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Phytochemical Composition, Antioxidant and Antimicrobial Activities of some *Dysphania ambrosioides* Extracts

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Abstract

The current study's objective was to examine the phytochemical makeup and antioxidant capacity of *D. ambrosioides* aerial parts that were gathered from the Albaha region, KSA. Forty-four compounds from various chemical classes were found in the volatile contents of the aerial portions of *D. ambrosioides*, according to the results of GC/MS analysis. After looking into the lipid components using GC/MS analysis, the fatty acid mixture, which is made up of 32 fatty acids, was isolated and identified. The main component is palmitic acid methyl ester ($C_{16:0}$), which accounts for 24.68% of the mixture. The main component, unsaturated fatty acids, linoleic acid methyl ester ($C_{18:2}$), makes up 14.48% of the composition overall. Sterols, diterpene, and a variety of hydrocabons are present in the unsaponifiable fraction. It was done to quantify phenolic compounds, to offer the scientific foundation for its biological action. HPLC was used to identify and quantify ten phenolic components and five flavonoidal compounds. The DPPH assay was used to screen for antioxidant activity. Strong antioxidant activity was demonstrated by the methanolic and ethyl acetate extracts of the aerial portions of *D. ambrosioides.* The modified Kirby-Bauer disc-diffusion method was utilized to assess the antimicrobial activity effectively. The extracts' levels of inhibition against the eight tested strains varied. The pet. ether extract demonstrated increased activity against Gram-positive bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus faecalis*, with a larger inhibition zone diameter of 14 mm. Against Gram-negative bacteria, such as *Escherichia coli*, *Neisseria gonorrhoeae*, and *P. aeruginosa*, the inhibition zone diameter ranged clearly from 13 to 14 mm. The plant may have potential antibacterial and antioxidant properties.

Keywords: *Chenopodiaceae*, *D. ambrosioides*, Antioxidant, Antimicrobial activity.

1. Introduction

The World Health Organization (WHO) supports, encourages, and helps the nations with successful herbal health initiatives. Higher plants have not yet been fully investigated for their potential as a source of novel medications (1). With over 102 genera and 1400 species, the family *Chenopodiaceae i*s a big one (2). More than 200 species of weedy herbs, native to much of Europe, Asia, India, China, and both North and South America, belong to the genus *Dysphania* (*Chenopodium)* (3). *Chenopodium* L. is well-known in Ayurveda for its use in treating a wide range of illnesses, including mental affections, cough, chest problems, abdominal pain, and pulmonary obstruction (4). Previously known as *Chenopodium ambrosioides* L., *Dysphania ambrosioides* is an annual hermaphrodite herb that grows in tropical and subtropical climates worldwide. It can be found in waste areas, gardens, cultivated fields, and disturbed environments, but it is most frequently seen on sand near rivers (5). As a vermifuge, emmenagogue, and abortifacient, *D. ambrosioides* L. is frequently employed in conventional medicine(6). *D. ambrosioides* treats wounds, respiratory issues, inflammatory and painful processes, bronchitis, TB, and rheumatism. It also functions as a diuretic and an anthelmintic (7). It is believed to have wound-healing qualities and has been applied externally as a wash for hemorrhoids and as a poultice to detoxify toxins and snake bites (8). Other filamentous fungi such as *Aspergillus, Fusarium, Colletotrichum*, and dermatophytes have been shown to be inhibited in growth by the essential oil of *D. ambrosioides* (9,10). It possessed antihelmintica worm expulsion (10, 11) and antiaflatoxigenic, antimalarial, and antioxidant properties (7).

D. ambrosioides' essential oil composition was primarily composed of monoterpenes, specifically *p*-cymene, iso-ascaridole, α-terpinene, and ascaridole. It has been used as an anthelmintic, particularly for ascariasis, and has demonstrated cytotoxic, antioxidant, and antifungal properties against the insecticidal activities of human colon adenocarcinoma HT29 cell line (12-17).

