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Original Article

Phytochemical Screening and Antibacterial Effect of Methanol Extracts of *Suaeda aegyptiaca* Leaves on *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*

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Abstract

The screening of plant extracts for natural products and antimicrobial activity has revealed the potential of higher plants as a source of new anti-infective agents. In the current study, the antibacterial activity of methanol extract of *Suaeda aegyptiaca* leaves was tested against four bacteria species: two Gram-positive bacteria (*Staphylococcus aureus* and *S. epidermidis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The Well diffusion method was used to assess the antibacterial activity of the plant extract. The results showed that the methanolic extract of *Suaeda aegyptiaca* leaves was more effective against Gram-positive bacteria, with an inhibition rate of 18.28 mm, as measured by the spectrum of antimicrobial activity against *S. epidermidis* bacteria, where the zone of inhibition ranged between (24.7- 12.7) mm and (20-13.3) mm against *S. aureus*, when compared to Gram-negative bacteria with a mean inhibition rate of 14.28 mm and indicated by zones of inhibition ranging between (18.7 and 11.3 mm) against *E. coli* and (16.3-9.3 mm) against *P. aeruginosa* at concentrations of 10%-2.5% since the mean of inhibition increases with increasing concentration of the extract. This suggests that this plant extract could be used for the treatment of a variety of diseases caused by these pathogens. The presence of alkaloids, flavonoids, saponins, sterols/terpenes, and tannins in the leaf was validated by phytochemical screening, confirming the potential good source of antibacterial agents with the highest sensitivity observed. This indicates that this plant extract could be used for the treatment of numerous diseases.

Keywords: Antibacterial activity, *Suaeda aegyptiaca*, Medicinal plants, Antibiotics, Plant extract

1. Introduction

Medicinal plants are thought to be a rich source of medicinal compounds that have been shown to treat infectious diseases. Traditional remedies are well documented and widely used in developing nations, where medicinal plants have been used as a replacement for antibiotics which have side effects and develop resistance to numerous therapies (1-5). Since the majority of plants include various active chemicals, such as flavonoids, tannins, and alkaloids, they provide a plentiful supply of antimicrobial agents against infectious diseases caused by bacteria, fungi, viruses, insects due to their anti-inflammatory activity and immunomodulatory effect (6-12).

Suaeda aegyptiaca, a member of the Chenopodiaceae family, is found throughout Europe, the Canary Islands, and the Mediterranean region, Australia, Asia, as well as Argentina, and North America. It can be found throughout Arabia in saline settings, particularly along the beaches, and grows in a variety of plant groups and

fields (13). Since this plant appears deeper or blackish when dried, Suaeda takes its name from the Arabic word suwaid (14). The herb *S. aegyptiaca* has been used in folk medicine to treat stomachache, wounds, and skin infections (15). Considering the significance of therapeutic herbs, the present study aimed to assess the antibacterial activity of a methanol extract of *Suaeda aegyptiaca* leaf in vitro by evaluating its effects on bacterial biological activity inhibition, as well as its screening phytochemical ingredients, in an attempt to find new compounds and alternative treatments for the diseases caused by harmful microorganisms.

2. Materials and Methods

Suaeda aegyptiaca leaves were collected in Thi-Qar from a variety of sources. The plants were powdered and stored in a sterile container until they were needed.

2.1. Preparation of Crude Extracts

A powdered sample of 20 g was extracted in a Soxhlet device with 200 mL of methanol at room temperature. Until the usage, the samples were maintained at 40°C. A 20 mg/mL stock solution was made. Stock solutions were prepared one day ahead of time. For initial testing and retests, several aliquots of each sample were stored (16, 17). Stock solutions were sterilized after being filtered. On the day of the assay, an aliquot of frozen stock solution was thawed in the refrigerator. By serial dilution, a concentration of 100 g/ml of extract was obtained. The crude extracts were then diluted to reach concentrations of 2.5%, 5%, and 10%.

2.2. Screening of Phytochemical Constituents

Suaeda aegyptiaca phytochemical components were qualitatively evaluated utilizing various standardized test methodologies (18, 19) as displayed in table 1.

