciocalteau[2]
Hexane	1.9	69.05 ± 0.00^{a}	3.4 ± 0.00^{d}
Ethyl ether	4.3	46.94 ± 1.64^{bc}	84.2 ± 2.06^{d}
Ethyl acetate	6.0	63.83 ± 3.06^{a}	340.1 ± 170.51^{b}
DCM	9.1	52.53 ± 2.14^{ab}	39.7 ± 0.70^{d}
Acetone	20.6	47.39 ± 3.76^{bc}	224.8 ± 47.30^{c}
Ethanol	22.4	59.38 ± 4.51^{ab}	456.7 ± 48.51^{a}
Methanol	32.6	57.34 ± 4.63^{ab}	19.1 ± 0.80^{d}
Water	79.7	29.64 ± 0.08^{c}	31.6 ± 3.10^{d}

[1]Results expressed as % free radical inhibition; [2]Results expressed as mg gallic acid equivalent/kg of plant; [a-d]. Mean values in the same column with different letter are significantly different ($p<0.05$ Anova post Tukey)

In table 2 we can observe the antioxidant properties of the extracts prepared at 0.1 g/mL, in the case of the inhibitory activity of the DPPH radical, an activity of the same level is presented for the extracts evaluated, obtaining the highest

levels for the extracts in ethyl acetate, hexane and ethanol, indicating that the feasibility of capturing free radicals is not dependent on polarity if we analyze at the same concentration, although in relation to the weight obtained, the extract in ethanol and ethyl acetate is of greater interest since have a high extraction ratio with high antioxidant capacity.

For the Folin test that quantifies total phenols, the extracts in acetone, ethyl acetate and ethanol have better results, indicating that the resulting phenols in these extracts are glycosylated given the polarity of acetone and ethanol, while those found in free form are extracted in ethyl acetate, however ethyl acetate and acetone extractions are not very popular in traditional medicine, indicating that the most feasible is the use of alcoholic extracts to take advantage of their antioxidant potential.

3.3. Antimicrobial activity

The antimicrobial evaluation (Table 3) was studied with Gram-negative and Gram-positive bacteria found in the environment and with mainly skin and airborne infection, denoting results for groups of bacteria. In the case of the extracts in hexane only in *S. saprophyticus* a minimal effect was presented, since in the rest of the bacteria no inhibition halo was observed. In the extracts prepared in ethyl ether, ethyl acetate, DCM and acetone, increasing the polarity increases the inhibition in most bacteria, this is associated with the increase in substances such as flavonoids, terpenoids and steroids in the extracts, with the exception of *A. baumanii* and *M. morganii* where the extract in ethyl acetate is the one that presents a greater halo of inhibition indicating a correlation between the flavonoids present in this extract and the antimicrobial activity.

Table 3 Halo inhibition (mm) at bacteria by extract at 1 mg/mL

Gram	**+**	**+**	**+**	**-**	**-**	**-**	**-**	**-**
Bacteria/Solvent	***E. faecalis***	***S. typhimurium***	***S. aureus***	***S. saprophyticus***	***A. baumanii***	***M. morganii***	***E. coli***	***E. coli BLEE***
Hexane	0.0000^{a}	0.0000^{a}	0.0000^{b}	0.0667^{b}	0.0000^{a}	0.0000^{b}	0.0000^{a}	0.0000^{a}
Ethyl ether	0.0000^{a}	0.0667^{a}	0.0000^{b}	0.0000^{b}	0.3333^{a}	0.2333^{ab}	0.0333^{a}	0.3000^{a}
Ethyl acetate	0.5000^{a}	0.2333^{a}	0.5333^{a}	0.2333^{ab}	0.6000^{a}	0.7333^{a}	0.3333^{a}	0.4667^{a}
DCM	0.1000^{a}	0.0333^{a}	0.1000^{ab}	0.0667^{b}	0.0333^{a}	0.3000^{ab}	0.1333^{a}	0.0000^{a}
Acetone	0.5000^{a}	0.5333^{a}	0.2667^{ab}	0.1333^{ab}	0.6667^{a}	0.5667^{ab}	0.8333^{a}	0.1667^{a}
Ethanol	0.1333^{a}	0.4000^{a}	0.2667^{ab}	0.0667^{b}	0.6333^{a}	0.4333^{ab}	0.4000^{a}	0.4667^{a}
Methanol	0.5667^{a}	0.2667^{a}	0.2667^{ab}	0.2333^{ab}	0.3333^{a}	0.3333^{ab}	0.3000^{a}	0.3667^{a}
Water	0.2667^{a}	0.3333^{a}	0.2000^{ab}	0.7000^{a}	0.5333^{a}	0.4333^{ab}	0.1667^{a}	0.7000^{a}

