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Research Article

Antimicrobial activities and metabolites profiling of *Heliotropium bacciferum* Forssk. methanolic extract

Ahmed ABDELKHALEK[1]*, Moustafa ELSHAER[2], Abdulaziz AL-ASKAR[3], Przemysław KOWALCZEWSKI[4], Said BEHIRY[5]

[1]*Plant Protection and Biomolecular Diagnosis Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Alexandria 21934, Egypt; abdelkhalek2@yahoo.com (*corresponding author)*
[2]*Mansoura University, Department of Microbiology at Specialized Medical Hospital, Mansoura 35516, Egypt; moustafaelshaer@mans.edu.eg*
[3]*Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; aalaskara@ksu.edu.sa*
[4]*Poznań University of Life Sciences, Department of Food Technology of Plant Origin, Poznań, Poland; przemyslaw.kowalczewski@up.poznan.pl*
[5]*Alexandria University, Faculty of Agriculture (Saba Basha), Agricultural Botany Department, Alexandria 21531, Egypt; said.behiry@alexu.edu.eg*

Abstract

In the present study, *Heliotropium bacciferum* Forssk. methanolic extract was tested for its antibacterial activities against *Pectobacterium carotovorum*, *P. atrosepticum, Ralstonia solanacearum,* and *Streptomyces scabiei* bacterial strains. The plant extract was also tested for its antifungal properties against strains of *Fusarium oxysporum*, *Botrytis cinerea*, and *Rhizoctonia solani*. The potent antibacterial activities were recorded against *R. solanacearum* after treatment with the extract at a concentration of 1000 μg/mL with an inhibition zone of 9.67 ±0.57 mm. The methanolic extract also showed promising antifungal effects against *B. cinerea* and *F. oxysporum*, with fungal growth inhibition percentages of 86.22 ±0.33% and 82.00 ±0.55%, respectively, which were higher than the standard antifungal drug. The plant extract's HPLC analysis identified 12 phenolic compounds and 6 flavonoid compounds, with HPLC peaks matching the used standards. Gallic acid, chlorogenic acid, coffeic acid, ellagic acid, coumaric acid, syringic acid, methyl gallate, ferulic acid, vanillin, pyrocatechol, and cinnamic acid were the phenolic compounds that were identified. The flavonoid compounds found in the extracts were daidzein, naringenin, quercetin, kaempferol, hesperetin, apigenin, and rutin, arranged from high to low abundance. Furthermore, according to the GC-MS analysis, the most abundant compounds detected in the plant extract were *n*-hexadecanoic acid, 9,12-octadecadienoic acid (z,z), á-sitosterol, betulin, phorbol, cis-13-octadecenoic acid, and octadecanoic acid. These compounds in HPLC and GC-MS analyses were proven to have antibacterial as well as antifungal properties. Based on the obtained antimicrobial results, *H. bacciferum* methanolic extracts could be recommended as a promising antibacterial and antifungal agent, as well as a safe alternative to many antimicrobial pesticides for the environment and human health.

Keywords: antibacterial; antifungal; *Heliotropium bacciferum*; HPLC; GC-MS; secondary metabolites

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Introduction

For thousands of years, plants have synthesized a wide range of secondary metabolites, crucial for their growth, development, and survival during challenging environmental conditions (Youssef *et al.*, 2021a; Elshafie *et al.*, 2023). Since plants are stationary organisms that can't change their environment, they need a huge battery of metabolites to ensure fitness, especially when stress conditions arise in their surroundings (Twaij and Hasan, 2022). Plant secondary metabolites are phytochemicals that are naturally produced in plant cells. They have been isolated and characterized from different plants for a long time because of their importance to both plant and human activities (Patra, 2012). These phytochemicals were classified into different groups, including phenolic acids, flavonoids, polyphenols, terpenoids, tannins, alkaloids, thiohydroximates, steroids, cyanogens, glucosinolates, salicylates, etc. (Patra, 2012; Twaij and Hasan, 2022; Elshafie *et al.*, 2023). These compounds are the adaptation tools that plants store in their cellular compartments so that they can be used as needed. Plant secondary metabolites also have a lot of antimicrobial properties that make plants biochemically flexible, so they can respond quickly and effectively to any new biotic or abiotic stresses (Behiry *et al.*, 2022b; Twaij and Hasan, 2022; Weiszmann *et al.*, 2023). The global environment has been facing great challenges, causing tremendous damage to the ecosystem. The use of chemically synthesized pesticides in agriculture is one of the factors that has negatively impacted the natural ecosystem. Scientists are trying to find alternative antimicrobial agents to replace pesticides (Abd El-Rahim *et al.*, 2003; Youssef *et al.*, 2021b; Behiry *et al.*, 2022a). In addition, the unbalanced usage of antibiotics has led to multidrug-resistant microbial strains. Therefore, the search for safe alternatives for synthetic antimicrobial compounds is a mandate to protect the environment from any further damage that might occur and secure new tools to protect human health from the lethal attacks of microbes (Jadimurthy *et al.*, 2022).

