Comparative Study of Biological Activities of Leaf Extracts from Wild *Verbascum sinuatum*, *Amaranthus spinosus*, *Carduus getulus* and *Heterotheca subaxillaris* in the Gaza Strip, Palestine

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Article

Comparative Study of Biological Activities of Leaf Extracts from Wild *Verbascum sinuatum, Amaranthus spinosus, Carduus getulus* and *Heterotheca subaxillaris* in the Gaza Strip, Palestine

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Abstract: Underexplored medicinal plants serve as valuable reservoirs of bioactive compounds that exhibit complex synergistic interactions whose therapeutic potential is increasingly recognized. In the current study, the antimicrobial activity, antioxidant potential, and Gas Chromatography - Mass Spectrometry (GC-MS) phytochemical screening of four medicinal plants (*Verbascum sinuatum*, *Amaranthus spinosus*, *Carduus getulus*, and *Heterotheca subaxillaris*) from the Gaza Strip in Palestine were investigated. For a comprehensive phytochemical characteristic, an analysis using GC-MS was conducted using the hexane extracts of each species. For the purpose of evaluating the antioxidant active ingredients, the total phenolic content (TPC) and total flavonoid content (TFC) were measured. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) test was utilized to assess antioxidant activity, while the method of disc diffusion was applied to evaluate antibacterial activity. GC-MS analysis revealed various lipophilic compounds in all species, including fatty acid esters, terpenoids, phenols, and polyacetylenes. *V. sinuatum* was dominated by three major constituents: saturated fatty acid esters, phenolic antioxidants, and a putative alkaloid. *A. spinosus* contained six compounds representing oxygenated monoterpenes, fatty acid derivatives, and aromatic compounds. Ten compounds in C. getulus represented predominantly monoterpenes, diterpenes, fatty acid derivatives, and phenolic antioxidants. *H. subaxillaris* had eight primary metabolites dominated by terpenoids, specialized fatty acid esters, and phenolic compounds. *V. sinuatum and C. getulus* showed moderate bioactivity, possibly due to the synergism of fatty acid esters and diterpenoid–phenol combinations, while the monoterpenoid-rich extract of A. spinosus possessed relatively weak effects. These results make *H. subaxillaris* a promising candidate for developing natural products, especially to combat resistant pathogens. These plants' chemical diversity and overall biological activity emphasize their potential for pharmaceutical applications and warrant further mechanistic exploration.

Keywords: *Verbascum sinuatum*; *Amaranthus spinosus*; *Carduus getulus*; *Heterotheca subaxillaris*; GC-Ms; antioxidant; antimicrobial; phenols; flavonoids

1. Introduction

The emergence of antimicrobial resistance and the prevalence of oxidative stress-related diseases underscore the need to explore novel bioactive compounds from medicinal plants. Synergistic metabolite interactions may provide superior therapeutic effects over single-compound drugs [1]. Assessing the phytochemical content and bioactivity of underutilized species is essential for identifying their medicinal and industrial potential [2]. Recently, the use of natural substances derived from traditional plants to develop pharmaceuticals, functional foods, and cosmetic products has increased significantly [3]. *Verbascum sinuatum* L. (Scrophulariaceae) is a Mediterranean herbaceous plant, traditionally valued for its medicinal properties. Its aerial parts are traditionally

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used as an infusion and comprising bioactive constituents, such as iridoids and polyphenols [3]. *Amaranthus spinosus* L is a globally distributed herb that belongs to the Amaranthaceae / Chenopodiaceae family [4]. This species naturally contains bioactive metabolites including flavonoids, phenols, terpenoids, tannins, and glycosides, which are involved in the human body's important physiological and metabolic functions [5]. Studies report that the plant species has diuretic, emollient, antipyretic, and diaphoretic properties and is traditionally used to treat internal bleeding, ulcers, menstrual disorders, snake bites, gastrointestinal problems, and various skin and mucous membrane wounds [6]. Additionally, the plant exhibits anti-leprotic, anti-diabetic, anti-inflammatory and antiandrogenic effects and serves as a valuable feed source for livestock [7]. *A. spinosus*, often regarded as an invasive weed in cattle pastures, shows potential as a resource for methane reduction in cow-calf production systems [8]. Carduus (Asteraceae / Compositae) is a Mediterranean genus used to treat colds, gastrointestinal disorders, and rheumatism. Ethnopharmacological and phytochemical studies have highlighted its diverse bioactivities [9]. Various species have provided flavonoids, lignans, alkaloids, sterols, and triterpenes [9]. Notably, *Carduus getulus* Pomel exhibits a high lipid content, which has been associated with enhanced biochemical and antioxidant defenses, justifying their use in the regulation of liver disease and microbial infections [7,10]. *Heterotheca* subaxillaris (Lam.) Britt. & Rusby (Asteraceae/Compositae) is a wide-ranging species that occurs in the USA particularly in sandy habitats [11]. It has become a dominant invasive species in Palestine along the coastal dunes. It has a strong camphoraceous odor derived from glandular trichomes covering its aerial parts due to high concentrations of monoterpenoids (camphor, bornyl acetate, borneol) and sesquiterpenoids, which serve as herbivore deterrents [12]. *H. subaxillaris* produces methylated flavonoids and phenolic compounds, which enhance resistance to abiotic stresses and contribute to its invasive success in arid regions. These secondary metabolites also exhibit allelopathic properties, explaining their ecological dominance in disturbed habitats like coastal dunes [12]. Notably, terpenoid production is influenced by soil nitrogen content, demonstrating phenotypic plasticity in different environments [11,13]. The current study aims to systematically evaluate the antibacterial and antioxidant potential of four medicinal plants (*V. sinuatum*, *A. spinosus*, *C. getulus*, and *H. subaxillaris*) through integrated phytochemical profiling and bioactivity assays. By elucidating metabolite-activity relationships, we seek to advance their therapeutic development as potential sources of novel bioactive compounds.

2. Results

2.1. GC-MS and Phytochemical Profiling of V. sinuatum

The GC-MS chromatogram of *V. sinuatum* revealed three dominant peaks, with the most abundant component eluting at 30.243 min, accounting for 65.90% of the total peak area (Table 1, Figure 1). The main compound was recognized methyl palmitate (MW: 270.45 g/mol; Figure 2A). Its electron ionization (EI) mass spectrum exhibited characteristic fragment ions at m/z 74.02 (McLafferty rearrangement) and m/z 87.00 (acyl ion), matching NIST library entries with a match score (total score minus threshold) of 71.0. The second peak (RT: 30.840 min, 13.34% relative abundance) was identified as 3,5-ditert-butylphenol (Figure 2B) (MW: 206.32 g/mol; match score: 77.2). The spectrum displayed key fragments at m/z 190.10 $[M–CH_3]^+$ and m/z 57.01 (tert-butyl ion). A third peak eluted at 34.752 min, accounting for 20.76% of the extract composition. This compound showed spectral similarity to phenethylamine derivatives, specifically a hordenine ($C_{10}H_{15}NO$; MW: 165.23 g/mol; Figure 2C), although the match was tentative with a lower confidence score (match score: 54.9). Fragmentation produced characteristic ions at m/z 57, supporting the tentative classification as a phenethylamine-type alkaloid. Due to its low spectral match, further confirmation via orthogonal techniques would be necessary to verify this assignment (Table 1).

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Table 1. Phytochemical constituents of *V. sinuatum* extract characterized by GC-MS analysis.

Peak No.	RT (min)	Area (%)	Chemical Compounds	MF	MW (g/mol)	Key m/z	Match score	Phytochemical Class
1	30.243	65.90	Methyl palmitate	$C_{17}H_{34}O_2$	270.45	74.02	71.0	Saturated fatty acid ester
2	30.840	13.34	3,5-ditert-butylphenol	$C_{14}H_{22}O$	206.32	190.57	75.6	Phenolic antioxidant
3	34.752	20.76	Hordenine	$C_{10}H_{15}NO$	165.23	190.57	75.6	Alkaloid (phenethylamine)

MF: Molecular Formula; MW: Molecular Weight.

