

## THE HALOPHYTIC PLANT, *SUAEDA VERMICULATA* FORSSK EXTRACTS REDUCE THE INFLAMED PAW EDEMA AND EXERT POTENTIAL ANTIMICROBIAL ACTIVITY

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### Abstract

*Suaeda vermiculata* is a halophytic plant widely distributed in central Saudi Arabia and traditionally used as a remedy for hepatitis, jaundice and viral infections. The study aimed to inspect the phytochemical constituents and evaluate the antimicrobial activity of *S. vermiculata* different extracts against broad-spectrum microbial strains collected from human blood and urine samples. Anti-inflammatory activity of *S. vermiculata* is also investigated for the first time in this study. Phytochemical constituents of *S. vermiculata* extracts were investigated by chemical tests and by using thin layer chromatography (TLC). Agar diffusion and minimal inhibitory concentration (MIC) methods were used to estimate the antimicrobial activity of *S. vermiculata* extracts. Anti-inflammatory effect of plant extracts was evaluated by Formalin-induced edema in rats' paw. Phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, saponins and sterols in the plant extracts. Tannins and flavonoids were strongly detected in ethanol and ethyl acetate while steroids were abundant in chloroform and *n*-hexane extracts. Among all extracts, the ethanol extract of *S. vermiculata* showed the best inhibition zone diameter (IZD) and MIC values against *Candida albicans* and *Klebsiella pneumoniae* with 12mm IZD (MIC 8.75mg/ml) and 13mm IZD (MIC 35mg/ml), respectively. Also, ethanol extract inhibits the growth of *E. coli* and *Proteus vulgaris* at MIC equal to 17.5mg/ml. All *S. vermiculata* extracts showed anti-inflammatory effect when they were compared with untreated vehicle group. Whereas, the anti-inflammatory activity observed for ethanol and ethyl acetate extracts were higher than diclofenac standard during all the intervals of the study. Ethanol extract of *S. vermiculata* showed potential antimicrobial activity with a remarkable anti-inflammatory effect which might be accounted for the phenolic and flavonoid constituents of the plant.

**Key words:** Antimicrobial; Anti-inflammatory;  $\beta$  sitosterol; Halophytes; *Suaeda vermiculata*;

### Introduction:

Herbal medicine nowadays has many advantages, with minimal side effects of herbal products as well as relatively lower cost than synthetic ones (Pu *et al.*, 2017). It was estimated by the World Health Organization (WHO) that more than 70 % of humans rely on herbals as a main source in diseases treatment (Chan, 2003). The medicinal plants still the most efficient source as antimicrobial agents in many countries due to presence of active secondary metabolites such as terpenoids, flavonoids, alkaloids, and volatile oils which drives the science to discover unlimited chances for new antimicrobial drug discoveries (Ahmed *et al.*, 2018; El-Shouny *et al.*, 2018; Mohammed *et al.*, 2019a).

Halophytes are old known plants, but they haven't been studied systematically until the 20<sup>th</sup> century (Flowers & Colmer, 2015). They are identified as plants that have the ability to survive, reproduce and normally thrive in salty environments (Mohammed, 2020b). Accordingly, halophytic plants are capable of conducting their regular functions under relatively unfavorable conditions (Moray *et al.*, 2015). *Suaeda vermiculata* is a halophytic plant belonging to family Amaranthaceae formerly classified under the Chenopodiaceae (Mohammed, 2020b). The growth of the *S. vermiculata* as C-4 evergreen shrub is in summer while the fruits and the flowers grow nicely in early falls (Al-Shamsi *et al.*, 2018). In addition, the plant can present in both high and low altitude from April to October (Chamkouri, 2015). Genera and species of Chenopodiaceae are present widely in Saudi Arabia with approximately 20 genera and 42 species (Al-Saleh *et al.*, 1997). In the Arabian Desert, *S. vermiculata* can grow in both salty and salt-free soil. However, factors

