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Research Article

Determination of the Antibacterial Activity of *Krameria pauciflora* (Rose)

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Abstract

In Mexico, the decoction of the rhizome *Krameria pauciflora* is taken against diarrhea and dysentery. Our objective was to determine the antibacterial activity of *Krameria pauciflora* on the growth of the causal bacteria of gastrointestinal infections.

The plant was dried and pulverized at room temperature, this included the leaves, the rhizome, and the complete plant (stem–leaves–rhizome). These were macerated consecutively 48 h in hexane, dichloromethane, methanol, and water. Also, a decoction was prepared of the complete plant. The minimal inhibitory concentration (MIC) was determined by the method of resazurin. Dilutions were prepared, ranging from 5-0.039 mg/mL of the extracts, and we added these to plates that contained 5 x 10^5 colony-forming units (CFU)/mL/, resazurin sodium 0.675% and Müeller–Hinton medium (3X). Negative and positive controls were included with dimethyl sulfoxide 1% and Penicillin–Streptomycin, respectively. The bacteria utilized were: *Escherichia coli*; *Proteus mirabilis*; *Shigella flexneri*; *Salmonella typhi*, and *Salmonella typhimurium.* At least three assays were carried out in triplicate.

The decoction of the complete plant presented the greatest antibacterial activity, with an MIC of 0.078 mg/mL. The methanolic and aqueous extracts of the rhizome and of the complete plant presented a MIC of 0.156 mg/mL on *Salmonella* and *Shigella flexneri*, respectively. The hexanic extract of the complete plant inhibited all of the strains. The extracts of the rhizome and of the leaf, as well as of the dichloromethanic extracts of the rhizome, stem, and the complete plant, inhibited only *S. typhi* and *S. flexneri*.

Keywords: Gastrointestinal infections, Antibacterial activity, Genus *Krameria.*

INTRODUCTON:

According to the World Health Organization (W.H.O), in the year 2015, respiratory airways infections and the infections that accompany diarrhea caused 3.2 and 1.4 million deaths, respectively, occupying third and eighth places as causes of death at the worldwide level.[1] Diarrheal infections and gastroenteritis of infectious origin caused 5.1% of deaths in children less than 5 years of age at the worldwide level. These gave rise to the death of 525,000 children every year. Malnourished and immunocompromised children are those who present the greatest risk of potentially mortal diseases.[2] The report of the National Institute of Geography and Statistics (INEGI) in Mexico shows that diarrheal infections and gastroenteritis of infectious origin caused 5.1% of deaths in preschool children aged 1-4 years during 2015.[3]

In Mexico, diarrheal disease has comprised an important public-health problem from the preHispanic era and the Viceroyalty. In the Cruz-Badiano Codices, the Florentine Codex, and Book of the Royal Protophysician Francisco Hernández, written in the XVI century, the existence is noted of "cocoliztli", an infection considered mortal, an infection that caused abdominal pains and evacuations with loose or liquid feces occasionally with blood.[4] The Mexican culture utilized between 40 and 79 plants for the treatment of such an affectation [5], which was responsible for 50-90% of the deaths that occurred during the XVI century, being identified today with infections caused by enteric *Salmonellas*.[5,6] Infectious diseases are treated with antibiotics, and at present many of these have lost their effectiveness due to that some bacteria have developed mechanisms of defense, expressing genes that confer on them resistance against antibiotics in clinical use, in addition to the secondary effects of these.[7]

It is estimated that by the year 2050, bacterial diseases will be the primary cause of death, all of this due to the excessive and indiscriminate use of antibiotics.[8] The latter has motivated the search for antimicrobials deriving from natural sources, such as medicinal plants and marine invertebrate organisms.[9] In

Mexico, plants of the genus *Krameria* are employed to alleviate diverse illnesses, among these, infections of the respiratory airways and gastrointestinal diseases. This genus, belonging to the Krameriaceae family, has 18 species. Plants from this genus can grow principally as herbs or as the hemoparasitic bushes of perennial plants; the shrubs reach one meter in height, and the stem in some species is spiny. The leaves are simple or alternate, and the inflorescence is solitary with axillary or terminal flowers.[10]