Natural plant secondary metabolites with bioactivity toward microbes include several classes of molecules, for example: aldehydes, alkaloids, amino acids, cyanogenic glucosides, flavonoids, polyketides, polyphenols, quinones, amides, saccharides, terpenes, and thiophenes (Patra, 2012; Elshafie *et al.*, 2023). In natural environments, certain compounds exhibit significant ecological properties. These traits include antimicrobial effects (like antifungal and antibacterial effects), anti-feedant effects, the ability to attract pollinators, nematocidal effects, insect repellent and insecticidal effects, controlling insect growth, and acting as allelopathic agents. Consequently, these compounds hold considerable potential as sources for the development of innovative pest control agents or biopesticides (Patra, 2012; Elshafie *et al.*, 2023; Qaderi *et al.*, 2023). However, several factors appear to affect plant production, so there is a strong need to explore their characteristics and biological functions. These metabolites have been commonly used in folk medicine for years, especially in underdeveloped communities, as medicinal treatments for diseases (Goyal and Sharma, 2014). The family Boraginaceae consists of 100 genera and more than 2000 species. The Egyptian flora combines 49 species belonging to 14 genera of the Boraginaceae family. *Heliotropium* is one of the genera in this family that grows in Egypt (El-Gazzar *et al.*, 2019). The plants in this genus are herbaceous in nature and are found in tropical and temperate regions around the world. They consist of around 300 plant species, commonly referred to as heliotropes. These plants show diverse therapeutic activities, including anti-inflammatory, antimicrobial, analgesic, and healing properties, etc. (Dresler *et al.*, 2017). For these reasons, heliotropes have been used for a long time in folk medicine to treat common diseases and symptoms, such as menstrual dysfunction, noxious bites, rheumatism, and biliary disorders (Modak *et al.*, 2007; Goyal and Sharma, 2014; Ghori *et al.*, 2016). A lot of phenolic compounds have been found in plants in this genus. These include phenolic acids, flavonoids, quinones (especially alkannins and shikonins), terpenoids, and pyrrolizidine alkaloids (Ahmad *et al.*, 2016; Najeeb *et al.*, 2020).

Heliotropium bacciferum Forssk. which belongs to the family *Boraginaceae*, is an upright herb with a perennial woody root. It grows in dry Sahel desert areas (Mauritania, Senegal, Mali, and North Nigeria), North Africa, Saudi Arabia, the Nile Valley, the Sinai, and tropical Asia (El-Gazzar *et al.*, 2019). Also, this plant is native to the Nile Valley and grows well in Egypt and Sudan (Najeeb *et al.*, 2020). It is grown in many Wadis and newly reclaimed fields in Saudi Arabia's Taif region and eastern province (Ahmad *et al.*, 2015, 2016). It has medicinal uses and is a rich source of pyrrolizidine alkaloids, which have antihyperlipidemic, antitumor, antidiabetic, and antimicrobial properties (Fayed, 2021). It is also good fodder for camels, and the dried powdered leaves are added to curdle milk or water to treat ringworms (Burkill, 1995). *H. bacciferum* plant extracts showed significant antibacterial and antifungal effects against different bacterial and fungal strains using crude, chloroform, and ethyl acetate extracts at 225 μg/mL. The authors indicated the potential curative effects of this plant (Ahmad *et al.*, 2015, 2016). Therefore, this plant draws special attention as a potent source of secondary metabolites that have the potential to produce antimicrobial secondary metabolites. The primary goal of the current work was to determine the chemical composition of methanolic extract of *H. bacciferum* using HPLC and GC-MS methods. Furthermore, the study aimed to investigate the antibacterial properties of the extract against diverse bacterial and fungal strains.

Materials and Methods

Source of bacterial and fungal strains

All bacteria and fungi utilized in this study were previously isolated and subjected to molecular identification, as indicated by the references (El-Bilawy *et al.*, 2022; Al-Askar *et al.*, 2023; Heflish *et al.*, 2023). Table 1 presents the microorganism isolate, along with its corresponding isolation source and GenBank accession number.

Table 1. List of microorganisms used in this study

Microorganism	Isolate	Isolation host	Accession number
Bacteria	*Pectobacterium carotovorum*	Potato	OQ878656
	Pectobacterium atrosepticum	Potato	MG706146
	Ralstonia solanacearum	Potato	OQ878653
	Streptomyces scabiei	Potato	OR437480
Fungi	*Fusarium oxysporum*	Potato	OQ820156
	Botrytis cinerea	Strawberry	MN398400
	Rhizoctonia solani	Bean	OQ880457

Preparation of plant extract

The *H. bacciferum* plants were obtained from Riyadh, Saudi Arabia, and subsequently subjected to the following procedures*:* Healthy plants with no plant disease infestations were selected and collected on paper pages. The collected plant materials were transferred to the lab and washed with tap water to remove any derbies or possible contaminants from the plant tissues. Then, the cleaned plants were left to dry in the shadow at room temperature until complete dryness. Subsequently, the air-dried materials were ground to a fine powder. Phenolic compounds were extracted from 50 g of ground plant material by incubating it overnight in 500 mL of 80% methanol in a rotary shaker (100 rpm) at room temperature. The following day, the resulting liquid was filtered through Whatman No. 1 filter paper to remove any plant debris. Subsequently, the methanol was subjected to evaporation under vacuum conditions using a rotary evaporator operating at a temperature range of 25-30 °C. Subsequently, the dry extractives were stored at a temperature of -20 °C until further analyses were conducted.

