Activity of Argemone Ochroleuca Extract On the in Vitro Viability of Cutaneous Leishmaniasis Promastigotes Comparison to Sodium Stibogluconate Drug

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Abstract: This study investigates the inhibitory activity of Argemone ochroleuca leaf plant extracts which was so prepared by using different solvents (ethanol, methanol, and distilled water) on the in vitro viability of cutaneous leishmaniasis promastigotes, in comparison with the established drug sodium stibogluconate.Cutaneous leishmaniasis, caused by Leishmania parasites, poses significant health challenges globally. Conventional treatments, like sodium stibogluconate, have limitations such as toxicity and drug resistance. Plant extracts, owing to their diverse bioactive constituents, present a potential alternative for disease management.Argemone ochroleuca plant extracts were prepared using ethanol, methanol, and distilled water as solvents. The inhibitory effect on promastigote viability was assessed using a time-based approach, measuring the duration in seconds that is required for inhibition to occur. Interestingly, at certain concentrations, the extract exhibited comparable or even superior efficacy to sodium stibogluconate. Results were compared with the inhibitory effect of sodium stibogluconate. The findings demonstrate the following inhibitory times for Argemone ochroleuca plant extracts: Ethanol: Inhibition occurred at 400 mg/ml in 9 seconds, at 200 mg/ml in 26 seconds, at 100 mg/ml in 60 seconds, and at 50 mg/ml in 128 seconds, Methanol: Inhibition occurred at 400 mg/ml in 13 seconds, at 200 mg/ml in 39 seconds, at 100 mg/ml in 91 seconds, and at 50 mg/ml in 210 seconds and Aquatic extract: Inhibition occurred at 400 mg/ml in 94 seconds, at 200 mg/ml in 150 seconds, at 100 mg/ml in 258 seconds, and at 50 mg/ml in 474 seconds. Comparatively, sodium stibogluconate showed its inhibitory effect at a specific concentration 100 mg/ml in 10 second.These results indicate that the Argemone ochroleuca leaves plant extracts, particularly those prepared using ethanol and methanol, exhibit substantial inhibitory effects on the viability of cutaneous leishmaniasis promastigotes. The variations in inhibitory times among the solvents suggest differences in the bioactive compound extraction and interactions. Ethanol and methanol extracts present potential alternatives to conventional treatments, with faster and efficient inhibitory action.Further research is warranted to elucidate the specific bioactive compounds responsible for these inhibitory effects and to explore the underlying.

1. INTRODUCTION

Leishmaniasis is among the most starting to appear and re-starting to appear zoonotic diseases that have beenrecognized, developed, noteworthy increased, and gradually expanded (Mostafavi E, 2021). Cutaneous leishmaniasis is the most widely distributed type, and the majority of cases occur in West and Southeast Asia, the Americas, and East and North Africa (Ruiz-Postigo JA,2017–2018). Leishmania parasites are transmitted by the bite of infected phlebotomine sand flies and 98 species of the genera Phlebotomus and Lutzomyia have been described as proven or suspected vectors for human leishmaniasis (Maroli M, 2013). In 2018, over 253,000 new cutaneous leishmaniasis (CL) cases were reported to World Health Organization (WHO). The region accounts for over 80% of the CL caseloads worldwide, only from the Eastern Mediterranean Region countries, representing a primary “hotspot”eco-epidemiological region.(WHO ,2020)

Sodium stibogluconat is compound that is highly effective and considered as the first-line treatment for most forms of leishmaniasis; these are SSG (Pentostam®) which are administered intravenously or intralesionally at the recommended dose of 20 mg/kg/day for 20 days., (Heras-Mosteiro J, Monge-Maillo B, Pinart M., 2017).

They have many adverse effects, the most frequent of which are cardiotoxicity, rise in liver function test, urea, and creatinine, anorexia, nausea, vomiting, myalgia, and arthralgia, whereas when applied intralesionally they may cause local irritation, pain, edema, erythema, or pruritus (Torres-Guerrero E, Quintanilla., 2017)

The 24 species in the genus Argemone include the bactericidal A. Mexicana and A. ochroleuca, which are regarded as medicinal plants (Sharma et al., 2011, 2017). Phytochemicals with bactericidal activity, such as the alkaloids sanguinarine and berberine, which have been tested against human pathogenic bacteria (Alamri and Moustafa, 2010; Bhattacharjee et al., 2010; Reyes et al., 2011), and flavonoid compounds with antioxidant activity (Al-Madhagi et al., 2016) are linked to these properties.

