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Research Article

Phytochemical, Pharmacological and Antimicrobial Properties of the Tissue Extracts of *Argemone* spp.

Margarita Jurado Pérez[1,2], Favela-Hernández JM[1,3], Guerrero GG[4]* and Concepcion Garcia-Lujan[1]

[1]*Universidad Juárez del Estado de Durango (UJED), Facultad de Ciencias Químicas, Fraccionamiento Filadelfia, Gómez Palacio, Mexico*

[2]*Universidad Autónoma Chapingo, Unidad Regional Universitaria de Zonas Áridas, Bermejillo, Mexico*

[3]*Instituto Multidisciplinario de Ciencias AVICENA, Torreón, Coahuila, Mexico*

[4]*Universidad Autónoma de Zacatecas, Campus II, Unidad Académica de Ciencias Biológicas, Mexico*

***Corresponding Author:** Concepcion Garcia-Lujan, Universidad Juárez del Estado de Durango (UJED), Facultad de Ciencias Químicas, Fraccionamiento Filadelfia, ómez Palacio, Mexico.

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Abstract

The medicinal plants of the genus *Argemone* spp are used for the alternative treatment of various pathologies and have a wide distribution in the Mexican territory. However, phytochemical knowledge about the genus *Argemone* spp is limited, and the scientific literature is scarce. Therefore, in the present work, we aimed to investigate the chemical composition, antioxidant, toxicological, and antimicrobial properties of *Argemone spp.* Ethanolic and acetic acid extracts of the different vegetal tissue parts of the plant were obtained. The Higher content of phenols than flavonoids in acetic and ethanolic extracts in petals and sexual organs. While the higher amount content of total alkaloids in the Acetic acid extracts of the leaf, stem, and sexual organs. Phenols and total flavonoids obtained from ethanol extracts showed a higher antioxidant activity than acetic extracts. However, lower toxicity in the Acetic acid extracts was found. The data show that ethanolic seed extracts are a good source of compounds with antifungal activity (*C. albicans). In contrast,* acetic Acid extracts of different tissues except seeds are a good source of secondary metabolites mostly with anti-bacterial properties.

Keywords: Phytochemical; Phytherapy; Secondary Metabolites; *Argemone* spp; *C. albicans*; *S. aureus*; *E. coli*; *P. aeruginosa*

Introduction

The Papaveraceae family, or poppy family, is an ethnopharmacological family of forty-four genera of plants and approximately seven hundred and sixty species of flowering plants. Taxonomic classification was difficult. Twenty-four and thirty-two species were identified and distributed in North and South America, Hawaii, Australia, India, and various parts of Africa and Asia [1-4]. *Argemone* L. plants with stems, leaves, and spiny capsules family of plants from whose *Argemone Mexicana* belong to this genus widely distributed in Mexico and the United States and used in traditional medicine for various pathologies, including the treatment of warts, skin infections, dropsy, and jaundice [4,5]. *Argemone* spp is considered an endemic plant of the North of the country, which has the advantage of being easy to grow,

does not require special care, and is easy to obtain; however, it is essential to clarify that phytochemical knowledge about the genus *Argemone* spp is limited and the scientific literature is scarce, so it is pertinent to carry out studies on the phytochemical profile, antioxidant, toxicological and antimicrobial activity of the plant for agrochemical, pharmaceutical, and therapeutic industry Approximately forty alkaloids compounds belonging to six groups with isoquinoline bases have been isolated from plants of the genus *Argemone*; however, the genus *Argemone* has been little studied by botanists.

Argemone mexicana is a thorny plant that grows mainly in subtropical states [4,5]. The medicinal application is in the traditional Indian medicine system for skin diseases, fever, diarrhea, and dysentery. *Argemone* spp. is a source of compounds such as alkaloids (protopine, sanguinarine, berberine, and benzophenanthridine), Flavonols (quercetin, rutin, and mexitin), Phenolic acids, and amino acids [6] (Singh., *et al*. 2012). In addition, the seed oil of the *Argemone Mexican* is composed almost entirely of sanguinarine, which shows anti-inflammatory and antibacterial activities [7-9].

The chemical composition of the plant includes various alkaloids, including protopine, sanguinarine, berberine, and benzophenanthridine, flavonols such as quercetin, rutin, and mexitin, as well as phenolic acids and amino acids [6,10,11]. Phenolic acids and flavonoids are naturally occurring phytochemicals in plants and foods that can regulate carbohydrate digestion, insulin secretion and signaling, and glucose uptake, in addition to epidemiological studies reveal that there is an inverse relationship between the consumption of a diet rich in these compounds and the development of chronic degenerative diseases. Alkaloids present in plants, animals, and fungi, are nitrogenous molecules, which have activity in the system central nervous system, which is why they are considered molecules of therapeutic interest [12-16]. Polyphenolic compounds are molecules from the secondary metabolism of plants with at least one aromatic ring to which one or more hydroxyl groups are attached. These compounds are present in fruits, vegetables, and cereals and exert protective functions against ultraviolet radiation and pathogen attacks [17,18].

The phenolic compounds occur most widely in plants, such as phenolic acids, flavonoids, coumarins, stilbenes, tannins, lignans, and lignins [19]. Phenolic acids are part of the polyphenolic compounds and have innumerable health benefits, such as anti-inflammatory, antibacterial, antiproliferative, anticancer, and antioxidant activities. In addition, they constitute a third of the phenolic compounds obtained from the diet [19-23]. More than 4000 flavonoids were identified and classified into anthocyanins, flavones, isoflavones, flavanones, and flavonols [24]. Flavonoids are antioxidants due to their high redox potential, which allows them to act as reducing agents, hydrogen donors, and oxygen inhibitors. In addition, they have metal chelation potential [24,25]. There is currently great interest in flavonoid research due to the possibility of improving public health through a diet of fruits and vegetables [26-28]. One of the current problems in public health is microbial resistance to antibiotics. The secondary metabolites extracted from different parts of the plants with antimicrobial activity represent a wealth of alternatives against bacterial, fungus, or viral pathogens. In recent years, *S. aureus* infections have increased thanks to the development of resistance against antimicrobial agents. These infections are already acquired outside the hospital environment, affecting the skin and soft tissues, and can cause necrotizing pneumonia. However, infections caused by *S. aureus*, one of the most frequent nosocomial diseases in immunosuppressed patients, are associated with 10% of catheter contamination [29]. In the case of *Pseudomonas aeruginosa*, this bacteria occurs in both abiotic and biotic environments, from soil and aquatic environments to plant and animal tissues. It can be isolated from several sources, including various nosocomial and life-threatening infections in patients with cystic fibrosis (CF), burns, urinary tract infections (UTIs), and pulmonary infections; medical equipment, such as inhalers, dialysis equipment, respirators, anesthesiology equipment, and vaporizers; and toilets and sinks. The infectious diseases caused by the enterobacteria, such as *Escherichia coli (E. coli),* colonize the human intestine a few hours after birth and are considered normal flora. However, six strains of *E. coli* are enteropathogenic that cause diarrhea, such as enterotoxigenic (ETEC), enterohaemorrhagic (EHEC), enteroinvasive (EIEC), and enteropathogenic (EPEC) [30,31].