These plants are native to the American continent, and they grow preferentially in tropical regions from 900-3,000 meters above sea level (masl). In Ecuador, Peru, and Bolivia, these plants are utilized as mouthwashes or for gargling in cases of periodontis. Popularly, they are known with the name of *ratania* (rhatany), the name given to the root of the plant, which is one of those most frequently employed in popular medicine. Other medicinal employments include their use as astringents, as anti-inflammatories, to combat cancer of the intestine, stomach, and tongue. The plants are additionally utilized as suppositories, hemostatics, and in the treatment of hemorrhoids.[11]

In Mexico, the medical use of the plants of the genus *Krameria* is linked to indigenous groups from the North of the country (Sonora state), such as the Seri and the Yaqui. The former use *Krameria grayl* to regulate menstruation, as a tonic, and to the clean the kidneys. Meanwhile, the Yaquis utilize *Krameria parvifolia* to purify the blood, against venereal disease in males, and to increase the red globules.[12] In the Mexican State of Hidalgo, on the center of the country, the decoction is ingested of the rhizome or the leaves of *Krameria pauciflora* to combat gastrointestinal infections, some types of cancer, and anemia. *K. pauciflora* is an herbaceous plant, a perennial, with a short main stem, with a woody base, with simple or trifoliate alternate leaves.[13] Due to that this plant is employed popularly against gastrointestinal diseases, the objective of this study was to determine the capacity of the rhizome, the leaves, and a rhizome–leaves–stem combination (the complete plant) to inhibit the growth of enterobacteria associated with gastrointestinal infections.

MATERIAL AND METHODS

Plant material

The aerial parts and the rhizome of *Krameria pauciflora* were collected in Tepeji del Río, Hidalgo state, Mexico, in July of 2017. The taxonomic identification of the samples was carried out by Professors Jorge Santana and Reyna Cerón of the "Ramón Riba y Nava Esparza" Metropolitan Herbarium of the Metropolitan Autonomous University (UAM), where the samples were deposited for safekeeping.

Preparation of the extracts The plant was left to dry at room temperature, including the rhizome, leaves, and the complete plant (leaves, stem, and rhizome). One hundred g of the pulverized material was macerated consecutively during 48 h in each of the following solvents: 1.2 L of hexane; dichloromethane; methanol (J.T. Baker, USA), and water during 48 h. The extracts were filtered, the organic solvents were eliminated at reduced pressure in a rotavapor (Buchii RII, Switzerland), and the water was eliminated by evaporation in a double boiler. Additionally, the decoction was prepared from the complete plant. With the solid extracts we prepared dissolution of dimethyl sulfoxide of 5-0.03 mg/mL 10% (DMSO)/H_2O (J.T. Baker, USA). A preliminary phytochemical study was carried out according that those reported by Alarcón and collaborators.[14]

Bacterial strains

The bacteria employed were as follows: *Salmonella typhimurium* ATCC 13311; *Shigella flexneri* ATCC 29003; *Salmonella typhi* ATCC 6539; *Escherichia coli SOS*, and *Proteus mirabilis*. To determine the antibacterial activity and to know the Minimal Inhibitory Concentration (MCI), the protocol of Drummond and Waigh, modified by Satyajit, was followed, in which 96-multi-well plates were utilized, with resazurin as viabity indicator. This method is based on the capacity of reduction of the resazurin to resorufin by the oxidoreductase enzymes of the surviving bacteria. When the extract inhibits the bacterial growth, there is no activity of bacterial oxidoreductases. Thus, the resazurin remains blue in color, while when they survive, the reduction to resorufin confers a pink color on the culture medium with the swerve of the indicator.[15]

Antibacterial activity of the extracts

The bacteria were seeded by crossed streak on plates with Müeller–Hinton medium (Bioxón, México) and were incubated for 24 h at 37°C. A colony was taken and was seeded in duplicate in 50 ml of Müeller–Hinton broth (Bioxón, México). The culture was incubated 24 h at 37°C. After this, a 2.5-ml aliquot was taken; it was centrifuged (SOL-BAT, México) at 2,500 *g* during 5 min, the supernatant was eliminated, and the cell pellet was suspended in the same volume of sterile physiological saline solution (PSS.) The bacterial concentration was adjusted to 4 x 10^6 CFU/mL, with the 0.5 McFarland-nephelometer turbidity pattern, and the concentration was incubated for 24 h at 37°C. With the solid extracts, solutions were prepared separately with an initial concentration of 5 mg/mL in Dimethyl sulfoxide (DMSO, J.T. Baker, USA) at 1.0% and double dilutions were carried out. Fifty µL/well was deposited on 96 multi-well plates (Nunclon, Germany), 10 µL of the bacterial suspension (4 x 10^6 CFU/mL) was added, in addition to 10 µL of Resazurin sodium (Sigma Chemical Co, St Louis MO, USA) 0.675% p/v in sterile distilled water and 30 µL of Müeller–Hinton 3X culture medium (320 mosm) (Bioxón, México). As negative control, DMSO 1.0% was utilized in sterile distilled water, and as positive control, we employed a Penicillin–Streptomycin solution 1 x 10^4 IU/mL-1 x 10^4 mg/mL (Sigma Chemical Co. St. Louis, MO, USA). The culture plates were Incubated at 37°C during 22 h. (LabLine, USA) Each extract was proved in triplicate on at least three ocassions. Each extract was proved in triplicate on at least three occasions.