About 80% of the essential oil of *D. ambrosioides* was recovered from its inflorescences, and the two main components that were extracted were ascaridole and *O*-cymene (18). According to Ávila-Blanco et al. (19), *D. ambrosioides'* essential oil exhibited antiamoebic action against *Entamoeba histolytica* both *in vitro* and *in vivo*. The fruits of *D. ambrosioides* were shown to contain a flavone glycoside (20). Kaempferol and quercetin from *D. ambrosioides'* fruits and aerial parts were

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isolated. It was found that *D. ambrosioides* fruits contained isorhamnetin. *D. ambrosioides* leaves contain a kaepmferol triglycoside known as ambroside (21).

From the butanol fraction, kaempferol 3-*O*-α-L-1C4-rhamnosyl-(1‴→2″)-β-D-4C1-xylopyranoside was isolated along with five other compounds that were identified as kaempferol 3-O-α-L-1C4–rhamnopyranoside (afzelin), kaempferol 7-O-α-L-1C4-rhamnopyranoside, caffeic acid, 1,2-benzopyrone (coumarin), and kaempferol (22). *D. ambrosioides* leaves were found to contain quercetin, myricetin, and rutin according to HPLC analysis (17).

The current study's objectives were to to examine the lipid components using GC/MS analysis, ascertain the phenolic and flavonoid contents, quantify the phenolic acids and flavonoids compounds using RP-HPLC, and analyze the antimicrobial and antioxidant properties of the various *D. ambrosioides* extracts.

2. Experimental

2.1. Plant material

In March 2024, aerial parts of *D. ambrosioides* plants were collected from the Albaha region, KSA. Dr. Heider Abdelkader, a taxonomist at the biology department of the Faculty of Science at Albaha University, identified the plant material. The plant samples were dried by air, ground into a powder, and stored for phytochemical and biological analysis.

2.2. Preparation of the plant extracts

Petroleum ether was used to extract roughly 500 g of the powdered, air-dried plant material of *D. ambrosioides* over the course of two days in a Soxhlet. After passing the pet. ether extract (1DA) over Fuller's earth to remove the colored pigments, it evaporated in a vacuum, leaving behind a yellowish residue (3 g). This residue was then dissolved in hot acetone to produce two fractions: acetone insoluble fraction which was filtered and analyzed by Gas chromatography-Mass spectrometry (GC/MS) and the acetone soluble fraction, which underwent saponification to produce the unsaponifiable materials, and the fatty acid methyl esters fraction, which was identified using Gas Chromatography-Mass spectrometry (GC/MS).

The defatted plant material was macerated in aqueous methanol (70%) for three days to produce a brownish extract (2DA), concentrated up to 250 mL which increased to 500 mL with hot distilled water, the precipitated material was filtered off and the filtrate was partitioned with successive portions of chloroform, ethyl acetate and butanol. The combined solvents was dried over anhydrous sodium sulfate and evaporated till dryness to afford the chloroform extract (3DA), ethyl acetate extract (4DA), n-butanol extract (5DA), and mother liquor (6DA), respectively which were investigated for biological activity.

2.3. GC/MS analysis

The pet. ether extract of *C. ambrosioides* aerial parts (1DA) was analyzed using Thermo Scientific GC-MS equipped with AS 3000 autosampler, trace ultra GC and ISQ detector. A nonpolar column consisting of 5% phenylpolysilphenylene siloxane (Thermo Scientific TR 5MS) with dimensions of 30 m x 0.25 mm (internal diameter) x 0.25 µm (film thickness) was used for separation of the components. Helium, at a flow rate of 1 mL/min (constant flow mode), was used as carrier gas. A volume of 2 µL of sample extracts was injected in splitless mode. The injection port was set at 260^{o}C and temperature of oven was initially set at 50^{o}C for 2 minutes. Then it was ramped to 140^{o}C at rate of 5^{o}C/min for 2 minutes and finally to 280^{o}C at rate of 3^{o}C/min for 50 minutes. The maximum oven temperature was set at 330^{o}C. The mass spectrometer was operated in an electron ionization (EI) mode within the mass range of 50-700 amu with 20 scan times (min). The MS transfer line temperature and ion source temperature were kept at 290^{o}C and 300^{o}C respectively with electron multiplier voltage of 1 Kv. The mass spectra were interpreted using the reference library of the National Institute of Standards and Technology (NIST), US, along with Willey 5 and mass finder, as well as data reported by Adams (23). The constituent percentages were measured based on the peak area.