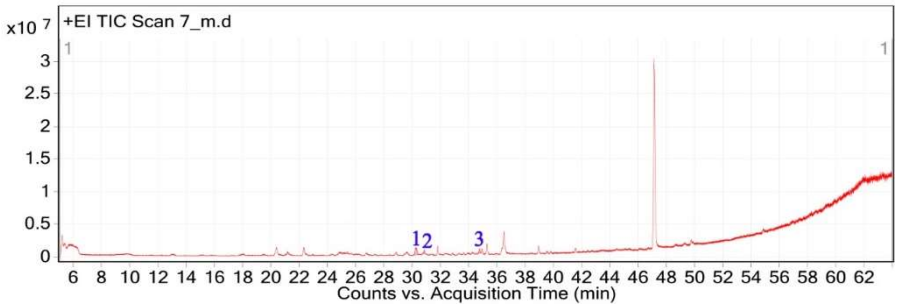

Figure 1. Total ion chromatogram (TIC) of *V. sinuatum* extract with labeled peaks. (1) Methyl palmitate (2) 3,5-ditert-butylphenol, (3) Hordenine.

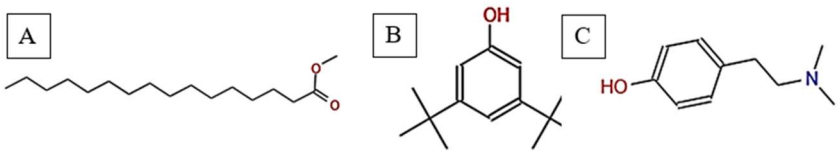

Figure 2. Chemical structures of key compounds identified in *V. sinuatum*: (A) Hexadecanoic acid methyl ester (B), (C) Hordenine.

2.2. GC-MS and Phytochemical Profiling of A. spinosus

The GC-MS analysis of the hexane extract of *A. spinosus* showed six prominent peaks representing key lipophilic phytochemicals (Table 2). The extract was dominated by monoterpenoid alcohols and fatty acid esters, constituting most of the chemical profile. The most abundant compound, eluting at 35.266 min (Peak 6) and accounting for 30.78% of the total area, was identified as isocitronellol ($C_{10}H_{20}O$; MW: 156.27 g/mol). The mass spectrum exhibited a dominant base peak at m/z 82.9, corresponding to [C6H10]+, formed via α-cleavage of the C–O bond in isocitronellol. Despite its moderate match score of 61.4, the compound's identification is supported by its retention time and co-occurrence with other structurally related terpenes, such as citronellol and geraniol. Further confirmation with reference standards is advised. Citronellol was identified at 20.510 min (Peak 2; 17.59%), with a match score 76.7. The mass spectrum displayed a characteristic base peak at m/z 69.01, derived from α-cleavage and other ions consistent with the hydroxylated side chain of this acyclic monoterpene. Geraniol, a structural isomer of citronellol, appeared at 22.395 min (Peak 3; 17.34%), with a high match score of 81.4. The spectrum is dominated by m/z 69.00 [C5H9]+, consistent with the α-cleavage of geraniol. Peak 5 (34.160 min; 21.75%) was identified as 11,14-Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$; MW: 294.47 g/mol), a polyunsaturated fatty acid ester. Its mass spectrum showed diagnostic ions at m/z 67.02 (due to diene cleavage) and m/z 81.96, typical of linoleic acid derivatives. The high match score of 87.6 supports its confident identification. Methyl elaidate was identified at 32.923 min (Peak 4; 9.51%), with a match score of 86.9. The compound displayed intense

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fragment ions at m/z 74.03 (McLafferty rearrangement) and m/z 55.03 (allylic cleavage), considered hallmark ions for mono-unsaturated fatty acid esters. The earliest eluting compound, Peak 1 (15.526 min; 3.03%), was identified as linalyl acetate ($C_{12}H_{20}O_2$; MW: 196.29 g/mol). The mass spectrum exhibited a base peak at m/z 71.01, corresponding to the $[C_5H_{11}]^+$ fragment (common for terpenes), along with other diagnostic ions at m/z 92.92 (likely the tropylium ion, $C_7H_7^+$) and m/z 120.74 (indicative of acetyl cleavage).

Table 2. Phytochemical constituents of *A. spinosus* extract characterized by GC-MS analysis.

Peak No.	RT (min)	Area (%)	Chemical Compounds	MF	MW (g/mol)	Key m/z	Match score	Phytochemical Class
1	15.526	3.03	Linalyl acetate	$C_{12}H_{20}O_2$	196.29	71.01	72.8	Monoterpene ester
2	20.51	17.59	Citronellol	$C_{10}H_{20}O$	156.27	69.01	76.7	Monoterpenoid alcohol
3	22.395	17.34	Geraniol	$C_{10}H_{18}O$	154.25	92.88	81.4	Monoterpenoid alcohol
4	32.923	9.51	Methyl elaidate	$C_{19}H_{36}O_2$	296.49	55.03	86.9	Fatty acid ester
5	34.16	21.75	11,14-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.47	67.02	87.6	Polyunsaturated fatty acid ester
6	35.266	30.78	Isocitronellol	$C_{10}H_{20}O$	156.27	152.77	61.4	Monoterpenoid alcohol

MF: Molecular Formula; MW: Molecular Weight.

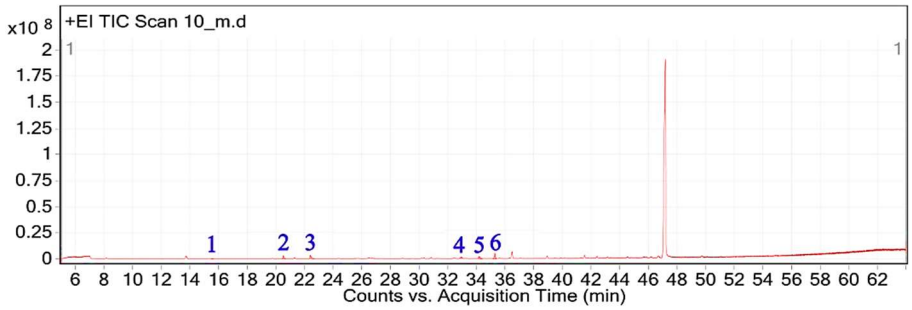

Figure 3. Total ion chromatogram (TIC) of *A, spinosus* extract with labeled peaks. (1) Linalyl acetate (2) 3,7-Dimethyloct-6-en-1-ol, (3) Geraniol (4) 9-Octadecenoic acid, methyl ester, (5) 11,14-Octadecadienoic acid, (6) Isocitronellol.

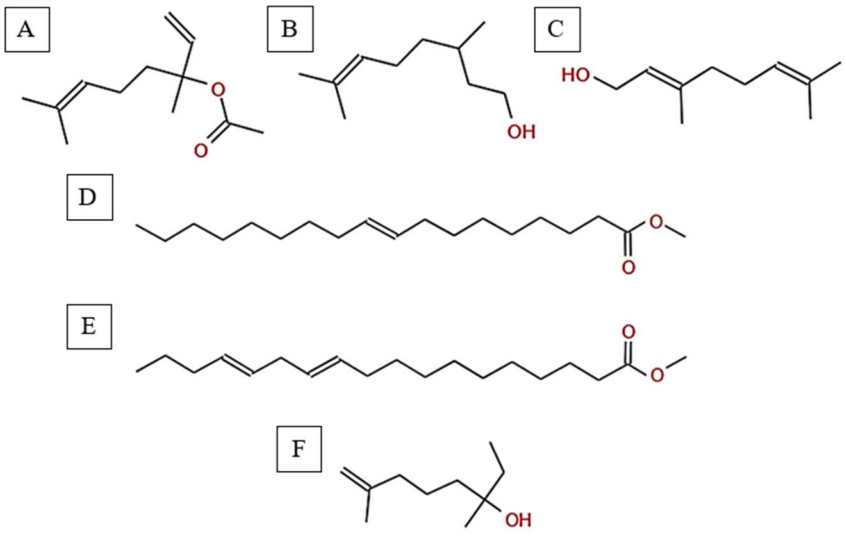

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Figure 4. Chemical structures of key compounds identified in *A. spinosus*: (A) Linalyl acetate (B) 3,7-Dimethyloct-6-en-1-ol, (C) Geraniol (D) 9-Octadecenoic acid, methyl ester, (E) 11,14-Octadecadienoic acid, (F) Isocitronellol.