Table 1. Phytochemical screening methods

Phytochemical	Methodology used
Alkaloids	A few drops of dilute HCL and 0.5 mL Wagner's reagent were added. A brown flocculent precipitate indicated the presence of alkaloid (18).
Anthraquinones	The plant extracts were given a few drops of 2% HCl. The generation of red precipitate revealed the presence of anthraquinones in the samples (19), while another test procedure (20) using a 10% ammonia solution pointed to the presence of anthraquinones in the samples. The presence of anthraquinones was illustrated by the production of a red tint.
Coumarins	One ml of a 10% NaOH solution was added to one ml of plant material. The presence of coumarins in the examined samples was confirmed by the production of a yellow color (19).
Flavonoids	1 g powder + 10 ml ethyl acetate was heated for 5 min over a steam bath (40ºC-50°C), filtrate+1 ml dilute ammonia. A positive test was indicated by yellow coloration (20).
Saponins	The extract was briskly agitated after boiling 1 g powdered in 10 ml distilled water for 15 min to record froth formation (19).
Sterols/ Terpenes	The test of Liebermann-Büchard: An aliquot of the extract was diluted in 1 ml of acetic anhydride in a test tube. Thereafter, 0.5 ml of concentrated H_2SO_4 was slowly poured on the walls of the tube. A positive reaction is indicated by the emergence of a purple tint changing blue to green (18).
Tannins	$FeCl_3$ test: 0.5 g powdered was heated in 20 ml distilled water with three drops of 5% $FeCl_3$ for a few minutes. A favorable sign was the emergence of a blue-black or brownish green color (20).

2.3. Test Microorganisms and their Sources

S. aureus, S. epidermides, E. coli, and *P. aeruginosa* isolates were obtained from the Department of Biology at Thi-Qar University College of Science. The API Staph. and API Enterobacteracea (21) were used to confirm the bacterial species. The bacteria were obtained from clinical specimens. Nutrient agar slants were used to subculture the pure cultures. They were

kept at 40°C until they were needed for the investigation.

2.4. Antibacterial Assay

The Kirby-Bauer test, known as the disk-diffusion method (22), was used to screen methanolic extracts of *Suaeda aegyptiaca* leaves for antibacterial activity in vitro. The sterile petri-plates were filled with sterilized nutritional agar medium (20 ml) and allowed to harden. The bacterial broth cultures were swabbed individually on an agar plates using a sterile bud. The wells (5 mm in diameter) were drilled into the agar with a sterile borer. Plant organic extracts were placed into each well aseptically and incubated at 37°C for 24 h. A measurement of the inhibitory zone was made. Antibacterial activity was measured using the diameter of the inhibition zone (DIZ) of the examined bacterium. The DIZ was measured in millimeters. All experiments were carried out in triplicate using the methods described in a previous study (23).

2.5. Statistical Analysis

Each experiment was carried out in triplicates, with the means from absolute data being reported. According to Box, Hunter (24). The data were analyzed in SPSS software using the ANOVA test.

3. Results and Discussion

The phytochemical screening of *Suaeda aegyptiaca* revealed the presence of alkaloids, tannins, sterols and/or terpenes, flavonoids, and saponins, but no anthraquinones or coumarins, as depicted in table 2.

Table 2. Phytochemical constituents of Suaeda aegyptiaca

Phytochemical	Results
Alkaloids	+
Anthraquinons	-
Coumarins	-
Flavonoids	+
Saponins	+
Sterols/Terpenes	+
Tannins	+

+ presence of phytochemicals; - absence of phytochemicals

According to the results of an antimicrobial assay using the agar diffusion method (wells of medium agar are filled with sample extracts), methanol extracts of the *S. aegyptiaca* plant showed potent antibacterial inhibitory zones against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis*. Crude methanolic extract of *S. aegyptiaca* has a significant inhibitory effect on *S. epidermidis*. Figure 1 illustrated that the inhibitory zones had diameters of 24.7%, 21%, and 12.7% mm at the concentrations of 10%, 5%, and 2.5 %, respectively. Based on figure 2, the sizes of the inhibition zones against *S. aureus* ranged from 20-13.3 mm at the concentrations of 10%, 5%, and 2.5 %, respectively. The inhibitory impact of the extract was stronger since the concentration of the extract was raised.