In referring specifically to the fungus infectious disease caused by *Candida albicans* (present in the vagina), the absence of immunosuppression or damaged mucosa is not associated with any clinical signs of infection and therefore is termed colonization. Unlike asymptomatic colonization, vulvovaginal candidiasis (VVC) shows signs and symptoms of inflammation in the presence of *Candida* spp. and the absence of another infectious etiology

[32]. Among the virulence factors in *Candida* are enzymes such as proteases, phospholipases, and lipases [32-34]. Adhesins, like ls, Hwp1p, Int1p, and Mnt1p promote the adhesion of *C. albicans* to host cells or their specific ligands. Moreover, proteins of *C. albicans* bind to various extracellular matrix proteins of mammalian cells, including fibronectin, laminin, fibrinogen, and type I and IV collagen [35]. Therefore, in the present work, we aimed to investigate the chemical, antioxidant, toxicological, and antimicrobial properties of *Argemone* spp. Herein, we are reporting that *seed* ethanolic extracts are a good source of compounds with antifungal activity (e.g. *C. albicans)*. W*hile* different tissues in acetic acid extracts of plants mostly show antibacterial activity.

Material and Methods

Biological material

Ten plants of *Argemone* spp were harvested on the Gómez Palacio - Tlahualilo highway, with coordinates N 250 36.975 W 1030 27.334 Km 5.87. Different plant tissues separated from the plant [2] (Figure 1). Each tissue was dried in a desiccator at 350C for an extended period. Dried tissues were grounded and sieved (with a sieve number one hundred). Fine powder was stored in a dark and dry environment.

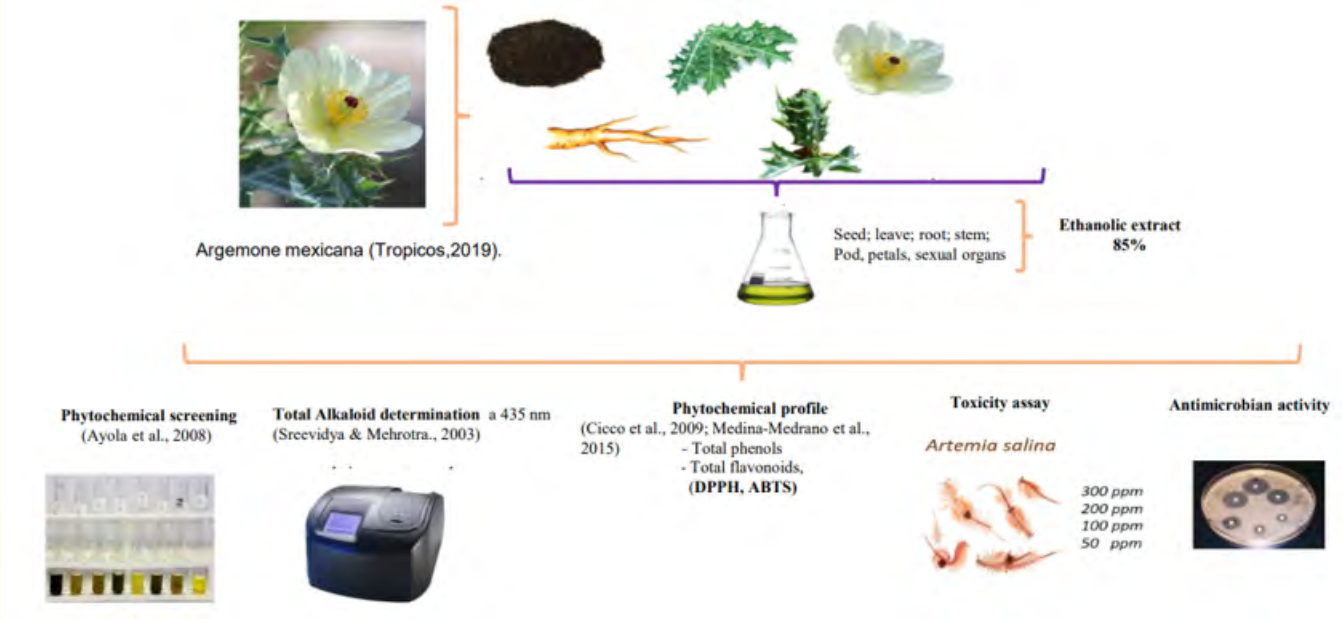

Figure 1: Pipeline for the phytochemical, antioxidant, toxicological and antimicrobial properties of the tissue extracts of *Argemone* spp, collected in the Gomez Palacio, Dgo town, ground and sieved. Ethanolic (85%) and Acetic acid (2%) extracts of leaf, pod, root, stem, seeds petals and sexual organs. Reports in the literature on *Argemone mexicana* has been focused in the aerial parts.

Plant extracts

Tissue grounded and sieved (0.5 g) added to five mL of ethanol (85%) or Acetic Acid (2.5%) [36-39]. The mixture was homogenized and then placed in an ultrasonic bath in the dark for 60 minutes. Centrifugation at 3500 rpm for 10 minutes, obtaining a tablet. The recovered supernatant of the previous step extraction (V= 10 ml) storing until use and repeating the extraction process.

Recovery index

For the recovery index acetic and ethanolic extracts of *Argemone* spp evaporated and concentrated. 1 mL of extract was put in a 35-mm diameter Petri dish (previously labeled and weighed on an analytical balance) and left for 24 hours at 37ºC and in the dark. The difference in weights indicated the concentration of each

extract. Then, the concentrated extract in sterile distilled water for the corresponding dilutions in the toxicity and antimicrobial activity tests.