2.3. GC-MS and Phytochemical Profiling of C. getulus

The GC-MS analysis of *C. getulus* hexane extract revealed a chemically diverse profile, with ten major peaks corresponding to different classes of lipophilic metabolites (Table 3). The extract was predominantly composed of fatty acid esters, terpenoid alcohols, and phenolic compounds, several of which are known for their pharmacological relevance. The most abundant compound, eluting at 30.127 min (Peak 5) and accounting for 66.26% of the total ion current, was identified as methyl palmitate ($C_{17}H_{34}O_2$; MW: 270.45 g/mol). This saturated fatty acid ester is a common component of plant cuticular waxes and has known antibacterial and anti-inflammatory properties. Its EI mass spectrum exhibited prominent fragment ions at m/z 74.02 (McLafferty rearrangement) and m/z 87.00, supporting its identification with a match score 73.8. 3,5-ditert-butylphenol, a phenolic antioxidant, was the second most abundant metabolite (12.67%; RT 30.800 min; Peak 6). The compound exhibited a high match score of 78.7 and a dominant fragment ion at m/z 190.59, reflecting the loss of a methyl group from the molecular ion. A third principal constituent was isophytol ($C_{20}H_{40}O$; MW: 296.5 g/mol), a diterpene alcohol, detected at RT 29.528 min (Peak 4) with 9.58% relative abundance and a base fragment at m/z 57.98. Other notable terpenoids included phytol ($C_{20}H_{40}O$; MW: 296.5 g/mol), eluting at 36.308 min (Peak 10; 1.05%), with a prominent fragment at m/z 70.92 and a match score of 76.1. Additionally, thymol, a monoterpenoid phenol, was identified at RT 31.776 min (Peak 7; 4.41%), characterized by m/z 134.76 and a match score of 74.1. Monoterpenoid alcohols were represented by citronellol (Peak 1; RT 20.292 min; 0.88%; m/z 68.98; match score 74.6) and Geraniol (Peak 2; RT 22.135 min; 1.29%; m/z 92.90; match score 77.6). The extract also contained unsaturated and polyunsaturated fatty acids, including oleic acid (Peak 3; RT 27.226 min; 0.69%; m/z 137.84; match score 65.3) and arachidonic acid (Peak 8; RT 33.083 min; 2.14%; m/z 78.85, match score 77.8). Undecanoic acid methyl ester (Peak 9; RT 33.798 min; 1.03%) was identified as a medium-chain fatty acid ester, with a central ion at m/z 73.94 and a match score of 64.6.

Table 3. Phytochemical constituents of *C. getulus* extract characterized by GC-MS analysis.

Peak No.	RT (min)	Area (%)	Chemical Compounds	MF	MW (g/mol)	Key m/z	Match score	Phytochemical Class
1	20.292	0.88	Citronellol	$C_{10}H_{20}O$	156.26	69.01	74.6	Monoterpenoid alcohol
2	22.135	1.29	Geraniol	$C_{10}H_{18}O$	154.25	92.90	77.6	Monoterpenoid alcohol
3	27.226	0.69	Oleic acid	$C_{18}H_{34}O_2$	282.5	137.84	65.3	Unsaturated fatty acid
4	29.528	9.58	Isophytol	$C_{20}H_{40}O$	296.5	57.98	70.3	Diterpene alcohol
5	30.127	66.26	Methyl palmitate	$C_{17}H_{34}O_2$	270.45	74.02	73.8	Saturated fatty acid ester
6	30.800	12.67	3,5-ditert-butylphenol	$C_{14}H_{22}O$	206.32	190.59	78.7	Phenolic antioxidant
7	31.776	4.41	Thymol	$C_{10}H_{14}O$	150.22	134.76	74.1	Monoterpenoid phenol
8	33.083	2.14	Arachidonic acid	$C_{20}H_{32}O_2$	304.5	78.85	77.8	PUFA
9	33.798	1.03	Methyl undecanoate	$C_{12}H_{24}O_2$	200.32	74.98	64.6	Fatty acid ester
10	36.308	1.05	Phytol	$C_{20}H_{40}O$	296.5	70.92	76.1	Diterpene alcohol

MF: Molecular Formula; MW: Molecular Weight.

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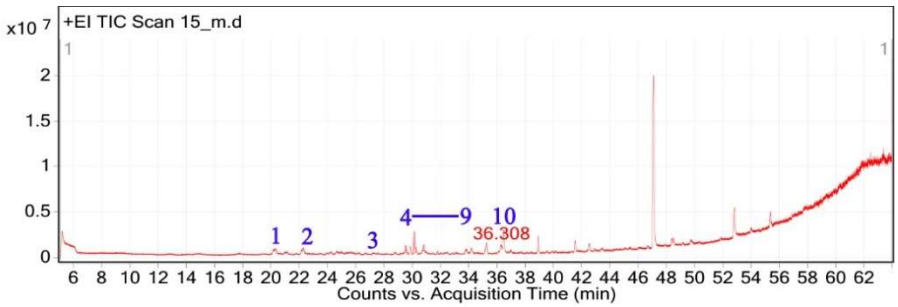

Figure 5. Total ion chromatogram (TIC) of *C. getulus* extract with labeled peaks. (1) Citronellol, (2) Geraniol, (3) Oleic acid (4-9) Isophytol, Methyl palmitate, 3,5-ditert-butylphenol, Thymol, Arachidonic acid, Methyl undecanoate respectivelly, (10) Phytol.

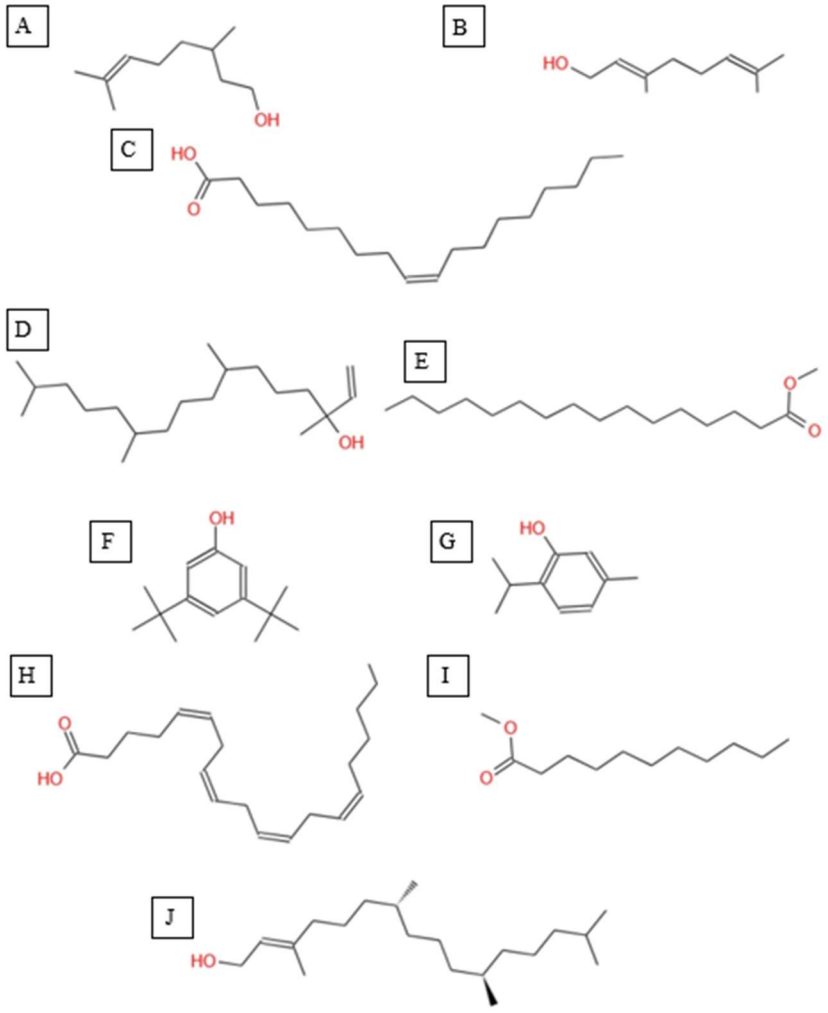

Figure 6. Chemical structures of key compounds identified in *C. getulus*: (A) Geraniol, (B) Citronellol, (C) Oleic acid (D) Isophytol (E) Methyl palmitate (F) 3,5-ditert-butylphenol, (G) Thymol (H) Arachidonic acid. (I) Methyl undecanoate (J) Phytol.