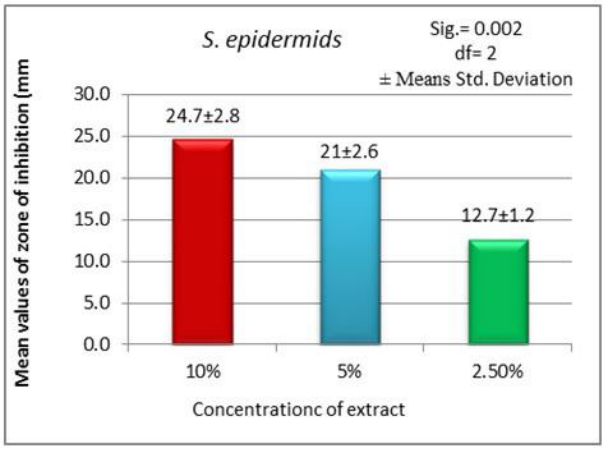

Figure 1. The effect of crude methanol extract of *S. aegyptiaca* against *S.epidermidis*

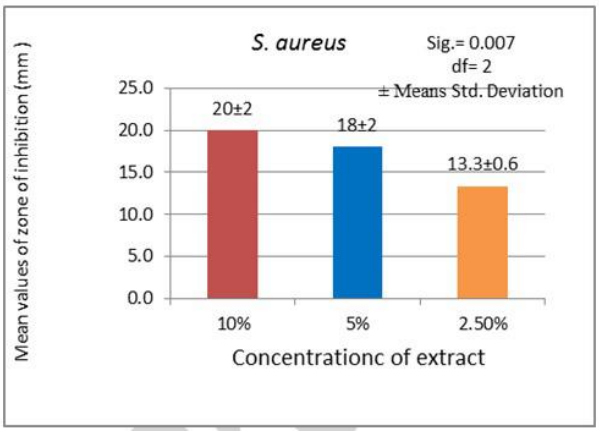

Figure 2. The effect of crude methanol extract of *S. aegyptiaca* against *S. aureus*

Figure 3 displays the effect of *S. aegyptiaca* extracts on *Escherichia coli*. The higher mean zone of inhibition was determined to be 18.7 mm at 10% con. At 5% and 2.5 %, the additional zones of inhibition are 16.7 and 11.3 mm, respectively. As illustrated in figure 4, the mean inhibition zone against *P. aeruginosa* was 16.3, 13.3, and 9.3 mm at 10%, 5%, and 2.5 % con, respectively.

The extract under investigation was examined for inhibitory effect against tested microorganisms that cause human diseases since the efficiency of the extract varied depending on the type of bacteria. As demonstrated in figure 5, *Staph. epidermids* had the highest inhibition zone of 19.4 mm, when compared to other bacteria with inhibition areas of 17.1, 15.6, and 13, respectively, for *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Figure 6 presents that ethanolic extracts of *S. aegyptiaca* have antibacterial efficacy against both Gram-positive and Gram-negative human pathogenic bacteria. The antibacterial efficiency of ethanolic extracts of *Suaeda aegyptiaca* against both Gram-positive and Gram-negative human pathogenic bacteria is displayed in figure 6 and 7. Positive bacteria were more effective, with a mean inhibition of 18.28 mm, as compared to negative bacteria which was inhibited by 14.28 mm.

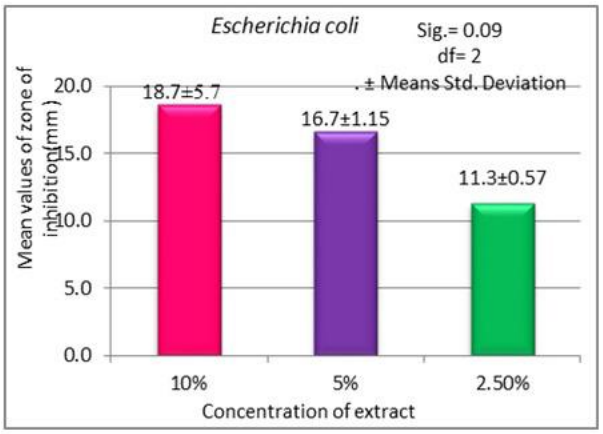

Figure 3. The effect of crude methanol extract of *S. aegyptiaca* against *E coli*

