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Chemical Composition, Antioxidant and Antimicrobial Activities of *Haloxylon salicornicum* Methanolic Extract

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Abstract

The hardest part of treating microorganism-related diseases is finding economical, effective, and environmentally friendly treatments. The bioactive chemical composition and biological application of the aerial portions of *Haloxylon salicornicum* were evaluated using GC-MS in this study. Eight components from the extracted plant were identified using this approach. 6H-[1]Benzopyrano [4,3-C]isoquinoline-6,11(5H)-dione (11.66%) was the main component after 17.71 minutes. After that, 3-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (6.23%), tetrahydro-4-hydroxy-4-methyl-2H-pyran-2-one (4.89%), 9(Z)-octadecenoic acid (4.60%), 2,5-octadecadiynoic acid, methyl ester (4.52%), and 1-deoxy-d-mannitol (4.12%). After examining retention time data at 13.99 and 17.71 minutes, the most abundant alkaloid components were determined. The above ground parts of *H. salicornicum* extract had the highest DPPH antioxidant potency, with an IC50 value of 71.35 mg/L and a radical scavenging activity percentage of 62.54%. The most effective zone of inhibition was shown by methanolic extract of *H. salicornicum* against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis,* and *Escherichia coli* (27.61, 20.68, 18.82, and 17.08 mm, respectively), while *Enterobacter cloacae, Listeria monocytogenes*, and *S. epidermidis* were less effective. The *H. salicornicum* plant may be a biological agent, and the next level of study involves pure biochemical isolation and bioactivity testing.

Keywords*: Haloxylon salicornicum;* GC-MS; bioactive component; DPPH, Antibacterial.

1. Introduction

Herbal and vegetative medical ingredients have often inspired hope. It has improved health and comfort [1]. Many infectious diseases require substance research due to their widespread use as treatments. Plants are antibacterial [2]. Tannins, terpenoids, alkaloids, and flavonoids are laboratory-identified plant secondary metabolites with antibacterial properties [3]. Plant chemical variety depends on microbial disease prevention [4]. Bacterial pathogen antimicrobial resistance causes high morbidity and mortality. Gram-positive and Gram-negative bacteria with multidrug resistance may not respond to antibiotics. New therapeutic and antimicrobial drugs are needed because there are no effective treatments, no prevention methods, and few novel medicines. Biofilms complicate infection control and multidrug resistance [5].

The Chenopodiaceae family, of which *Haloxylon salicornicum* is a member, is widely dispersed over both Northern Africa and Asia, with 25 species growing in sandy and stony desert environments [6,7]. It is a perennial herb that can be found in abundance in Egypt and consists of four distinct species: a woody, upright, perennial shrub with no leaves. Stem and branches of this plant are a pale yellow and are gnarled; the leaves, which are actually two short triangular points at the joints, are fuzzy on the inside, and neither flowers nor fruits are seen [7,8]. The plant is eaten by domestic animals and wild animals alike, and it also stabilizes the soil surface, creates a comfortable microclimate, serves as

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camouflage, and serves as a safe haven for many different kinds of animals [9,10]. One of the most promising species for sand dune fixing and revegetation [11,12]. Unfortunately, data on this species is scant and incomplete. This species has not received a lot of research, which has slowed its progress and prevented it from being used in a sustainable way [13].

The species has the adaptability that enables it to thrive in environments that are low in nutrients and have a limited supply of water, such as those found in desert areas. One of the most important structural components of the vegetation in Eastern Arabia is the *H. salicornicum* [9,14]. The antimicrobial and anti-inflammatory properties of *H. salicornicum* are known in the realm of traditional herbal medicine. It is being used as a treatment for intestinal ulcers by traditional healers [15]. Piperidine alkaloid [16], haloxynine, and haloxine are just some of the alkaloids that have been found in it [17]. In addition to that, it has pyranones [18], tannins, saponins, and a variety of glycosides [19]. Furthermore, the biological properties and bioactive constituents of *H. salicornicum* are little understood. Surprisingly little research has been done on the plant, given its obvious importance. This work employed gas chromatography mass spectrometry to analyses the biochemical components of a methanol extract of the Egyptian ecotype of *H. salicornicum*, responsible for biological effects. The DPPH free radical scavenging test was used to measure the plant extract's antioxidant activity, and in vitro antibacterial properties against several pathogenic bacteria were evaluated.