Biochemical screening

The phytochemical screening to obtain a general overview of the secondary metabolites in each part of the plant consisted of fifteen tests with the methodologies accordingly to different authors from the literature. For the Alkaloids, three techniques as described by Ayoola., *et al*. 2008 [40]. One relies on the precipitation of alkaloids with protonated tertiary nitrogens and quaternary nitrogens [41]. The modified saponin [42] consisted: of 1 mL of extract in 1 mL of boiling distilled water and shaking vigorously for 45 seconds. The presence of saponin is positive with foam formation. With Agitation forms persistent foam. The measurement of the foam made with a Vernier. Steroids and terpenes. Thirty microliters (30 µL) of sulfuric acid was added to a volume (30 µL) of tissue extract. A color change to yellow o red is positive. No color change or another color change is negative. For Coumarins. 100 µL of 10% sodium hydroxide was added to a 500 µL of tissue extract. A color change to yellow is positive. A negative result for coumarins was no change of color when adding sodium hydroxide. To confirm the presence or absence of coumarins. No color change after adding 10 µL of sulfuric acid.

Quantification of total flavonoids

For the quantification of the total flavonoids, is used the method described by Medina-Medrano., *et al*. 2015 [43], and Woisky and Salatino., 1998 [44] which is based on the formation of complexes between aluminum chloride (ALCl3) and flavonoids to develop a yellow coloration measured at an absorbance of 420 nm. 141 µL of the acetic acid or ethanolic extract sample mixed with 60 µl of aluminum chloride (AlCl3) at 5% (p/v, prepared in distilled water) in an Eppendorf tube, after which it was shaken in a vortex for 15 seconds and incubated for 20 minutes at room temperature. Then, absorbance in a UV-VIS spectrophotometer at a longwave of 350 to 500 nm. The blank for the readings (sample and solvent). The number of total flavonoids with a calibration curve using quercetin standard at 1 µg/ml. The results of total flavonoids as equivalent to milligrams of quercetin per gram of fresh dry food (mg EQ/g PS). The data analysis was in triplicate for each of the samples. The results are as the measurement of ± SD.

Determination of alkaloids using the Dragendorff's reagent (DR)

Total alkaloids precipitated by Dragendorff's reagent (DR) [45-47]. This method consist in the yellow bismuth complex formation in a medium acidified with nitric acid and thiourea following the Lambert-Beer law in the concentration range of 0.06 to 50 µg/mL with a maximum of 465 nm. Acetic acid tissue extract (2.5 ml) of *Argemone* spp to 50 µL of 10% HCl until a pH of 2 to 2.5. 1 mL of DR was added and centrifuged for 15 minutes at 3500 rpm. Precipitation of the Alkaloids was verified by adding a drop of DR which should precipitate immediately. After this, the supernatant was decanted several times. The precipitated was washed with 96% ethanol by adding 3.5 mL and centrifuged at 3500 rpm for 10 minutes. The supernatant was discarded, and the residue was treated with 1 mL of 1% sodium sulfide solution. The precipitated formation of a brownish-black precipitate was subsequently centrifuged at 3500 rpm for 15 min and verified by adding one drop of sodium sulfide. The supernatant was discarded, and the residue was dissolved in 1 mL of concentrated nitric acid (solution in 5 ml of distilled water). For the spectrophotometric determination, 500 µL of this solution was added to 2.5 mL of thiourea solution. The absorbance was measured at 465 nm with a blank solution containing 500 µL of 20% nitric acid and 2.5 mL of thiourea. The number of total alkaloids is determined based on the amount of bismuth present in the solution, calculated by multiplying the absorbance values of the sample with the factor, taking into account the appropriate dilution factor. The factor obtained from the standard curve with bismuth nitrate pentahydrate ($Bi(NO_3)3.5H_2O$) as standard with an initial concentration of 0.25 mg/mL.

Factor = concentration/absorbance Dragendorff's reagent was prepared by mixing two solutions, the first consisting of 0.8 g of $Bi(NO_3)3.5H_2O$ in 40 mL of distilled water together with 10 mL of glacial acetic acid, and then adding the second solution which

consisted of 0.8 g of drinking iodide (KI) dissolved in 20 mL of distilled water.

Antioxidant capacity

The term antioxidant capacity is the ability to eliminate or neutralize free radicals associated with the presence of compounds capable of protecting a system against complete biological oxidation [48]. The antioxidant capacity is a measurement of two factors, the rate of radical removal and the number of radicals an antioxidant molecule can remove [49-52]. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical blocking capacity. One of the most widely used methods for antioxidant capacity in food and plant extracts is the DPPH radicals test [53]. This technique is based on DPPH radical reduction, which, in solution, has an intense violet color. When in contact with an antioxidant, an oxidation-reduction reaction occurs, causing DPPH change to a pale yellow color, indicating, thus, DPPH reduction, and can be measured at an long wave of 515-520 nm [36,48]. The test used as the method described by Carboleda-Velasco., 2017 [36]. A calibration curve prepared in triplicate for each tissue extract and incubated for 20 minutes in the dark, followed by lectures at a wavelength of 523 nm. DPPH solution with 0.625 mg per 100 mL of 85% ethanol. Data analysis based on the percentage of entrapment of the DPPH radical.

Toxicological properties

The toxicity of *Argemone* spp extracts. For the toxicity of *Argemone* spp, tissue extracts underwent treatment before the test. Artemia salina cysts were incubated for 24 hours to cause their hatching in a brine solution prepared with 20 g of sea salt for each liter of distilled water, and 0.16 mg of yeast was added, with a pH of 7. After hatching, the larvae were in vials with a capacity of 250 µL (10 larvae per vial) and immediately exposed to different concentrations of the extracts, 300, 200, 100, and 50 µg/mL. After 24 h of exposure to the tissue extract, surviving or dead larvae were registered, and Probit analysis was to obtain the fifty lethal concentration (LC50) in µg/mL.