2.3.1. GC/MS of unsaponifiable matters, acetone insoluble and fatty acid methyl esters

The GC-MS analysis for unsaponifiable matters and acetone insoluble fractions were carried out using the following specifications, Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 50^{o}C for 3 min; rising at 5^{o}C/min to 300^{o} C and held for 20 min. The injector and detector were held at 280^{o}C. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. While the conditions for FAMEs are: Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 80^{o}C for

1 min; rising at 4.0°C/min to 300°C and held for 1 min. The injector and detector were held at 240°C. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of *m/z* 40-450.

2.4. HPLC analysis

HPLC is used to separate, identify, and quantify the constituents of a mixture A sample is introduced into the system to start the HPLC procedure (24).

HPLC analysis that was carried out using an Agilent 1260 series. The separation was carried out using Zorbax Eclipse Plus C8 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0-1 min (82% A); 1-11 min (75% A); 11-18 min (60% A); 18-22 min (82% A); 22-24 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solutions. The column temperature was maintained at 40°C. Quantification in each gram of sample was carried out using the external standard method. The amount of each phenolic compound was expressed as µg/mL and µg/g**.**

2.5. Assay for antioxidant activity

DPPH (2, 2-diphenyl-1-picryl hydrazyl hydrate) was used to measure the radical scavenging activity of various plant extracts. This method was previously published (25), and quercetin was used as a reference.

2.6. Antimicrobial Activity

A modified Kirby-Bauer disc diffusion method was used to assess the antibacterial activity against various species (26). Eight microorganisms have been studied, including bacterial and fungal strains that were obtained from the Micro Analytical Center at Cairo University's Faculty of Science. These were categorized as Gram-negative bacteria included *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 10145), *Neisseria gonorrhoeae* (ATCC 19424), and Gram-positive bacteria included *Staphylococc*us *aureus* (ATCC 12600), *Bacillus subtilis* (ATCC 6051), and *Streptococcus faecalis* (ATCC 29212). As fungal strains, *Aspergillus flavus* (ATCC 9643) and *Candida albicans* (ATCC 71102) were employed. Standard antibacterial and antifungal medications, Ampicillin and Amphotericin B (Bristol-Myers Squibb, Switzerland), have been used; however, as a negative control, filter discs coated with 10 µl of solvent (distilled water, chloroform, or DMSO) were utilized. The zone of inhibition, also known as the clear zone, is the area of the disc that is devoid of development. On agar plates, 100 µl of the microbial suspension has been applied. Eventually, Bauer et al. (27) assessed the inhibition zone sizes.

3. Results and discussion

3.1. Identification of lipoidal matter in *D. ambrosioides* pet. ether extract

The results of GC/MS analysis of the volatile constituents of *D. ambrosioides* aerial parts (Figure 1 and Table 1) revealed the presence of 44 compounds belonging to different chemical classes.

Monoterpenes made up the largest percentage (43.70%), whereas cis-piperitone oxide represented the main compound (21.17%). Saturated and unsaturated hydrocarbons made up to 39.81% of the total Hydrocarbons (saturated and unsaturated) were up to 39.81% with trans-2-oxabicyclodecane($C_9H_{16}O$) accounting for the largest share (18.20%), followed by nitrogenous compounds (11.93%)(Table 2).