2. Materials and Methods

2.1. Plant material and extraction process

During the blossoming season in April 2022, many populations of *H. salicornicum* were gathered from Egypt's northeastern desert. The identification was based on Tackholm [6] and Boulos [7]. The samples were cleaned and air-dried after being washed. Ten grams of dried plant material were combined with 150 ml of methanol in a 250 ml conical flask. This was followed by two hours of continuous shaking in a water bath shaker at room temperature (Memnert WB18, Schwabach, Germany). The mash was filtered using Whatman filter sheets (no. 1, 125 mm, Cat. No. 1061 135, Germany). The concentrations of the final plant extracts were determined and kept at 4 degrees Celsius [20].

2.2. GC-MS analysis

A Trace GC-TSQ mass spectrometer (Thirmo Scientific, Austen, TX, USA) with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 m film thickness) evaluated H. salicornicum plant extract [21]. The column oven was originally held at 50 °C, then elevated by 5 °C per minute to 250 °C and maintained for 2 minutes, and then raised by 30 °C per minute to 300 °C for 2 minutes. MS transfer line and injector temperatures were 260 and 270 °C. Helium carried inert gas at 1 mL/min. After 4 minutes, the solvent was removed, and an Autosampler AS1300 supplied 1 L diluted samples into the GC in split mode. At 70 EV, packed scan EI mass spectroscopy data from 50–500 m/z was obtained. 200 °C ion source. GC-MS revealed five components for each peak in each extracted plant material's mass spectrum data to WILEY 09 and NIST 14. Probability factors and primary structure fragmentation patterns determined suggested component structures.

2.3. Antioxidant DPPH Assay

H. salicornicum was diluted in methanol from a plant stock solution (5, 10, 20, 30, 40, and 50 mg L^{-1}). DPPH solution spiked sample solutions of various concentrations (1 mL, 0.135 Mm). Sample catechol levels were the standard. A UV/Vis spectrophotometer at = 517 nm assessed absorbance after 30 minutes of undisturbed sitting at room temperature in the dark (Spekal 11 spectrophotometer, analytic Jiena AG, Jiena, Germany). Using DPPH in methanol as the standard, the following equation computed antioxidant scavenging percentages.

$$\% \text{ Inhibition} = (\text{Ab. control} - \text{Ab.sample}) / (\text{Ab. control}) \times 100$$

The technique was implemented with little changes to previous experiments [22,23]. The exponential curve [24] showed the relationship between sample concentration and residual DPPH• radical to determine inhibitive concentrations (IC_{50}, mg L^{-1}).

2.4. Antimicrobial Activity Procedure

H. salicornicum extract's antibacterial activity was determined by agar well diffusion [25]. AMP (Ampicillin), CTX (Cefotaxime), TCN (Tetracycline), CM (Clindamycin), AZI (Azithromycin), and OFX (Ofloxacin) were standard antibiotics. The human pathogenic bacteria were obtained from Microbiology Department in Specialized Medical hospital, Mansoura University. *Acinetobacter* spp. (NA013254.1), *Escherichia coli* (ND-112558.1), *Enterobacter cloacae* (NR-118568.1), *Enterobacter cowanii* (KX-876243.1), *Salmonella typhimurium* (ND-078910.1), *Klebsella pneumonius* (NR-119588.1), and *Pseudomonas aeruginosa* (CP050335.1) were examined. Gram-positive bacteria: *Bacillus cereus* (EMND-1254), *Bacillus subtilis* (NR-027552.1), *Staphylococcus aureus* (ND-115627.1), *Listeria monocytogenes* (NR-044823.1), *Listeria innocua* (NR-022156.1), and *Staphylococcus epidermidis* (ND-113359.1). After 18–24 hours at 37 °C, plates were measured for inhibitory zone diameters (mm).

