Research Article

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HPLC and GC–MS analyses of phytochemical compounds in *Haloxylon salicornicum* extract: Antibacterial and antifungal activity assessment of phytopathogens

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Abstract: The present study investigated the phytochemical constituents and antimicrobial effects of aqueous methanolic extract of *Haloxylon salicornicum* against some phytopathogenic bacterial and fungal strains. The selected bacterial strains were *Pectobacterium carotovorum*, *Pectobacterium atrosepticum*, *Ralstonia solanacearum*, and *Streptomyces scabiei*, while fungal strains were *Fusarium oxysporum*, *Botrytis cinerea*, and *Rhizoctonia solani.* The extract demonstrated significant efficacy against *P. atrosepticum* and *P. carotovorum* at a concentration of 1,000 µg/mL, resulting in inhibition zones measuring 12.3 and 11 mm, respectively. Furthermore, the extract demonstrated considerable effectiveness against fungal strains, achieving an impressive fungal growth suppression rate of 68.8% against *R. solani* at a concentration of 5,000 µg/mL. The high-performance liquid chromatography analysis identified nine notable phenolic compounds and six common flavonoid compounds in the extract. The identified phenolic compounds in the highest quantities were gallic acid (6427.5 µg/g), vanillin (1145.4 µg/g), chlorogenic acid (498.1 µg/g), and syringic acid (322.5 µg/g). Apigenin (1155.9 µg/g), daidzein (460.9 µg/g), quercetin (382.7 µg/g), and naringenin (160.4 µg/g) exhibited the most significant concentrations of flavonoid compounds. Gas chromatography–mass spectrometry analysis revealed that *n*-hexadecanoic acid (53.7%), 9-octadecenoic acid (26.9%), 9,12-octadecadienoic acid (*Z*,*Z*) (8.67%), palmitic acid, and TMS derivative (4.36%) were the predominant compounds in the extract. Consequently, the *H. salicornicum* aqueous methanolic extract could be used for the first time as an environmentally safe antimicrobial pesticide agent against plant pathogens to reduce the excessive use of chemical pesticides.

Keywords: *Haloxylon salicornicum*, secondary metabolites, HPLC, GC–MS, antibacterial, antifungal

1 Introduction

Plant diseases are caused by a wide range of organisms, including fungi, bacteria, viruses, and nematodes, and have significant economic impacts on agricultural production [1–3]. Phytopathogenic bacteria and fungi are primary agents of plant diseases, generate toxic compounds harmful to humans, and result in significant yield losses in numerous economically vital crops worldwide [4,5]. Synthetic pesticides are extensively employed in various sectors of traditional agriculture due to their efficacy in managing plant diseases and enhancing the profitability. Nonetheless, the excessive application of artificial components has sparked growing public apprehension regarding environmental contamination, food residues, and potential health risks [6,7]. Furthermore, the increasing resistance of plant pathogens to existing synthetic compounds has led to investigations into novel fungicides and bactericides [8]. Therefore, using biopesticides and biological control methods instead of harmful synthetic

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pesticides has led to the rise of integrated pest management as a strategy to control plant diseases in sustainable agriculture [9].

The Environmental Protection Agency of the United States categorizes biopesticides into three specific groups: (I) biochemical biopesticides, (II) biocontrol organisms, and (III) plant-incorporated protectants [10]. At the European level, there is currently no official definition for biopesticides. However, biopesticides can generally be classified into two broad groups: (I) biopesticides derived from living organisms and (II) biopesticides made from natural products [11]. Plants and their extracts serve as valuable sources of natural products, encompassing a diverse array of secondary metabolites like alkaloids, terpenes, phenolics, flavonoids, polyketides, phytosterols, and resins. Such compounds can exhibit antibacterial, antifungal, herbicidal, and insecticidal properties against plant pathogens [12,13]. Consequently, identifying effective botanical pesticides that are safe for mammals and the environment, as well as being cost-effective, is crucial to mitigating the over-reliance on chemically synthesized pesticides in agriculture and reducing toxic residues in food consumption [1,14].

Haloxylon salicornicum is a desert shrub that belongs to the Chenopodiaceae family. It is typically found in the desert and semi-desert regions of several nations, such as Jordan, Kuwait, Iraq, Egypt, Pakistan, and Iran [15]. *H. salicornicum* and its family are acknowledged in traditional medicine for their antibacterial, antituberculosis, antidiabetic, and anti-inflammatory properties, as well as their effectiveness in treating liver illness, digestive disorders, and jaundice [16–18]. It was found that the plant *H. salicornicum* has several bioactive substances, such as piperidine alkaloids [19], β-amyrin, ursolic acid, β-sitosterol, and a few others [20]. However, only one study has described the isolation of a bioactive flavonoid from this plant [21]. The plant extracts were found to have antibacterial properties against several human pathogenic bacteria, including *Staphylococcus aureus*, *Salmonella typhi*, *Micrococcus luteus*, *Bacillus subtilis*, and *Sarcina ventriculi* [22,23]. The plant extract exhibited antifungal properties against several human pathogenic fungi, including *Aspergillus flavus, A. fumigates, Candida albicans, C. tropicalis,* and *Penicillium chrysogenum* [22,23]. In addition, the antibacterial properties of *H. salicornicum* plant extracts were observed not only against human diseases but also against animal pathogens [24]. Such antimicrobial properties may be attributed to various bioactive components identified in the extracts from *H. salicornicum*, including piperidine alkaloids, β-amyrin, ursolic acid, and β-sitosterol [20]. Nevertheless, only one study has thoroughly investigated the extraction of a bioactive flavonoid from this plant [21].