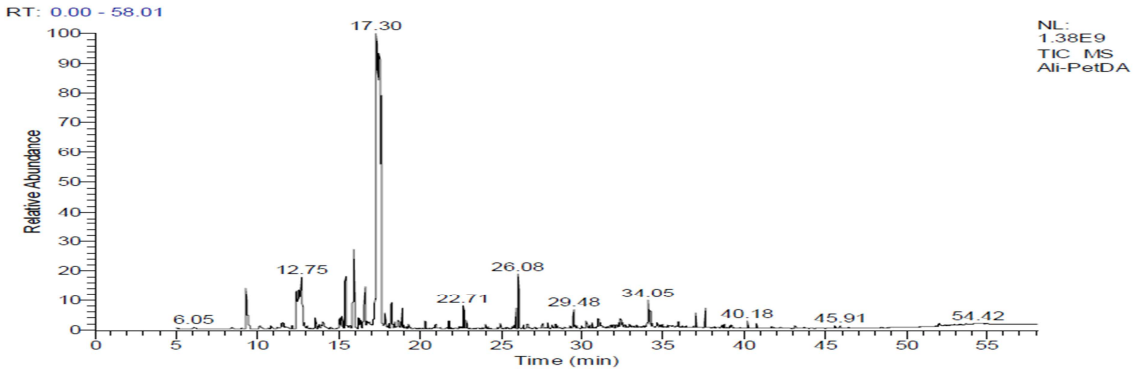

Figure 1: GC chromatogram of pet. ether extract of *D. ambrosioides*

Table 1: GC/MS analysis of Pet. ether extract of *D. ambrosioides*

Peak No.	Ret. Time	%	Mol. Wt.	Chemical formula	Compounds
1	9.28	3.36	134	$C_{10}H_{14}$	*p* -Cymene
2	11.44	0.30	140	$C8H_{12}O_2$	1-Acetyl-2-(isobutanoyl)ethylene
3	11.58	0.24	142	$C_9H_{18}O$	Nonanal
4	12.55	1.00	126	$C_8H_{14}O$	3-Hepten-2-one,4-methyl
5	12.74	2.27	168	$C_{11}H_{20}O$	Amyl cyclohexanone
6	13.54	0.61	152	$C_{10}H_{16}O$	Trans-2-caren-4-ol
7	13.80	4.43	168	$C_{10}H_{16}O_2$	Ascaridole
8	14.02	2.30	150	$C_{10}H_{14}O$	Thymol
9	15.07	0.65	212	$C_{12}H_{20}O_3$	Acetoxy-*p*-menth-3-one
10	15.21	0.58	170	$C_{10}H_{18}O_2$	endo,endo-2,3-Bornanediol
11	15.42	1.74	136	$C_{10}H_{16}$	α-terpinolene
12	15.93	4.55	168	$C_{10}H_{16}O_2$	Carvenone oxide
13	16.23	0.54	224	$C_{13}H_{20}O_3$	2-Propenoic acid, 3(2,2,6 trimethyl -7-oxabicyclo[4.1.0]hept1yl), methyl ester
14	16.36	0.33	184	$C_{13}H_{28}$	Decane, 5-ethyl-5-methyl
15	16.53	0.26	182	$C_{11}H_{18}O_2$	Nona-2,3-dienoic acid, ethyl ester
16	16.62	1.78	127	$C_7H_{11}O_2$	(3S,4S) Hept-1-en-6-yne-3,4-diol
17	17.31	10.92	306	$C_{17}H_{14}N_4O_2$	4-H-6-*p*-methoxyphenyl-3-phenyl v(1,2,3)triazolo[1,5d][1,3,4]oxadiazine
18	17.36	18.20	140	$C_9H_{16}O$	Trans-2-Oxabicyclo[4.4.0]decane
19	17.49	21.17	168	$C_{10}H_{16}O_2$	cis Piperitone oxide
20	17.59	9.60	194	$C_{14}H_{26}$	Tetradecyne
21	17.84	0.67	168	$C_9H_{16}N_2O$	2,2-Dimethyl-1(1-methyl-1H imidazol-2-yl)propan-1-ol
22	18.25	1.17	212	$C_{13}H_{24}O_2$	3,7-Dimethyl-6-nonen-1-ol acetate
23	19.29	0.21	256	$C_{17}H_{36}O$	1-Hexadecanol, 2-methyl
24	20.33	0.29	198	$C_{12}H_{22}O_2$	Heptyl(E)-2-methylbut-2-enoate
25	22.71	0.72	212	$C_{13}H_{24}O_2$	Octyl (E)-2-methylbut2enoate
26	22.87	0.34	208	$C_{12}H_{20}N_2O$	Cyclododecaneone,2-diazo
27	24.97	0.25	226	$C_{14}H_{26}O_2$	Nonyl(E)2methylbut-2-enoate
28	25.94	0.69	196	$C_{12}H_{20}O_2$	Lavandlyl acetate
29	26.08	3.16	136	$C_{10}H_{16}$	dl-Limonene
30	27.58	0.25	152	$C_{10}H_{16}O$	Cis -*p*-Mentha-2,8-dien1ol
31	29.48	0.62	268	$C_{18}H_{36}O$	2-Pentadecanone,6,10,14trimethyl
32	30.24	0.42	224	$C_{16}H_{32}$	Cyclopentane, undecyl
33	30.61	0.21	192	$C_{13}H_{20}O$	Ionone
34	31.00	0.49	262	$C_{18}H_{30}O$	Farnesyl acetone
35	31.09	0.36	270	$C_{17}H_{34}O_2$	Pentadecanoic acid,13-methyl, methyl ester
36	32.27	0.22	266	C19H38	1-Nonadecene
37	32.37	0.46	284	$C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester
38	34.06	1.38	270	$C_{18}H_{38}O$	1-Octadecanol
39	34.20	0.63	296	$C_{21}H_{44}$	Heneicosane
40	34.60	0.28	296	$C_{20}H_{40}O$	Phytol
41	35.93	0.23	310	$C_{22}H_{46}$	Docosane
42	36.99	0.53	352	$C_{25}H_{52}$	Pentacosane
43	37.61	0.68	394	$C_{28}H_{58}$	Octacosane
44	40.18	0.27	352	$C_{22}H_{24}O_4$	Taxamairin B