RESULTS

The preliminary phytochemical analysis of the hexanic and dichloromethanic extracts revealed the presence of terpenoid-type and fatty-acid compounds, while in the methanolic and aqueous extracts, soluble tannin compounds and flavonoids were detected.

Antibacterial Activity of the Rhizome

In Table 1, it is shown that the methanolic and aqueous extracts of the rhizome presented better antibacterial activity than those of hexane and dichloromethane. The latter was that of least activity, solely inhibiting *Shigella flexneri* and *Salmonella typhi*, bacterial strains that were inhibited by all of the extracts. The MIC obtained was 0.156 mg/mL of the methanolic and aqueous extracts on *S. typhi* and *S. flexneri*, respectively (Table 1).

Table 1. Antibacterial activity of the extracts of the rhizome of *Krameria pauciflora*

Extract:	*Escherichia coli*	*Proteus mirabilis*	*Shigella flexneri*	*Salmonella typhi*	*Salmonella typhimurium*
1. Hexanic	----	----	5	2.5	----
2. Dichloromethanic	----	----	2.5	2.5	----
3. Methanolic	5	0.312	0.625	0.156	1.25
4. Aqueous	5	1.25	0.156	0.312	2.5

-- Signifies negative.

The extracts of the leaves presented similar activity to that of the rhizome. However, it can be observed that the activity of the leaf is lesser with respect to that of the rhizome. With the methanolic extract, an MIC was obtained of 0.312 on *S. typhi* and with the aqueous extract on *S. flexneri* and *S. typhi* (Table 2).

Table 2. Antibacterial activity of the extracts of the leaf of *Krameria pauciflora*

Extract:	*Escherichia coli*	*Proteus mirabilis*	*Shigella flexneri*	*Salmonella typhi*	*Salmonella typhimurium*
1. Hexanic	----	----	2.5	2.5	----
2. Dichloromethanic	----	----	2.5	2.5	----
3. Methanolic	5	1.25	2.5	0.312	2.5
4. Aqueous	5	1.25	0.312	0.312	5

---- Signifies negative.

Antibacterial Activity of the Extracts of the Complete Plant

Different from the hexanic extracts of the rhizome and of the leaves, that obtained from the complete plant inhibited the growth of the strains employed. The hexanic, methanolic, and aqueous extracts of the complete plant inhibited all of the bacteria in concentrations lesser than those of the rhizome and the leaves, in particular on *S. flexneri* and *S. typhi*. However, it is important to highlight that the decoction presented the lowest MIC, that is, 0.078 mg/mL, of the study (Table 3).

Table 3. Antibacterial activity of the extracts and decoction of the complete plant of *Krameria pauciflora*

Extract:	*Escherichia coli*	*Proteus mirabilis*	*Shigella flexneri*	*Salmonella typhi*	*Salmonella typhimurium*
1. Hexanic	5	2.5	2.5	0.312	5
2. Dichloromethanic	----	----	5	2.5	----
3. Methanolic	5	0.312	1.25	0.156	1.25
4. Aqueous	5	1.25	0.156	0.312	2.5
5. Decoction	5	0.625	0.078	0.156	1.25

---- Signifies negative.