2.5. Statistical Analysis

Experiments measuring antioxidant and antibacterial potency were repeated three times with three replicates using the Costat software (CoHort Software, Monterey, CA, USA). One-way analysis of variance (ANOVA) was then performed on the results to see whether there were statistically significant differences between samples.

3. Results and Discussion

3.1. GC-MS Spectroscopy

Using GC-MS (Gas-Chromatography Mass Spectroscopy), researchers were able to describe the chemical structures and components that make up the *H. salicornicum* extract. According to the findings shown in Table 1, eight different components were deduced to have been taken from the extracted plant. After 17.71 minutes of analysis, the principal components were determined to be 6H-[1]Benzopyrano [4,3-C]isoquinoline-6,11(5H)-dione, which accounted for 11.66 percent of the total. In general, this compound is regarded as the most important component. After that, additional constituents that had a higher percentage of total composition for example, 3-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (6.23%), Tetrahydro-4-hydroxy-4-methyl-2H-pyran-2-one (4.89%), 9(Z)-Octadecenoic acid (4.60%), 2,5-Octadecadiynoic acid, methyl ester (4.52%), and 1-Deoxy-d-mannitol (4.12%) (Figure 1). Retention periods for the terpene and hydrocarbon components were measured as 17.44 and 10.35 minutes, respectively, while the fatty acid derivative components were recorded at 18.52 and 31.76 minutes. Retention times of 13.99 and 17.71 minutes were used to determine the alkaloid's most prevalent components. Numerous naturally existing classes were applied to the identified components, including hydrocarbons (9.01%), fatty acid derivatives (12.03%), alkaloids (14.67%), and terpenes (6.23%).

Table 1. Chemical constituents identified by GC-MS technique from the extracted aerial parts of *Haloxylon salicornicum*.

No.	Chemical name	Classification	Rt (min.)	MW	MF	Composition%
Oxygenated hydrocarbon						
1	1-Deoxy-d-mannitol	Oxygenated hydrocarbon	10.35	166	$C_6H_{14}O_5$	4.12
2	Tetrahydro-4-hydroxy-4-methyl-2H-pyran-2-one		11.43	130	$C_6H_{10}O_3$	4.89
Alkaloid						
3	1-(2-Methoxy-6-methylphenyl)-N-methylpropan-2-amine	Alkaloid	13.99	193	$C_{12}H_{19}NO$	3.01
4	6H-[1]Benzopyrano [4,3-C] isoquinoline-6,11(5H)-dione		17.71	263	$C_{16}H_9NO_3$	11.66
Terpene						
5	3-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	Sesquiterpene	17.44	206	$C_{14}H_{22}O$	6.23
Fatty acid derivatives						
6	2,5-Octadecadiynoic acid, methyl ester	Fatty acid derivatives	18.52	290	$C_{19}H_{30}O_2$	4.52
7	17-Octadecynoic acid		25.47	280	$C_{18}H_{32}O_2$	2.91
8	9(Z)-Octadecenoic acid		31.76	282	$C_{18}H_{34}O_2$	4.60