In-vitro antibacterial assay

In-vitro antibacterial assays were accomplished using the agar disc diffusion method. Briefly, a single colony of each purified bacterial strain was transferred to 100 mL of nutrient broth medium, and the inoculated flasks were incubated overnight at 27 °C. After incubation, the bacterial growth concentration was adjusted to 10^8 CFU/mL using a nutrient broth medium. 100 µl of 10^8 CFU/mL from each bacterial strain was spread on the surface of each glycerol nutrient agar plate. The recovered dried extract was dissolved in 10% DMSO to prepare 10 mL of different concentrations (300, 500, 700, and 1000 µg/mL) of plant extract. The control group was set using 10% DMSO (without the extract). An aliquot of 15 µL of each concentration was loaded on 5.0 mm diameter filter paper discs. After drying at 4 °C for 24 h, the discs were placed on the surface of the bacteria-inoculated plates, and the plates were incubated at 27 °C for 24 h. Amoxicillin was used as a standard antibiotic drug (positive control) at a concentration of 25 µg/disc. The inhibition zone diameter was measured in millimeters (in triplicate) and compared with the control treatment (untreated strains) groups.

Antifungal assay

The study examined the linear growth of fungal isolates at different methanolic extract concentrations, including 1000, 2000, 3000, 4000, and 5000 µg/mL. The antifungal activity was determined according to the poisoned media method as described by Heflish *et al.* (2023). Briefly, circular fungal specimens with a diameter of 5 mm were surgically removed from a culture that had reached maturity after 6 days. The discs were subsequently transferred to the centers of Petri dishes that had been filled with extract solutions of different concentrations. Subsequently, the dishes were subjected to a one-week incubation period at a temperature of 25 °C. Each treatment was replicated three times. Copper hydroxide at a concentration of 250 µg/mL was used as a positive control. At the end of incubation, the fungal mycelial growth inhibition was expressed as growth inhibition% and calculated according to the following equation:

Mycelial growth inhibition % = [(A0 - At)/A0] X 100,

where, A0 is the average diameter of the un-treated fungal growth and At is the average diameter of the fungal growth post treatment.

Identification of phenolic compounds using HPLC

HPLC (Agilent 1260 Infinity HPLC Series, Santa Clara, CA, USA) equipped with a Zorbax Eclipse plus C18 column (100 mm × 4.6 mm i.d.) (Agilent Technologies, Santa Clara, CA, USA), a VWD detector, and quaternary pump was used to identify the phenolic compounds detected in the plant extract using the following parameters: 20 µL injection volume; 30 °C; linear elution gradient of water, 0.2% H_3PO_4 (HPLC grade v/v), methanol, and acetonitrile. The resulting peaks were detected using the VWD detector at 284 nm. Several common phenolic compounds were used in the identification process. These included caffeine, vanillic acid, syringic acid, vanillin, *p*-coumaric acid, ellagic acid, benzoic acid, salicylic acid, and cinnamic acid. To identify each compound, we compared the retention times of the authentic compounds, and the unknown phenolics in the plant extract were compared to identify each compound.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The composition of the methanolic plant extract was studied using gas chromatography coupled with mass spectrometry (GC-MS). The analysis was conducted using an Agilent 6890 GC-MS machine (Agilent, USA) that was equipped with an Agilent mass spectrometric detector. The machine was fitted with a direct capillary interface and a fused silica capillary column HP-5MS (30 m × 0.32 mm × 0.25 µm film thickness). The temperature of the column was initially set to 50 °C and then increased at a rate of 5 °C per minute until reaching 230 °C. It was held at this temperature for 2 minutes before being raised to the final oven temperature of 290 °C. The samples were injected, and the separation and detection program was configured according to the previously established protocol as described in the previously published study (Abdelkhalek *et al.*, 2022).

The constituents in the extracts were identified using a search in the MS library, specifically the NIST and Wiley databases. The mass spectra and retention times were cross-referenced with the databases in the Wiley and NIST MS libraries.

Statistical analysis

The experiments were performed in triplicate or more, and the resulting means (M) and standard deviations (SD) were denoted as M ± SD. The data's significance was assessed by conducting an analysis of variance (ANOVA) using CoStat software. Tukey's honest significant differences (H.S.D.) method was employed, with a probability value (*P*-Value) threshold set at ≤0.05. The observed distinctions between the groups were arranged in alphabetical order, with ascending levels of significance (a > b > c). Additionally, no significant differences were found among the groups denoted by the same letters.