Antimicrobial activity of the tissue extracts of *Argemone* spp. Bacteria's (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli)* and fungi (*Candida albicans*) of medical importance were obtained from clinical samples. The strains were identified and isolated from the Strain Collection of the Faculty of Chemical Sciences of the Juárez University of the State of Durango Gómez Palacio Unit. Bacteria or fungi on Petri dishes maintained at 4°C until use. Briefly, 2 to 5 colonies of each culture were resuspended in tubes with 3 ml of sterile saline solution, adjusted to 0.5 of the Mac Farland standard equivalent to 1.5 X108 CFU/mL 100 µl, and placed on the center of the Petri dish with the agar indicated for each microbial species and homogenized with a sterile swab. For stock solutions of ethanolic or acetic acid tissue extract of *Argemone* spp (2 mg/ml) prepared in distilled water. Serial dilutions at 1000, 500, 250, 125, and 62.5 µg/ml. Determination of the Minimum Inhibitory Concentration (MIC) by the plate diffusion method with sensidisks The assay to test the antifungal activity by the agar diffusion method where the bacteria evenly distributed with a loop on the surface of the plate. Once the Petri dishes were inoculated, sterile Whatman No.4 filter paper discs with a diameter of 6mm were placed in them equidistant and impregnated with 12.5 µL of each concentration of the treatments. The plates were incubated at a temperature of 35°C for 24 hours. The zone of inhibition is measured as the radiated surface area of the disc that is free of bacterial growth. Two measurements perpendicular to each of the edges of the paper disk to the outer edge of the inhibition zone averaged.

Statistic data analysis

A correlation of the variation of phenols and flavonoids made with the development and the analyzed tissues of *Argemone* spp. The data analysis with a variance Tukey test at $p < 0.05$. To verify the normal distribution, the Shapiro-Wilk test and the statistical package used Minitab 17.

Results

The Phytochemical profile in the tissue extracts of *Argemone* spp represented mostly by Alkaloids, Flavonoids and Phenols.

To investigate and evaluate different properties, such as chemical profile, antioxidant, or toxicity in the plan*t Argemone* spp, we follow *a* pipeline of the work (Figure 1). The phytochemical screening (Mats and Methods) in Ethanolic and Acetic acid extracts are characterized by flavonoids, tannins, and sesquiterpenes in some tissue extracts (Table 1). Of note is that alkaloids are present in all tissue extracts as constant with three different determinations. In general, a higher content of phenols than flavonoids in all tissues. However, ethanolic extracts (85% v/v) have a higher concentration of phenols and total flavonoids than 2% Acetic acid extracts. Moreover, the highest content of total phenols in the ethanolic extract in petals with a total of 34.37 mg EQ AG/g dry weight of the sample ($P < 0.05$). (Figure 2A), and in general, it produced the highest content of secondary metabolites, followed by sexual organs (B) with a total of 22.60 mg EQ AG/g dry weight of the sample. In third place was the leaf extract (C), while that seed and pod (D) do not present significant differences between them, but they do with the other groups, like stem and root (E) that are statistically the same but different from all the other groups. Furthermore, total flavonoids in the ethanolic extract

of petals (group a) were the highest amount (16.62 mg EQ Q/g dry weight), followed by the sexual organs extracts (group b). No significant difference ($P < 0.05$) between the leaf and seed extracts (group c), and no differences between pod, stem, and root (group d) (Figure 2A) (yellow bars) ($P < 0.05$).