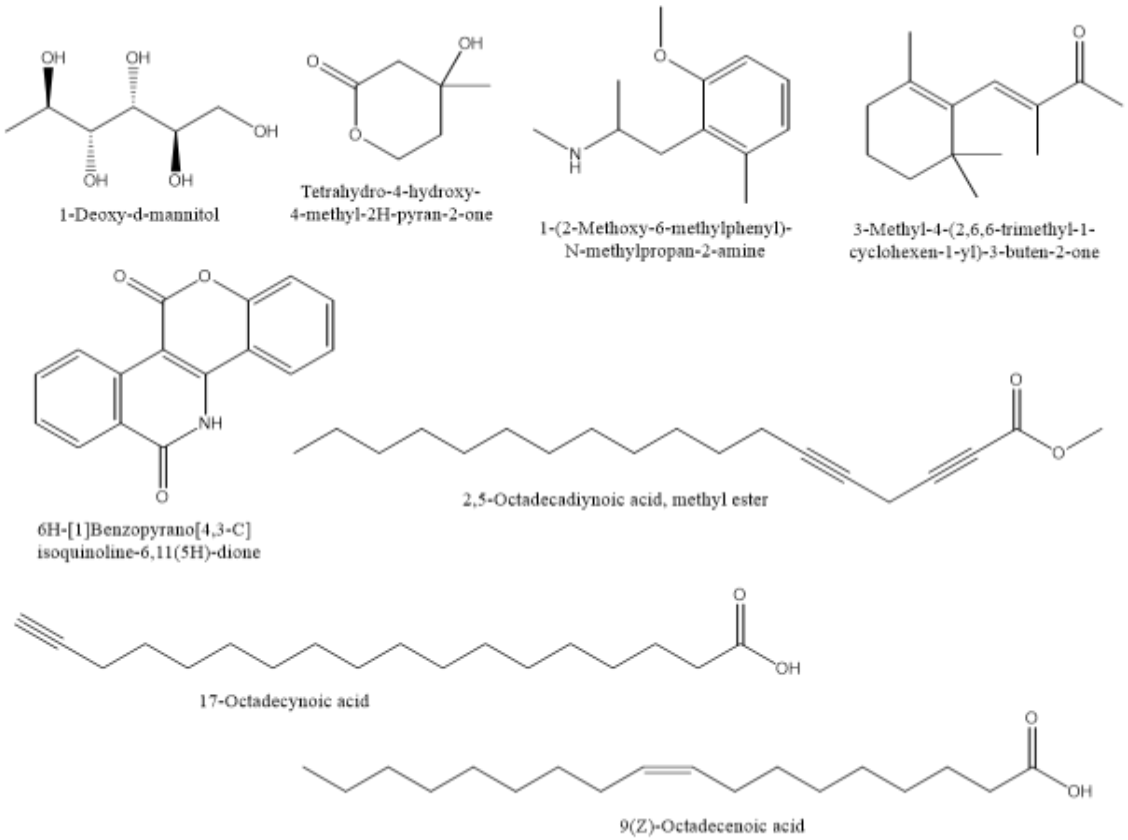

Figure 1. Chemical structure of the main component that were found in the extract of *Haloxylon salicornicum* made with MeOH alcohol.

3.2. Biological Application of H. salicornicum extracts

3.2.1. Antioxidant activity - DPPH assay

The antioxidant power of *H. salicornicum* methanolic extract was measured by its ability to scavenge DPPH free radicals relative to that of ascorbic acid. The scavenging effects of plant extracts and the standard on the DPPH radical were expressed as half maximal inhibitory concentration (IC_{50}) values, and the results are reported in Table 2. When it comes to scavenging DPPH radicals, a lower IC50 value suggests a higher capability. Table 2 shows that the extract from the above ground parts is the most effective in scavenging free radicals (with an IC_{50} of 71.35 mg/L), confirming earlier findings. The predominant fatty acid derivatives (12.03%), alkaloids (14.67%), hydrocarbons (9.01%), and terpenes (6.23%) of total separated components are the primary factor governing the mechanism of the reactions involved in assessing the antioxidant potential of the investigated extract. These findings for *H. salicornicum* accord with those of Salama et al. [26, 27], Sultan et al. [28], and El-Amier et al [29].

On the other hand, fatty acids, lipids, alkaloids, and terpenes that were extracted from *Cytisus multiflorus*, *Filipendula ulmaria*, *Reichardia tingitana*, and *Rumex vesicarius* reflect potent antioxidant capabilities for scavenging the free radicals in the solution [30-31, 26]. Numerous studies have shown that the amount of phytochemicals present in plants, notably phenolic compounds like carotenoids, ascorbic acid, flavonoid, and phenolic acids has a direct bearing on their capacity to fight free radicals and prevent oxidative damage [32]. According to the findings of our research, this plant is composed of non-volatile chemicals such tannins, flavonides, and phenolics].