Results

In-vitro antibacterial assay

The antibacterial activity of *H. bacciferum* plant extract was tested against *P. carotovorum*, *P. atrosepticum*, *R. solanacearum*, and *S. scabiei*. Figure 1 shows that plant extract at 1000 µg/mL had a strong antibacterial action against *P. carotovorum*, with an inhibition zone of 8.97±0.57 mm. However, the standard antibacterial drug (Amoxicillin) treatment against *S. scabiei* at a concentration of 25 µg/disc resulted in a higher inhibition zone diameter (IZ) that reached 9.93 mm. The difference in inhibition value between the 700 µg/mL and 1000 µg/mL plant extract treatments was not significant. The plant extract treatments had considerably different antibacterial effects on *P. atrosepticum* compared to the usual 25 µg/disc antibacterial medication (Amoxicillin). The methanolic extract was more effective at inhibiting *R. solanacearum* bacteria at 1000 µg/mL, with an IZ of 9.67±0.57 mm compared to 7.33±0.57 mm for Amoxicillin. The methanolic extract had a moderate effect on both *P. carotovorum* and *P. atrosepticum* at a dose of 500 µg/mL (IZ =8.33±0.57). The standard antibiotic Amoxicillin (IZ = 17.33±0.53 and 14.33±0.92 mm, respectively), and higher concentrations of plant extract did not differ significantly from each other. This suggests that these bacterial strains are more sensitive to the standard antibiotic treatment (25 µg/mL of Amoxicillin).

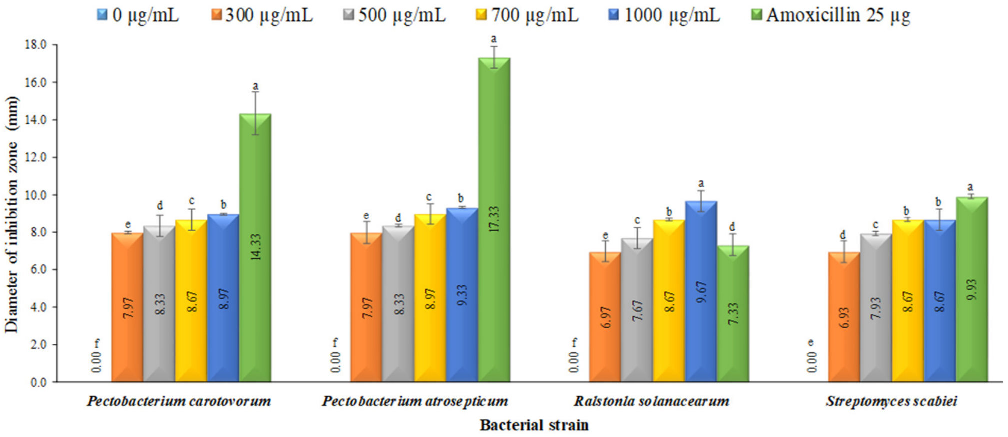


Figure 1. Antibacterial activities of different concentrations of *H. bacciferum* methanolic plant extract
The diameter of the inhibition zone (mm) was measured including the disc diameter (5.0 mm). Error bars represent the standard deviation value calculated for three replicates.

Antifungal activity of the methanolic plant extracts

The antifungal activity of the plant extract against *F. oxysporum, B. cinerea*, and *R. solani* is shown in Figure 2. The obtained data indicate that the methanolic extract has a strong antifungal effect against the three tested fungal strains, with a potent antifungal action against *F. oxysporum* and *R. solani* compared to the positive control (copper hydroxide 250 µg/mL). The lowest percentage of fungal growth inhibition was found for *B. cinerea* and *R. solani* when they were exposed to 2000 and 1000 µg/mL of methanolic extract, respectively (Figure 2). The extract treatment caused the highest inhibition percentage for fungal growth, with values of 86.20±0.33 and 82.00±0.55% for *B. cinerea* and *F. oxysporum*, respectively. The copper hydroxide treatment inhibited the growth of *B. cinerea* and *F. oxysporum* fungi by 61.80±1.03% and 50.40±0.42%, respectively (Figure 2). This suggests that the methanolic plant extract has a stronger inhibitory effect against the tested fungi. On the other hand, the methanolic extract treatment at a concentration of 2000 µg/mL showed antifungal effects against *R. solani* (66.00±1.69% growth inhibition) without significant differences from the higher concentrations, but still lower than the positive control (89.3% growth inhibition).

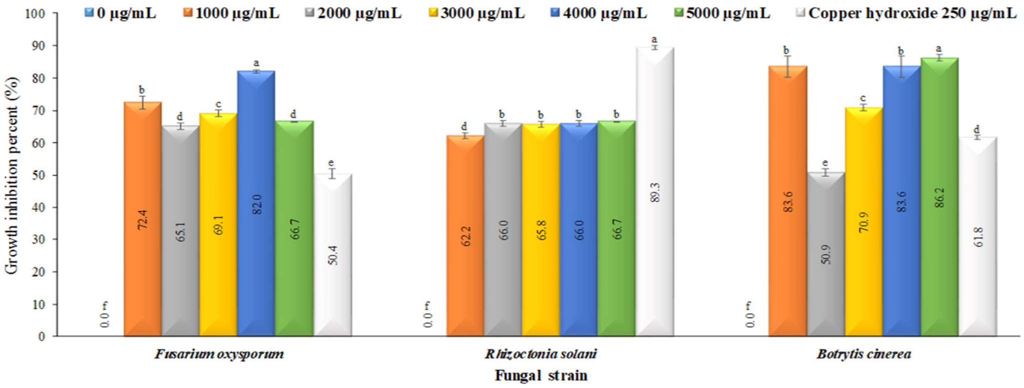


Figure 2. Antifungal activities of different concentrations of *H. bacciferum* plant methanolic extract. The activity was expressed as a growth inhibition percentage (%)

Each column represents the average value of three replicates, while the bars indicate the standard deviation. According to Tukey's HSD test with a significance threshold of 0.05, the values in each column assigned the same letter (a/b/c/d/e) do not exhibit any statistically significant differences.