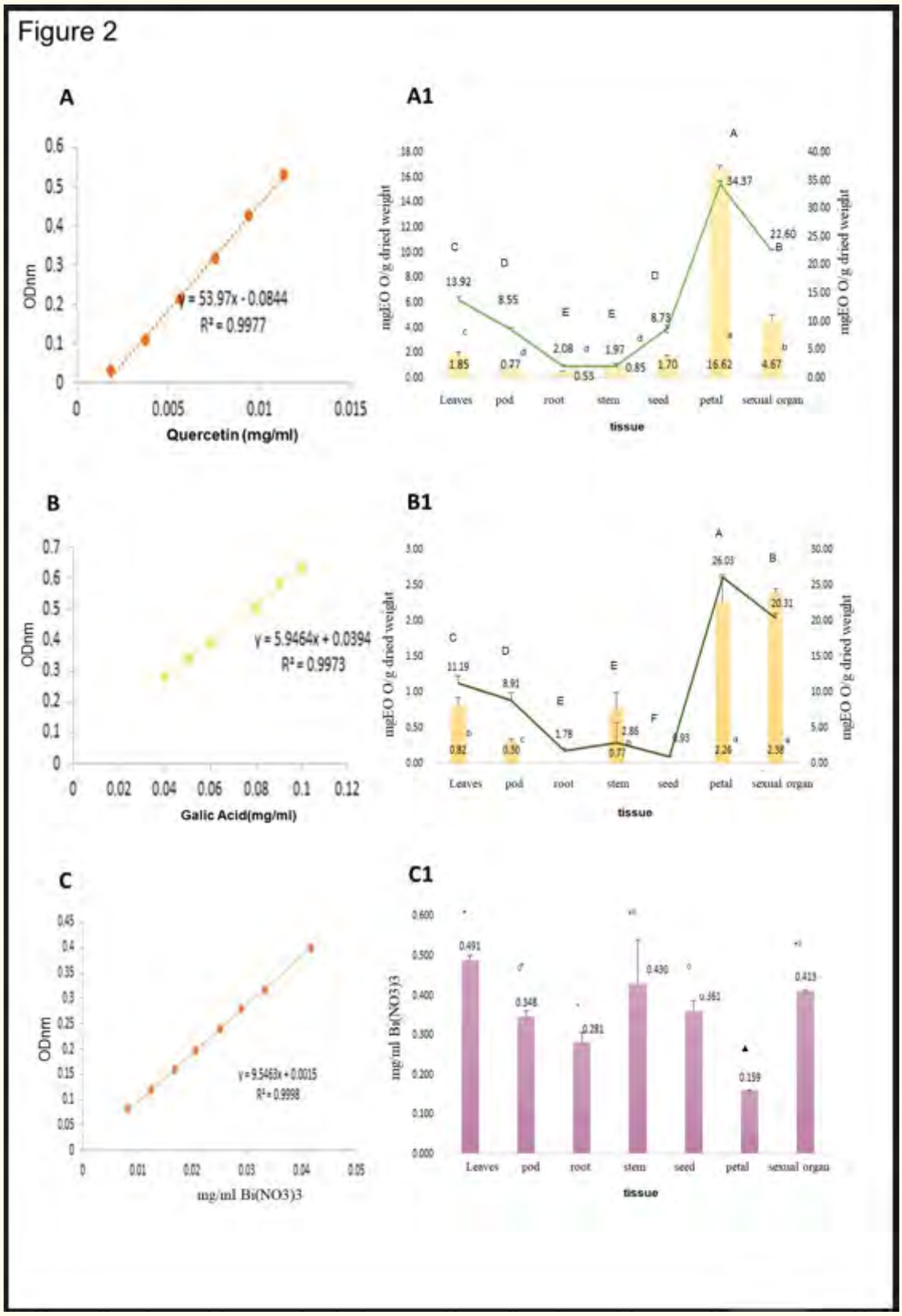

Figure 2: The phytochemical profile of the *Argemone* spp. For the phytochemical profile in the ethanol (85%), and Acetic acid(2%), Thirty four tissue extracts obtained and tested as described in material and methods. The amount of secondary metabolites (phenols, flavonoids) calculated and extrapolated from the calibration curves of Quercetin (A) for Flavonoids (A1); Galic Acid (B) for Phenols (B1); Bi(NO3)3 (D) for total alkaloid in acetic acid tissue extracts (D1) . Letter in uppercase represent the statistics analysis for the total phenols. The lower case letters represent the total flavonoids. Differential letter, and symbols indicate significative differences at $p < 0.05$ (post hock Tukey ANOVA analysis).

Tabla 1. Phytochemical screening of the different ethanolic extracts of Argemone spp.

Metabolite	Leaves	pod	root	stem	seed	petal	Sexual Organ
Alkaloids	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Steroids and Terpens	-	-	+	-	-	-	-
Flavonoids (Flavons/Flavonoids)	-	-	+/+	+/+	+/+	+/+	+/+
Tanins	- -	- -	- +	+ +	+ +	+ -	- -
Flavonoids	- +	- -	- -	+ -	+ -	+ +	+ -
Sesquiterpens	+ -	- +	- -	+ +	+ +	+ -	- -
Carbohydrates	+	+	+	-	+	-	+

-The phytochemical screening in the tissue extracts of Argemone spp made in thirthy four exttracts. The methods used to carry out as described in material and methods. In particular all the tissue extracts either in ethanolic or Acetic Acid solvent showed alkaloids, followed by flavonoids (Flavones, Flavonoids), carbohydrates.

By another hand, 2% acetic acid extracts have a higher content of total phenols than total flavonoids. Thus the total phenols (Figure 2B1, green line)in petals, sexual organs, leaf, and pod showed to be different among themselves and with all the extracts, the petal being the one with the highest amount of total phenols, with a total of 26.3 mg AG/g dry weight of the sample. Seed-pod and seed-root are not statistically different ($P < 0.05$). The extracts of the petals of *Argemone* spp showed the highest content of phenols, followed by the tissue extract of sexual organs. However, for total flavonoids, there is a difference with the ethanolic tissue extracts (Figure 2A1). The tissue extracts of sexual organs showed a higher content of total flavonoids. In addition, the tissue extract of stems in acetic acid showed a higher amount of flavonoids than the tissue extracts of pod, root, and seeds, whose concentrations are lower or null (Figure 2B1). Total phenols content in acetic acid extract (2%) of petal, sexual organs, leaf, or pod were different. Tissue extracts of petals has the highest content of total phenols, 26.3 mgEQ AG/g dry weight of the sample, and the tissue extracts of seeds, pod and roots were not statistically difference ($P < 0.05$) (Figure 2B1). Total flavonoids in tissue extracts of petals and sexual organs were not statistically different. However, both tissue extracts showed the highest flavonoid content, 2.38 and 2.26 mg Q/g of sample dry weight. Tissue extracts of seed and leaf did not show a significant difference between them but the other plant tissues. Tissue extracts of pod showed a lower content of flavonoids statistically different ($P < 0.05$) from other plant tissues (Figure 2B1, yellow bars). There were no total flavonoids in tissue extracts of the root and stem. Furthermore, analysis of flavonoid content in ethanolic or acetic acid tissue extracts showed differences in flavonoid content in all tissues analyzed. The ethanolic (85%) tissue extract of leaves is different from the Acetic acid (2&) tissue extract of leaves ($p \leq 0.000$). For the total phenols content in the ethanolic or acetic acid tissue extracts of leaves, stems, seeds, petals, or sexual organs, there were significant differences between both types of solvents at $P < 0.005$) However, in the tissue extracts of pod and root, no difference between solvents ($p = 0.419$ and 0.130) respectively. Alkaloid content. The highest content of total alkaloids found in the acetic acid tissue extracts of leaf, stem, and sexual organs were 0.491, 0.430, and 0.413 mg/ml $Bi(NO_3)3$. There was no significant difference between them. The lowest content in the tissue extracts of petals ($P < 0.05$) (Figure 2C1), a significant difference was observed compared to the other groups.

Differential antioxidant properties of the ethanolic versus acetic acid tissue extracts of *Argemone* spp.

The antioxidant activity was determined using the DPPH free radical inhibition method described in the material and methods.

For the determination, all plant tissues, solvents, and three concentrations (4, 7, and 10 mg/ml). The higher inhibition of the DPPH free radical entrapment was at 10 mg/ml. The antioxidant properties determination in ethanolic and acetic acid extracts of the different tissues of the Argemonas plants showed that ethanolic tissue extracts of Argemonas spp tissues showed the highest antioxidant activity versus acetic acid tissue extracts. However, it was not too high, except in the tissue extracts of petals with 2% acetic acid, where it has been reported a lower percentage of entrapment of DPPH radical. However, not all tissues are a marked difference. Acetic acid tissue extracts of root and seed showed a lower antioxidant capacity (free radical entrapment) of 39.13 and 24.19% than leaf, pod, and stem (Figure 3A). The antioxidant activity for ethanolic tissue extracts of leaf, pod, and stem showed the highest antioxidant activity, and the tissue extracts of root and seed showed lower antioxidant activity. The percentage of entrapment for each of the tissue extracts in ethanolic or acetic acid is shown (Figure 3A). Since the Antioxidant activity of the petal and sexual organ extracts was the highest at a higher dilution factor than used for the previous tissue extracts. Therefore, the final concentration for these tissues was 0.5 mg/ml.