2.4. GC-MS and Phytochemical Profiling of H. subaxillaris

The GC-MS analysis of *H. subaxillaris* hexane extract revealed a chemically diverse and bioactive phytochemical profile consisting of eight major peaks, dominated by fatty acid esters, terpenoid alcohols, polyacetylenes, and phenolic antioxidants (Table 4). The most abundant compound, eluting at 30.130 min (Peak 4), was identified as methyl 8-methyl-nonanoate, constituting 36.84% of the total peak area. The EI mass spectrum exhibited a dominant McLafferty rearrangement fragment at m/z

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74.03, confirming its identity as a saturated fatty acid methyl ester with a match score 74.3. Methyl palmitate ($C_{17}H_{34}O_2$; MW: 270.5 g/mol) was the second most abundant metabolite (18.03%, RT 30.153 min; Peak 5), showing a characteristic base ion at m/z 74.02 and a match score of 71.9. Among the early-eluting volatile terpenoids, Geraniol (Peak 1; RT 22.256 min; 3.22%) was identified with a high match score of 78.6. It exhibited key fragment ions at m/z 69.02 (from α-cleavage) and m/z 92.87 (tropylium ion). Cubebol (Peak 2; RT 27.538 min; 18.52%). The spectrum is dominated by m/z 118.76 [C8H14O]+, indicating vinyl ether cleavage in geranyl vinyl ether and a strong match score (78.0). Falcarinol (Peak 3; RT 29.072 min; 3.56%), a long-chain fatty alcohol with a polyacetylene moiety, was confirmed by ions at m/z 128.67, a typical fragment of polyyne cleavage. A unique class of polyunsaturated fatty acid esters was represented by 13,16-octadecadienoic acid methyl ester (Peak 6; RT 30.242 min; 4.31%). The spectrum exhibits a base peak at m/z 74.08 [C3H6O2]+, consistent with McLafferty rearrangement of the methyl ester group in 13,16-octadecadienoic acid methyl ester. Additional peaks at m/z 86.92 [C5H10O]+ and hydrocarbon fragments (m/z 55–69) support the assignment. 2,5-octadecadiynoic acid methyl ester (Peak 8; RT 34.889 min; 6.24%). The spectrum is dominated by m/z 83.95 [C5H7O]+, indicating cleavage near the diyne group. 3,5-ditert-butylphenol (Peak 7; RT 30.772 min; 9.28%) is a well-documented phenolic antioxidant, identified by its base peak at m/z 190.65.

Table 4. Phytochemical constituents of *H. subaxillaris* extract characterized by GC-MS analysis**.**

Peak No.	RT (min)	Area (%)	Chemical Compounds	Molecular Formula	MW (g/mol)	Key m/z	Match score	Phytochemical Class
1	22.256	3.22	Geraniol	$C_{10}H_{18}O$	154.25	69.03	78.6	Monoterpenoid alcohol
2	27.538	18.52	Cubebol	$C_{15}H_{26}O$	222.37	81.87	78.0	Sesquiterpenoid alcohol
3	29.072	3.56	Falcarinol	$C_{17}H_{24}O$	244.38	114.75	80.1	Long-chain fatty alcohol
4	30.153	36.84	Methyl 8-methyl-nonanoate	$C_{11}H_{22}O_2$	186.29	74.02	74.3	Branched fatty acid ester
5	30.130	18.03	Methyl palmitate	$C_{17}H_{34}O_2$	270.5	114.75	71.9	fatty acid methyl ester
6	30.242	4.31	methyl octadeca-13,16-diynoate	$C_{19}H_{30}O_2$	290.45	190.59	78.7	Polyunsaturated fatty acid ester
7	30.772	9.28	3,5-ditert-butylphenol	$C_{14}H_{22}O$	206.32	55.04	72.9	Phenolic antioxidant
8	34.889	6.24	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290.45	190.59	78.7	Polyunsaturated fatty acid ester

MF: Molecular Formula; MW: Molecular Weight.

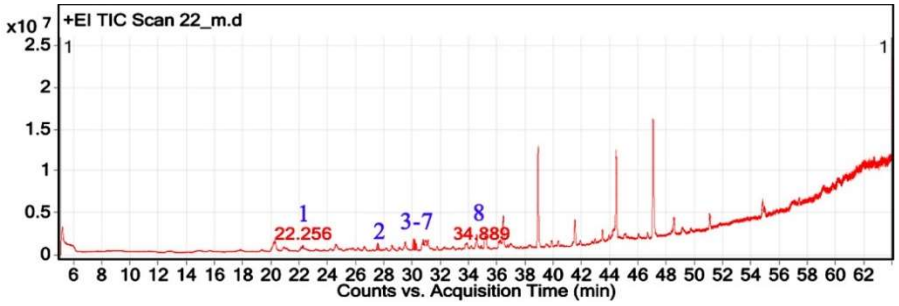

Figure 7. Total ion chromatogram (TIC) of *H. subaxillaris* extract with labeled peaks. (1) Geraniol, (2) Cubebol (3-7) Falcarinol, Methyl 8-methyl-nonanoate, Methyl palmitate, methyl octadeca-13,16-diynoate, and 3,5-ditert-butylphenol respectively, (8) 2,5-Octadecadiynoic acid, methyl ester.

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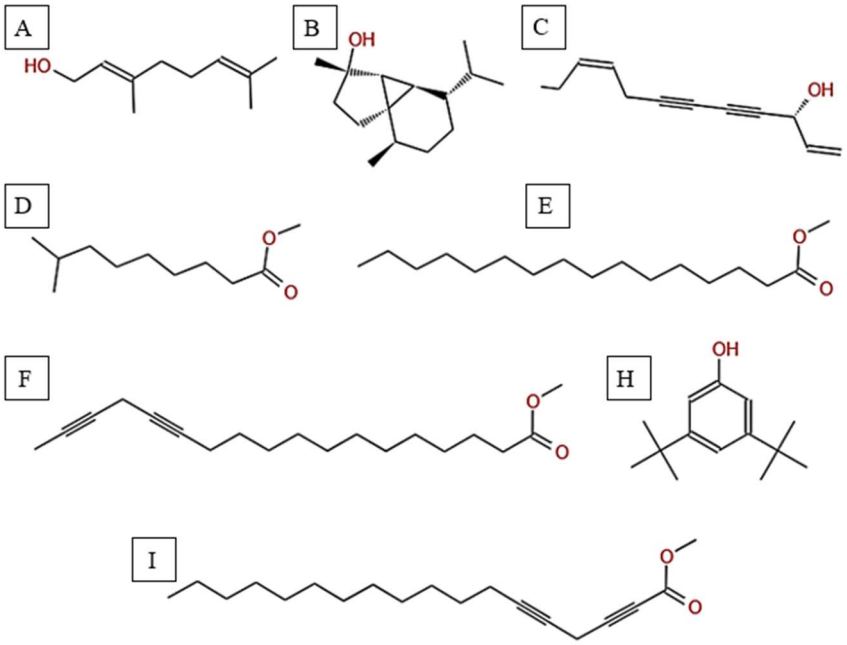

Figure 8. Chemical structures of key compounds identified in *H. subaxillaris*: (1) Geraniol, (2) Cubebol (3) Falcarinol, (4) Methyl 8-methyl-nonanoate, (5) Methyl palmitate, (6) methyl octadeca-13,16-diynoate, (7) 3,5-ditert-butylphenol, (8) 2,5-Octadecadiynoic acid, methyl ester.