Table 2: Classes of the volatile constituents of *D. ambrosioides*

No.	Compounds	%
1	Monoterpenes	43.70
2	Sesquiterpenes	0.49
3	Hydrocarbons(saturated and unsaturated)	39.81
	Diterpenes	0.55
4	Esters	2.88
5	Nitrogenous compounds	11.93

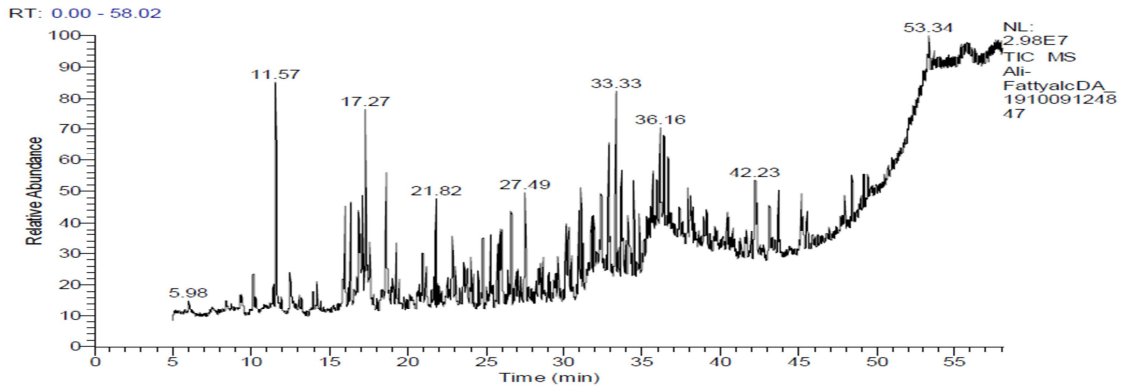

Figure 2: GC Chromatogram of acetone insoluble fraction of *D. ambrosioides*

Table 3's GC/MS results of *D. ambrosioides'* acetone insoluble materials demonstrated that it is a mixture of hydrocarbons, with docosane (14.18%) serving as the primary hydrocarbon and 2,6,10,14 hexadecatetraen-1-ol (6.61%) serving as the principal fatty alcohol(Figure 2).