Argemone ochroleuca (Figure 1) is an herbaceous, annual or short-lived perennial plant with glaucous, yellow or orange latex, straight spines of various lengths, widely spaced, and perpendicular to the surface from which they originate; a simple or branched stem at the top; and leaves that are frequently arranged in a rosette in the base of the plant and are oblanceolate to elliptic in shape. The cylindrical floral buttons on A. ochroleuca are 4 to 11 mm wide and range in length from 8 to 18 mm. Its sepals have triangular-subulated, plump, or somewhat flattened apical horns with at least three fine spines each. Including the terminal spine, flowers have a length of 5 to 12 mm. Cream or occasionally white, obcunei form to elliptical, orbicular petals are present.

Stamens range in number from 20 to 75 and have yellow anthers and filaments. Purple stigma measures 2.0 to 3 mm in width and 1 to 1.5 mm in length. They display bluish non-receptive zones between their expanded lobes, which are typically very noticeable. The fruits are capsuled with 3 to 6 carpels, measuring 1 to 2.5 cm in width (without the spines) and 2 to 5 cm in length, including the style and stigma. The fruit's spines are dispersed throughout the fruit, occasionally mixed up with smaller spines and measuring between 6 and 12 mm in length. The tiny seeds have a diameter of 1.5 to 2 mm, are dark brown in color, have a sphere form, and have a rough surface (Calderón, 1991).

Figure 1. A, B) Plant and flower of Argemone ochroleuca, C) Floral buttons and ramified stems collected in Irapuato, Guanajuato, Mexico.

This herb is designated an invasive species in Africa and Asia (Berhanu, 2007). The genus Argemone contains 24 species, including A. Mexicana and A. ochroleuca, which are considered medicinal species with bactericidal properties (Sharma et al., 2011, 2017) These properties are associated with phytochemicals, such as the alkaloids , which are responsible for bactericidal activity and have been tested against human pathogenic bacteria (Alamri and Moustafa 2010; Bhattacharjee et al., 2010 and Reyes et al., 2011),and flavonoid compounds with antioxidant activity (Al-Madhagi et al., 2016).Traditional use of Argemone ochroleuca

Natural products, especially plants, are an important source of metabolites with diverse biological properties that can be used as active principles for the treatment of diseases, and the therapeutic use of medicinal plants is the fundamental basis for the discovery and development of new active principles, as such plants contain metabolites with diverse biological properties (patel et al ;2011). Popular knowledge of medicinal plant use is a powerful tool in the search for new active principles and, the WHO estimates that between 65% and 80% of the population in developing countries depends on traditional medicine (sharanappa R.et al 2014). Mahmoud et al.,

(2013): showed that antifungal activities of crude latex of Argemone ochroleuca Sweet against four clinical isolates of Candida (Candida albicans, Candida glabrata, Candida krusei and Candida tropicalis) and six isolates of plant pathogenic fungi (Alternaria alternate, Drechslera halodes, Fusarium oxysporum Macrophomina phaseolina, Pythium ultimum and Rhizoctoina solani) were assessed using well diffusion method. Antiasthmatic (María Elena Sánchez-et al, 2007) showed the relaxant effect of the aerial parts of Argemone ochroleuca (Papaveraceae), which is used in Mexican traditional medicine for the treatment of various respiratory diseases such as cough, bronchitis and asthma. Antibacterial (Alamri S, et al. 2010) showed the potent antibacterial effect on all bacterial strains examine antimicrobial (Reyes F. et al ,2011) showed antimicrobial activity. S. aureus (MIC= 125 µg/mL) and C. neoformans (MIC= 500 µg/mL) were the most sensitive strains. Antifungal (Moustafa et al,2013) showed antifungal activity against D. halodes (10.60 mm) and Candida spp

2. EQUIPMENT AND APPARATUS: (MATERIAL AND METHOD)

The following equipments and apparatus were used in our research: Autoclave, Automatic micropipette, Electric balance, Electric Oven, Incubator, Light microscope, Bensen burner, Flask,Slid and Petri dish.