$^{a-b}$Mean values in the same column with different letter are significantly different (p<0.05 Anova post Tukey)

In contrast for polar solvents from ethanol to water the growth inhibitory effect is very dispersed. In the case of ethanol in *S. typhimurium*, *E. coli*, *E. coli* BLEE, *M. morganii* and *A. baumanii* present significantly higher halos. In contrast, the aqueous extract has a higher effect on *S. saprophyticus*, *A. baumanii* and *E. coli* BLEE only, while methanol has an effect at the same level on all except *E. fecalis*, which is slightly higher. Demonstrating that the antimicrobial activity is dependent on the extract used to isolate the secondary metabolites.

4. Conclusion

The great potential of the fractions of secondary metabolites, obtained from their polarities, as antioxidants and antimicrobials with a wide field of application due to their activity on gram negative and positive bacteria, for which the fractions of greater polarity such as those obtained with ethanol and acetone present the best performance from the extraction as well as in the evaluation as inhibitors of free radicals, the total content of phenols and their effects on bacterial strains, as well as in less polar solvents such as ethyl acetate, allowing to support the potential medicine of the endemic plants of each country.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest between the authors oh this manuscript.

References

[1] Joshee N, Dhekney SA, Parajuli P. Antioxidant, antimicrobial, analgesic, anti-inflammatory and antipyretic effects of bioactive compounds from passiflora species. In: Medicinal Plants: From Farm to Pharmacy. Springer; 2019.

[2] López-Patiño EJ, Vibrans H, Moctezuma-Pérez S, Chávez-Mejía MC. Ecological apparency, ethnobotanical importance and perceptions of population status of wild-growing medicinal plants in a reserve of south-central Mexico. Journal of Ethnobiology and Ethnomedicine. 2022; 18(1):1–13.

[3] Carrillo-Galván G, Bye R, Eguiarte LE, Cristians S, Pérez-López P, Vergara-Silva F, et al. Domestication of aromatic medicinal plants in Mexico: Agastache (Lamiaceae)- A n ethnobotanical, morpho-physiological, and phytochemical analysis. Journal of Ethnobiology and Ethnomedicine. 2020; 16(1):1–16.

[4] WFO: The World Floral Online. Loeselia mexicana Brand. 2022.

[5] Rzedowski J, Calderón de Rzedowski G. POLEMONIACEAE. In: Flora del Bajío y de regiones adyacentes. instituto de ecologia, A.C.; 1995. p. 1–41.

[6] Farfán-Heredia B, Casas A, Moreno-Calles AI, García-Frapolli E, Castilleja A. Ethnoecology of the interchange of wild and weedy plants and mushrooms in Phurépecha markets of Mexico: Economic motives of biotic resources management. Journal of Ethnobiology and Ethnomedicine. 2018; 14(1):1–19.

[7] Navarro García V, Rojas G, Gerardo Zepeda L, Aviles M, Fuentes M, Herrera A, et al. Antifungal and antibacterial activity of four selected Mexican medicinal plants. Pharmaceutical Biology. 2006; 44(4):297–300.

[8] Tan Y, Zhou K, Tang X, Kudinha T, Wang L, Guo Z, et al. Bacteremic and non-bacteremic pneumonia caused by Acinetobacter baumannii in ICUs of South China: A Clinical and Microbiological Study. Scientific Reports. 2017; 7(1):1–9.

[9] Golan Y. Current treatment options for acute skin and skin-structure infections. Clinical Infectious Diseases. 2019; 68(Suppl 3):S206–12.

[10] He H, Wunderink RG. Staphylococcus aureus Pneumonia in the Community. Seminars in Respiratory and Critical Care Medicine. 2020; 41(4):470–9.