Table 2. Radical scavenging activity percent (RSA%), and IC_{50} values (mg/L) at diverse concentrations of the MeOH-extracted of *Haloxylon salicornicum* and the standard ascorbic acid by DPPH assay.

Treatment	Conc. (mg/L)	RSA (%)	IC_{50} (mg/L)
Haloxylon salicornicum	1000	62.54 ± 0.58^{A}	71.35
	800	59.08 ± 1.61^{B}	
	600	44.52 ± 2.81^{C}	
	400	32.62 ± 1.95^{D}	
	200	21.14 ± 1.05^{E}	
	100	10.74 ± 0.85^{F}	
	$LSD_{0.05}$	1.78***	
Ascorbic acid	500	2.81 ± 0.01^{F}	12.02
	400	11.38 ± 0.03^{E}	
	300	38.57 ± 0.19^{D}	
	200	47.92 ± 0.51^{C}	
	100	61.34 ± 1.42^{B}	
	500	72.61 ± 1.55^{A}	
	$LSD_{0.05}$	1.55***	

Values are average (n = 3) ± standard deviation. $LSD_{0.05}$ reflected the computed least of the minimal significance between two means as each test was run on those two means (calculated by Factorial ANOVA).

3.2.2. Antibacterial Activity

In this work, the antibacterial activity of a 10 mg/L methanolic extract of *H. salicornicum* was tested using an agar disc diffusion assay against seven Gram-negative and six Gram-positive bacterial strains. The findings shown that a plant's methanolic extract suppresses the development of harmful microorganisms (Table 3). This study found that the methanolic extract of *H. salicornicum* was most effective against *S. aureus, P. aeruginosa, B. subtilis*, and *E. coli* (with zones of inhibition of 27.61, 20.68, 18.82, and 17.08 mm against these organisms, respectively), while *E. cloacae, L. monocytogenes*, and *S. epidermidis* were the most resistant (Table 3).

On the basis of how sensitive they are, the pathogenic organisms that have been examined may be grouped in the following order: *Staphylococcus aureus* > *Pseudomonas aeruginosa* > *Bacillus subtilis* > *Escherichia coli* > *Klebsiella pneumonia* > *Bacillus cereus* > *Salmonella typhi* > *Acinetobacter* spp. > *Listeria innocua* > *Enterobacter cowanii*. The common antibiotics, including ampicillin (AMP), cefotaxime (CTX), tetracycline (TCN), clindamycin (CM), azithromycin (AZI), and ofloxacin (OFX) had varying degrees of activity. *Enterobacter cloacae* was totally resistant to AMP, AZI and OFX, but *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* was completely resistant to CM, *Bacillus subtilis* and *Staphylococcus aureus* was entirely resistant to AMP and TCN, *Listeria* monocytogenes was totally resistant to AMP, CTX and CM, *Enterobacter cowanii* and *Salmonella typhi* was completely resistant to TCN and AMP, respectively (Table 3).

In the present study, the phenols extract from Iraq ecospecies of *H. salicornicum* showed roughly one-third less activity against *B. subtilis, S. epidermis, E. coli*, and *K. pneumonia* than did the MeOH extract of *H. salicornicum* [33]. According to the findings of Yousif et al. [34], the antibacterial potency of *H. salicornicum* extract from Egyptian ecospecies shown a considerable inhibition against the growth of *E. coli* and *S. aureus*. The antibacterial activity of *H. salicornicum* extract from Morocco ecospecies shown a significant suppression against the spreading of *S. aureus, E. coli, P. aeruginosa*, and *K. pneumoniae*, according to Lamchouri et al. [35]. In the past, crude extracts of the aerial parts of *H. salicornicum* were tested with a variety of different solvents and solvent combinations, including methanol, chloroform, ethyl acetate, petrol ether, and water. The results of these tests showed that the crude extracts were highly effective antibacterial agents against Gram positive and Gram negative bacteria [34,35]. Variations in the chemical composition of the extracts, which are created by environmental factors such as geographical variability, the type of soil, and temperature, might be the cause of these shifts in the antibacterial capability of the substance [36, 37, 26,27].