Chemical and biological Materials: Ethanol 95 %, Ethanol 30%, Methanol 95 %, Distilled water, Geimesa staining, Normal saline 0.9 % and Gentamycin 80/2mg.

Drug use in this study: Sodium stibogluconate (GlaxoSmithKline, Vai, CDC Drug Service or USAMMDA for military health care beneficiaries)

Sample collection: Five samples were collected from patients arriving at the health center in Ibb Governorate for 25/1/2023 to 30/1/2023

3. CULTURE MEDIA

The cutaneous leishmaniasis parasite was obtained from five patients suffering from CL after being diagnosed by specialist doctor in the health center in Ibb Governorate, then we took needle aspiration from the areas of skin lesions of the patients and multiplied them on culture media combined from three culture media (Cooked meat media, sabouroud dextrose agar (SDA) and Novy-MacNeal-Nicolle (NNN)medium this media contain to phase solid phase and liquid phase.

Components of solid phase: cooked meat media 47g, sabouroud dextrose agar 16.25g, Agar 20g, DW 1000ml, blood (AB-) 100ml (Instead of defibrinated rabbit blood modified by the research center at the University of Nahrain), gentamycine 80mg/ml 1.25ml. Preparation of media: Mixing the mentioned substances except (antibiotic and blood) and measure the PH and set it to a value of 7. 4 Sterilize the medium by pressure at 121 °C for 15 minutes by autoclave Then it was cooled in a water bath at(-5055°C) We added the blood and the antibiotic under sterile conditions and we poured 5 ml of Media into each sterile tube of 25 ml, taking into account the placement of the tubes diagonally to obtain a larger surface area of the medium for the growth of parasites, and then we put it at 37C° for 24 hours to make sure that the medium is free of contamination, then we kept it in the refrigerator at 4 C° until use.

Components of liquid phase: sodium chloride (NaCl) 9g, potassium chloride (KCl) 0.42g, calcium chloride (CaCl2.2H2O) 0.32g, sodium bicarbonate (NaHCO3) 0.2g, D. Glucose 2g, D.W 1000 ml, Gentamycin 1.25ml. The substances previously mentioned were dissolved in distilled water, except for the antibiotic, the pH value of which is 7.4 The medium was sterilized and cooled as in the previous medium, then the antibiotic was added in small sterile tubes with a capacity of 25ml *0.5-1 ml of them were added to the solid phase and then incubated at 37C° to make sure Free of contamination then we kept it in the refrigerator at 4 C° until use.

Plant collection: The plant was collected from Jiblah city of Alkhashaba area, Ibb governorate, Yemen for 1/1/2023 to 5/1/2023.

Extraction: The extract of the leaves of A. ochroleuca was prepared which was dried and ground at 25 °C. 15 g of leaf powder was added to 300 ml of distilled water in a glass beaker and then shaken by manual shaking for intermittent periods for two days at 25 °C and the extract was filtered through filter paper (0.45) micrometer. The extract was evaporated in an oven at 45 °C for 48 hours and the obtained solid extracts were weighed and stored in the dark at 4 °C until subsequent use (oqba nafie abdul-aziz et al ,2009). The total crude solids extract was dissolved in 5 ml of ethanol30% (Ana Maria cevallos et al, 2017) by magnetic stirring for 10 minutes at 25 °C and the solution was filtered through filter paper (0.45) micrometer (Joel Horacio Elizondo-Luevano et al,2020). The alcoholic extract was obtained in the same way and using 95% ethanol and methanol as a solvent.

4. PREPARATION OF EXTRACT SOLUTIONS

The stock solution was prepared for each extract with a concentration of (400 mg / milliliter) and from this solution a series of solutions were prepared for each extract from the crude, aqueous and alcoholic extracts for concentrations 200, 100, 50 mg/ml (oqba nafie abdul-aziz et al ,2009).

RESULTS

The results showed a dose-dependent decrease in promastigote viability in both Argemone ochroleuca extract and sodium stibogluconate treatments. The extract demonstrated significant antiparasitic activity, with viability decreasing as the concentration increased. Interestingly, at certain concentrations, the extract exhibited comparable or even superior efficacy to sodium stibogluconate.