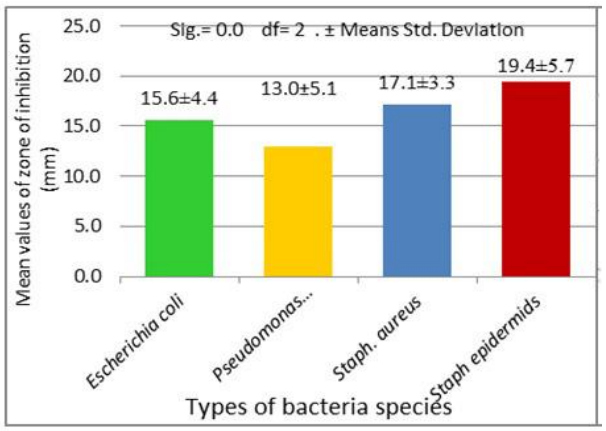

Figure 5. Effect of crude methanol extract *of S. aegyptiaca* against types of bacteria species

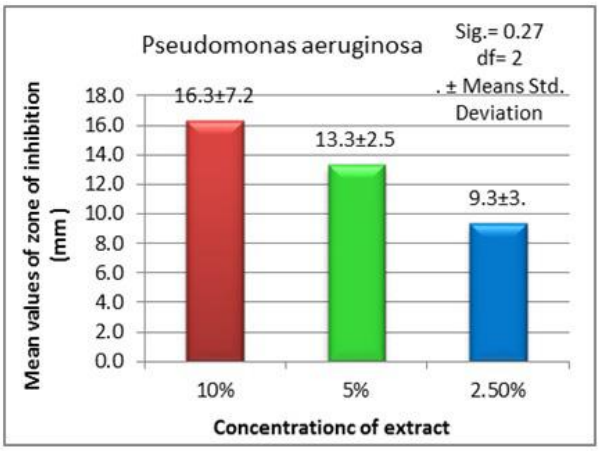

Figure 4. The effect of crude methanol extract of *S. aegyptiaca* against *P. aeruginosa*

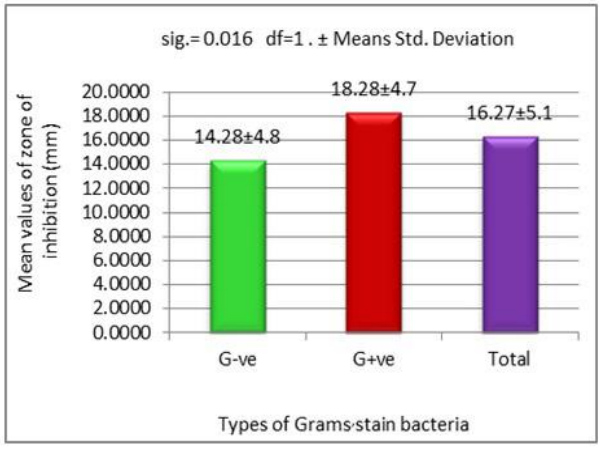

Figure 6. Effect of crude methanol extract of *S. aegyptiaca* against types of Grams·stain bacteria