Currently, there is a scarcity of research studies that detail the effects of *H. salicornicum* extracts on plant pathogens. Consequently, this investigation evaluated the antimicrobial properties of the aqueous methanolic extract of *H. salicornicum* against a range of phytopathogenic bacteria and fungi. Furthermore, we examined the phytochemical constituents of *H. salicornicum* extract through high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS).

2 Materials and methods

2.1 Source of bacterial and fungal strains

The phytopathogenic bacterial strains used in this study were *Pectobacterium carotovorum* (OQ878656), *Pectobacterium atrosepticum* (MG706146), *Ralstonia solanacearum* (OQ878653), and *Streptomyces scabiei* (OR437480). In addition, the three phytopathogenic fungi used in the current study were *Fusarium oxysporum* (OQ820156), *Botrytis cinerea* (MN398400), and *Rhizoctonia solani* (OQ880457). All of these microorganisms have been isolated, characterized, and identified at the molecular level previously [25–27].

2.2 Preparation of plant extract

Healthy plant samples of *H. salicornicum* were collected from the town of Saint Catherine in the South Sinai Governorate, Egypt (coordinates 28°33′55.1″N 33°56′56.8″E). We confirmed that the chosen plants exhibited no signs of morphological disease symptoms. The specimens gathered were classified by the Department of Plant Production, affiliated with the Faculty of Agriculture at Saba Basha, Alexandria University, Egypt. The specimen voucher for the plant was submitted to the herbarium, recorded under the number 07-93-ALX. The plant materials were subsequently moved to the laboratory, where they underwent a thorough washing process with running tap water for 30 min to eliminate any debris or contaminants. The botanical specimens, after being rinsed, were air-dried at a temperature of 28 ± 3°C in a shaded location for 10 days until they were fully desiccated. The desiccated plant specimens were pulverized into a fine powder using the DSP Powder Grinder Silver 650 W Model KA3025 (Yiwu DSP Electric Appliance Co., Ltd., Zhejiang, China). The extract was acquired by shaking 50 g of plant powder in 500 mL of

80% methanol at 100 rpm and 25°C for 12 h using a REMI RS-36 BL rotary shaker (Remi Elektrotechnik Limited, Mumbai, India). The extract-free supernatants were obtained by filtering through the Whatman No. 1 paper. The methanol was subsequently evaporated in a GWSI rotary evaporator R-3001 (Zhengzhou Greatwall Scientific Industrial and Trade Co., Ltd., Zhengzhou, China) at temperatures ranging from 35 to 40°C. The dried extract was subsequently utilized in various assays to investigate its phytochemical composition.

2.3 Antibacterial assay

The efficacy of the extract against bacteria was determined using the agar disc diffusion method. A single colony of the purified bacterial strain was added to 100 mL of nutrient broth medium and incubated overnight at 28 ± 2°C. After incubation, bacterial growth concentrations were adjusted to 10^8 CFU/mL using a nutrient broth medium. About 100 µL was then equally distributed on a glycerol nutrient agar plate. Different concentrations of the dried extract were prepared in 10% dimethyl sulfoxide (DMSO) to final concentrations of 300, 500, 700, and 1,000 µg/mL. An aliquot of 15 µL of each extract concentration was added to 5 mm diameter filter paper discs, and the filter discs were left to dry for 24 h. The plant extract-loaded discs were placed on the surface of the bacterial culture plates, and the plates were incubated for 24 h at 28 ± 2°C. The negative control group of the experiment was adjusted using 10% DMSO-treated discs (without the plant extract). The positive control groups were set up using amoxicillin as a standard antibiotic drug with a concentration of 25 µg/disc. The antibacterial efficacy of *H. salicornicum* extract was assessed by measuring the diameter of the inhibition zone (IZ) in triplicate against the tested bacterial strains and comparing it to the control group.

2.4 Antifungal assay

The antifungal efficacy of the extract against various strains of plant pathogenic fungi was evaluated following the methodology described by Heflish et al. [25]. In summary, potato dextrose agar (PDA) plates were amended with varying concentrations of the plant extract to achieve final concentrations of 0, 1,000, 2,000, 3,000, 4,000, and 5,000 µg/mL. A 5 mm diameter disc was removed from 7-day-old cultures of *F. oxysporum*, *B. cinerea*, and *R. solani* and placed centrally on each treated PDA plate. The plates were incubated for 7 days at 25°C. Positive controls were established using copper hydroxide at a concentration of 1.5 g/L. Following incubation, antifungal efficacy was assessed by calculating the percentage of fungal mycelial growth inhibition using the following formula:

$$\text{Mycelial growth inhibition (\%)} = \left[\frac{A_0 - A_t}{A_0}\right] \times 100,$$

where A_0 is the average diameter of the untreated fungal growth and A_t is the average diameter of the fungal growth after treatment.