2.5. Antibacterial Activity Analysis

The hexane crude extracts of the selected medicinal plants showed a statistically significant difference ($p < 0.05$) in their activity against the tested bacterial strains (Table 5). *H. subaxillaris* exhibited the most potent inhibition against B. cereus (19.8 ± 0.8 mm), surpassing all other species in activity. Both *V. sinuatum* (15.2 ± 1.5 mm) and *C. getulus* (13.5 ± 1.2 mm) showed moderate activity and did not differ significantly from each other, while *A, spinosus* exhibited the weakest activity (8.3 ± 0.6 mm). For *S. aureus* (MRSA), *V. sinuatum* (12.6 ± 0.9 mm), *C. getulus* (11.8 ± 0.9 mm), and *H. subaxillaris* (12.5 ± 0.7 mm) exhibited statistically similar and potent inhibitory effects, while *A. spinosus* had significantly lower activity (7.0 ± 1.1 mm). In the case of *E. coli* O157:H7, both *V. sinuatum* (9.3 ± 0.7 mm) and *H. subaxillaris* (10.1 ± 0.3 mm) displayed comparable and significantly higher antibacterial activity than *C. getulus* (6.2 ± 0.5 mm) and *A. spinosus* (5.5 ± 0.8 mm). Regarding *P. aeruginosa*, *H. subaxillaris* (8.4 ± 0.9 mm) and *V. sinuatum* (7.0 ± 0.5 mm) showed the most effective activity, while *C. getulus* (5.0 ± 0.4 mm) and *A. spinosus* (4.2 ± 0.6 mm) were significantly less active. These results highlight the stronger efficacy of *H. subaxillaris* and *V. sinuatum* against this highly resistant Gram-negative *P. aeruginosa* pathogen (Table 5).

Table 5. Comparative inhibition zones (mm) of various plant species, hexane crude extract against pathogenic bacteria.

	V. sinuatum	***A. spinosus***	***C. getulus***	***H. subaxillaris***
B. cereus	15.2 ± 1.5^{b}	8.3 ± 0.6^{c}	13.5 ± 1.2^{b}	19.8 ± 0.8^{a}
S. aureus	12.6 ± 0.9^{a}	7.0 ± 1.1^{b}	11.8 ± 0.9^{a}	12.5 ± 0.7^{a}
E. coli	9.3 ± 0.7^{a}	5.5 ± 0.8^{b}	6.2 ± 0.5^{b}	10.1 ± 0.3^{a}
P. aeruginosa	7.0 ± 0.5^{a}	4.2 ± 0.6^{b}	5.0 ± 0.4^{b}	8.4 ± 0.9^{a}

Mean values labeled with different superscript letters (a–c) are significantly different ($p < 0.05$), according to Tukey's HSD test. SD: Standard deviation of triplicate experiments.

2.6. Assessment of Antioxidant Capacities in Plant Extracts

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Antioxidant activity measured by DPPH assay significantly varied across species ($p < 0.05$), with *H. subaxillaris* demonstrating the highest activity (80.1 ± 2.4%, $p < 0.05$) compared to the other plants, followed by *V. sinuatum* (53.9 ± 8.6%). *A. spinosus* and *C. getulus* showed moderate activity (32.5 ± 7.4% and 43.7 ± 9.5%, respectively) (Figure 9A).

2.7. Total Phenolics Content

Quantification of bioactive compounds revealed distinct compositional profiles among the four species (Figure 9). *H. subaxillaris* exhibited the highest total phenolic content (TPC = 1.9 ± 0.4 mg GAE/g DW), significantly surpassing *A. spinosus* (0.6 ± 0.3 mg GAE/g DW; $p < 0.05$) and showing comparable levels to *V. sinuatum* (1.5 ± 0.2 mg GAE/g DW) and *C. getulus* (1.3 ± 0.5 mg GAE/g DW; $p > 0.05$, $F = 7.2$). Notably, *A. spinosus* demonstrated consistently lower metabolite accumulation across both assays (Figure 9B).

2.8. Total Flavonoids Content

In the terms of total flavonoid content (TFC), *H. subaxillaris* (0.62 ± 0.2 mg QE/g DW) and *V. sinuatum* (0.61 ± 0.1 mg QE/g DW) formed a statistically homogeneous group ($p = 0.87$). In contrast, *A. spinosus* (0.27 ± 0.1 mg QE/g DW) showed significantly reduced values ($p < 0.01$). *C. getulus* displayed TFC (0.49 ± 0.2 mg QE/g DW), which did not differ significantly from either group ($p > 0.05$, $F = 4.8$) (Figure 9C).

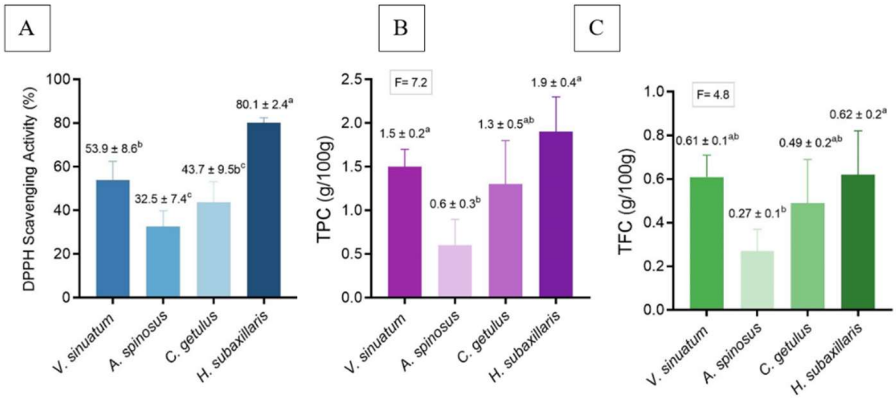

Figure 9. (A) Antioxidant activity; (B) TPC; (C) TFC of *V. sinuatum*, *A. spinosus*, *C. getulus*, and *H. subaxillaris* hexane extracts. Values are expressed as mean ± standard deviation (n = 3, from independent experiments). Different lowercase letters designate significant differences among plants based on one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

3. Discussion

Medicinal herbs are the focus of interest as they may provide alternative pharmacological agents in the face of increasing antibiotic resistance and diseases caused by oxidative stress. In this study, the hexane crude extracts of *V. sinuatum*, *A. spinosus*, *C. getulus*, and *H. subaxillaris* were studied for their antioxidant and antimicrobial properties as well as phytochemical content.

3.1. GC-MS Analysis and Phytochemical Characterization

GC-MS profiling of the hexane extract of *V. sinuatum* revealed a phytochemical profile dominated by bioactive and lipophilic metabolites, confirming its use in traditional medicine and corroborating recent evidence of its biological potential. The extract was dominated by three main constituents: Hexadecanoic acid methyl ester, Phenol, 3,5-bis(1,1-dimethyl ethyl)- and a putative hordenine, each belonging to a different phytochemical class with documented pharmacological activity. The most abundant compound, hexadecanoic acid methyl ester, exhibits antibacterial,