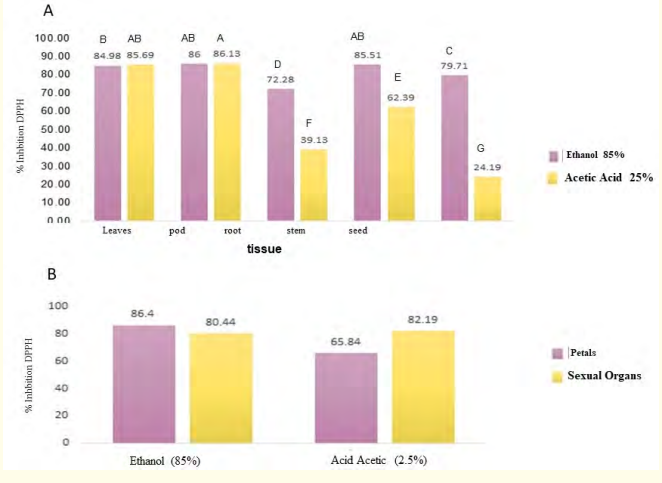

Figure 3: Inhibition percentages of the DPPH radical with different extracts of Argemone spp to a concentration of 10 mg/ml. Different letters indicate significative differences at $p < 0.05$ (post hock Tukey ANOVA analysis). Percentages of inhibition of the DPPH radical with different extracts of Argemone spp to a concentration of 0.5 mg/ml. Different letters indicate significative differences at $p < 0.05$ post hock Tukey ANOVA analysis.

Lower toxicity of the acetic acid tissue extracts of *Argemone* spp

In general, we found that the tissues extracts of *Argemone* spp are not toxic against larvae of Artemia salina, The toxicity of the acetic acid tissue extracts of *Argemone spp* in larvae (one day) of Artemia salina showed lower toxicity compared to the ethanolic tissue extracts. However, most of the tissue extracts have relatively low or no toxicity according to the toxicity scale established by Sánchez and Neira., 2005 [54] Contrary to data from the literature, the lowest LC_{50}, or the most toxic extract, was found in the ethanolic tissue extract of the root (the highest LC_{50}) (Table 2), while the lowest toxicity was showed by acetic acid tissue extract of seed with a total of 117.839 ± 0.246 and 1046.81 ± 0.375 mg/ml respectively (Table 2).

Table 2: Letal Concentration 50 (CL50) of the ethanolic tissue extracts (A), and acetic acid (B) of *Argemone* spp in larvae of Artemia salina.

Tissue	(CL50)	Toxicity level
A		
Leaves	192.548 ± 0.309	Moderate toxicity
Pod	404.389 ± 0.171	No toxicity
Root	117.839 ± 0.246	Moderate toxicity
Seed	206.571 ± 0.972	Moderate toxicity
Petals	244.033 ± 0.643	Moderate toxicity
B		
Leaves	175.497 ± 0.186	Moderate toxicity
Pod	775.175 ± 0.279	No toxicity
Root	992.064 ± 0.183	No toxicity
Seed	1046.810 ± 0.375	No toxicity
Petals	658.991 ± 0.345	No toxicity

Toxicity assays as described in material and methods. Tissue extracts of leaf, stem, root,pod, petals, and tested in larvae of Artemia salina of one day. The letal concentration (CL50) calculated using Probit analysis. Toxicity scale expressed as the one established by Sanchez and Neir., (2005).

Antifungal and antibacterial properties in the tissues extracts of *Argemone* spp.

Acetic acid tissue extract of seeds showed higher anti-fungicidal than antibacterial properties. The highest inhibition halos (material and methods) found were in the concentration of 62.5 µg/ml. Interestingly, halos up to 18 mm against *C. albicans* (Figures 4A-B) and inhibition halos induced by acetic extracts of root, leaf, and pod (Figure 4B. orange bars). In addition, *the C. albicans* strain

was susceptible to the ethanolic tissue extracts of seed in a higher proportion ($P < 0.05$), followed by leaf, stem, and petal (Figure 4A, orange bars). Acetic acid tissues extracts of leaf, pod, root, stem, and petal against *the P. aeruginosa* strain (Figure 4B, dark green bars). While ethanolic tissue extracts of the leaf, pod, and root against *P. aeruginosa* (Figure 4A, dark green bars). Furthermore, antibacterial activity against *Staphylococcus aureus* (*S. aureus*) showed by the ethanolic tissue extracts of petal and sexual organs and by the acetic acid tissue extracts of leaf, root, stem, and seeds. In addition, antimicrobial activity against *Escherichia coli*, an inhibition halo produced only by the ethanolic tissue extracts of the stem. *E. coli* showed halos of intermediate inhibition, mainly with the ethanolic extract of the root and the acetic acid tissue extract of the sexual organs with a diameters of up to 15 mm (Figure 4C). *P. aeruginosa,* and *S. aureus did not show* significant intermediate inhibition halos (data not shown). In *C. albicans,* no significant inhibition halos were found, except with the 85% ethanolic extract of the petal and only with the concentrations of 1000 µg/ml, 500 µg/ml, and 125 µg/ml, where intermediate inhibition halos of 12.5, 14.5 and 19.5 mm respectively. As positive controls for the antimicrobial activity: for the *S. aureus* strain, 500 mg ciprofloxacin, and the mean inhibition halos was 38 ± 3 mm. For *P. aeruginosa*, the antibiotic used was again 500 mg ciprofloxacin, and the inhibition halos obtained were 20 ± 1 mm. For *E. coli,* 500 mg ciprofloxacin producing inhibition halos of 24 ± 2 mm. For *C. albicans*, Fluconazole 150 mg as a control, and an average inhibition halos of 20 ± 2 mm. In marked contrast ATCC strains of *C. albicans*, *S. aureus*, *E. coli*, and *P. aeruginosa* did not show susceptibility to the tissue extracts of *Argemone* spp (data not shown).