2.5 Analysis of the aqueous methanolic extract using HPLC

The polyphenolic components of the aqueous methanolic extract were identified using an Agilent 1260 Infinity HPLC Series system with a quaternary pump. Specific conditions were employed to conduct the HPLC. The separation process was conducted using a Zorbax Eclipse Plus C18 column with a 4.6 mm inner diameter and a 100 mm length. The separation was conducted at 30°C. To achieve the separation procedure, a tertiary linear elution gradient was employed. The gradient was composed of water, 0.2% H_3PO_4 (HPLC grade, v/v), methanol, and acetonitrile. Twenty microliters of the mixture were injected. Individual compounds were identified using a VWD detector designed to detect at a wavelength of 284 nm. A variety of polyphenolic compounds were utilized as standard compounds. These included vanillin (VA), syringic acid, caffeine, vanillic acid, ferulic acid, ellagic acid, benzoic acid, salicylic acid, and cinnamic acid. The retention times (RTs) of the discovered compounds were compared to those of the authentic standard compounds [28].

2.6 Analysis of the aqueous methanolic extract using GC–MS

We utilized an Agilent 6890 GC–MS apparatus to analyze the aqueous methanolic extract of *H. salicornicum* for the presence of bioactive compounds. The GC–MS apparatus was fitted with an Agilent mass spectrometry detector featuring a direct capillary interface. In addition, a fused silica capillary column HP-5MS was used. It was 30 m long, 0.32 mm wide, and had a film thickness of 0.25 µm. The temperature of the column was increased gradually from an initial value of 50°C to a final value of 230°C, at a step of 5°C/min. After being held at this temperature for 2 min, it was then increased to 290°C, which served as the ultimate column temperature. The bioactive metabolites were identified through MS library searches, utilizing the NIST and Wiley databases. The identification process involved

comparing the mass spectra and RTs to the data available in the Wiley and NIST MS laboratory databases [29].

2.7 Analysis of data

For the statistical analysis of data, we employed CoStat software (version 6.4, CoHort Software, Monterey, CA, USA) to perform the necessary computations. To determine the significance of differences between groups, one-way analysis of variance was conducted, followed by Tukey's post hoc test.

3 Results

3.1 Antibacterial activity assay

Figure 1 illustrates the antibacterial effects of the aqueous methanolic extract of *H. salicornicum* against selected pathogenic bacteria, specifically *P. atrosepticum*, *P. carotovorum*, *R. solanacearum*, and *S. scabiei*. The data indicate that *P. atrosepticum* exhibited the highest susceptibility to the plant extract, with an IZ diameter of 12.3 mm. This was followed by *P. carotovorum*, showing an IZ diameter of 11 mm when treated with a concentration of 1,000 µg/mL aqueous methanolic extract. In contrast, *R. solanacearum* and *S. scabiei* demonstrated limited sensitivity to amoxicillin, with IZ diameters of 6.3 and 6 mm, respectively. However, they responded more significantly to the plant extract treatments, with IZ diameters reaching 10 mm following the application of a 1,000 µg/mL extract and 8 mm at a concentration of 500 µg/mL (Figure 1). The results demonstrate that the 1,000 µg/mL concentration of *H. salicornicum* extract was effective across all bacterial strains tested, showing superior antibacterial activity relative to other concentrations. While the 500 µg/mL concentration displayed notable antibacterial effects against *S. scabiei*, differences between this and the higher concentrations (700 and 1,000 µg/mL) were not statistically significant.