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antioxidant, hypocholesterolemic, nematicidal, pesticidal, lubricating, hemolytic, and antiandrogenic activity [14,15]. Its lipophilicity enhances penetration into Gram-positive bacteria [16], which is consistent with the observed inhibition of *Bacillus cereus* and MRSA. Phenol,3,5-bis(1,1-dimethylethyl)- (a sterically hindered phenolic antioxidant) suggests a possible role in the free radical scavenging activity detected in the DPPH assay. The compound belongs to the family of tert-butylphenol (TBP) antioxidants (TBP-AO), which exhibit increased stability and antioxidant activity due to their massive tert-butyl substituents that hinder oxidative degradation [17]. The extract contained lower levels of 3,5-di-tert-butylphenol, a compound known to inhibit acid production and biofilm formation in *Streptococcus mutans* [18], as well as certain sesquiterpenes that exhibit antioxidant, prooxidant, and other biological activities [19]. 3,5-ditert-butylphenol is a phenolic antioxidant characterized by two tert-butyl substituents [17]. Tert-butyl phenol (TBP), which consists of a phenol ring bonded to a tert-butyl group, is widely utilized as an antioxidant, UV stabilizer, and chemical precursor, owing to its high stability [20]. Its identification in *V. sinuatum* confirms the plant's high DPPH radical scavenging activity. Identifying a hordenine adds a neuroactive and potentially bioactive property to the phytochemical profile of *V. sinuatum* [21]. Hordenine and its related alkaloids are associated with central nervous system stimulation, antimicrobial activity and enzyme inhibition [22–24]. Although the spectral agreement was relatively low, their abundance is consistent with the documented ability of various medicinal plants to biosynthesize nitrogenous secondary metabolites, especially under stress conditions [25]. Ecologically, the presence of fat-soluble fatty acid esters and phenolic antioxidants could indicate an adaptation to abiotic stress, especially to oxidative environments or UV irradiation in arid or semi-arid habitats [26,27]. The phytochemical composition of *V. sinuatum*, with a high proportion of lipophilic esters and phenols, distinguishes it from the other species in this study, such as *H. subaxillaris*, which, on the contrary, store methylated polyacetylenes and sesquiterpenoids as diagnostic metabolites. These differences illustrate Verbascum species' interspecific metabolic specialization and ecological plasticity [28]. From a chemotaxonomic basis, phenolics, fatty acid esters, and putative alkaloids, make *V. sinuatum* a metabolically complex species [29]. These compounds most likely enhance protection against pathogens and oxidative stress, with bioactivity directed toward multiple targets [30]. This is consistent with natural product drug discovery trends that focus on the synergy of chemically distinct classes in crude extracts rather than single compound therapy. The hexane extract of *A. spinosus* contained a complex mixture of lipophilic constituents, falling into three major chemical categories: oxygenated monoterpenes, aromatic compounds, and fatty acid derivatives. Six compounds were found to be the major constituents: Linalyl acetate, 3,7-Dimethyloct-6-en-1-ol, Geraniol, 9-Octadecenoic acid, methyl ester, 11,14-Octadecadienoic acid, methyl ester, and Isocitronellol. The extract contained high levels of oxygenated monoterpenes, with citronellol and geraniol being the predominant compounds. Antimicrobial activities were attributed to these acyclic monoterpene alcohols, possibly through membrane-disrupting mechanisms [31,32]. High concentrations of isocitronellol, a structural isomer of 3,7-Dimethyloct-6-en-1-ol, and linalyl acetate, an ester of linalool [33], were also present in the extract. This is consistent with the moderate antibacterial activity previously reported for A. spinosus against MRSA and E. coli O157:H7 detected in our bioassays. The fatty acid derivatives were characterized as 9-octadecenoic acid, methyl ester, and 11,14-octadecadienoic acid, methyl ester. These molecules are responsible for the extract's lipophilic properties and can increase membrane permeability in the target microorganisms [34]. Such actions may be partially responsible for the extract's moderate activity against MRSA and its weak but measurable effect. The *C. getulus* hexane extract exhibited a dense array of lipophilic constituents that fall into four general categories: (1) monoterpenoids were responsible for citronellol and geraniol, (2) diterpenoids such as phytol and isophytol; (3) fatty acid derivatives such as methyl palmitate, oleic acid, arachidonic acid and methyl undecanoate; and (4) phenolic compounds such as 3,5-ditert-butylphenol and thymol. This concordance in composition suggests a potential antioxidant [35], synergistic antimicrobial effect [36–40] and a dual ecological role in both direct inhibition of pathogens and plant defense signaling [41]. The hexane extract of *H. subaxillaris* comprised a diverse

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array of lipophilic compounds classified into three main categories: (1) terpenoids, including the monoterpenoid geraniol and the sesquiterpenoid cubebol; (2) unique fatty acid derivatives such as methyl 8-methyl nonanoate, hexadecanoic acid methyl ester, and the polyacetylenic esters falcarinol, 13,16-octadecadienoic acid methyl ester and 2,5-octadecadienoic acid methyl ester; and (3) phenols represented by Phenol,3,5-bis(1,1-dimethyl ethyl)-. The extract was characterized by high levels of terpenoids and rare acetylenic fatty acid esters, which are rarely found in plants. In recent years, numerous novel terpenes and terpenoids have been isolated or synthesized, leading to the discovery of further new terpene-derived compounds with potential chemotherapeutic activity, some of which are already being tested in clinical trials [42]. Monoterpenes and sesquiterpenes are released from aerial parts of plants, while others are produced as part of the plant's defense mechanisms [43–45]. The simultaneous occurrence of branched-chain (methyl 8-methyl-nonanoate) and polyacetylene derivatives of classical fatty acid esters (palmitate) in the species indicates a higher-order lipid metabolism. The health-promoting effects are caused by these compounds with high biological activities [46–48]. Scientific studies have shown that fatty acid esters of hydroxy fatty acids have anti-diabetes [49–52], anti-cancer [53,54], anti-inflammatory [51,55], cardiovascular protective [56] and hepatoprotective activities [57,58] in mammals [59]. The presence of methyl 8-methyl-nonanoate in *H. subaxillaris* could indicate similar roles in lipid metabolism modulation, which is worthy of investigating its ecological or pharmaceutical role. The phenolic antioxidant 3,5-di-tert-butylphenol will possess a further protective role in shielding from oxidative stress [60].

3.2. Comparative Phytochemical and Pharmacological Profiling of Four Medicinal Plant Species

The phytochemical profile of H. subaxillaris stands out among the four species for its unique combination of sesquiterpenoid alcohols, polyacetylenic fatty acid esters, and phenolic antioxidants, in contrast to the dominance of monoterpenoids in A. spinosus, the simpler phenolic ester profile of V. sinuatum and the diterpenoid-phenol synergy of C. getulus. The acetylenic compounds, which are rarely found in the other species, reflect specialized adaptations at the ecological level, e.g., survival on dry land or protection against pathogens [61], while the high content of geraniol indicates an insect-repellent effect [62]. Pharmacologically, the diverse profile is reflected in the greater antibacterial efficacy of H. subaxillaris due to synergistic membrane disruption by terpenoid-polyacetylene mixtures [63]. *A. spinosus* is less effective antibacterially but has a composition enriched with terpenoids and fatty acid esters, to which anti-inflammatory and hepatoprotective activities are attributed [64]. *V. sinuatum* is particularly niche in its high methyl palmitate content and phenolic esters, consistent with traditional respiratory use and moderate antibacterial potency [65]. In parallel, *C. getulus* combines diterpenoids with antimicrobial phenolics, a novel strategy that suggests anti-inflammatory potential and previously untapped synergies against resistant pathogens [66]. The chemically diverse matrix of *H. subaxillaris*- a balance between volatile defenses and lipid-based storage- suggests its pharmacognostic potential. At the same time, the other species offer complementary bioactivities that merit targeted investigation.

3.3. Total Phenolics Content and Total Flavonoids Content

Quantitative phytochemical profiling showed significant differences in the accumulation of total phenolic and flavonoid compounds in the four medicinal plant species. Among them, *H. subaxillaris* showed the highest TPC content, significantly higher than A. spinosus and with comparable values to *V. sinuatum* and *C. getulus*. Polyphenols are a large group of secondary metabolites that have an important function in the detoxification of hydrogen peroxide from plant cells and make an important contribution to the cellular antioxidant defense system [67]. Phenolic compounds are well-known as key factors in plant defense and pharmacological action and function as antioxidants, metal chelators, and enzyme modulators [68]. Their hydroxylated aromatic scaffolds facilitate the effective neutralization of free radicals, disrupt bacterial membranes, and inhibit microbial enzyme systems [69–71]. Their high TPC content in *H. subaxillaris* and *V. sinuatum* is likely the basis for their greater activity in free radical scavenging assays. It contributes synergistically to their antimicrobial