Table 3: GC/MS analysis of acetone insoluble fraction of *D. ambrosioides*

Peak No.	Ret. time	Rel. %	Mol. Wt.	Molecular formula	Compounds
1	11.57	5.13	142	$C_9H_{18}O$	Nonanal
2	15.99	2.33	154	$C_{10}H_{18}O$	Decenal
3	16.37	2.03	212	$C_{15}H_{32}$	Dodecane, 2,7,10-trimethyl
4	16.44	2.63	226	$C_{16}H_{34}$	Hexadecane
6	16.61	1.91	268	$C_{19}H_{40}$	Nonadecane
7	16.87	3.91	296	$C_{21}H_{44}$	Heneicosane
8	16.96	14.18	310	$C_{22}H_{46}$	Docosane
9	18.66	3.62	168	$C_{11}H_{20}O$	2-Undecenal
10	19.28	2.77	256	$C_{17}H_{36}O$	1-Hexadecanol, 2-methyl
11	22.44	2.21	490	$C_{35}H_{70}$	17-Pentatriacontene
12	25.31	1.42	536	$C_{37}H_{76}O$	1-Heptatriacotanol
13	27.49	3.06	220	$C_{15}H_{24}O$	Farnesal
14	34.46	6.61	290	$C_{20}H_{34}O$	2,6,10,14-Hexadecatetraen-1-ol

3.2. Unsaponifiable fraction

GC/MS was used to examine the unsaponifiable fraction of *D. ambrosioides*. The total ion chromatogram was shown in Figure 3, and the various components of the unsaponifiable fraction were listed in Table 4. The unsponifiable matter's GC/MS examination showed the existence of 33 identified chemicals, which account for 89.68% of the material's overall composition. The unsaponifiable fraction's GC/MS analysis revealed that it contained a combination of sterols, diterpene, and hydrocarbons. The predominant hydrocarbon was 1-octadecanol (C_{18}), which accounted for 7.59% of the hydrocarbons in the range of C_{10} to C_{44}. Additionally present were stigmasterol, 24-methylene, 25 homocholesterol and 9,19 cyclolanost-24-en-3-ol, 24methyl,(3α), of which stigmasterol constituted the predominant sterol (1.77%).

3.3. Fatty acid methyl esters

The methyl esters of *D. ambrosioides's* fatty acids were examined using the GC/MS method. The analysis and fatty acid content were presented by the chromatogram (Fig. 4). The results of the fatty acid analysis (Table 5) indicated that 32 methyl esters of fatty acids were found, making up 92.11% of the total acids.

Saturated fatty acids, which make up 58.85% of the total fatty acids, were discovered to be abundant in this oil. Palmitic acid methyl ester ($C_{16:0}$) accounted for 24.68% of the saturated fatty acids, while arachidic acid methyl ester ($C_{20:0}$) came in second at 15.51%.

It was discovered that 33.26% of all fatty acids are unsaturated fatty acids. The main component of linoleic acid, which is an unsaturated fatty acid, is its methyl ester ($C_{18:2}$), which accounts for 14.48% of the composition.