The mean ± SEM of the time required for inhibiting the viability of leishmania parasites produced by the different concentrations of the ethanolic, methanolic, and aquatic extracts of the plant were 55.75 ± 13.75, 88.25 ± 22.8, and 244.0 ± 43.8 seconds, respectively. This indicates that the ethanolic extract had the shortest inhibitory effect time, followed by the methanolic extract, while the aquatic extract exhibited the longest inhibitory effect time figure 1.

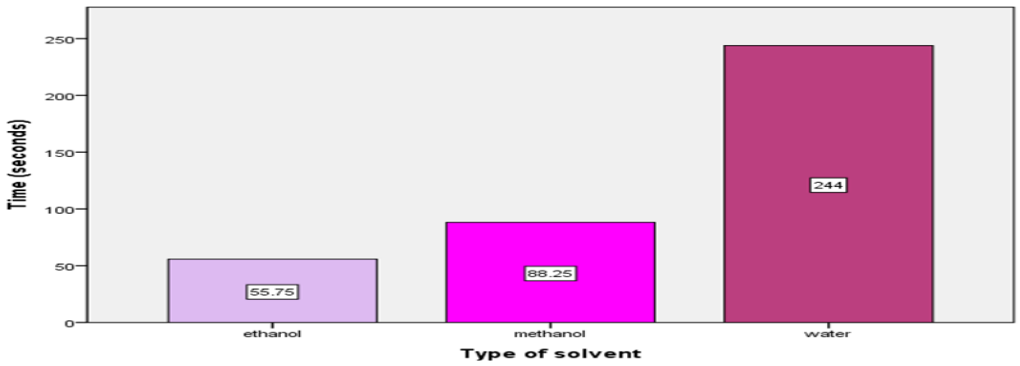

Figure 1: Means of time (second) of all the concentrations of the plant extract of each type of the solvents

Table (1) and figure (2), shows that there are statistically significant variations between inhibition promastigote of leishmania and different concentrations of the plant extracts; ethanolic, methanolic and aquatic with P-value (0.000, 0.000, and 0.000 respectively). Also, table (1) showed that the minimum mean ± SD of time for each concentration to inhibition the promastigote of leishmania; first the ethanol extract; a mean ± SD of time required for inhibition promastigote of leishmania was 9.0±2.0 seconds for the concentration of 400 mg/ml, compared with others concentrations; 26.0±2.0 seconds for the concentration of 200 mg/ml, 60.0±3.0 seconds for the concentration of 100 mg/ml, and 128.3±3.0 seconds for the concentration of 50 mg/ml.

Secondary, methanol extract showed that a mean ± SD of time required for inhibition promastigote of leishmania a viability was 13.0±3.0 seconds for the concentration of 400 mg/ml, compared with other

concentrations; 39.0±2.0 seconds for the concentration of 200 mg/ml, 91.0±3.0 seconds for the concentration of 100 mg/ml, and 210.0±3.0 seconds for the concentration of 50 mg/ml.

Third; water solvent (aquatic extract) showed that a mean ± SD of time required for inhibition promastigote of leishmania viability was 94.0±1.0seconds for the concentration of 400 mg/ml, compared with other concentrations150.0±2.0 seconds for the concentration of 200 mg/ml, 258.0±4.0 seconds for the concentration of 100 mg/ml, and 474.0±1.0 seconds for the concentration of 50 mg/ml.

These results suggest that the inhibitory effects of the plant extracts on the viability of promastigote form of leishmania parasites varied based on the concentration and the solvent used. Ethanol and methanol extracts generally exhibited faster inhibitory effects compared to the aqueous extract, and higher concentrations of the extracts tended to result in faster inhibition times.