such as darkness and high temperatures at higher salinity can decrease the final plant production (El-Keblawy *et al.*, 2018). As a halophytic plant, *S. vermiculata* has a notable mechanism to withstand high Na<sup>+</sup> and Cl<sup>-</sup> concentrations by regulating ion intra-cellular transport and perform compatible solution to overcome reactive oxygen species (ROS) (Carillo *et al.*, 2011; Flowers & Colmer, 2015; Shabala & Mackay, 2011). In addition to physiological conditions, anatomical changes such as hairs or salt glands are responsible to reduce salinity levels within the plant (Moray *et al.*, 2015). Accordingly, the plant is capable to overcome toxic ROS because it is supplied with a strong antioxidant systems including enzymatic and non-enzymatic mechanisms (Flowers & Muscolo, 2015). Vitamins, terpenoids (carotenoids and essential oils) and phenolic compounds are also represented in *S. vermiculata*, which are considered as an important part in the defense mechanism against ROS and also important for normal growth and development of the plant (Al-Tohamy *et al.*, 2018; Zahran & Al-Ansari, 1999). The phenolic compounds of the plant e.g. simple phenolic and flavonoid constituents also contribute in the protection mechanism for plants against ultraviolet radiation and microbial infections (Mohammed, 2020a; Moray *et al.*, 2015). Coumarins, saponins, sterol/terpenes, tannins, volatile oils and high level of alkaloids were identified in the plant (Mohammed *et al.*, 2019b; Moray *et al.*, 2015). Minerals such as magnesium, sodium and a slight amount of calcium and potassium represent the main mineral constituents of *S. vermiculata* (Zahran & Al-Ansari, 1999).

*S. vermiculata* can be considered as an potential alternative source in searching for a new antimicrobial and antioxidant agents with possible importance in food and biomedical uses (Al-Tohamy *et al.*, 2018). In addition, *S. vermiculata* is used in the Bedouins traditional medicine as a remedy for hepatitis and as antiviral agent (Al-Tohamy *et al.*, 2018; Sefidanzadeh *et al.*, 2015). Furthermore, *S. vermiculata* is an edible halophyte that has antioxidant, hypolipidemic and hypoglycemic activities (Mohammed *et al.*, 2019b, Cybulska *et al.*, 2014).

The resistant bacteria to different types of antibiotics are now well developed. For instance, in Saudi Arabia resistance for penicillin G reaches 33% against *Streptococcus pneumonia* (*S. pneumonia*) while the same microbe is resistant by 26% to erythromycin. In addition, 32% of *Staphylococcus aureus* (*S. aureus*) is methicillin-resistant (MRSA) (Zowawi, 2016). On the other hand, WHO has encouraged the use of herbals for developing and screening of therapies against Pan-drug resistant (PDR) and Multi-drug resistant (MDR) that can cause a difficult, severe and untreatable condition (El-Shouny *et al.*, 2018). Therefore, efforts are currently underway to find an herbal agent effective against such types of bacterial resistance. Moreover, Inflammation is a degenerative disease caused by oxidative stress and affects different body tissues including brain, heart, liver, and skin (Bickers & Athar, 2006; Hald & Lotharius, 2005; Kang *et al.*, 2005; Rudnicki *et al.*, 2007). Accordingly, Plants rich in secondary metabolites specially the antioxidant phenolic and flavonoids constituents such as *S. vermiculata* might play an important role in reducing inflammation (García-Lafuente *et al.*, 2009; Kim *et al.*, 2004).

The present work includes phytochemical and chromatographic identification for *S. vermiculata* growing in Qassim region of Saudi Arabia. The study estimates the antimicrobial activity of *S. vermiculata* against a group of microbial organisms including resistant strains collected from the microbiology laboratory of Alrass General Hospital. The anti-inflammatory activity of *S. vermiculata* extracts were investigated for the first time in the current work.

## Material and Methods

**Plant materials:** The plant was collected during the flowering stage in September 2018 from the Salt mining area on the Airport road near Qassim University (The plant growing area in Buraidah is usually used in summer as salt evaporation pond for the production of natural salt GPS; 26°20'24.1"N 43°45'12.0"E) and has been identified as *S. vermiculata* Forssk. by Prof. Dr. Ahmed El-Oglah, a taxonomist from Yarmouk University, Irbid, Jordan. The plant was dried in shade and ground by mechanical mixer before used for extraction process. A specimen of the plant under a number of 78 is deposited at College of Pharmacy herbarium, Qassim University.

**Extraction of *S. vermiculata*:** Accurately, 800 grams of the dried *S. vermiculata* whole herb (aerial parts including flowers, stems and roots) were extracted by maceration method (Kaneria *et al.*, 2012). *S. vermiculata* powder was extracted three successive times with *n*-hexane, chloroform, ethyl acetate and ethanol (1.5L each) in sequence. Each extraction procedure was carried out on 120 rpm shaker for overnight. Then the extracts were filtrated and vacuum-dried at 40°C. The obtained extracts were kept in -20°C freezer for further process.