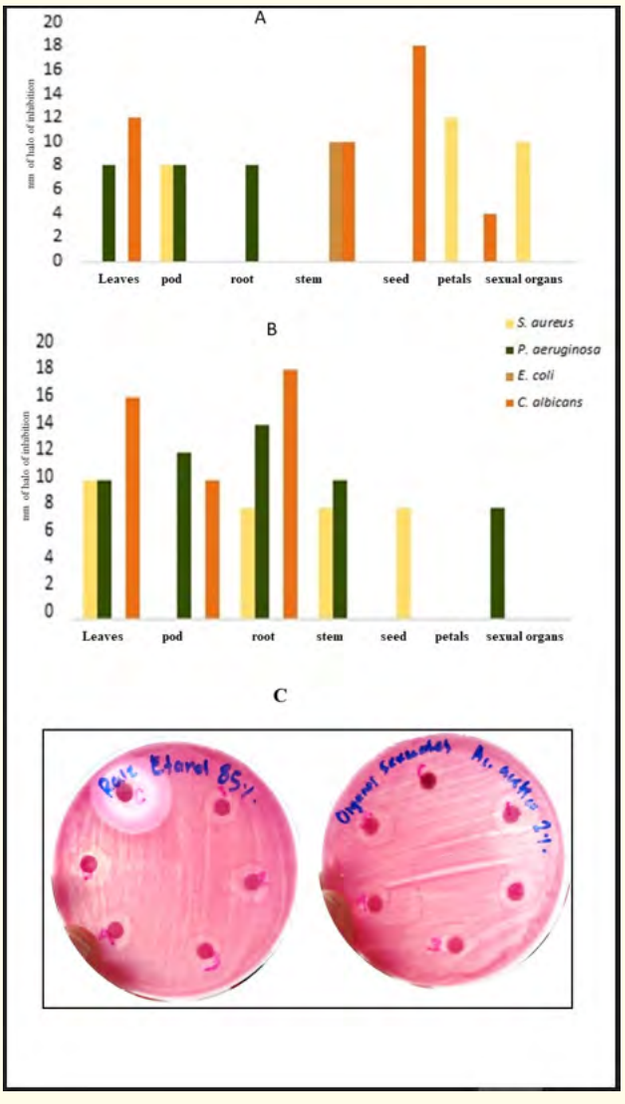

Figure 4: Antimicrobial activity of different tissue extracts (65.5 mg/ml) of *Argemone* spp in ethanol (85%) (A), and acetic acid (2%) (B). The antimicrobial properties of the different tissues extracts of Argemone spp as described in Mat and Methods, measured against *Staphylococcus aureus* (light yellow bars); *Pseudomonas aeruginosa* (dark green bars); Escherichia coli, (light brown bars) and *Candida albicans* (orange bars). Halos of bacterial or fungi cell growth inhibition induced by the different tissue extracts (ethanolic or acetic) tested and measured in diameter (mm). As positive controls, ciprofloxacin (500 mg) (antibacterial), and Fluconazol (antifungal) (150 mg). C. Representative picture of the halos of inhibition (24 ± 2 mm) induced by the tissue extracts of roots and sexual organs against *Escherichia coli* (C).

Discussion

The present study aims to evaluate and characterize the chemical, antioxidant, toxicity, and antimicrobial properties of the *Argemone* spp. collected in Gomez Palacio, Durango. From the data, ethanolic tissue extracts of petals, sexual organs, and seeds showed higher flavonoids, tannins, and alkaloids. There was no toxicity in ethanolic or acetic acid tissue extracts. Higher antioxidant activity in the ethanolic and acetic acid tissue extracts of leaf, pod, and stem. Ethanolic tissue extracts showed higher antifungal (against *C. albicans)* properties than antibacterial (*P. aeruginosa, S. aureus)*. However, acetic acid tissue extracts showed higher activity against *P. aeruginosa* and *S. aureus* than antifungal (*C. albicans*).

The phytochemical profile reported for the *Argemone* spp some year ago (Apu., *et al*. 2012) [55], for example, reported the presence of alkaloids, flavonoids, and anthraquinones in a methanolic

extract of the leaves of *Argemone* spp. Khan and Bhadauria., 2018 [56] analyzed ethanolic and methanolic extracts of leaves, seeds, and flowers of *Argemone Mexican* and reported the presence of alkaloids, phenols, flavonoids, and sesquiterpenes, as well as saponins. Sakar., *et al*. 2018 [57] reported alkaloids, flavonoids, phenols, and tannins in ethanolic extracts of the arterial parts of *Argemone mexicana.* Sahu., *et al*. 2012 [58] reported alkaloids, steroids, saponins, and flavonoids in methanolic and ethanolic extracts of the seed of *Argemone* spp. The production of secondary metabolites is related to the defense mechanism against dangerous agents, such as pathogens or the presence of herbivorous animals or to attract pollinating insects [59]. The authors showed that the highest content of phenols and flavonoids is in the ethanolic and acetic acid tissue extracts of the petal and sexual organs, followed by the leaf and the seed, while the lowest content is in the tissue extracts of pod, root, and stem extracts. Khan and Bhadauria., 2018 [56] reported a similar chemical profile with plants collected from India. They analyzed ethanolic tissue extracts of leaves, seeds, and flowers, and they found the highest content of flavonoids in flowers with a total of 41.76 ± 0.74 mg QE/g of plant extract, as well as the highest content of phenols in seeds with a total of 28.5 ± 1.15 mg GAE/g of plant extract. Although the results vary regarding the predominance of metabolites in the tissues, the amounts obtained in both studies oscillate between the same ranges.

In contrast, Arcos-Martínez., *et al*. 2015 [60] reported a higher content of total flavonoids with a total of up to 190.90 ± 26.70 mg EQ/g of plant extract, compared to total phenols, of which they only reported a total of 12.31 ± 1.20 mg EAG/g of plant extract, all of the above in ethanolic extracts of leaves of the *Argemone mexicana.* Apu., *et al*. 2012 [55] carried out an analysis of phenols in methanolic, hexane, and ethyl acetic tissue extracts of leaves of the *Argemone Mexican* (collected in Bangladesh). He found the highest content of total phenols with ethyl acetate up to 106.65 ± 1.39 mg EQ AG, followed by the methanolic extract and finally the hexane extract, in plants collected in Bangladesh. Our data are different from the above data. We think the difference might be due to the method and the geographical region for the plant collection. Our findings agree with those reported by Khan and Bhadauria [56] in 2018.