Table (1): A comparison between the times (seconds) of inhibiting the viability of the promastigote form of leishmania parasites by various concentrations of Argemone ochroleuca leaves plant extracts of each one of various

Table (1): A comparison between the times (seconds) of inhibiting the viability of the promastigote form of leishmania parasites by various concentrations of Argemone ochroleuca leaves plant extracts of each one of various solvents

Concentration / **Solvent**	400 mg/ml (n=3 for each one)	200 mg/ml (n=3 for each one)	100 mg/ml (n=3 for each one)	50mg/ml (n=3 for each one)	*f*-test	*P*-value
Ethanol	9.0±2.0[a]	26.0±2.0[b]	60.0±3.0[c]	128±3.0	1278.269	0.000*
Methanol	13.0±3.0[a]	39.0±2.0[b]	91.0±3.0[c]	210.0±3.0	2957.258	0.000*
aqueous extract	94.0±1.0[a]	150.0±2.0[b]	258.0±4.0[c]	474.0±1.0	33772.8	0.000*

*: P ≤ 0.001

a) time is significantly less than those of the other concentrations.

b) time is significantly less than those of the 100 and 50 mg/ml concentrations.

c) time is significantly less than that of the 50 mg/ml concentration

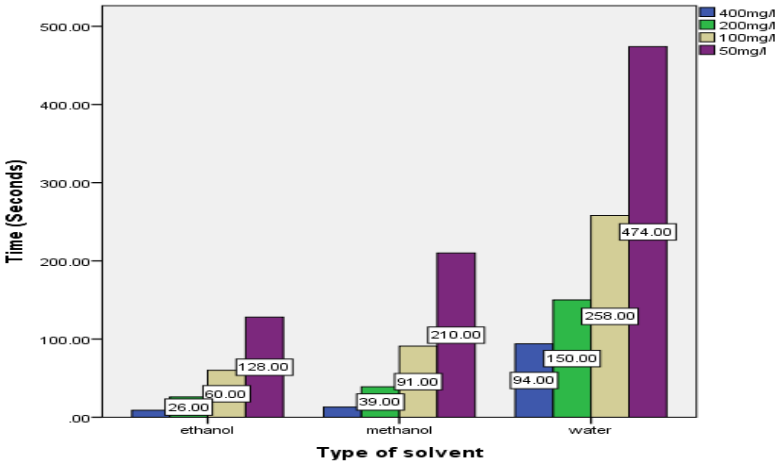

Figure 2: The inhibitory time for the viability of promastigote form of leishmania parasites produced by the different concentrations of Argemone ochroleuca leaves plant extracts of each type of the solvents (ethanol, methanol and aqueous).

Table (2): A comparison between the times (seconds) of inhibiting the viability of the promastigote form of leishmania parasites by the same concentrations of *Argemone ochroleuca* leaf plant extracts of various solvents.

Concentration	Solvents			*f*-test	*P*-value
	Ethanol (n=3 time for each one)	Methanol (n=3 time for each one)	Water (n=3 time for each one)		
400 mg/dl	9.0±2.0[a]	13.0±3.0[a]	94.0±1.0	1478.786	0.000*
200 mg/dl	26.0±2.0[b]	39.0±2.0[a]	150.0±2.0	3483.250	0.000*
100 mg/dl	60.0±3.0[b]	91.0±3.0[a]	258.0±4.0	4640.045	0.000*
50mg/dl	128.3±3.0[b]	210.0±3.0[a]	474.0±1.0	15484.421	0.000*

Table (2) and figure (3) shows that the inhibitory time for the viability of promastigote form of leishmania parasites produced by each concentration of Argemone ochroleuca plant extracts of the various extracts (ethanol, methanol and aquatic). However, there is a statically significant variation between promastigote form of leishmania parasites and plant extracts (ethanol, methanol and water).

As shown in table (2), the mean ± SD of time for concentration 400 of ethanolic was estimated with less time (9.0±2.0 seconds) for inhibiting the viability of promastigote form of leishmania parasites, compared with more time for aquatic (94.0±1.0 seconds respectively) with p-value of (0.000). While the methanolic extract was higher a statistically significant with minimum mean ± SD of time (13.0±3.0 seconds) to inhibiting the viability of promastigote form of leishmania parasites, then aquatic extracts (94.0±1.0 seconds) with P-value (0.000).

Regarding the concentration 200 mg/dl it was obvious that the minimum mean ± SD of time to inhibiting the viability of promastigote form of leishmania parasites for ethanolic was (26.0±2.0 second), compared to methanolic and aquatic extracts (39.0±2.0 and 150.0±2.0 seconds) respectively with P-value (0.000).

Also, the methanolic extract was statistically significant higher statistically significant with minimum mean ± SD of time to inhibiting the viability of promastigote form of leishmania parasites (39.0±2.0 seconds), than aquatic extracts (150.0±2.0 seconds) with P-value (0.000).