DISCUSSION

Neto and collaborators studied the effect of an ethanolic extract of *Krameria trianda* and its hexane, ethyl acetate, and methanol fractions on the growth of 18 bacterial strains, among these *Escherichia coli*. The raw extract inhibited such bacteria. However the hexane fraction did not inhibit any strain. In our study, the hexanic extract, particularly that of the leaf–stem–rhizome, inhibited all of the bacteria utilized, including *E. coli*. The difference in activity would be attributed to that, in the Neto study, the hexane fraction procedes from an ethanolic extract, a solvent that can extract some chemically non-polar compounds that are dissolved in hexane, while in our work, the hexanic extract was obtained directly with hexane as first extraction solvent, hence a greater number and variety of compounds.[16]

Bussmann and collaborators [17] prepared ethanolic extracts and an aqueous decoction of 52 plants from the North of Peru and evaluated their effect on the growth of *Escherichia coli* and *Staphylocccus aureus.* These authors reported that 38 of the ethanolic extracts inhibited *S. aureus* and that only two extracts inhibited *E. coli*, one of these being *K. lappaceae.* The aqueous extract solely inhibited *S. aureus*, but not *E. coli.* These latter results are in contrast with ours, in that the aqueous extracts of the rhizome, leaf, and complete plant inhibited *E. coli.* This difference could be attributed to that our aqueous extract derives from another species. It is the residue of a

material treated previously with three organic solvents chemically growing in polarity, thus less contaminated or more enriched with compounds that inhibit the proliferation of *E. coli.*

With respect to *K. lappacea,* Genovese and collaborators[18] reported the sensitivity of Methicillin-resistant *S. aureus* (MRSA) to the aqueous and ethanolic extracts of the root. Both extracts diminished the adhesive properties of the bacteria and their capacity to form biofilms. In results not included in this study, we evaluated the activity of the extracts of *K. pauciflora* on *S. aureus* ATTC 6538, observing susceptibility to the hexanic, dichoromethanic, methanolic, and aqueous extracts of the rhizome and to the decoction of the complete plant with an MIC of 0.156 mg/mL of the hexanic extract,[19] which confirms the susceptibility of *S. aureus* to species of the genus *Krameria*.

The antibacterial and antifungal activity of some species of the genus *Krameria* has been associated with the lignans and neolignans present in the plant's root. Also reported have been catechin tannins, ratanitanic acid, and N-methyltyrosine, and cycloartanes, among other compounds.[20,21]

Lignans and neolignans are chemically non-polar compounds that could preferentially affect the lipids of the external membrane of Gram-negative bacteria, achieving the disorganization of the membrane, which would explain the antibacterial activity of our extracts of hexane and dichloromethane.[22,23] The lignans of the root of *Krameria lappacea* have been linked with anti-inflammatory effects *in vitro* and *in vivo,* inhibiting acute inflation. Anti-inflammatory activity has also been reported in *Krameria pauciflora*, associated in this case with the cycloartanes isolated from the root of the plant.[24]

Other compounds detected in the genus *Krameria* are the tannins. Our study showed the presence of tannins in the methanolic and aqueous extracts and in the decoction. Due to that tannin compounds are soluble in water and in organic polar compounds, it is possible that the antibacterial activity demonstrated by the methanolic and aqueous extracts and by the decoction could be related with the presence of tannins in these, because it is known that tannins precipitate proteins, including structural or enzymatic, impeding the microorganism from feeding and reproducing itself.[25] Although the majority of the phenolic compounds are hydrophobic, the hydroxyl group (-OH) allows them to introduce aryl and alkaline groups into the proteins, modifying the three-dimensional (3D) structure of the latter. The OH-group can also affect the stability of the DNA, when such an interaction with amino and carbonyl groups of the pyrrhic and pyrimidine bases form new hydrogen bridges, rendering impossible the functionality of the microorganisms, in that they can disorganize the lipids of the bacterial membrane.[26]

In the literature, the antibacterial and antifungal activity is principally reported of the genus *Krameria* in extracts of the root. However, in our study, we demonstrated that other structures also possess antibacterial activity. In popular Mexican medicine, the decoction is recommended of the rhizome of *K. pauciflora* for the treatment of gastrointestinal infections. It is observed that the antibacterial activity improves when the extract is prepared with the rhizome–leaves–stem of the plant (the complete plant). It is additionally observed that this activity is even greater if, instead of preparing the extract, a decoction is utilized of the complete plant (rhizome–leaves–stem). Our results are in agreed with the popular use of plant in the zone of its collection.

CONCLUSIONS:

The minimal inhibitory concentration was obtained with the rhizome–stem–leaves decoction.

The methanolic extract demonstrated better antibacterial activity than the remaining extracts.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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