Chemical composition of the extract using HPLC analyses

In order to investigate the chemical composition of *H. bacciferum* plants, HPLC analysis was conducted. The HPLC chromatogram shown in Figure 3 shows that the methanolic extract of *H. bacciferum* contains a lot of phenolic and flavonoid compounds. Table 2 provides a comprehensive list of each compound, along with its corresponding retention time (RT) and any potential nomenclature in relation to the standard compounds. The most abundant phenolic compounds in the plant extract were gallic acid (4824.7 µg/g), coffeic acid (2330.3 µg/g), chlorogenic acid (2294.07 µg/g), coumaric acid (1178.5 µg/g), and syringic acid (996.2 µg/g). However, the identified compounds in the extracts were daidzein (3935.5 µg/g), naringenin (3003.4 µg/g), querectin (519.4 µg/g), and kaempferol (441.3 µg/g).

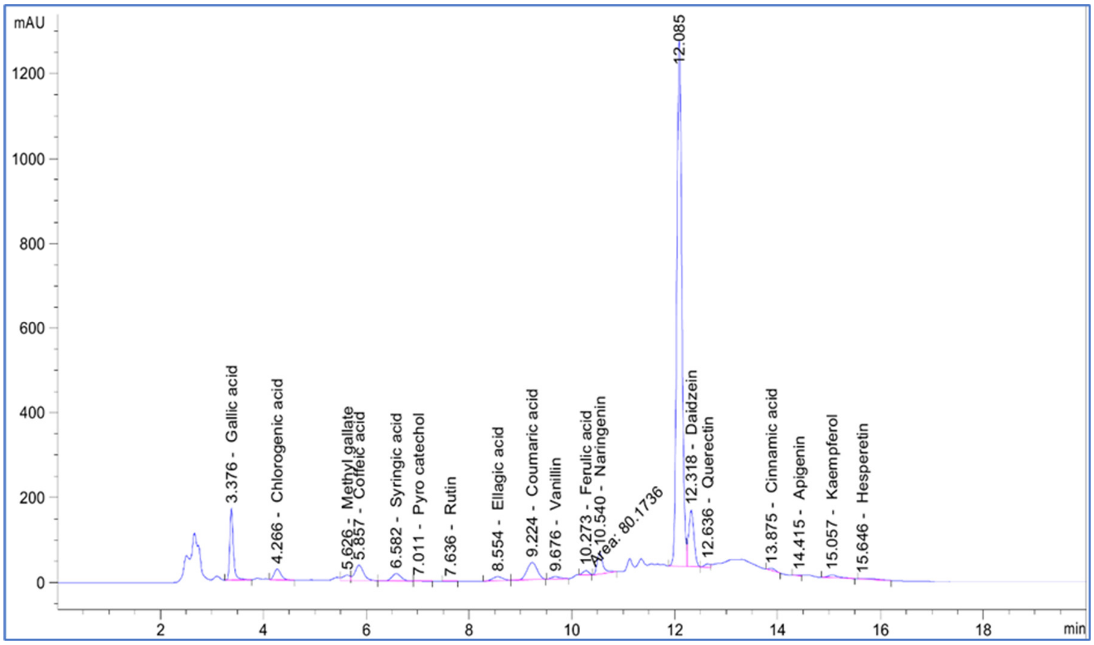


Figure 3. HPLC chromatogram of phenolic secondary metabolites content of the *H. bacciferum* methanolic extract

Table 2. Chemical composition of *H. bacciferum* plant phenolic and flavonoid compounds in its methanolic extract using HPLC

Polyphenolic compounds		Area under curve	Conc. (μg/g)
Phenolic compounds	Gallic acid	792.88	4824.75
	Chlorogenic acid	248.63	2294.07
	Coffeic acid	424.35	2330.30
	Ellagic acid	131.85	1673.13
	Coumaric acid	553.22	1178.53
	Syringic acid	211.97	996.22
	Methyl gallate	130.53	487.04
	Ferulic acid	80.17	345.62
	Vanillin	62.19	181.64
	Pyro catechol	6.7512	68.37
	Cinnamic acid	46.99	58.77
Flavonoid compounds	Daidzein	961.24	3935.56
	Naringenin	413.09	3003.41
	Querectin	57.40	519.45
	Kaempferol	95.30	441.39
	Hesperetin	32.86	118.19
	Apigenin	13.30	66.62
	Rutin	1.52	11.93