**Phytochemical investigation of *S. vermiculata*:** *S. vermiculata* extracts were chemically examined for the presence of alkaloids, flavonoids, tannins, anthraquinones, saponins and sterols by methods described in literatures (Auwal *et al.*, 2014; Bankole *et al.*, 2016) using Mayer's and Dragendorff's reagents for the identification of alkaloids, aluminum chloride reagent for flavonoids, aqueous ferric chloride reagent for tannins, Borntrager's test for anthraquinone, froth test for saponins and Liebermann-Burchard's reagent for the steroids identification. Furthermore, thin layer chromatographic (TLC, Merck 60 F<sub>245</sub>, 0.2 mm diameter) technique was used to screen the extracts constituents using different mobile phases according to the extract nature. Chloroform: methanol (95:5 to 80:20) was employed as a developer for ethanol and ethyl acetate extracts while *n*-hexane: ethyl acetate in different proportions mobile phase was employed as a developer for others. The spots were detected by 1% vanillin in sulfuric acid general spearing reagent (Bauer *et al.*, 1988).

### Antimicrobial Assays

**Microbial strains:** Microbial microorganisms include *S. aureus* (Methicillin resistance staphylococcus aureus), *Enterococcus faecium* (*E. faecium*), *Escherichia coli* (*E. coli*), *Klebsiella pneumonia* (*K. pneumonia*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Proteus mirabilis* (*P. mirabilis*), *Candida albicans* (*C. albicans*) and *Proteus vulgaris* (*P. vulgaris*) were kindly provided from the microbial laboratory of Alrass General Hospital; the microorganisms were isolated from the patients' blood and urine samples and were identified in the microbiology laboratory, College of Applied Medical Sciences, Qassim University.

**Agar diffusion assay:** According to Cooper and Woodman, agar diffusion assay was carried out to test the antimicrobial activity of *S. vermiculata* (Cooper & Woodman, 1946). The required number of holes was cut on the agar plate using the back of a sterile glass dropper with 6 mm diameter. The microbial suspensions were prepared by taking a sample from the culture and mixed it with Muller Hinton Broth (MHB). The turbidity/growth corresponding to a 0.5 McFarland was used as a standard. Mueller Hinton Agar (MHA) plates were inoculated with the culture of each microbial suspension by spreading it evenly using sterile cotton swabs. DMSO was used to dissolve the residues of the extracts to get the concentration of 70 mg/ml and 100 µl of each extract was micro pipetted into the agar cups. Positive controls drug disks vancomycin (30 µg/disc), gentamicin (10 µg/disc) and polymixin B (300 U/disc) were used against gram-positive, gram negative bacterial and fungal growth, respectively. The plates were incubated at  $37 \pm 1^\circ\text{C}$  and checked after 24 and 48 hours to record the degree of inhibition in the microbial growth. Inhibition zone diameter (IZD) around agar cups was recorded to the nearest mm according to Mohammed *et al.*, (Mohammed *et al.*, 2019d). The process was performed in a triplicate and mean IZD was recorded in table 2.

**Determination of Minimum Inhibitory Concentration (MIC):** A broth dilution test was used to determine the MIC for selected microorganisms according to the positive results obtained from well-diffusion method. The MIC test was conducted by the procedure of Mohammed *et al.*, (Mohammed *et al.*, 2019b). Bacterial strains were incubated 24 hrs. at  $37^\circ\text{C}$  in Mueller Hinton broth while *Candida albicans* was cultured overnight at  $30^\circ\text{C}$  in Sabouraud dextrose broth-SDB + Tween 80. Serial dilutions of extracts were prepared in sterile test tubes. The tubes containing bacterial strains were incubated at  $37^\circ\text{C}$  for 24 h while tubes containing *C. albicans* were incubated at  $30^\circ\text{C}$  for 48 h. White turbidity in well bottom indicates the bacterial growth.

### Anti-inflammatory Assay

**Animals:** Healthy young Sprague Dawley male rats of around three months old weighing 150 gm to 200 gm were accommodated at normal laboratory conditions for a period of 5 days before start the anti-inflammatory test. The animals were maintained at half day dark and light normal cycle and at a temperature of  $20 \pm 3^\circ\text{C}$  with 67 % humidity. Standard Rat Chow diet supplied from First Milling Company in Qassim, Buraidah, Saudi Arabia and water *ad libitum* were given for animals. The experiment was conducted according to ethical guidelines of Qassim University for experimental animals.

