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SECONDARY METABOLITES AND IN VITRO ANTIMICROBIAL ACTIVITY OF ROOTS OF CUBAN ARGEMONE MEXICANA LINN

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ABSTRACT

Introduction: *Argemone mexicana* Linn is a native plant of the north of Mexico, belongs to the genus *Argemone* of the family *Papaveraceae*; it has a high concentration of benzylisoquinolinic alkaloids and medicinal properties in all its organs; however, no information was available on the phytochemical and antimicrobial characterization of the roots of plantations growing in Cuba. **Objective:** to determine phytochemical screening and antimicrobial activity of *A. mexicana* roots. **Methods**: the roots of the plant under study were collected; the samples at room temperature and stove were dehydrated, and then at 1 mm particle size were pulverized. A phytochemical screening on the alcoholic and aqueous extracts was performed. Also, the antibacterial activity (*Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella* Typhimurium and *Bacillus subtilis*) and antifungal (*Candida albicans*) were determined from the dried extract with a concentration of 250mg/ml. **Results**: the phytochemical screening of *A. mexicana* roots showed a high coalification of alkaloids in both extracts, likewise, it detected triterpenes, free amino acids, phenols and/or tannins, anthocyanidins and sugars. Also, the roots have antibacterial activity against *B. subtilitis*, *S. aureus* and *S. epidermidis* and antifungal activity against *Candida albicans.* However, no activity was found against Gram negative. **Conclusion**: *A. mexicana* roots have abundant alkaloids, as well as the presence of other beneficial secondary metabolites. In addition, the dried extract has antibacterial activity against Gram positive and antifungal against pathogenic yeast.

KEYWORDS: *Argemone mexicana*, root, secondary metabolite, antibacterial, antifungal.

INTRODUCCIÓN

It is recognized by the international scientific community that the indiscriminate use of antibiotics in humans and animals can increase the number of resistant strains and transfer cross-resistance to other microorganisms.[1] Specifically, in intensive animal production, antibiotics have been used since 1950 as growth promoters (APCs) to control enteric processes of a subclinical nature at an early age. Although many countries restrict their use, developing countries use it extensively.[2]

On the other hand, every day natural and traditional medicine is more used in human and veterinary medicine. Many studies have been developed with local plants with medicinal properties in order to find effective alternatives to reduce or eliminate the use of sub-therapeutic antibiotics in farm animals.[3] The studies focus on physico-chemical characterization, *in vitro* biological activity and levels of use in animals. In this sense, the phytochemical screening and antimicrobial activity of a new plant under study provides a perception about the possible medicinal benefits, especially to be used as a nutraceutical additive in animal diets.[4,5]

Argemone mexicana L. is a plant native to the north of Mexico, near the border with the United States, belongs to the genus *Argemone* of the family *Papaveraceae*. It is commonly known as holy thistle or chicalote. This plant has been widely used by native cultures for its healing

properties; its medicinal effects are attributed to the secondary metabolites, specifically the benzylisoquinoline alkaloids.[6] Other plants of the family *Papaveraceae* have high concentrations of these beneficial alkaloids such as *Macleaya cordata*, *Sanguinaria canadensis*, *Argemone ochroleuca* Sweet and *Chelidonium majus*.[7]

The benzylisoquinoline alkaloids are heterocyclic aromatic organic compounds and are a structural isomer of quinoline. These alkaloids include benzophenanthridines (sanguinarine, chelerythrine), protoberberines (berberine) and protopines (protopine, allocryptopine).[8] These chemical compounds have various medicinal properties, such as analgesic, expectorant, purgative, sedative, antimicrobial and immunomodulator.[6-9]

In this sense, extracts of leaves and seeds of *A. mexicana* have been used as antimalarial, larvicidal against larvae of *Aedes aegypti* and *Culex pippiens*, molluscicide against *Lymnaea acuminata* and anti-inflammatory in cases of conjunctivitis and psoriasis.[10-13] Moreover, aqueous extract of the aerial parts reduces serum glucose in laboratory mice, as well as the use of barberine in patients with hypercholesterolemia decreases the harmful serum lipids[14] and sanguinarine and chelerythrine increases the apoptosis in tumor cells.[15] Studies in poultry, pigs, fish and calves demonstrated that dietary supplementation of *Macleaya cordata* extract with high concentrations of benzylisoquinoline alkaloids improves immunological status and biological response and decreases intestinal inflammation and diarrheal syndrome.[16] However, oral use of high contents of this plant causes intoxications and vomiting in cattle and epidemic dropsy syndrome on humans.[17]

Although the leaves, flowers, seeds, fruits, latex and roots of *A. mexicana* have a great diversity of secondary metabolites (especially alkaloids); to our knowledge no studies have been reported to address their use in animal diets, with prior chemical characterization and antimicrobial activity, specifically in the root, organ of the plant that concentrates the largest number of these secondary metabolites.[6]

The objective of this work was to determine the secondary metabolites by phytochemical screening and the *in vitro* antimicrobial activity of the roots of *Argemone mexicana* L.