Chemical composition of the extract using GC-MS analysis

GC-MS analysis was also used to find secondary metabolites in the methanolic extract of the *H. bacciferum* plant (Figure 4). The main 19 secondary metabolites were identified and listed in Table 3. The table shows secondary metabolite chemical structure, retention time (RT), and chemical formula. The most abundant molecule in the extract was discovered at 26.35 min and identified as n-hexadecanoic acid ($C_{16}H_{32}O_2$) with a molecular weight of 256. The compounds that were found second in the methanolic extract secondary metabolites were octadecadienoic acid and sitosterol, which were found at 29.30 min and 45.17 min, respectively. The two compounds showed molecular weights of 280 and 414, respectively. Phorbol and betulin were the third-most abundant secondary metabolites in the methanolic extract. They appeared at 43.94 min and 36.88 min with molecular weights of 364 and 422, respectively. Furthermore, cis-13-octadecenoic acid and octadecanoic acid were infrequent in the methanolic extract of *H. bacciferum*, with RT values of 29.46 min and 29.96 min, and molecular weights of 282 and 284, respectively. The other chemicals, such as octadecatrienoic acid, gorgost-5-en-3-ol, 1,4-benzenediol, 2-(1,1-dimethylethyl)-5-(2-propenyl), tetradecanoic acid, pentadecanoic acid, 14-methyl-, methyl ester, 2,2,7,7tetra, hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, androstan-17-one,3-ethyl-3-hydroxy-, (5à)- and tibolone were detected at different RT values with low levels of relative abundance (Table 3).

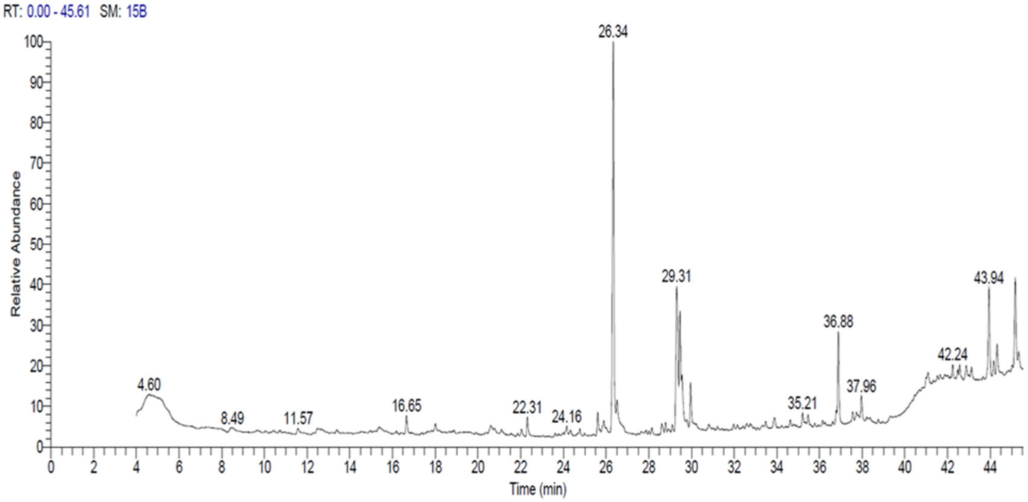
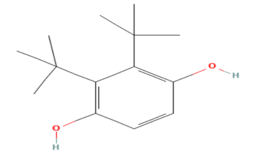
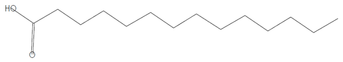
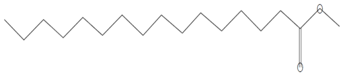
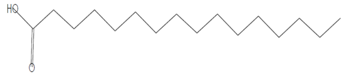
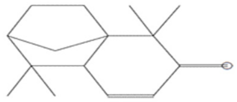
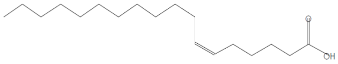
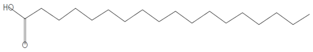
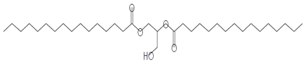
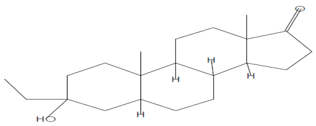
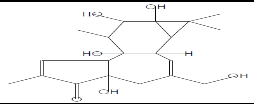
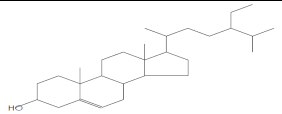
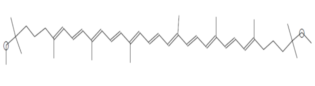
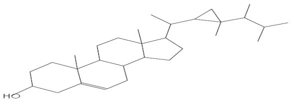
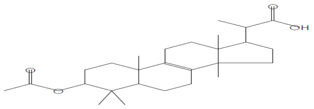
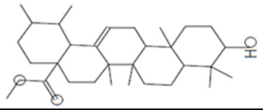
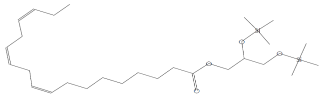
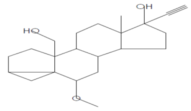
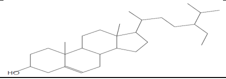