**Vehicle:** Formaldehyde was diluted with distilled water to 5 % v/v. Carboxymethylcellulose (0.5% w/v) was used to suspend *S. vermiculata* extracts in addition to sodium diclofenac tablets obtained from local pharmacy. The prepared vehicles were administered orally to animals.

**Formalin-induced paw edema in rat:** The animals were randomly allocated into 6 groups (n=6). Initially, the plethysmometer (IITC Life Sciences, U.S.A.) was used to determine the (Basal Paw Volume) volume of intact paw. The negative control group (first group) received carboxy methyl cellulose 0.5 % at the dose of 20 ml/kg p.o. while the second, third, fourth and fifth groups received different extracts of *S. vermiculata*, chloroform, ethanolic, ethyl acetate and *n* hexane at doses of 400 mg/kg p.o. The positive control group (sixth group) received sodium diclofenac (10 mg/kg p.o.) as a standard anti-inflammatory drug. Inflammation was induced on the right hind paw of the rat by sub-plantar injection of formalin (0.1ml of 5%), 15 minutes before oral administration to all groups (Kumar and Jain, 2014). Swelling of the formalin-injected paw was determined using a plethysmometer at first, third, sixth and twenty-fourth hours (Mubashir *et al.*, 2014). The ability of anti-inflammatory agents to suppress paw inflammation was expressed as paw edema in milliliters (ml) (Shejawal *et al.*, 2014).

### Statistical Analysis

All the results were expressed as mean  $\pm$  standard error (S.E.M.). Data were analyzed using Tukey's multiple comparison test were  $p < 0.05$  were thought to be statistically significant.

## Results and Discussion

**Phytochemical investigation of the plant extracts:** Phytochemical investigation of *S. vermiculata* secondary metabolites was conducted qualitatively using specific chemical reaction for each metabolite. Flavonoids and tannins were identified in large quantities in ethanol and ethyl acetate extracts as observed by the color intensity obtained from aluminum chloride and ferric chloride reactions for flavonoids and tannins, respectively. In addition, flavonoids were faintly detected in the chloroform extract while they were absent in *n*-hexane extract of the plant. Moreover, Alkaloids were only detected in ethanol extract while sterols were detected in *n*-hexane and chloroform extracts (Table 1). The TLC observation of the extracts confirms the results obtained from the chemical investigation of the plant constituents. Flavonoids gave a yellow spot with vanillin sulfuric acid reagent on TLC plates of ethanol and ethyl acetate extracts. In addition, steroidal compounds and pigments of chlorophyll were detected in *n*-hexane and chloroform extracts (Steroidal compounds were UV inactive and gave a dark olive spot when they were heated with vanillin sulfuric acid reagent; while pigment compounds were acquired intense blue color on the TLC chromatogram) (Gruber *et al.*, 2004; Mohammed *et al.*, 2019c). The presence of large amount of phenol and flavonoid constituents in *S. vermiculata* was inconsistency with the halophytic nature of the plant (Fig. 1).

**Table 1. Phytochemical constituents of *Suaeda vermiculata* herb**

Secondary metabolites*	<i>Suaeda vermiculata</i> extracts				Test of Identification
	n-Hexane	Chloroform	Ethyl acetate	Ethanol	
Sterols	++++	+++	nd	nd	Liebermann-Burchard's test (Acetic anhydride and sulfuric acid)
Alkaloids	nd	nd	nd	++	Mayer's and Dragendorff's reagents
Flavonoids	nd	+	++++	++++	Aluminum chloride reagent
Tannins	nd	nd	++	++++	Ferric chloride reagent
Anthraquinones	nd	nd	nd	nd	Borntrager's reaction
Saponins	nd	nd	+	+	Froth test

\*Secondary metabolites were qualitatively measured, nd= not detected in the extract.

+, ++, +++, +++++ were referring to faintly, mild, strong and intensely detection of secondary metabolites in the extracts, respectively.