MATERIALS AND METHODS

Samples and preparation

The roots of *A. mexicana* were collected from the surrounding urban areas of Santiago de Cuba province, Cuba, figure 1. The plants were identified and placed in the collection of the Department of Botany, Faculty of Agronomy at the University of Granma, Cuba. These territories are characterized by a flat topography and brown soil with carbonate.

Figure 1: Dried roots of cuban *A. mexicana.*

The roots for 14 days were dehydrated in the shade on perforated cardboard and twice per day were removed. Then, in a hammer mill with parallel blades at 1 mm in particle size were crushed, figure 2. Samples at room temperature in amber flasks were stored to avoid decomposition of the active substances by light.[18]

Figure 2: Root powder of cuban *A. mexicana.*

Preparation of ethanolic and aqueous extracts

The experiment was carried out at the Center for Applied Chemistry Studies (CEQA) of the Faculty of Technical Sciences of the University of Granma, Cuba.

The dried powders obtained from the roots of the plant under study were taken, then 5 g into an analytical balance (BS 2202S Sartorius, China) were weighed and 50 mL of ethanol of 70% was added for the preparation of the alcoholic extract and 50 mL of distilled water to obtain the aqueous extract, followed by extraction in an analogous manner was done.

Phytochemical screening

Simple, rapid and selective techniques to determine the different secondary metabolites were used. Phytochemical screening according to the methodology described by Miranda and Cuellar[19] was performed. The tests were: Liebermann-Burchard (triterpenes), foam (saponins), ninhydrin (free amino acids), Mayer, Wagner and Drangerdoff (alkaloids), Baljet, Fehling (reducing carbohydrates), ferric chloride (phenols and/or tannins), Borntrager (quinones), Shinoda, (flavonoids), Kedee (glycosides), Molish (sugars), Mucilages (polysaccharides), resins, anthocyanins and catechins.

The crossing system as a measurement criterion for the identification of the secondary metabolites was used.

Antibacterial and antifungal activity

The experiment was carried out in the Microbiology Laboratory of the Studies Center of Plant Biotechnology, Faculty of Agronomy, University of Granma, Cuba.

The dry extract of *A. mexicana* roots from the ethanolic extract was obtained. A sample of 100 mL on a 500mL balloon was deposited and in a rotary evaporator (IKA LABORTECHNIK HB4, Germany) and a consistent mass by removing the solvent at 50°C at a rotational speed of 60rpm was obtained.

To evaluate the antibacterial activity of the dry extract, the Bauer-Kirby disc diffusion agar method was used.[20] A battery consisting of two international reference strains deposited with the American Type Culture Collection (ATCC)[21] and three wild strains isolated in the Center of Hygiene and Epidemiology of Manzanillo, Granma, Cuba were used, Table 1.

The bacterial strains separately in 90mm diameter Petri dishes (Alumbra, China) with 20mL of Mueller-Hinton agar (BioCen, Cuba, lot: 2500005) (pH 7.3±0.2) were seeded and for 16-18 hours at 35±2°C in an incubator (Boxun BG-80, China) were incubated.

Table 1: Bacterial strains used in the battery.

Name	Type	ATCC No.
Bacillus subtilis	Gram positive	ATCC 3366
Staphylococcus aureus	Gram positive	Wild
Staphylococcus epidermidis	Gram positive	Wild
Escherichia coli	Gram negative	Wild
Salmonella Typhimurium	Gram negative	ATCC 14028

From the dry extract dissolutions with a concentration of 250mg/mL were made using dimethylsulfoxide (DMSO). To obtain the inoculum, 1-2 colonies from each of the cultures obtained were isolated and by separately in test tubes with a physiological saline (NaCl 0.89%) were resuspended. The turbidity to a standard of 0.5 McFarland for a concentration of approximately 1.5 x 10^8 UFC/mL was adjusted.