Figure 4. GC-MSMS chromatograms of detected compounds in the *H. bacciferum* methanolic extract

Table 3. Chemical composition analysis of the methanolic extract of *H. bacciferum* using GC-MS

Compound name	RT	Area %	MF	Molecular Formula	M.wt	Chemical Structure
1,4-benzenediol, 2-(1,1-dimethylethyl)-5-(2-propenyl)-	16.65	1.49	963	C13H18O2	206	
Tetradecanoic acid	22.31	1.63	843	C14H28O2	228	
Pentadecanoic acid, 14-methyl-, methyl ester	22.61	1.80	814	C17H34O2	270	
n-hexadecanoic acid	26.35	32.76	930	C16H32O2	256	

2,2,7,7-tetramethyltricyclo [6.2.1.0(1,6)]undec-4-en-3-one	26.52	1.35	6.99	C15H20O2	232	
9,12-octadecadienoic acid (z,z)-	29.30	13.68	913	C18H32O2	280	
cis-13-octadecenoic acid	29.46	6.46	902	C18H34O2	282	
Octadecanoic acid	29.96	3.55	846	C18H36O2	284	
Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	35.20	1.20	770	C35H68O5	568	
Androstan-17-one,3-ethyl-3-hydroxy-, (5à)-	36.78	0.89	774	C21H34O2	318	
Phorbol	36.88	7.37	745	C20H28O6	364	
Tibolone	37.96	1.99	701	C21H28O2	312	
psi.,.psi.-carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	42.23	1.06	709	C42H64O2	600	
Loperamide	42.56	1.44	630	C29H33ClN2O2	476	
Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost -8-en-17-yl)-	42.87	1.2	66.8	C27H42O4	430	
Betulin	43.94	7.31	737	C30H50O2	442	
9,12,15-octadecatrienoic acid,	44.16	1.22	766	C27H52O4Si2	496	
Gorgost-5-en-3-ol, (3á)	44.32	2.63	790	C30H50O	426	
á-Sitosterol	45.17	8.13	8.65	C29H50O	414	

Discussion

H. bacciferum, one of the heliotropes, is an important medicinal plant in the family Boraginaceae. This genus was extensively studied for its healing activity, as it is rich in secondary metabolites, which makes it a common plant in folk medicine (Modak *et al.*, 2007; Goyal and Sharma, 2014). It is generally composed of phenolics (flavonoids, phenolic acids, and polyphenols), terpenoids, and pyrrolizidine alkaloids (PA) groups. This composition makes it very important as an antioxidant, inflammatory, antimicrobial, and antifungal agent (Oznatmar-Pouloglou *et al.*, 2023). In the current study, this plant showed antibacterial and antifungal activity when the plant methanolic extract was tested against plant pathogenic bacterial and fungal strains. Also, we investigated the secondary metabolite profiles of this important plant using two advanced chromatographic approaches, HPLC and GC-MS. The results showed that the methanolic extract of the air-dried plant material was effective against the pathogenic bacteria *P. carotovorum*, *R. solanacearum, P. atrosepticum,* and *S. scabiei.* Also, the antibacterial effect was significantly stronger than the standard antibiotic drug Amoxicillin, which was used as a control treatment to limit *P. atrosepticum's* growth. In addition, different concentrations of the extract showed different strengths in controlling bacterial growth, as indicated in Figure 1. The concentration of 1000 μg/mL was enough to stop the bacterial growth. This significant antibacterial effect could be attributed to the high phenolic content in *H. bacciferum* plants, particularly the high concentrations of daidzein, chlorogenic acid, gallic acid, quercetin, and kaempferol. These compounds belong to the phenolics group of plant secondary metabolites and were previously proven to have antimicrobial activities against different microbes (Patra, 2012).

For instance, Gañan *et al.*, (2009) reported that both gallic acid and *p*-hydroxybenzoic acid reduced the viability of *Campylobacter jejuni* at low concentrations of 1 mg/L. Caffeic acid, vanillic acid, and synaptic acid were also found to be microbicidal at concentrations of 10 mg/L. On the other hand, a concentration of 100 mg/L demonstrated the activity of ferulic and coumaric acids (Gañan *et al.*, 2009). Furthermore, the effect of the plant methanolic extract of *H. bacciferum* showed similar effects on the four tested bacterial strains, even though the sensitivity of the strains to the standard drug treatment, Amoxicillin was variable. This could be explained by the fact that the plant extract may affect the bacterial strains through multiple mechanisms that target general components of the bacterial cells, which are not different from one strain to another. On the other hand, amoxicillin affects bacterial cells through a single mechanism, which one strain may develop to counteract the effects of this antibacterial compound. It is well-documented that phenolic compounds affect plant growth by different mechanisms (Jadimurthy *et al.*, 2023). The antibacterial properties of flavonoids are thought to be the result of their ability to complex with both extracellular and soluble proteins of the bacterial membranes. For example, quercetin disrupts of cell membrane integrity and, subsequently, cell lysis (Wang *et al.*, 2018). Meanwhile, apigenin and quercetin were found to target D-alanine ligase as a new targeting mechanism for *Helicobacter pylori* (Wang *et al.*, 2018). Kaempferol inhibits a crucial enzyme (the PriA helicase) in *S. aureus* that functions in the initiation of DNA replication and subsequently bacterial survival (Huang *et al.*, 2015).