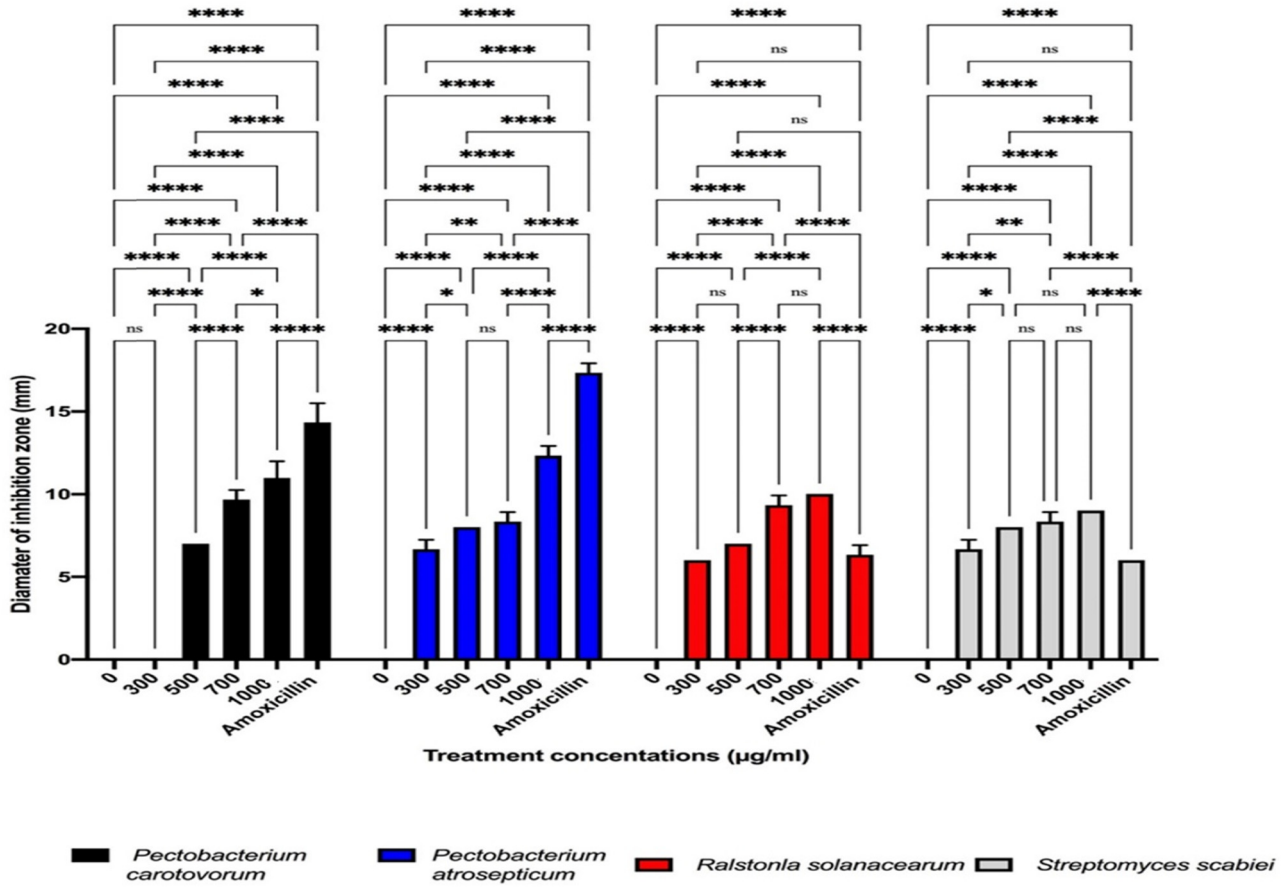


Figure 1: Antibacterial activities of *H. salicornicum* aqueous methanolic extract. The diameter of the IZ (mm) includes a disc diameter of 5 mm.

3.2 Antifungal activity of *H. salicornicum* aqueous methanolic extract

The antifungal activity of *H. salicornicum* aqueous methanolic extract against *F. oxysporum*, *B. cinerea*, and *R. solani* was assessed, as illustrated in Figure 2. The findings reveal that the aqueous methanolic extract exerted significant inhibitory effects on the tested fungal strains, showing particularly strong efficacy against *R. solani*. Specifically, the growth diameter of *R. solani* was reduced to 28 mm with the *H. salicornicum* extract, compared to 9.0 and 12.2 mm for the untreated control and copper hydroxide treatments, respectively (Figure 2a). The growth inhibition rate for *R. solani* reached 68.8%, although slightly lower than that of the positive control (copper hydroxide at 1.5 g/L), which achieved an inhibition rate of 86.4% (Figure 2b). Furthermore, *B. cinerea* exhibited the highest resistance to copper hydroxide among all the fungi tested. However, treatment with the extract at 5,000 µg/mL effectively inhibited *B. cinerea* growth by 45.7%, a significantly higher rate than that of the positive control (33.3%). Similarly, the extract at 5,000 µg/mL concentration also inhibited *F. oxysporum* growth by 42%, a rate statistically comparable to the 41.55% inhibition observed with copper hydroxide treatment.

3.3 Chemical composition of *H. salicornicum* extract

Figure 3 shows the plant of *H. salicornicum*, while Figure 4 presents the HPLC chromatogram of the aqueous methanolic extract of *H. salicornicum*. The detected flavonoid and phenolic compounds are listed in Table 1. The obtained results indicated that gallic acid (GA, 6427.54 µg/g), VA (1145.39 µg/g), chlorogenic acid (498.09 µg/g), and syringic acid (322.52) µg/g were the most abundant phenolic compounds in the plant extract. On the other hand, apigenin (1155.92 µg/g), daidzein (460.87 µg/g), quercetin (382.70 µg/g), and naringenin (160.37 µg/g) were the most abundant flavonoid compounds in the extract (Figure 4). Also, the main secondary metabolites in the extract were identified using the GC–MS analysis (Figure 5); the analyzed data confirmed the presence of six main different compounds in the