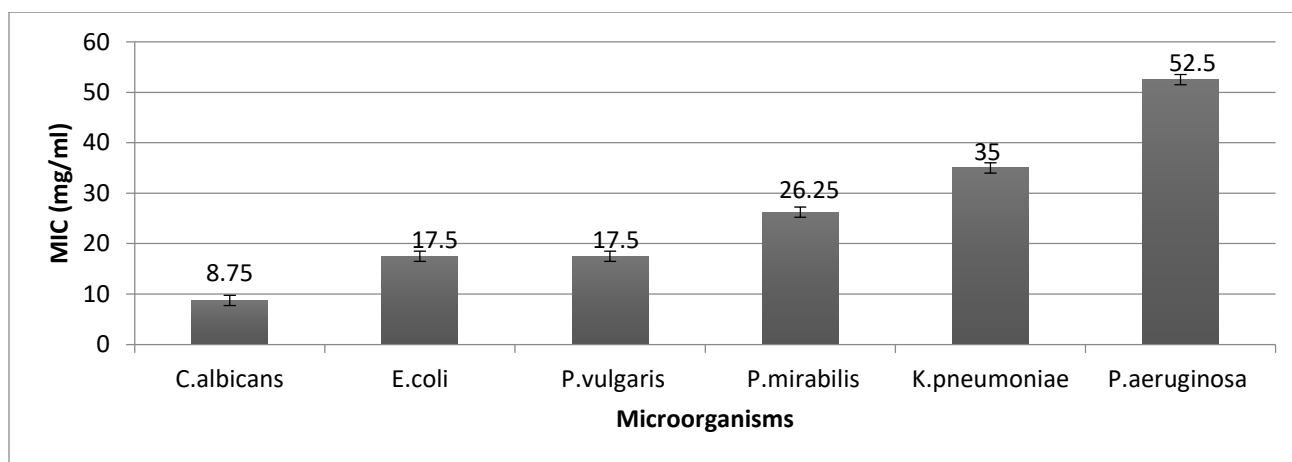


Fig. 1. MIC values of *S. vermiculata* ethanol extract against tested microorganisms.

**Antimicrobial activity of *S. vermiculata* extracts:** The activity of *S. vermiculata* different extracts against microbial pathogens isolated from patient's blood and urine samples were investigated for the first time in the present work. The extracts were evaluated for antimicrobial activity by two different methods; determination the inhibition zone diameter using agar diffusion method and measuring the minimal inhibitory concentration (MIC) assay previously mentioned in many publications (Al-Saleh *et al.*, 1997; Al-Tohamy *et al.*, 2018; Mahasneh *et al.*, 1996). The results which are shown in table 2 for the IZD indicated that *S. vermiculata* ethanol extract was active against all microbial pathogens except for *Enterococcus faecium* with IZD ranged from 11 to 13 mm diameter. The best antimicrobial activity obtained by ethanol extract of *S. vermiculata* compared with positive control was against *Candida albicans* fungal strain (polymixin B positive control showed 13 mm IZD) and the Gram -ve bacteria *Klebsiella pneumoniae* (gentamicin positive control showed 14 mm IZD) with IZD values equal to 12 and 13 mm, respectively (Table 2). The important observation here is that the antimicrobial activity of all other extracts was almost less active than ethanol extract. For instance, the *n*-hexane and ethyl acetate extracts were only active against *Klebsiella pneumonia* and *Pseudomonas aeruginosa* with IZD not more than 11 mm. However, the chloroform extract was inactive against all microbial strains (Table 2). The antimicrobial activity of ethanol extract might be related to the phenolic and flavonoid constituents which were detected as major

proportion in this extract. In addition, the alkaloids which were detected only in ethanol extract might play an additional role in the antimicrobial activity of the extract. Furthermore, the activity of ethanol extract against microbial pathogens might be considered as a primary investigatory point for further biological and clinical trials for the plant as a natural antimicrobial agent.