The quantitative and qualitative yield of the extraction of secondary metabolites depends on the solvent polarity and the physicochemical factors such as solvent concentration, temperature, contact time, and mass-solvent ratio [61]. The extraction depends on the chemical composition, the quantity and position of their hydroxyl groups, and molecular size. The amount of secondary metabolite synthesized and obtained might depend on the plant's growth requirements t in conjunction with the oxidative stress that the plant has at the time of its growth [59]. The method used in the present work for the total alkaloids determination by Sreevidya and Mehrotra., 2003 [62] is easy and based on Dragendorff's reagent (DR) precipitation (Mats and Methods). From the data (Figure 2C), the tissues with the highest presence of total alkaloids were leaf, stem, and sexual organs, with up to 0.491 ± 0.011 mg/ml $Bi(NO_3)3$, followed by pod and seed, while the tissue with less presence of total alkaloids was the petal.

There is not much literature that serves as a comparison in terms of total alkaloids. Arcos-Martínez., *et al*. 2015 [60], reported a concentration of 0.96 ± 0.04 mg/g of extract that is, 1% of the composition of the ethanolic tissue extract of the *Argemone mexicana*, are alkaloids. Of relevance is that in contrast to this author, which analyzed aerial parts of the plant. In the present work, we evaluated each part of the plant issue of *Argemone* spp (Table 1, Figure 2, and Figure 3) and gave us more precise data. On the other hand, according to the literature, the alkaloid contained in the *Argemone mexicana* is sanguinarine, present in a higher proportion in the seed oil (Shing and Shing, 2010; Shing., *et al*. 2010) [63,64], data that contrasts with the results obtained. , since the seed did not appear among the highest alkaloid contents. However, according to the authors of the method used by Sreevidya and Mehrotra, 2003 [62] this was developed for quaternary alkaloids, such as the case of berberine and protopine, but not for sanguinarine, which could explain why sanguinarine was not with the highest amount of total alkaloids. On the contrary, it is one of the tissues with the least amount of secondary metabolite. The antioxidant activity in the plant tissue extracts of the *Argemone s*pp with a concentration of 10 mg/ml the inhibition percentage is around 80 to 90% in almost all the tissue extracts with both solvents, except for root and seed (Figure 3A-B). Similarly, in the petal and sexual organs, a high percentage of entrapment of the DPPH free radical is appreciated, the above with a concentration of 0.5 mg/ml, that is, 20 times less quantity of extract than those of more tissue extracts. However, the previous fact is expected, since the antioxidant activity, in

this case, is given by the polyphenolic compounds present in the tissue extracts, that is, by the concentration of total phenols and flavonoids, so that, in the evaluations of phenols and total flavonoids, these two tissues mentioned above were the ones with the highest concentration.

On the other hand, there is currently no other work in the available literature where the antioxidant capacity of each *Argemone* spp tissue. However, there are some references where the antioxidant activity of the plant, in general, was evaluated. Arcos-Martínez., *et al*. 2015 [60] determined the antioxidant activity by calculating the IC_{50} with the DPPH technique. They found that the concentration necessary to inhibit 50% of the DPPH free radical in solution was 1218.5 µg/ml of ethanolic extract of the arterial parts of the *Argemone mexicana* [5,65,66]. Babu., *et al*. 2008 [67] evaluated the antioxidant activity of the arterial parts of *Argemone mexicana* with different solvents [5,65-67] They found the highest antioxidant activity in the ethyl acetate extract, which at 39.91 µg/ml, inhibits 50% of free radicals in the solution. As already mentioned in the results of this investigation, most of the extracts presented an inhibition percentage of between 70 and 80%, with the average being 83%b at 10 mg/ml. However, at the concentration of 4000 µg/ml, the average inhibition percentage was 50.3%. The antioxidant capacity of the extracts evaluated in the present study is lower than that reported by other authors. However, this may be due to many factors, such as the effectiveness of the extraction, the solvent, the growth conditions of the plant, and the number of phenolic compounds present in the extracts.

Accordingly to Zhu., *et al*. 2017 [68], in recent years, there has been an increase in toxicity studies carried out with Artemia salina larvae due to physiological characteristics, such as availability, multiple life stages, and the culture of simple laboratory, and ideal for carrying out toxicological studies. Secondary metabolites present in different parts of the plant express high toxicity [63-65]. According to the results (Table 2), the extracts evaluated in this study showed no or moderate toxicity in most cases (ethanolic tissue extracts). In general, acetic acid tissue extracts were less toxic than ethanolic extracts. Various authors agree with the results obtained of the toxicity of the aerial ethanolic parts of the *Argemone mexicana*. The authors found that the ethanolic extracts of the *Argemone mexicana* are safe for therapeutic use with doses up to 400 mg/kg of weight. Apu., *et al*. 2010 [69] reported that the methanol, ethyl acetate, and hexane tissue extracts of leaves of the *Argemone mexicana* are not toxic at doses greater than 100 µg/ml, indicating that they are safe and innocuous. Possibly due to the low presence of sanguinarine in the extracts of the different parts of *Argemone* spp, as has been found for the seed oil extracts [70].

Regarding the antimicrobial activity, the most susceptible strains were the clinical, contrary to what we expected inhibition halos induced by seed extracts halos against *C. albicans* [71-75]. This type of registered activity may be mainly due to the secondary metabolites present in the plant, more predominantly in the seed, since it contains a high amount of liposomes, which can promote the perforation of plasmatic membranes. Results similar to those obtained were reported by Singh., *et al*. 2012 [6] but with ethanolic seed extracts against *S. aureus* and *P. aeruginosa,* with inhibition halos of 13 mm, apparently the halos of inhibition found are not of clinical relevance [30,76,77].

Conclusion and Future Directions

All the tissue extracts analyzed showed alkaloids. In general, tissue extracts made with both solvents (ethanolic and acetic acid) have a higher content of phenols than flavonoids. While in the petal and sexual organs tissue extracts, there was a higher amount of both secondary metabolites than in the other tissues. Petal tissues and sexual organs exhibit higher antioxidant activity because they have a higher content of phenols and flavonoid content. However, almost all the tissues (except the seed and stem) have a good antioxidant capacity. The highest content of total alkaloids occurred in the leaf, stem, and sexual organs, which is an interesting point for future studies on the valuations of the biological activity of the alkaloids present in these extracts, as well as their chromatographic identification. The tissue extracts have zero or are innocous. Although we don't find a relevant clinical antibacterial activity, the tissue extracts of *Argemone* spp showed antifungal activity against the *C. albicans. Another* perspective of this work is to assess whether this activity is fungus specific or another antimicrobial activity.

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Conflict of Interest

The authors declare not conflict of interest.

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