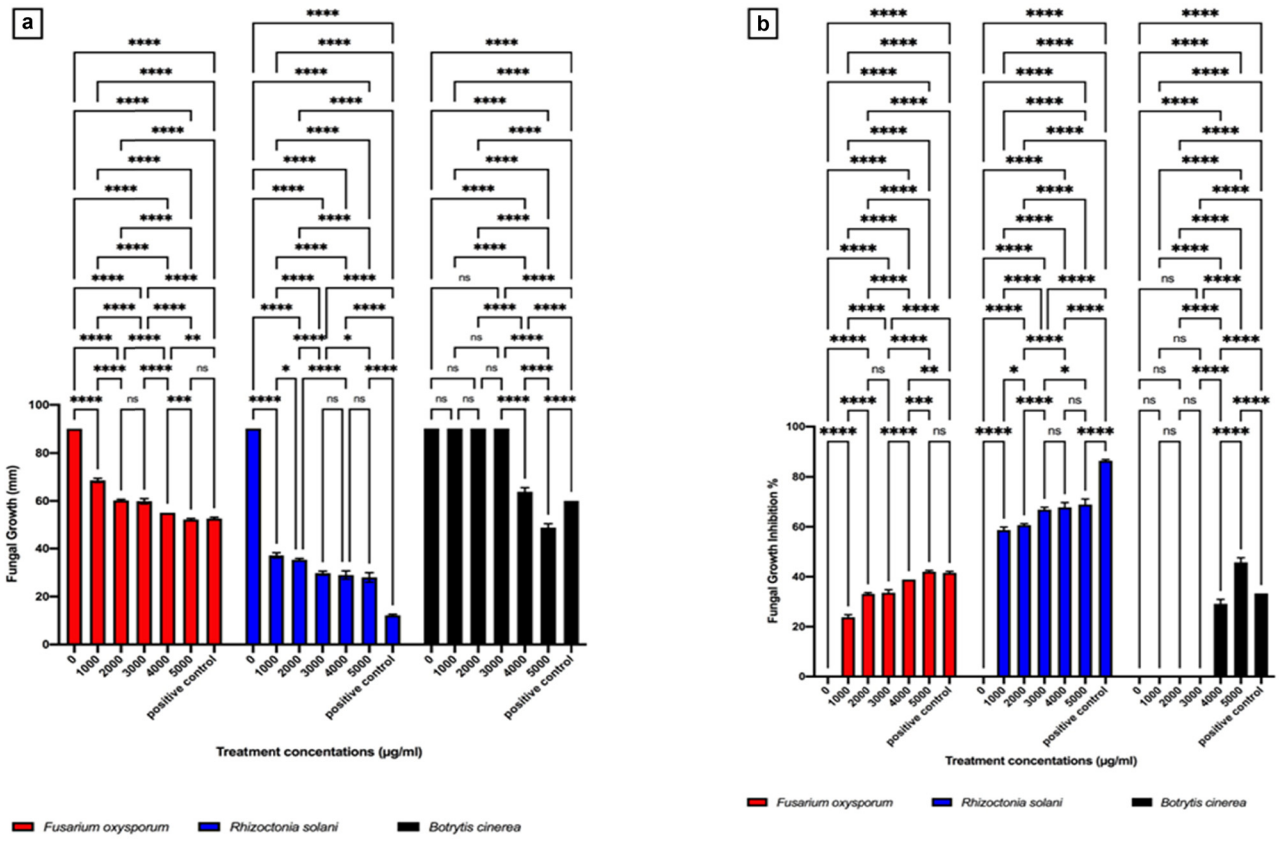


Figure 2: Antifungal effects of *H. salicornicum* aqueous methanolic extract against plant pathogenic fungi. (a) Mycelial growth diameters expressed in mm and (b) antifungal activity expressed as fungal growth inhibition %.

Figure 3: *H. salicornicum* plant.

Table 1: Analysis of *H. salicornicum* phenolics and flavonoids of the plant aqueous methanolic extract using HPLC

Compound	Area	Conc. (µg/g)
Phenolic compounds		
GA	1056.27	6427.54
VA	392.17	1145.39
Chlorogenic acid	53.98	498.09
Syringic acid	68.63	322.52
Caffeic acid	37.85	207.83
Ellagic acid	15.72	199.45
Ferulic acid	33.55	144.63
Cinnamic acid	25.07	31.35
Methyl gallate	4.44	16.58
Flavonoid compounds		
Apigenin	230.75	1155.92
Daidzein	112.56	460.87
Quercetin	42.29	382.70
Naringenin	22.06	160.37
Catechin	6.63	110.01
Rutin	6.30	49.57

extract (Table 2) with different chemical structures and RTs. The most abundant compound was *n*-hexadecanoic acid reported at a RT of 26.35 min with the molecular formula $C_{16}H_{32}O_2$ and molecular weight 256. Additionally, 9-octadecenoic acid (*E*), 9,12-octadecadienoic acid (*Z*,*Z*), and palmitic acid were the second highest compounds, which were detected at RTs of 29.4, 29.3, and 28.15 min with molecular weights of 282, 280, and 328, respectively. While octadecanoic acid and 9,12-octadecadienoic acid (*Z*,*Z*)-, 2,3-bis[(trimethylsilyl)oxy]propyl ester were the least abundant compounds were detected at RTs of 29.9 and 42.8 min with molecular weights of 284 and 498, respectively (Table 2).