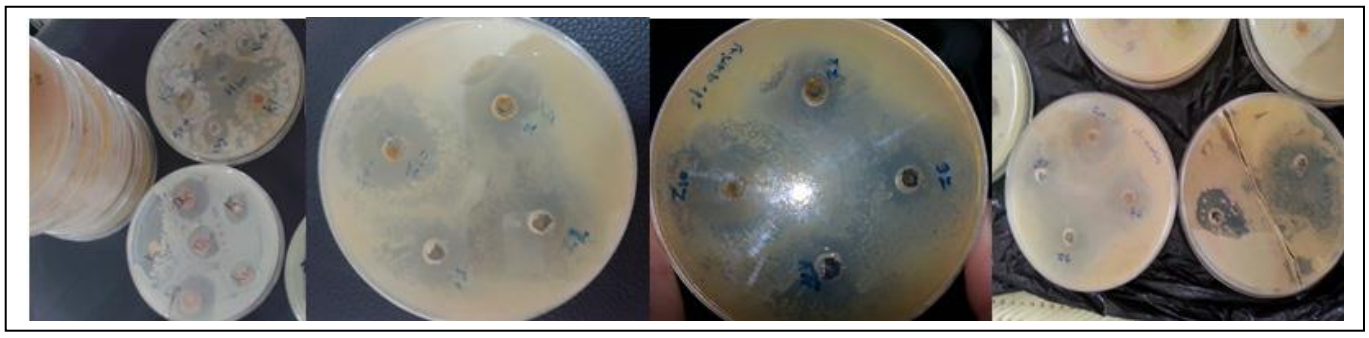

Figure 7. Inhibition zone created by *S. aegyptiaca*

The results of the present research revealed that the current extracts were more efficient against Gram positive bacteria. This finding is consistent with those obtained in earlier studies (25-27) which indicated that the presence of outer membrane lipopolysaccharides is responsible for higher resistance of Gram-negative bacteria to plant extracts. Many investigations have found that herbal antimicrobial compounds are unable to stop the growth of Gram-negative bacteria due to the presence of a sophisticated cell wall construction that prevents herbal extracts from penetrating bacterial cells. Nevertheless, in the current investigation, the extracts inhibited the growth of numerous bacteria, demonstrating their potential to penetrate bacterial cells (28, 29). *Pseudomonas aeruginosa (P. aeruginosa)* as a Gram-negative bacterium has a high level of natural resistance to nearly all known antimicrobials, antibiotics, and even manufactured medicines owing to its outer membrane barrier.

In the current results, nonetheless, the extracts prevented the growth of this bacterium, revealing its ability to permeate bacterial cells (30). Plant-derived flavonoids, terpenoids, and steroids have sparked a lot of attention in recent years due to their wide range of pharmacological effects, such as antibacterial, antioxidant, and anticancer activities. Consistent with previous studies, the effectiveness of this extract is due to its active chemical compounds, pointing to the presence of alkaloids, stimulants, coumarins, catechins, tannins, phenols, flavonoids, saponins, glycosides, and the bioactive xanthoprotein of the leaves of *Suaeda monoica*, which were used as a treatment for hepatitis and as an ointment for wounds and antiviral (31, 32).

As evidenced by the results of the present study, this extract inhibited both Gram positive and negative bacteria, indicating that they have a broad spectrum inhibitory effect. Gram positive bacteria were more susceptible to the extracts than the Gram-negative bacteria, indicating that it could be utilized to treat infections caused by resistant germs.

Authors' Contribution

Study concept and design: S. R. N.
Acquisition of data: D. H. M.
Analysis and interpretation of data: Z. K. H.
Drafting of the manuscript: S. R. N.
Critical revision of the manuscript for important intellectual content: D. H. M.
Statistical analysis: Z. K. H.
Administrative, technical, and material support: S. R. N.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Jose J, Raju D, Nayak P. Microspheres-Novel Drug Delivery Carrier for Plant Extracts for Antibacterial Activity. Res J Pharm Technol. 2018;11(4):1681-4.
2. Pintado C, de Miguel A, Acevedo O, Nozal L, Novella JL, Rotger R. Bactericidal effect of saffron (Crocus sativus L.) on Salmonella enterica during storage. Food Control. 2011;22(3-4):638-42.