[11] Bastien S, Meyers S, Salgado-Pabón W, Giulieri SG, Rasigade JP, Liesenborghs L, et al. All Staphylococcus aureus bacteraemia-inducing strains can cause infective endocarditis: Results of GWAS and experimental animal studies. Journal of Infection. 2023; 86:123–33.

[12] Urish KL, Cassat JE. Staphylococcus aureus Osteomyelitis: Bone, Bugs, and Surgery. Infection and Immunity. 2020;88(7).

[13] Narciso A, Nunes F, Amores T, Lito L, Melo-Cristino J, Duarte A. Persistence of uropathogenic Escherichia coli strains in the host for long periods of time: Relationship between phylogenetic groups and virulence factors. European Journal of Clinical Microbiology and Infectious Diseases. 2012; 31(6):1211–7.

[14] Santos ACM, Santos-Neto JF, Trovão LO, Romano RFT, Silva RM, Gomes TAT. Characterization of unconventional pathogenic Escherichia coli isolated from bloodstream infection: virulence beyond the opportunism. Brazilian Journal of Microbiology. 2022; 54(1):15–28.

[15] Salze M, Giard JC, Riboulet-Bisson E, Hain T, Rincé A, Muller C. Identification of the general stress stimulon related to colonization in Enterococcus faecalis. Archives of Microbiology. 2020; 202(2):233–46.

[16] Dahl A, Bruun NE. Enterococcus faecalis infective endocarditis: focus on clinical aspects. Expert review of cardiovascular therapy. 2013; 11(9):1247–57.

[17] Zheng J, Wu Y, Lin Z, Wang G, Jiang S, Sun X, et al. ClpP participates in stress tolerance, biofilm formation, antimicrobial tolerance, and virulence of Enterococcus faecalis. BMC Microbiology. 2020; 20(1):1–18.

[18] Hashemzadeh M, Dezfuli AAZ, Nashibi R, Jahangirimehr F, Akbarian ZA. Study of biofilm formation, structure and antibiotic resistance in Staphylococcus saprophyticus strains causing urinary tract infection in women in Ahvaz, Iran. New Microbes and New Infections. 2021; 39:100831.

[19] Nishimura S, Matsuyama S, Yamamoto K. Staphylococcus saprophyticus native valve endocarditis possibly originating from the lower gastrointestinal tract. IDCases. 2020; 19:e00713.

[20] Yang Y, Mei F, Lin J, Liao J, Wu K, Duan F. Comparison of causative microorganisms of posttraumatic endophthalmitis with and without retained intraocular foreign bodies. BMC Ophthalmology. 2021; 21(1):1–7.

[21] Seribelli AA, Cruz MF, Vilela FP, Frazão MR, Paziani MH, Almeida F, et al. Phenotypic and genotypic characterization of Salmonella Typhimurium isolates from humans and foods in Brazil. PLoS ONE. 2020;15(8 August):1–18.

[22] Assimakopoulos SF, Kraniotis P, Gogos C, Marangos M. Renal vein thrombosis complicating severe acute pyelonephritis with renal abscesses and associated bacteraemia caused by extended-spectrum beta-lactamase producing Escherichia coli. CEN Case Reports. 2018; 7(1):90–3.

[23] Farzin A, Akter R, Rahman A, Nasir S, Rahman M. The Pattern of Aerobic Microorganisms Causing Hospital : Acquired Urinary Tract Infection. 2022; 4(1):2021–3.

[24] Jabłońska-Trypuć A, Pankiewicz W, Czerpak R. Traumatic Acid Reduces Oxidative Stress and Enhances Collagen Biosynthesis in Cultured Human Skin Fibroblasts. Lipids. 2016; 51(9):1021–35.

[25] Kaur C, Kapoor HC. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int J Food Sci Tech. 2002 Feb; 37(2):153–61.

[26] Fukumoto LR, Mazza G. Assessing Antioxidant and Prooxidant Activities of Phenolic Compounds. J Agric Food Chem. 2000 Aug 1; 48(8):3597–604.

[27] Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. American Journal of Clinical Pathology. 1966 Apr 1; 45(4_ts):493–6.

[28] Borges A, José H, Homem V, Simões M. Comparison of Techniques and Solvents on the Antimicrobial and Antioxidant Potential of Extracts from Acacia dealbata and Olea europaea. Antibiotics. 2020 Jan 28; 9(2):48.

[29] MiniTab, LLC.