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activities, as our GC-MS evidence shows for detecting several phenolic and terpenoid metabolites. *H. subaxillaris and V. sinuatum* showed statistically insignificant values, which placed them in the high flavonoid category. Flavonoids, a large subgroup of phenolic compounds, exhibit various bioactivities ranging from antioxidants and antimicrobial and anti-inflammatory effects to inhibition of enzymes [72]. Various food plants have flavonoids, which are considered important nutritional components for humans [73,74]. Flavonoids are one of the most ubiquitous secondary molecules that greatly benefit humans, not only because of their function in plant coloration but also because of their various physiologically active constituents [75]. The finding of the beneficial effects of flavonoids, such as cancer prevention, has generated much research interest, including consuming foods containing flavonoids [76]. Their planar polyphenolic molecular structure enables interactions with bacterial proteins and nucleic acids and confers versatile antimicrobial activities. Polyphenols from plant parts act against bacteria via proteins, DNA, cell wall, membrane and mechanisms based on energy metabolism are involved [77]. *C. getulus* had a medium flavonoid concentration, and its TPC and TFC values were only moderately high and did not show a significant difference from the high and low groups. The moderately high TPC and TFC values in conjunction with the GC-MS profile dominated by lipophilic terpenoids such as phytol and sesquiterpenes suggest that non-phenolic lipophilic constituents may be primarily responsible for the reported bioactivities of *C. getulus*. In contrast, *A. spinosus* exhibited the lowest levels of phenolics and flavonoids, consistent with its relatively weak antioxidant and antibacterial activity. Such a lack of phytochemicals could be caused by lower expression of genes for phenylpropanoid biosynthesis or by ecological traits that are less reliant on polyphenolic defense mechanisms. Interestingly, despite low levels of TPC and TFC, A. spinosus was characterized by high levels of monoterpenoid alcohols and fatty acid esters, suggesting a metabolic shift towards volatile or lipid-based defense mechanisms. Overall, these results emphasize the importance of both the chemical class and the concentration of the compound for the prediction of biological activity. While the levels of phenols and flavonoids are strongly correlated with antioxidant activity, the relationship with antimicrobial activity appears to be more complex and less direct. It often depends on a synergistic relationship between phenols, terpenoids, and fatty acid derivatives. The phytochemical richness shown here demonstrates the chemotaxonomic uniqueness of each species and confirms their different uses in ethnopharmacological practice.

3.4. Antioxidant Activity

Plants are a major source of natural antioxidants, with many of their bioactive properties attributed to the presence of hydroxyl groups in their structures [78]. The strongest free radical scavenging activity, based on the DPPH assay, was observed in *H. subaxillaris*, followed by *V. sinuatum*. This superior activity correlates with their high total TPC and TFC. Phenolic compounds identified in *V. sinuatum* such as Phenol,2,4-bis(1,1-dimethylethyl)-, are recognized for their free radical scavenging and antitumor properties [79]. The antioxidant activity of 2,4-bis(1,1-dimethylethyl)- is attributed to its tert-butyl groups, which enhance the stability of the aromatic hydroxyl group by facilitating phenoxyl radical formation and donating hydrogen atoms to neutralize active free radicals to stop lipid peroxidation [80,81]. This observation was supported by a previous study through in silico molecular docking analysis [82]. The antioxidant activity of *H. subaxillaris* may also stem from its unique polyacetylenes, including monoterpenoid alcohols, sesquiterpenoid alcohols, and falcarinol, which have been reported to diminish oxidative stress[83–85]. Furthermore, the synergistic interaction between citronellol and geraniol emphasizes their potential role as prooxidants [86]. In contrast, the comparatively lower antioxidant activity observed in *A. spinosus* and *C. getulus* is consistent with the lower TPC and TFC levels. These results emphasize the critical role of flavonoids and related polyphenols in contributing to the free radical scavenging activity observed in medicinal plants [87,88].

3.5. Antimicrobial Activity

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The hexane extracts of *H. subaxillaris*, *A. spinosus*, *V. sinuatum*, and *C. getulus* exhibited measurable inhibitory effects against four clinically significant bacterial strains, including *B. cereus*, MRSA, *E. coli* (O157:H7 serotype), and *P. aeruginosa*. Notably, *B. cereus and MRSA* were more susceptible to these lipophilic plant extracts compared to *E. coli* O157:H7 and *P. aeruginosa*, consistent with previous findings that Gram-positive bacteria are more sensitive to plant-based hydrophobic compounds [89–92]. Among all extracts, *H. subaxillaris* demonstrated the most significant antibacterial activity across all tested strains, followed by *V. sinuatum*, *C. getulus*, and *A. spinosus*. While *H. subaxillaris* and *V. sinuatum* were effective against both Gram-negative and Gram-positive bacteria, *C. getulus* and *A. spinosus* exhibited markedly weaker activity, especially against *P. aeruginosa*, a pathogen known for its multidrug resistance, biofilm formation, and active efflux mechanisms [93–95]. Phytochemicals are influenced by their chain length, degree of unsaturation, and isomerism, with *cis*-isomers generally exhibiting more potent activity than their trans counterparts [96]. For example, *cis*-6-hexadecenoic acid showed inhibitory effects at low concentrations by disrupting the proton gradient, altering membrane fluidity, and inhibiting electron transport [97]. Additionally, esterified fatty acids frequently show enhanced antibacterial potency compared to their free acid counterparts, likely contributing to the effectiveness of these extracts [98]. The vigorous antibacterial activity of *H. subaxillaris* is likely attributed to its unique phytochemical profile, which includes sesquiterpenoid and monoterpenoid alcohols (with known membrane-disrupting properties and anti-inflammatory properties) [99–102] , polyunsaturated and branched-chain esters (which interfere with bacterial cell wall synthesis and trigger autolysis through endogenous enzyme activation) [96], and phenolic compounds (which induce oxidative stress and cause protein denaturation) [99–101,103,104]. These multi-targeted effects likely synergistically enhance antimicrobial efficacy while potentially reducing resistance development. *V. sinuatum* also exhibited noteworthy antibacterial activity, particularly against *B. cereus* and MRSA. This may be due to its phytochemical composition, which includes saturated fatty acid esters, phenolic antioxidants, and alkaloids [105]. While these components share some antimicrobial properties with those in *H. subaxillaris*, their distinct molecular structures may offer complementary or even unique modes of action. The exceptional performance of *H. subaxillaris*, followed by *V. sinuatum*, highlights their potential for further investigation in natural product-based drug discovery targeting resistant pathogens. The synergistic combination of diverse bioactive constituents in these crude extracts may offer a strategic advantage over monotherapeutic agents in combating bacterial resistance.

4. Materials and Methods

4.1. Collection and Identification of Plant Material

Fresh and healthy leaves of various plant species, including *V. sinuatum* L., *A. spinosus* L., *C. getulus* Pomel and *H. subaxillaris* (Lam.) Britt. & Rusby were collected at various locations in the Gaza Strip, Palestine, during the flowering period between March and July 2023. The Gaza Strip (31°25′N, 34°20′E), an arid coastal region on the south-eastern Mediterranean coast, is about 365 km² in extent. With around 2.3 million inhabitants, it is one of the most densely populated regions in the world. It is bordered to the southwest by Egypt (11 km) and to the east and north by the occupied Palestinian territories (51 km). The climate is Mediterranean, with hot summers (average 25°C), mild winters (13°C) and between 200 mm in the south and 400 mm in the north per year. Plant species were taxonomically identified by the Botany Department, Al-Aqsa University, Gaza. The leaves were washed with tap water, air-dried at room temperature for ten days, then ground and stored in a cool, dry environment. Phytochemical studies were carried out at the Analytical Chemistry and Desert Soils Laboratories of Cairo University Research Park, Faculty of Agriculture, Cairo University, Egypt.