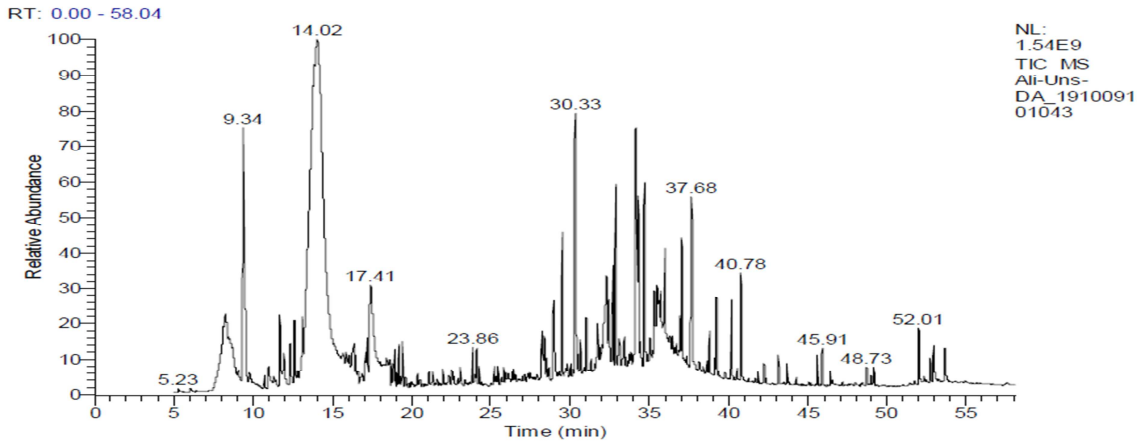

Figure 3: GC Chromatogram of unsaponifiable fraction of *D. ambrosioides*

Table 4: GC/MS of unsap. fraction of *D. ambrosioides*

Peak No.	Ret. time	Rel. %	Mol.Wt.	Molecular formula	Compounds
1	8.25	1.65	168	$C_{10}H_{16}O_2$	7-Oxabicyclo[4.1.0]heptan-2-one
2	8.66	0.75	212	$C_{13}H_{24}O_2$	1-Cyclohexanol,1[5hydroxy4methyl2hexenyl]
3	9.34	5.88	134	$C_{10}H_{14}$	Benzene,1-methyl-3-(1methylethyl)
4	10.98	0.80	168	$C_{11}H_{20}O$	transUndec4enal
5	11.92	1.54	152	$C_{10}H_{16}O$	Trans *p*-Mentha-2,8-dienol
6	12.59	1.19	168	$C_{11}H_{20}O$	α -Cyclohomogeraniol
7	13.09	0.83	152	$C_{10}H_{16}O$	Trans-2-Caren4ol
8	14.02	16.66	112	$C_6H_8O_2$	Staffane-3-carboxylic acid
9	16.36	1.15	170	$C_{10}H_{18}O_2$	4-Isopropyl-5-methylhex-2- yne-1,4-diol
10	17.11	0.73	154	$C_{10}H_{18}O$	3-Cyclohexen1ol,4methyl1(1methylethyl)
11	17.39	3.54	282	$C_{19}H_{38}O$	2-Nonadecanone
12	18.64	0.62	168	$C_{10}H_{16}O_2$	Ascaridole
13	28.24	0.97	252	$C_{18}H_{36}$	1-Octadecene
14	28.34	1.35	354	$C_{24}H_{50}O$	n-Tetracosanol-1
15	28.95	2.24	296	$C_{17}H_{28}O_4$	Nerolidolepoxy acetate
16	29.50	3.03	268	$C_{18}H_{36}O$	2-Pentadecanone,6,10,14 trimethyl
17	30.32	7.94	174	$C_{10}H_{22}O_2$	1,10-Decanediol
18	31.02	0.96	262	$C_{18}H_{30}O$	(E,E)Farnesyl acetone
19	32.14	0.66	234	$C_{15}H_{22}O_2$	Isocurcumenol
20	32.30	2.27	266	$C_{19}H_{38}$	1-Nonadecene
21	32.41	0.97	282	$C_{20}H4_2$	Eicosane
22	32.70	5.14	298	$C_{21}H_{30}O$	5-Methyl-3-phenyl2,4dipentylfuran
23	34.13	7.59	270	$C_{18}H_{38}O$	1-Octadecanol
24	34.31	1.44	380	$C_{27}H_{56}$	Heptacosane
25	34.69	3.82	296	$C_{20}H_{40}O$	Phytol
26	35.99	4.70	310	$C_{22}H_{46}$	Docosane
27	37.67	4.83	324	$C_{23}H_{48}$	Tricosane
28	39.24	1.15	338	$C_{24}H_{50}$	Tetracosane
29	40.78	1.78	352	$C_{25}H_{52}$	Pentacosane
30	45.91	0.58	618	$C_{44}H_{90}$	Tetratetracontane
31	52.01	1.77	412	$C_{29}H_{48}O$	Stigmasterol
32	52.95	0.93	412	$C_{29}H_{48}O$	24-Methylene, 25 Homocholesterol
33	53.66	0.76	440	$C_{31}H_{52}O$	9,19-Cyclolanost24en3ol, 24methyl,(3α)