As negative controls, 6mm diameter filter paper disks loaded with 5µL of DMSO were used and as positive controls, commercial antibiotic disks of Gentamicin, Ciprofloxacin and Amoxicillin of 30, 5 and 30 µg/disc, respectively, were used (Sensi-Disc TM, France). Each treatment had three replicates. Finally, Petri dishes at 37±0.1°C in an incubator (Boxun BG-80, China) were incubated. After 24 hours of incubation, the surface of Petri dishes was examined. The results by reading in millimeters of diameter the halo of inhibition of the growth of microorganisms were evaluated.[22]

In order to determine the antifungal activity of the extract obtained from of *A. mexicana* roots against the yeast *Candida albicans*, malt extract agar as culture medium for the seeded and inoculation of the yeast (BioCen, Cuba, lot: 7500007) (pH 5.6±0.2) was used; the incubation period after inoculation was 48 hours at 29±1°C. As negative controls 6mm diameter filter paper disks loaded with 5µL of DMSO were used and as a positive control, antifungal Fluconazole discs of 30µg/disc (Sensi-DiscTM, France) were applied.

The data using the descriptive statistics module were analyzed and the mean was determined. Statistical software SPSS version 22.1 was used.

RESULTS

Table 2 shows the phytochemical screening of the *A. mexicana* roots grown in Cuba. Both extracts (ethanolic and aqueous) have a high coalification of alkaloids (+++) detected by the three analytical techniques. In the ethanolic extract triterpenes, free amino acids, phenols and/or tannins, anthocyanidins and sugars were identified, but not resins, coumarins, quinones, flavonoids, glycosides and catechins. Also, in the aqueous extract were observed the reducing carbohydrates without presence of saponins and polysaccharides.

Table 2: Phytochemical screening of the roots of Cuban *A. mexicana.*

Compounds	Ethanolic extract	Aqueous extract
Resins	-	
Triterpenes	+	
Saponins		-
Free Amino Acids	+	
Alkaloids (Mayer)	+++	+++
Alkaloids (Wagner)	+++	+++
Alkaloids (Drangerdoff)	+++	+++
Coumarins	-	
Reducing carbohydrates		+
Phenols and/or Tannins	+	
Quinones	-	
Flavonoids	-	
Anthocyanidins	+	
Glucosides	-	
Sugars	+	
Mucilages (Polysaccharides)		-
Catechins	-	

Legend: *(-) Absence (+) Presence (+++) Abundance*

Data on *in vitro* antibacterial activity with a concentration of 250mg/mL in DMSO from dry extract of *A. mexicana* roots against five bacterial strains are presented in table 3. This concentration has activity against the Gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, but not against Gram negative bacteria (*Escherichia coli* and *Salmonella* Typhimurium). The results of the positive controls showed better results than the *A. mexicana* root extract.

Table 3: *In vitro* antibacterial activity of the dry extract of the roots of *A. mexicana*.

Items	Inhibition halos (mm) against bacterial strains				
	B. subtilis	***S. aureus***	***S. epidermidis***	***E. coli***	***S.* Typhimurium**
A. mexicana	10.7±1,15	12,7±1.53	10.3±0.58	-	-
Gentamicin	-	19.3±0.58	23.0±1.00	20.7±1.53	18.7±0.58
Ciprofloxacin	17.0±1.00	22.7±1.53	23.0±1.00	31.3±1.53	16.0±1.00
Amoxicillin	18.7±0.58	30.0±2.00	29.3±1.53	19.0±1.00	19.0±1.00

(-) Negative result in the inhibition of the bacterial growth.

The *in vitro* antifungal activity of the dry extract of the roots of *A. mexicana* is shown in table 4. The alternative treatment (*Argemone mexicana* extract) against *Candida albicans* showed 8mm less than fluconazole indicating 20.33mm in the halos of inhabitation.

Table 4: *In vitro* antifungal activity of the dry extract of the roots of *A. mexicana*.