3. Rammo RN. Bactericidal and anti-biofilm formation of aqueous plant extracts against pathogenic bacteria. Asian J Pharm. 2017;7(1):25-9.
4. Soureshjan EH, Heidari M. In vitro variation in antibacterial activity plant extracts on Glaucium elegans and saffron (Crocus sativus). Bangladesh J. Pharmacol. 2014;9(3):275-8.
5. Thorat Sheela S, Patil Poonam J. Phytochemical Analysis and Pharmacological Screening of Caralluma fimfriata wall. Whole Plant Extract. Res J Pharmacognosy Phytochem. 2019;11(4):217-21.
6. Bouafia A, Laouini SE, Ouahrani MR. A review on green synthesis of CuO nanoparticles using plant extract and evaluation of antimicrobial activity. Asian J Res Chem. 2020;13(1):65-70.
7. Das PK, Sahoo S, Sethi R, Nayak PS, Nayak S. Pharmacological Investigation of Protective Effects of Euphorbia hirta Whole Plant Extract, Against Duodenal Ulceration in Wistar Rats. RJPPD. 2009;1(3):150-3.
8. El-Sayed MA, Kamel MM, El-Raei MA, Osman SM, Gamil L, Abbas HA. Study of antibacterial activity of some plant extracts against Enterohemorrhagic Escherichia coli O157: H7. Res J Pharm Technol. 2013;6(8):916-9.
9. Malviya K, Shivhare U, Srivastav P, Shivhare S. Evaluation of Anti-inflammatory Potential of Cyathocline lyrata Cass Plant Extract by using Carrageenan Induced and Formalin Induced Rat Paw Edema. Asian J Res in Pharm Sci. 2013;3(2):90-4.
10. Nazzaro F, Caliendo G, Arnesi G, Veronesi A, Sarzi P, Fratianni F. Comparative content of some bioactive compounds in two varieties of Capsicum annuum L. sweet pepper and evaluation of their antimicrobial and mutagenic activities. J Food Biochem. 2009;33(6):852-68.
11. Palaksha M, Ravishankar K, Nandini V. Evaluation of the immunomodulatory effect of Eclipta prostrata whole plant extract on albino rats. RJPPD. 2017;9(2):41-5.
12. Sravanthi K, Ramana K, Sumalatha G, Neeraja K. Evaluation of Anti-inflammatory and Analgesic potency of Whole Plant Extract of Solanum surattense in Experimental Animals. RJPPD. 2013;5(2):101-5.
13. Waisel Y. Biology of halophytes: Elsevier; 2012.
14. Torkelson AR. Cross Name Index of Medicinal Plants: CRC Press; 1995.
15. Ghazanfar SA. Handbook of Arabian medicinal plants: CRC press; 1994.
16. Akujobi C, Anyanwu B, Onyeze C, Ibekwe V. Antibacterial activities and preliminary phytochemical screening of four medicinal plants. J Appl Sci. 2004;7(3):4328-38.
17. Esimone C, Adikwu M, Okonta J. Preliminary antimicrobial screening of the ethanolic extract from the lichen Usnea subfloridans (L). J Pharm Res Dev. 1998;3(2):99-101.
18. Bekro Y-A, Mamyrbekova J, Boua BB, Bi FT, Ehile EE. Etude ethnobotanique et screening phytochimique de Caesalpinia benthamiana (Baill.) Herend. et Zarucchi (Caesalpiniaceae). Sci Nat. 2007;4(2):217-25.
19. Harborne A. Phytochemical methods a guide to modern techniques of plant analysis: springer science & business media; 1998.
20. Evans WC. Trease and evans' pharmacognosy E-book: Elsevier Health Sciences; 2009.
21. Collee J, Dugmid J, Fraser A, Marmion B. Practical medical microbiology, Mackie and Mc Cartney. Pearson professional limited. 14thed; 1996.
22. Wayne P. National committee for clinical laboratory standards. Performance standards for antimicrobial disc susceptibility testing. 2002;12:01-53.
23. Cheesbrough M. Medical laboratory Manual for Tropical countries 2nd edition. Topical health Technology and Butterworth Scientific limited. 1991:494-529.
24. Box GE, Hunter WH, Hunter S. Statistics for experimenters: John Wiley and sons New York; 1978.
25. Abhipsa V, Manasa M, Poornima G, Rekha C, Kekuda T. In vitro antibacterial efficacy of selected plant extracts, streptomycin and their combination. Asian J Res Chem. 2012;5(6):791-3.
26. Kelmanson JE, Jäger AK, van Staden J. Zulu medicinal plants with antibacterial activity. Journal of Ethnopharmacology. 2000;69(3):241-6.
27. Masika P, Afolayan A. Antimicrobial activity of some plants used for the treatment of livestock disease in the Eastern Cape, South Africa. J Ethnopharmacol.. 2002;83(1-2):129-34.
28. Mann C, Cox S, Markham J. The outer membrane of Pseudomonas aeruginosa NCTC 6749 contributes to its tolerance to the essential oil of Melaleuca alternifolia (tea tree oil). Lett Appl Microbiol. 2000;30(4):294-7.
29. Walsh SE, Maillard JY, Russell A, Catrenich C, Charbonneau D, Bartolo R. Activity and mechanisms of action of selected biocidal agents on Gram-positive and-negative bacteria. J Appl Microbiol. 2003;94(2):240-7.
30. Ravikumar S, Gnanadesigan M, Serebiah J,

Inbaneson S. Hepatoprotective effect of an Indian salt marsh herb Suaeda monaica Forrskex . J Gmelagainst concanavalin. 2010:1-9.

31. Alhdad GM, Seal CE, Al-Azzawi MJ, Flowers TJ. The effect of combined salinity and waterlogging on the halophyte Suaeda maritima: the role of antioxidants. Environ Exp Bot. 2013;87:120-5.

32. Moghaieb RE, Saneoka H, Fujita K. Effect of salinity on osmotic adjustment, glycinebetaine accumulation and the betaine aldehyde dehydrogenase gene expression in two halophytic plants, Salicornia europaea and Suaeda maritima. Plant Sci. 2004;166(5):1345-9.