**Table 2. The antimicrobial activity of *S. vermiculata* extracts evaluated as inhibition zones diameters.**

Test Organism	Inhibition zone diameter (IZD) in millimeter				
	Standard Disc	n-Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Ethanol Extract
<i>MRSA</i> <sup>a</sup>	20±0.57 <sup>b</sup>	Nd	Nd	Nd	9.6±1.4
<i>Enterococcus faecium</i>	20±0.57 <sup>b</sup>	Nd	Nd	Nd	Nd
<i>Escherichia coli</i>	21±1.15 <sup>c</sup>	Nd	Nd	Nd	11.5±0.57
<i>Proteus vulgaris</i>	22±0.57 <sup>c</sup>	Nd	Nd	Nd	11.5±0.57
<i>Proteus mirabilis</i>	22±0.57 <sup>c</sup>	Nd	Nd	Nd	11±0
<i>Klebsiella pneumoniae</i>	14 ±0.57 <sup>c</sup>	11 ±0.57	Nd	10±1	13 ±0.57
<i>Pseudomonas aeruginosa</i>	20±0 <sup>c</sup>	10 ±0.57	Nd	11±1.15	13±0
<i>Candida albicans</i>	13 ±0 <sup>d</sup>	Nd	Nd	11±0.57	12 ±0.57

<sup>a</sup> *MRSA*: (Methicillin resistance staphylococcus aureus), <sup>b</sup> Standard vancomycin disc, <sup>c</sup> Standard gentamicin disc,

<sup>d</sup> Standard polymixin B disc.

Nd= Indicates inhibition zone is not detected at tested concentration,

Data represented as the mean of three replications ± Standard Deviation adjusted to the nearest millimeter.

**Anti-inflammatory activity of *S. vermiculata* extracts:** Oxidative stress is considered as the major cause for inflammatory diseases and cell injury. Also, inflammation of tissues is usually accompanied by the reduction in the cellular antioxidant capacity (Khansari *et al.*, 2009, Mohammed *et al.*, 2020). The ability of halophytes to overcome oxidative stress induced by salinity environment makes them a potential utilizing source for the treatment of oxidative-related diseases such as inflammation (Cybulska *et al.*, 2014; Mohammed *et al.*, 2013). Therefore, we investigated the anti-inflammatory activity of *S. vermiculata* as one of the halophytes. The results showed in Fig. 2 revealed that all extracts of *S. vermiculata* demonstrated a remarkable anti-inflammatory effects to the inflamed paw edema of rats at dose of 400 mg/kg p. o. compared with the vehicle control group; i.e. significant differences ( $p < 0.001$ ) in paw volume were observed from the first hour of the experiment when compared with vehicle-treated group (Figure 2). Furthermore, ethanol and ethyl acetate extracts (400 mg/kg) were significantly ( $p < 0.001$ ) reduced the paw edema better than sodium diclofenac standard anti-inflammatory drug (10 mg/kg dose as p.o.) at all intervals of the experiment. In addition, the effect of these extracts was exaggerated during the experiment after one, three and six hours of the dose and the effect was gradually decreased after that, i.e. the paw edema was decreased in the group of animals treated by ethanol and ethyl acetate extract from about 0.9 ml after one hour of the treatment to 0.85 ml and 0.75 ml after three and six hours of the treatment, respectively (Figure 2). The anti-inflammatory effect of ethanol and ethyl acetate extracts is mostly attributed to the presence of tannins and flavonoids which are known for their anti-oxidant and anti-inflammatory activities (García-Lafuente *et al.*, 2009; Zhang *et al.*, 2011). The results shown in Figure 2 also revealed that chloroform and *n*-hexane extracts were significantly weak in the anti-inflammatory effect compared to other extracts of *S. vermiculata*. In addition, chloroform extract was exerting similar anti-inflammatory effect to sodium diclofenac standard after six hours and one day of the treatment. The anti-inflammatory effect of chloroform extract might be due to having a steroidal compounds, e.g.  $\beta$ -sitosterol which has been reported for its anti-inflammatory activity (Gupta *et al.*, 1980). Furthermore, *n*-hexane extract of *S. vermiculata* had similar anti-inflammatory activity to the standard only after 24<sup>th</sup> hour of study and its effect was weaker than the standard after one and three hours of drug administration. The weak activity of *n*-hexane extract after one and three hours of administration might be related to the physico-chemical properties (solubility and dissolution) which may affect the absorption of extract constituents.