Table 3. Antibacterial activity of a 10 mg/ml methanol extract of *H. salicornicum* aerial parts in comparison to a few carefully chosen reference antibiotics.

Microbes	*H. salicornicum* (10 mg/ml)	Standard antibiotic (10 mg/L)					
		AMP	CTX	TCN	CM	AZI	OFX
Gram-negative bacteria							
Acinetobacter spp.	11.21	13.14	12.41	13.42	12.18	16.07	14.41
Enterobacter cloacae	-	-	15.22	16.73	13.64	-	-
Enterobacter cowanii	10.68	13.93	14.05	-	14.51	15.43	18.71
Escherichia coli	17.08	23.84	13.37	23.48	-	23.88	28.06
Klebsiella pneumonia	14.41	13.06	12.62	23.26	8.77	16.73	23.19
Pseudomonas aeruginosa	20.68	11.46	10.42	12.05	-	16.05	13.35
Salmonella typhi	13.54	-	13.61	14.82	11.57	13.4	10.56
Gram-positive bacteria							
Bacillus cereus	14.32	21.73	26.19	24.05	11.29	24.34	24.32
Bacillus subtilis	18.82	-	21.27	-	31.42	21.17	15.91
Listeria innocua	10.78	12.52	9.28	7.43	7.57	10.31	11.47
Listeria monocytogenes	-	-	-	11.05	-	8.68	8.09
Staphylococcus aureus	27.61	-	21.34	-	10.94	21.03	21.05
Staphylococcus epidermidis	-	11.62	14.57	21.07	-	24.07	24.51

AMP: Ampicillin, CTX: Cefotaxime, TCN: Tetracycline, CM: Clindamycin, AZI: Azithromycin, OFX: Ofloxacin

In general, as compared to the ecospecies found in Iraq and Morocco, the Egyptian ecospecies had a higher level of antibacterial activity. One possible explanation for this disparity is because the extract's chemical make-up is inconsistent from plant to plant. The bioactivities of a plant extract are directly correlated with the chemical component compositions of that extract, either alone or in combination [38]. And research has demonstrated that the chemical components found in MeOH extracts of wild plants are rather diverse. Phenolic substances and their derivatives, weak acid esters, fatty acid esters, terpenes, and many other chemical molecules fall under this category. As a consequence of this, these chemical components have the potential to effect many target locations on the bacterial cells, although with some slight variances [39]. Researching the bacterial species' make-up is necessary in order to ascertain whether or not it is effective against the extracts that have been taken into account. Regardless of whether the bacteria were Gram-negative or Gram-positive species, the methanol extract of H. salicornicum seemed to have a greater antibacterial impact against a specific sort of bacterial species than it did against other kinds of bacteria.

4. Conclusions

GC-MS analysis revealed eight phytocomponents in the MeOH extract of *H. salicornicum* aerial parts. After 17.71 minutes, 6H-[1]Benzopyrano [4,3-C]isoquinoline-6,11(5H)-dione (11.66%) was the main component. Subsequently, 3-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (6.23%), Tetrahydro-4-hydroxy-4-methyl-2H-pyran-2-one (4.89%), 9(Z)-Octadecenoic acid (4.60%), 2,5-Octadecadiynoic acid, methyl ester (4.52%), and 1-Deoxy-d-mannitol (4.12%). *H. salicornicum* shoots were antioxidant and antibacterial. The extract of above ground parts had the strongest antioxidant potency, trapping free radicals in the DPPH solution with an IC50 of 71.35 mg/L. E. cloacae, L. monocytogenes, and S. epidermidis were the most resistant to *H. salicornicum's* methanolic extract, which inhibited S. aureus, P. aeruginosa, B. subtilis, and E. coli. The *H. salicornicum* extract's excellent bioactive content and biological results suggested future investigation for medication development from natural sources.

5. Conflicts of interest

"There are no conflicts to declare."

6. Acknowledgments

"None"

7. References

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