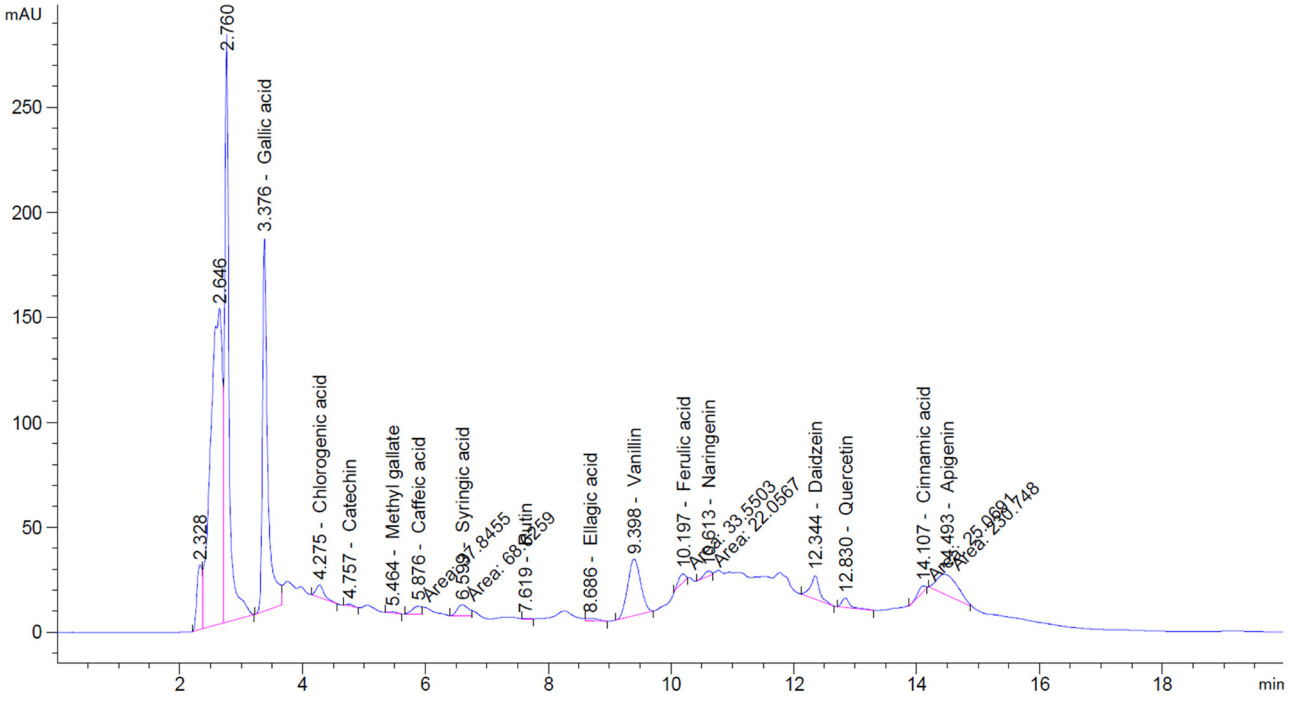


Figure 4: HPLC chromatogram of phenolic flavonoid compounds of *H. salicornicum* aqueous methanolic extract.

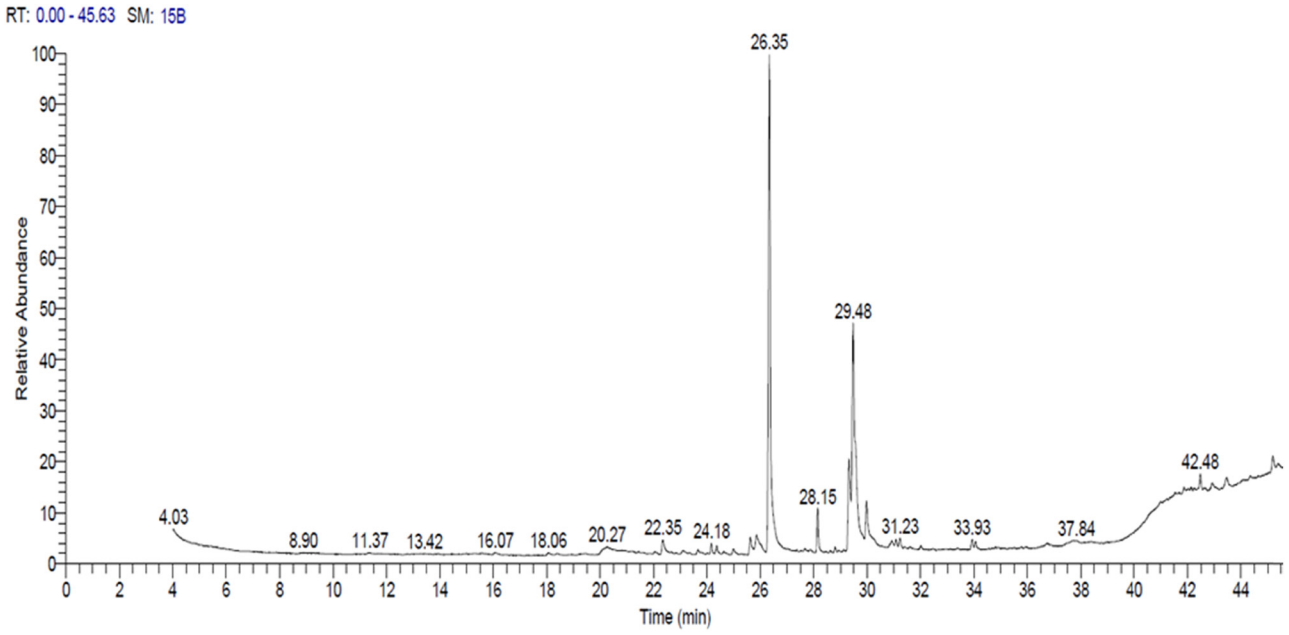
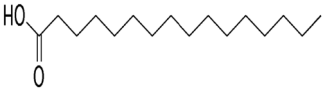
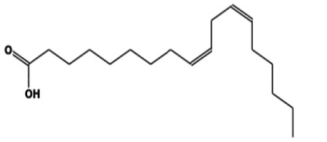
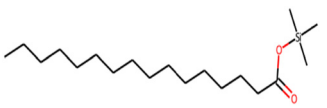
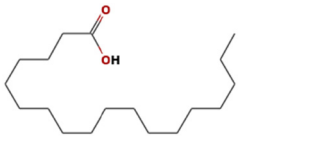
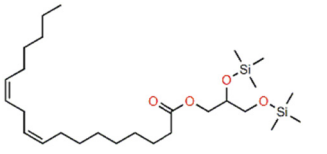


Figure 5: GC–MS chromatogram of *H. salicornicum* aqueous methanolic extract.