Concerning the concentration 100 mg/dl; the ethanolic extract revealed a lower time for inhibiting the viability of promastigote form of leishmania parasites with mean ± SD of time (60.0±3.0 seconds) as compared to those of the methanolic and aquatic extracts (91.0±3.0 and 258.0±4.0 seconds, respectively). On the other hand, the methanolic extract was higher significant with minimum mean ± SD of time to inhibiting the viability of promastigote form of leishmania parasites (91.0±3.0 seconds), than aquatic extracts (258.0±4.0 seconds) with P-value (0.000).

Finally, the concentration 50 mg/dl of the ethanolic extract revealed a significantly lower time for inhibiting the viability (128±3.0 seconds) as compared to those of the methanolic and the aquatic extracts (210.0±3.0 and 474.0±1.0 seconds, respectively), with p-value of (0.000) of the variation between the three solvents. While methanolic extract was higher significant with minimum mean ± SD of time to inhibiting the viability of promastigote form of leishmania parasites (210.0±3.0seconds), than aquatic extracts (474.0±1.0 seconds) with P-value (0.000).

Table (2): A comparison between the times (seconds) of inhibiting the viability of the promastigote form of leishmania parasites by the same concentrations of *Argemone ochroleuca* leaf plant extracts of various solvents.

Concentration	Solvents			*f*-test	*P*-value
	Ethanol (n=3 time for each one)	Methanol (n=3 time for each one)	Water (n=3 time for each one)		
400 mg/dl	9.0±2.0[a]	13.0±3.0[a]	94.0±1.0	1478.786	0.000*
200 mg/dl	26.0±2.0[b]	39.0±2.0[a]	150.0±2.0	3483.250	0.000*
100 mg/dl	60.0±3.0[b]	91.0±3.0[a]	258.0±4.0	4640.045	0.000*
50mg/dl	128.3±3.0[b]	210.0±3.0[a]	474.0±1.0	15484.421	0.000*

*: P ≤ 0.001

a) time is significantly less than that of the distilled water extract

b) time is significantly less than that of the methanol and distilled water

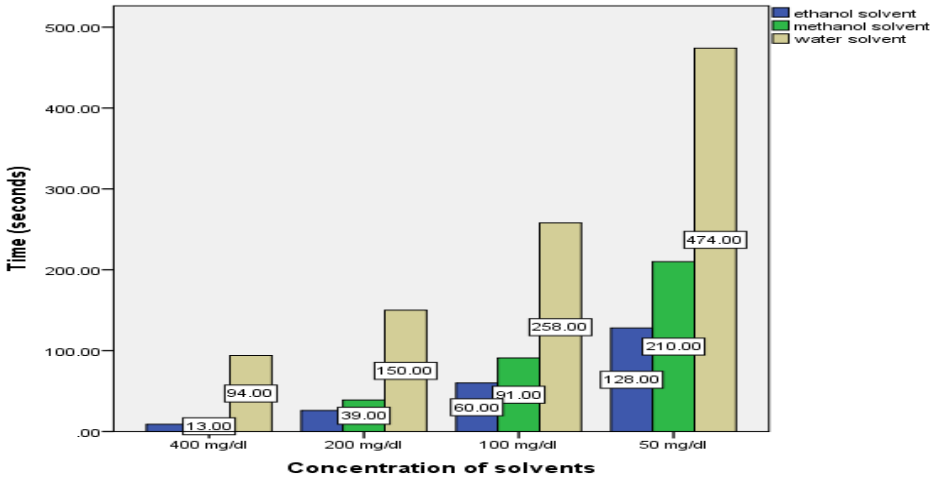

Figure 3: The inhibitory time for the viability of promastigote form of leishmania parasites produced by each concentration of Argemone ochroleuca plant extracts of various solvents (ethanol, methanol and water).