Items	Inhibition halos (mm)
	Candida albicans
A. mexicana	8.00±0.01
Fluconazole	20.33±1.52

DISCUSSION

Phytochemical screening of medicinal plant extracts provides an idea of its possible preventive and therapeutic effects.[5,18] Thus, the presence of beneficial secondary metabolites in the *A. mexicana* roots could confirm their medicinal properties. The abundant presence of alkaloids coincides with other authors,[8.23] who identified benzophenanthridine alkaloids in root extracts of *A. mexicana* from Mexican cultivars. Also, these alkaloids were detected in the leaves and seeds of wild Indian plantations.[24] Likewise, Rubio-Piña and Vázquez-Flota[6] reported that sanguinarine is the most found benzylisoquinoline alkaloid in roots and seeds, while barberine and protopine are identified in all the organs of this plant. Besides, dihydrosanguinarine, dihydrochelerythrine, nor-sanguinarine, nor-chelerythrine, cop-coptisine, isocoridine, oxyhydrastine, (α; β)-hydroxymethylstylopine, 6-acetonylsanguinarine, cryptopine, and cheilantifoline were other alkaloids identified in the tissues of *Argemone*.[6,25]

The presence of these alkaloids gives the plant analgesic, anti-inflammatory, antioxidant, expectorant and sedative properties.[26,27] In this sense, use of 200 and 400mg/kg of methanolic extract of *A. mexicana* decreases the acute inflammation in mice[28], as well as the concentration of 100mg/mL of the ethanolic extract of the roots and 200mg/mL of several extracts of leaves reduces oxidative stress *in vitro*.[29,30]

The identification of tannins in this study coincides with other authors, [23] who reported on the anti-peseudomonal activity of the ethanolic extract of *A. mexicana*. In addition, this metabolite has astringent properties because it dries and decreases the inflammation of the mucosa of the intestinal tract and the diarrheal syndrome in humans, calves, poultry and pigs, being a very important characteristic to counteract the animal stress.[5,9,31]

Non-existence of flavonoids in the plant under study is contradictory; indistinctly investigations have reported the presence or not of this metabolite in the methanolic, chloroform and aqueous extracts of the seeds.[23,24,32] It seems that the low presence of flavonoids in the roots and extracts studied could determine this result. However, other investigations are necessary to confirm this hypothesis.

On the other hand, the triterpenes qualified in the of *A. mexicana* roots present antibacterial and anti-inflammatory activities in the intestinal mucosa, which improves the intestinal health and therefore the absorption of nutrients.[23] In addition, Singh and collaborators[32] detected glycosides in methanol and saponins in aqueous extract, these compounds exhibit antiprotozoal activity.[30] It is noted that the extraction of the biologically active compounds from the plant material depends on the type of solvent, the part of the plant used and the procedure employed.

Apparently, the antibacterial activity of *A. mexicana* is due to the presence of benzylisoquinolinic alkaloids, including sanguinarine and chelerythrine.[32] According to the results obtained, the *A. mexicana* roots have antibacterial activity only against bacteria Gram positive. In this sense, investigations using extracts of leaves and seeds of this plant[10,24,32] showed similar responses. However, other authors[24,33] found antibacterial properties against Gram positive and Gram negative with the chloroform extract of the seeds of *A. mexicana*. It appears that higher concentrations than those used in this

study are needed to inhibit the growth of Gram negative bacteria.

Antimicrobial effect of benzylisoquinolinic alkaloids has been attributed to bacterial cell lysis, as these secondary metabolites forms pores in the cell membrane and causes the loss of cytoplasmic components. It has also been shown to inhibit nucleic acid biosynthesis, membrane proteins and phospholipids.[23,24]

On the other hand, this experiment demonstrated the antifungal action of the ethanolic extract of the roots of *A. mexicana*. Vaghasiya and others[28] using various extracts reported that the methanolic extract has the highest antifungal activity in this plant. In addition, other investigations have found that the seed shows toxicity against a number of fungal strains.[6] In addition, the latex of the plant diminishes the colonization of *Trichophytan mentagrophytes*, as well as, the extract of the leaves presents significant toxic activity against pathogenic fungi of fruits like *Alternaria alternata*, *Dreschlera halodes* and *Helminthosporium speciferum*, and against *Curvularia tuberculata*, responsible for different diseases.[1] It is important to note that antimicrobial few studies have been developed with the roots of *Argemone mexicana* grown in Cuba, which this study could lay the scientific groundwork for further research with this organ of the plant.

CONCLUSION

According to phytochemical screening, powder roots of *A. mexicana* have abundant alkaloids, and the presence of other beneficial secondary metabolites. In addition, it has antimicrobial activity against Gram-positive bacteria and a fungal strain.

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CONFLICTS OF INTEREST

The authors declare that there is no potential conflict of interest regarding the publication of this article.

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