Table 2: Chemical composition of *H. salicornicum* aqueous methanolic extract using GC–MS analysis

Compound name	RT	Area %	MF	Molecular formula	Molecular weight	Chemical structure
n-Hexadecanoic acid	26.35	53.74	919	$C_{16}H_{32}O_2$	256	
9-Octadecenoic acid (*E*)	29.48	26.97	923	$C_{18}H_{34}O_2$	282	
9,12-Octadecadienoic acid (*Z*,*Z*)	29.32	8.67	855	$C_{18}H_{32}O_2$	280	
Palmitic acid, TMS derivative	28.15	4.36	876	$C_{19}H_{40}O_2Si$	328	
Octadecanoic acid	29.98	4.39	825	$C_{18}H_{36}O_2$	284	
9,12-octadecadienoic acid (*Z*,*Z*), 2,3-bis [(trimethylsilyl)oxy]propyl ester	42.84	1.88	758	$C_{27}H_{54}O_4Si_2$	498	

RT: retention time; MF: match factor.

4 Discussion

Plant pathogenic microorganisms are recognized as harmful agents that cause substantial damage to crop productivity, both quantitatively and qualitatively, thus posing a significant threat to agriculture worldwide [30,31]. With increasing awareness of the drawbacks associated with synthetic pesticides "including off-target toxicity, environmental residues, and challenging biodegradability," there has been a heightened demand for safe, sustainable, and economically viable pest control alternatives [32]. Recently, plant-based extracts have gained considerable attention as effective and environmentally friendly solutions for managing microbial pathogens due to their viability, efficacy, and relatively fewer adverse effects [7,33]. Historically, botanical pesticides were widely employed in both subsistence and commercial farming before the widespread use of synthetic pesticides [34]. These botanical pesticides contain naturally occurring bioactive compounds that are extracted from plants using organic solvents [35]. Although synthetic pesticides became dominant in agriculture due to their high efficacy, the emergence of concerns regarding environmental persistence and human health impacts has renewed interest in botanical alternatives [36]. In light of these developments, the present study explores for the first time the potential application of the *H. salicornicum* methanolic extract as a botanical pesticide.

H. salicornicum, a shrub belonging to the family Chenopodiaceae, is commonly found in various regions across Asia and Africa [37,38]. Each part of this plant, which is highly tolerant to environmental stress, such as water scarcity, saline soil, and high temperatures, is utilized in India for diverse purposes: the fruiting tops and seeds are used as animal feed and for human consumption [17]. While, to the best of our knowledge, no studies have specifically examined the efficacy of *H. salicornicum* extracts against plant pathogens, various research studies have demonstrated the potency of its extracts in controlling human pathogenic bacteria and fungi [22,23]. This suggests a promising foundation for the use of *H. salicornicum* as a plant-based pesticide in agriculture, aligning with the pressing need for sustainable pest management solutions.

Previous studies have established the antimicrobial efficacy of *H. salicornicum* extracts against a variety of human and animal pathogens. For instance, antibacterial activity has been demonstrated against human pathogens such as *M. luteus*, *B. subtilis*, and *S. typhi*, as well as antifungal effects against human pathogens like *A. flavus*, *P. chrysogenum*, and *C. tropicalis* [22,23]. Furthermore, *H. salicornicum* extracts have shown efficacy against animal pathogens, including *Pseudomonas aeruginosa* and *S. typhimurium*, with a particular advantage seen in acetone and ethanol extracts over ether and aqueous extracts [24]. In this study, we expand on these findings by exploring the effects of *H. salicornicum* aqueous methanolic extract against major plant pathogens: *P. carotovorum*, *P. atrosepticum*, *R. solanacearum*, *S. scabiei*, *F. oxysporum*, *B. cinerea*, and *R. solani*. Chemical analysis revealed the presence of various phenolic compounds, including GA, VA, chlorogenic acid, and syringic acid, along with flavonoids such as apigenin, daidzein, quercetin, and naringenin. The antimicrobial activity observed may be attributed to these compounds, particularly GA and VA, which are well-documented for their pathogen-suppressive properties [39–41]. GA, found naturally in many fruits and vegetables, has been widely studied for its inhibitory effects on microbes, including *F. oxysporum* [42–44]. GA, one of the several phenols found in the leaf extracts of grapevines and *Coccoloba uvifera*, has been found to suppress the growth of *B. cinerea* and *R. solani* [45,46]. VA, a phenolic aldehyde derived primarily from vanilla orchids, exhibits potent antibacterial activity through membrane disruption mechanisms [47,48]. Its hydrophobic nature enables interactions with microbial cell membranes, which disrupts ionic gradients and impairs respiration [49].

Several phenolic compounds have been shown to exhibit varying levels of antifungal activity against key plant pathogens. Chlorogenic acid, for instance, is produced in grafted watermelon roots and has been found to inhibit the growth of *F. oxysporum*, *R. solani*, and *B. cinerea* [50–52]. Similarly, caffeic acid has demonstrated limited antifungal effects against *F. oxysporum* but has a notable inhibitory impact on *B. cinerea* [53,54]. Syringic acid, however, shows mixed results; while it has been observed to increase the prevalence of *Fusarium* in the rhizosphere of cucumber seedlings, it does not significantly affect the growth of *B. cinerea* [55]. Ferulic acid, commonly found in the leaf extracts of *Vitis* spp., exhibits inhibitory effects on both *F. oxysporum* and *B. cinerea*, potentially by disrupting fungal cell structures [45,56]. VA and trans-cinnamic acid further contribute to antifungal activity by increasing cell membrane permeability in *B. cinerea*, thereby inhibiting its mycelial growth [57,58]. When applied as a VA-chitosan coating on tomato fruit, VA extends the shelf life and protects against *F. oxysporum*, even under ambient storage conditions [59]. Free cinnamic acid also significantly suppresses *F. oxysporum* by disrupting the structure of its mycelia [60]. Ellagic acid, present in the peel extracts of *Musa paradisiaca*, has been shown to inhibit *R. solani*, while catechin, a polyphenolic compound identified in *Pinus wallachiana* leaf extracts, suppresses the mycelial growth of *F. oxysporum f. sp. cubense* [46,61]. Epicatechin, another related compound, inhibits *B. cinerea* growth by regulating phenylpropane metabolism, a pathway important for fungal defense mechanisms [54]. Additionally, catechol-type siderophores