DISCUSSION

The findings suggest that the inhibitory effect of Argemone ochroleuca leaves plant extracts on the promastigote form of the Leishmania parasite is influenced by both the concentration of the extract and the solvent used for extraction. Ethanol extract exhibited the fastest inhibitory action across all concentrations, while methanol extract also demonstrated relatively quick inhibition times. On the other hand, distilled water extract showed slower inhibitory effects compared to the organic solvent extracts. Although there are no reports on the use of extracts of A. ochroleuca against cutaneous leishmaniasis promastigotes, some studies have evaluated extracts of this plant against other organism such as antiparasitic (as insect larvae) Martínez et al., (2017), antifungal, Mahmoud et al., (2013) and Antibacterial (Alamri S, et al. 2010).

The variations observed can be attributed to differences in the solubility of bioactive compounds within the plant extract, as well as potential interactions between these compounds and the solvents which were used. Ethanol and methanol are known to efficiently extract a wide range of bioactive components from plant materials, possibly contributing to their quicker inhibitory effects. This corresponds to the study (Suad Aqrab 2016), which proved that the use of distilled water as a solvent partially extracts the active substances from the plant, on the contrary, when using alcohols, they extract the active substances well. This explains that the aqueous extract needs a longer time to have an effect and a greater concentration and, showed that ethanol extraction gives the best results compared to other solvents (Suad Aqrab 2016 and Suad Shallal 2017). Also in a study, (Joel H.et all 2018), absolute methanol was adopted in the extraction process of the plant Argemone ochroleuca, and in a study (Martinez et al 2017) absolute ethanol was adopted 96%in the process of extracting the Argemone ochroleuca plant. It was reported (Al-Hayyan 2006), that alkaloids in Argemone ochroleuca have good extractability in Organic solvents (ethanol, methanol and other solvent) ethyl acetate) also (Torres Gonzalez Omar Rickard et al.2018) confirmed the existence of phytochemicals of great pharmacological importance, for example alkaloids such as isoquinoline, from where compounds such as allocryptopine and protopine and berberine were isolated

The study examines the varying inhibitory effects for the viability of leishmania parasites produced by the different concentrations of three solvents, named ethanol, methanol, and distilled water, on the activation of

Leishmania parasite promastigotes, as indicated by the time taken in seconds for inhibition to occur. The method of calculating the time of the effect was adopted according to the study (Suad Aqrab 2017), which was applied to glass tubes in the study of the effect of girls on the vitality of the whip form of mania and relied on the time in minutes to kill the parasite and obtained a 100% death rate at 180 minutes, until in this study, direct viewing and direct application on glass slides instead of tubes was adopted because the impact time is very short only in seconds, as ethanol extract at concentration 400 had a parasite death time of only 9 seconds .

The findings suggest that Argemone ochroleuca extract possesses promising antiparasitic activity against cutaneous leishmaniasis promastigotes in vitro. This alternative treatment option could address some of the limitations associated with conventional drugs. The potential benefits of using the extract include reduced toxicity, cost-effectiveness, and a lower likelihood of drug resistance development. Further studies are needed to elucidate the mechanism of action, assess its efficacy in animal models, and evaluate its safety profile. Natural products have potential in the search for new and selective agents for the treatment of important tropical diseases caused by protozoans (Wright and phillpson 1990). The in vivo efficiencies of drugs have been reported to be under the control of different parameters, such as pharmacokinetic parameters (Sereno and Iemesre, 1997), so that for various reasons, including simplicity in vitro culture maintenance, routine screenings of antileishmanial chemotherapeutic agents are often based on promastigote susceptibility assays (Gupta et al., 2001). Therapeutic evaluations for medicinal plants are essential because of the growing interest in alternative therapies and the therapeutic use of natural products. Natural products can be lead compounds, allowing the design and rational planning of new drugs biomimetic synthesis development, and the discovery of new therapeutic properties not yet attributed to known compounds (Hamburger and Hostettmann, 1991)

CONCLUSION

The study's findings indicate the varying inhibitory effects of different concentrations of Argemone ochroleuca leaves plant extracts dissolved in ethanol, methanol, and distilled water. Ethanol and methanol extracts exhibited faster and more potent inhibitory responses compared to the water extract. Although these plant extracts showed significant activity against Leishmania major promastigotes in vitro, further synthesis and in vivo studies are indicated to validate these results. These observations emphasize the importance of solvent choice and concentration when evaluating the inhibitory potential of plant extracts against Leishmania parasite promastigotes. Further research is warranted to identify the specific bioactive compounds responsible for these effects and to elucidate the underlying mechanisms of action.

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