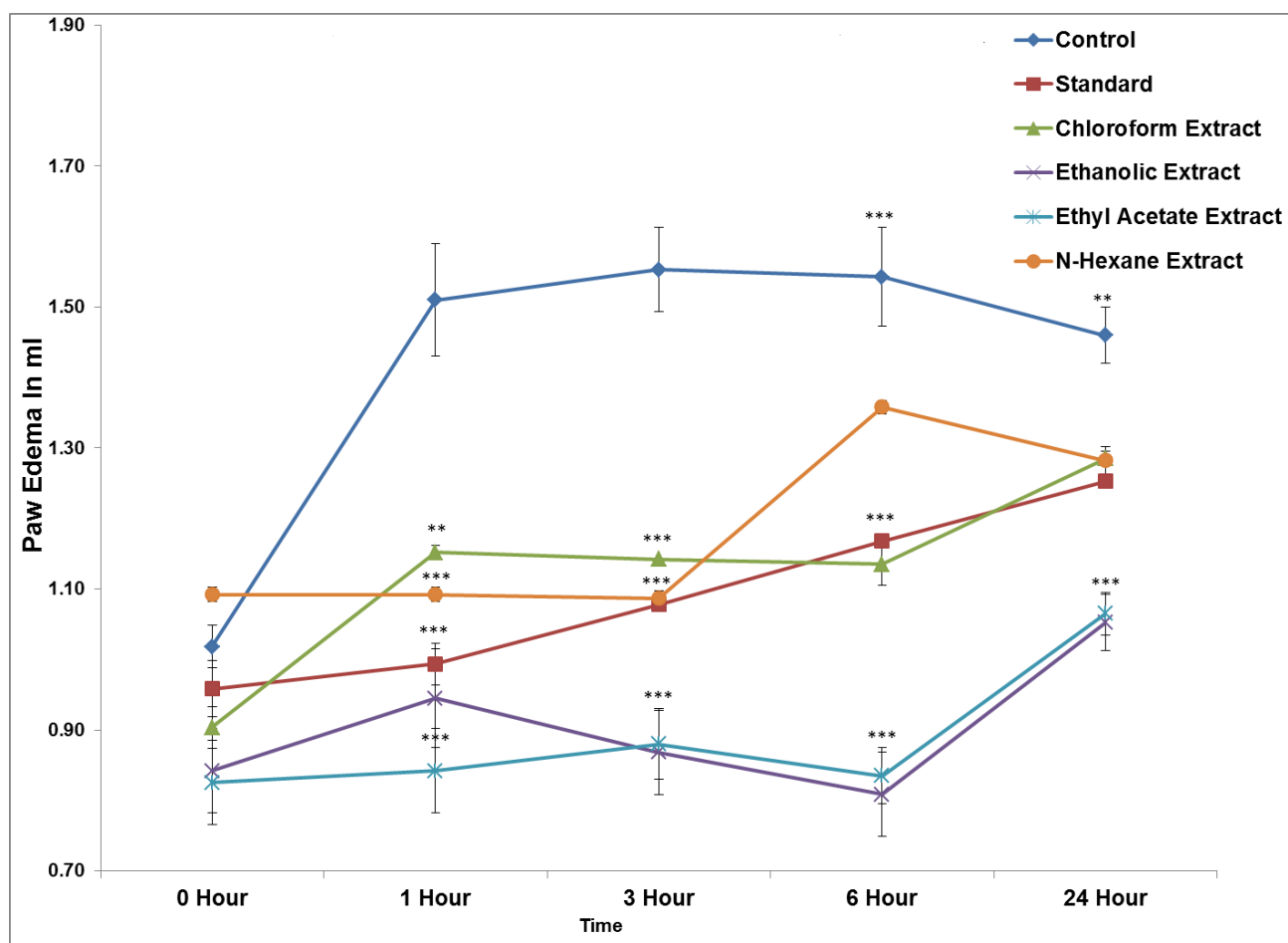


Fig. 2. Effect of vehicle control, diclofenac (10 mg/kg p.o.) and different *S. vermiculata* extracts (400 mg/kg p.o.) on rat paw edema induced by formalin. Points are means S.E.M. (6 animals) of paw volumes between different groups of animals.

## Conclusion

The phytochemical investigation results of *S. vermiculata* were consistent with the plant halophytic behavior. Among all extracts of *S. vermiculata*, ethanol and ethyl acetate showed the best antimicrobial and anti-inflammatory activities. These activities were mostly attributed to the phenolic and flavonoid constituents of the plant. Also, the noticeable anti-inflammatory effect of the chloroform and *n*-hexane extracts is mainly due to steroidal content of these extracts. Further preclinical and clinical investigations are required to estimate the antimicrobial and anti-inflammatory effects of *S. vermiculata* in higher animals and human. Also, further chromatographic and spectroscopic studies will be continued to identify the phenolic and flavonoid constituents of the plant.

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