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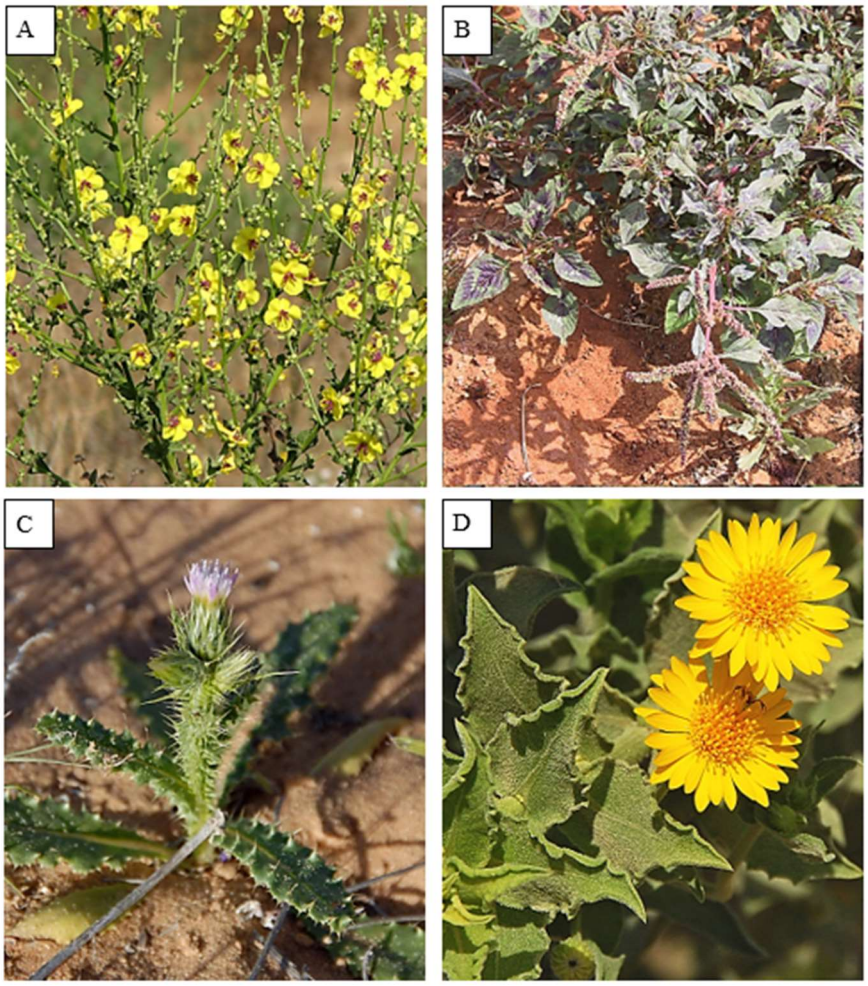

Figure 10. Illustrative photographs of the studied plant species recorded in the Gaza Strip, Palestine: (A) *V. sinuatum*; (B) *A. spinosus*; (C) *C. getulus*; (D) *H. subaxillaris*. (https://flora.org.il/en/plants).

4.2. Extraction of Plant Material

Ten grams (g) of each sample of leaf powder was extracted in hexane, ethyl acetate and methanol (1:10 w/v) using a Soxhlet apparatus for 6 hours for each solvent. The resulting filtrates were concentrated under reduced pressure at 45 °C, and the remaining solvents were evaporated under nitrogen stream. The extracts were stored at −20 °C in amber vials for later analysis [106]

4.3. Gas Chromatography-Mass Spectrometry Analysis

The chemical profile was generated on an Agilent 7000 Triple Quadrupole GC–MS instrument using an Elite-5MS column. The analytical conditions were electron ionization (70 eV), helium as carrier gas (1 mL/min, 30:1 split) and a temperature ramp from 110 °C to 280 °C in 36 minutes. Mass spectra in the range of m/z 45–450 were matched to the NIST library, and compound abundance was determined from the percent peak area using Turbomass software [107].

4.4. Analysis and Characterization of Compounds of Plant Extracts

A combined approach identified the compounds: spectral matching with the mass spectral libraries of NIST (2017) and Wiley (11th edition), calculation of the retention index (RI) in comparison to n-alkanes (C8–C40). Only compounds with > 70% agreement were considered confidently identified. Quantification was based on individual peak areas (% of total ion chromatogram), processed using Mass Hunter Qualitative Analysis, which provided comprehensive results, including retention times, peak areas, and fragmentation profiles. Triplicate analysis of leaf extracts

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under identical conditions enabled reproducibility with a relative standard deviation (RSD) of less than 5%. Sample preparation, extract treatment, and GC-MS analysis were combined in one step for data interpretation based on spectral libraries, mass Fragmentation, and RI confirmation.

4.5. Measurement of Total Phenolic Content

Total phenolic compounds in hexane extracts were quantitated by Folin–Ciocalteu assay [108]. 0.5 g of fresh material was extracted with 10 mL hexane, filtered, and stored in refrigerator for a week. A gallic acid stock solution (100,000 ppm) was prepared, and a calibration curve (10–200 ppm) was prepared. To determine, 200 µL of the extract was mixed with 400 µL of the 10% Folin–Ciocalteu reagent, followed by 800 µL of 10% Na_2CO_3 after 3 minutes. Samples, standards, and blanks were stored in the dark for 1 hour. The absorbance at 725 nm was measured. The TPC from the standard curve was calculated and expressed as % gallic acid equivalents (%GAE) as follows: TPC (%GAE) = (standard curve concentration/sample weight) × dilution factor × 10,000

4.6. Measurement of Total Flavonoid Content

Total flavonoids were quantitated according to the aluminum chloride colorimetric assay reported by [109]). Plant material (0.5 g) was homogenized in hexane (10 mL) and filtered. A quercetin stock solution (1000 ppm) was prepared (0.1 g in 100 mL), and a working solution (100 ppm) was diluted. A standard curve (20–640 ppm) was created from the standard solutions. For the assay, 125 µL of extract was mixed with $NaNO_2$ (75 µL of a 5% solution) and $AlCl_3$ (150 µL of a 10% solution), followed by NaOH (750 µL of 1 M) addition, and the volume adjusted to 2.5 mL. Standard solutions and blanks underwent the same treatment. After 15 min in darkness, absorbance was measured at 510 nm. Flavonoid content was calculated as quercetin equivalents (%QE) using a calibration curve and the formula: TFC (%QE) = (standard curve concentration/sample weight) × dilution factor × 10,000.

4.7. DPPH Radical Scavenging Assay

Antioxidant activity of the extracts was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method on the basis of violet DPPH radical reducibility to the yellow DPPH-H form when interacting with antioxidants. All the tested extracts at various concentrations were examined. In brief, 0.5 mL of DPPH solution (50 mg/100 mL) was mixed with 4.5 mL of hexane, and 0.1 mL of extract was added. The mixtures were shaken and incubated in the dark for 45 minutes and subsequently assayed for 515 nm absorbance against a blank [110].

4.8. Measurement of Antimicrobial Activity

The antimicrobial activity of hexane crude extracts was tested against two Gram-positive bacteria (*Staphylococcus aureus* MRSA and *Bacillus cereus*) and two Gram-negative bacteria (*Escherichia coli* O157:H7 and *Pseudomonas aeruginosa*) from Cairo University bacterial culture collection using the agar well diffusion method [111]. Bacterial cultures were grown to logarithmic phase at 37°C in nutrient broth, adjusted to 1–5 × 10^5 CFU/mL, and inoculated onto Mueller-Hinton agar plates. Wells (6 mm) were filled with 100 µL of each extract (dissolved in ≤1% DMSO), and the plates were incubated at 37°C for 24 hours. Zones of inhibition were measured with a digital caliper. Streptomycin (10 µg/mL) served as a positive control, and DMSO (1% v/v) as a negative control.

4.9. Statistical Analysis

All experimental data were analyzed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). Continuous variables were tested for normality distribution using the Shapiro-Wilk test. Parametric data were compared using a one-way ANOVA followed by Dunnett's test. Results are presented as mean ± SD (standard deviation) of at least three independent experiments. Statistical significance was set at $p < 0.05$.

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5. Conclusions

This study provides a comprehensive phytochemical analysis of four Mediterranean medicinal plants (*V. sinuatum*, *A. spinosus*, *C. getulus*, and *H. subaxillaris*), revealing a rich diversity of lipophilic metabolites. *H. subaxillaris* emerged as the most potent antibacterial and antioxidant agent, linked to its unique combination of sesquiterpenoids, polyacetylenic fatty acids, and phenolic antioxidants. *V. sinuatum* and *C. getulus* also demonstrated considerable activity due to their phenolic and diterpenoid profiles. The weak performance of *A. spinosus* underscores the role of phytochemical diversity in bioactivity. These findings underscore the value of phytochemical profiling for bioprospecting and pave the way for future pharmacognostic exploration of structurally diverse natural compounds from underexplored medicinal flora. Future work should isolate key metabolites (e.g., falcarinol, methyl 8-methyl-nonanoate) to mechanisms of action and elucidate structure-activity relationships.

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