produced by *Pseudomonas syringae* BAF.1 have been effective in inhibiting *F. oxysporum*, and free catechol reduces the bulk and size of *R. solani* mycelia after 3 days of incubation [62,63].

Nevertheless, certain flavonoids identified through HPLC analysis in the examined extract exhibited the capacity to inhibit the growth of all three fungi. Multiple investigations have verified the antibacterial characteristics of apigenin, a plant flavonoid compound found in various plants, including parsley, chamomile, and celery. Research suggests that it possesses antibacterial properties, making it potentially useful in combating bacterial infections. Studies have shown that apigenin can inhibit the growth of various bacteria, including those responsible for common infections like *S. aureus* and *Escherichia coli* [64]. This comparative analysis underscores the variable antifungal activities of different phenolic compounds, each employing distinct mechanisms such as membrane permeability alteration, mycelial growth inhibition, and metabolic pathway regulation. Together, these mechanisms underscore the potential of plant-derived compounds as targeted treatments against specific pathogens. Notably, the combined presence of these phenolic and flavonoid compounds in *H. salicornicum* extract presents a promising, multifaceted approach for effectively managing a range of plant pathogens. Furthermore, we reported the presence of some other bioactive antimicrobial compounds in the methanolic extract using GC-MS analysis revealing that the studied extract contained saturated fatty acids (SFAs) such as *n*-hexadecanoic acid (C16:0), and octadecanoic acid (C18:0). These fatty acids were found to possess inhibitory effects on the growth of the tested fungal isolates. Guimarães and Venâncio [65] validated our idea by explaining that SFAs include a significant amount of hydrophobic groups, which enhance their contact with the cell membrane. However, the extract contains high levels of unsaturated fatty acids (UFAs) such as 9-octadecenoic acid (*Z*)- (C18:1) and 9,12-octadecadienoic acid (*Z,Z*)- (C18:2), these fatty acids are believed to have inhibitory effects on the tested isolates. This hypothesis aligns with the previous evidence that UFAs, which are more abundant but less thermodynamically stable than trans-UFAs, have a greater impact on the cell membrane of specific bacteria [65]. In addition to phenolics and flavonoids, various reports explain the positive direct actions of fatty acids such as linoelaidic acid and palmitic acid and their derivatives in controlling plant pathogens [66–68]. Palmitic acid is an SFA compound, and different reports have indicated that palmitic acid can prevent the growth of plant soil-borne pathogens and enhance the growth of seedlings [66,69,70]. Also, the antimicrobial activities of *n*-hexadecanoic acid were confirmed against *S. aureus*, *B. subtilis*, and *E. coli* [71].

The current study provides a detailed comparison with several other investigations into the chemical composition and bioactivities of *H. salicornicum* from various geographical regions. Our findings revealed a distinct phytochemical profile, particularly the high content of GA and apigenin, as well as a significant presence of fatty acids like *n*-hexadecanoic acid and 9-octadecenoic acid, which were consistent with the findings of Yousif et al. [72]. However, unlike Yousif et al. [72], who employed microwave-assisted extraction and focused on antibacterial and anticancer activities, our study uniquely highlighted the antimicrobial efficacy of the methanolic extract against a range of phytopathogens, including *Pectobacterium* spp., *R. solanacearum*, and *F. oxysporum*. This is not in contrast with the work of Elagamy et al. [73], who identified alkaloids as the major compounds and investigated the antibacterial effects against human pathogens. Furthermore, while Rugaie et al. [74] explored the antimicrobial and antioxidant potentials of various halophytes, including *H. salicornicum*, their study lacked a specific focus on phytopathogens and comprehensive phytochemical profiling seen in our research. Additionally, Ashraf et al. [75] provided only a preliminary phytochemical screening, which did not detect flavonoids, diverging from the results of both our study and others. The regional variation in chemical composition, as suggested by Ullah et al. [76] from Saudi Arabia, further emphasizes the unique bioactive potential of *H. salicornicum* from the Saint Catherine region in Egypt, particularly in the context of agricultural pathogen management. Continued research on the separation and identification of these biologically active substances is crucial for the continuous progress of plant-based fungicides, offering environmentally friendly and health-conscious alternatives to conventional chemical fungicides.

5 Conclusions

This study demonstrates the antimicrobial potential of the *H. salicornicum* aqueous methanolic extract against plant pathogens, indicating its promise as a natural alternative to synthetic pesticides. The bioactive compounds, including phenolics, flavonoids, and fatty acids, likely contribute to its antibacterial and antifungal effects. These findings support the development of *H. salicornicum* extract as a botanical pesticide. However, further research is needed to evaluate its performance under real agricultural conditions, ensuring its effectiveness and practical applicability in sustainable crop protection.

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