The results showed that increasing the amount of *H. bacciferum* extract used in the treatments made the methanolic plant extract much more effective at inhibiting bacteria. At a concentration of 1000 μg/mL, the plant extract had the greatest effect. It is worth noting that phytochemicals have a greater MIC than antibiotics, owing to the synergistic impact of several metabolites in the extract (Buchmann *et al.*, 2022). Meanwhile, multiple studies have demonstrated that the effectiveness of these substances has grown in synergistic activity when taken with antibiotics (Cho *et al.*, 2011; Amin *et al.*, 2015; Ayaz *et al.*, 2019). Buchmann *et al.*, (2022) investigated the synergistic effect of different phytochemicals against antibiotic-resistant bacteria, among them gallic acid and daidzein. They reported strong synergistic effects between antibiotics and some of the tested phytochemicals including, daidzein against the tested antibiotic-resistant bacteria. Furthermore, the results obtained in our research are consistent with those obtained by other researchers who investigated the secondary metabolite profile of this group of medicinal plants. On the other hand, Ahmad *et al.*, (2016) looked into the presence of various secondary metabolites in *H. bacciferum* and found that it contained amines, carboxylic acids, amides, esters, alcohols, phenols, nitro compounds, energetic compounds, and alkyl halides. Najeeb *et al.*, (2020) studied the chemical composition of *H. bacciferum* growing in the Nile valley in Sudan. Scientists discovered many groups of metabolites that were antimicrobial. These included a lot of flavonoids, tannins, carbohydrates, amino acids, and polyphenols; they also found some alkaloids, sterols, triterpenes, and saponins.

On the other hand, when tested against *F. oxysporum* and *R. solani*, the methanolic extract showed a remarkable ability to inhibit fungal growth compared to the standard fungicide. The potent antifungal properties of *H. bacciferum* could be attributed to its rich content of natural compounds known for their natural antifungal effects, such as polyphenols, alkaloids, phytosterols, phenolics, and fatty acids. In the current study, HPLC analysis of the plant methanolic extract showed the presence of several compounds known for their antiviral, antibacterial, and antifungal activities, such as gallic acid, quinic acid, caffeic acid, chlorogenic acid, naringenin, quercetin, apigenin, daidzein, catechin, and hesperidin. Also, GC-MS testing of the plant showed that the methanolic extract of the air-dried plant parts contained a lot of n-hexadecanoic acid, also known as palmitic acid. This compound has been shown to have antibacterial properties, specifically against *K. pneumoniae*, *B. subtilis*, *E. coli*, and *S. aureus* (Ganesan *et al.*, 2022). The second abundant compound in the GC-MS analysis was sitosterol and with less abundance, gorgost-5-en-3-ol (3á). These compounds belong to the phytosterol group and are reported to have antimicrobial activity (Patra, 2012). Betulin and phorbol were also detected in moderate abundance in the *H. bacciferum* plant extract. They are pentacyclic triterpenoid and diterpene compounds, respectively. This group of compounds is known for its antiviral, antifungal, and antibacterial activities. Phorbol ester has shown antifungal activity against key fungal phytopathogens, including *Fusarium oxysporum*, *Pythium aphanidermatum*, *Lasiodiplodia theobromae*, *Curvularia lunata*, *F. semitectum, Colletotrichum capsici,* and *C. gloeosporioides* (Saetae and Suntornsuk, 2010). Similar antifungal activity of *H. bacciferum* was found by Najeeb *et al.*, (2020) when plant extract was tested against fungal strains.

Conclusions

In this study, we used HPLC and GC-MS chromatography to determine the secondary metabolite content of *H. bacciferum* methanolic plant extract. The plant extract was also screened for its antibacterial and antifungal activity against different pathogenic bacterial and fungal strains. The methanolic extract was very good at inhibiting *R. solanacearum*, *B. cinerea*, and *F. oxysporum* strains. The HPLC and GC-MS tests showed that *H. bacciferum* has a lot of different types of secondary metabolites that might be able to inhibit different phytopathogenic microorganisms. These include phenolics, flavonoids, fatty acids, phytosterols, diterpenes, and pentacyclic triterpenoids. These results indicate that *H. bacciferum* can be used as a potent antimicrobial agent, a biocontrol agent, and a safe alternative to many pesticides in the agricultural field.

Authors' Contributions

Conceptualization: AA and SB; Data curation: AA; Formal analysis: MEl; Funding acquisition: AAl; Investigation: SB; Methodology: MEl and SB; Project administration: AA and AAl; Resources: AA and SB; Software; Supervision: PK; Validation: SB and PK; Visualization: AA and MEl; Writing - original draft AA and SB; Writing - review and editing: PK and AAl. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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