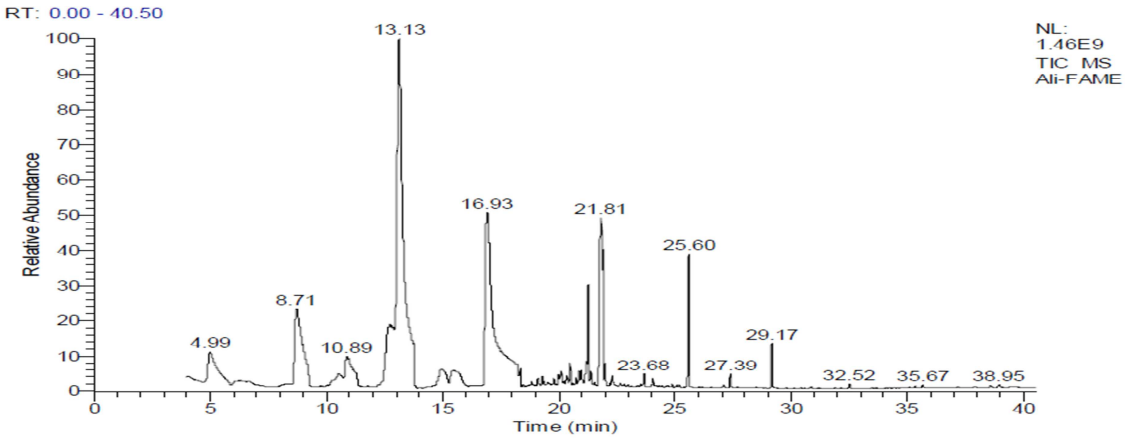

Figure 4: GC Chromatogram of fatty acid methyl ester of *D. ambrosioides*

Table 5: GC/MS analysis of FAME of *D. ambrosioides*

Peak No.	Ret. time	Rel. %	Mol. Wt.	Molecular formula	Compounds
1	4.97	3.05	214	$C_{13}H_{26}O_2$	Lauric acid, methyl ester ($C_{12:0}$)
2	8.71	6.58	242	$C_{15}H_{30}O_2$	Myristic acid, methyl ester ($C_{14:0}$)
3	9.19	0.51	242	$C_{15}H_{30}O_2$	Methyl isomyristate (iso- $C_{14:0}$)
4	10.50	0.67	296	$C_{19}H_{36}O_2$	4-Octadecenoic acid, methyl ester ($C_{18:1}$)
5	10.85	1.63	256	$C_{16}H_{32}O_2$	Pentadecanoic acid, methyl ester ($C_{15:0}$)
6	12.64	4.70	268	$C_{17}H_{32}O_2$	Palmitoleic acid, methyl ester ($_{16:1}$)
7	13.12	24.68	270	$C_{17}H_{34}O_2$	Palmitic acid, methyl ester($C_{16:0}$)
8	15.02	1.24	282	$C_{18}H_{34}O_2$	8-Heptadecenoic acid methyl ester ($C_{17:1}$)
9	15.51	2.34	284	$C_{18}H_{36}O_2$	Hexadecanoic acid,14-methyl, methyl ester ($C_{17:0}$)
10	16.89	14.48	294	$C_{19}H_{34}O_2$	Linoleic acid, methyl ester ($C_{18:2}$)
11	18.20	2.01	296	$C_{19}H_{36}O_2$	5-Octadecenoic acid, methyl ester ($C_{18:1}$)
12	18.34	0.48	298	$C_{19}H_{38}O_2$	Stearic acid, methyl ester ($C_{18:0}$)
13	19.11	0.34	294	$C_{19}H_{34}O_2$	6,9-Octadecadienoic acid, methyl ester ($C_{18:2}$)
14	19.29	0.32	310	$C_{20}H_{38}O_2$	Cis-10-Nonadecenoic acid, methyl ester ($C_{19:1}$)
15	19.37	0.10	310	$C_{20}H_{38}O_2$	10-Nonadecenoic acid, methyl ester ($C_{19:1}$)
16	19.79	0.21	312	$C_{20}H_{40}O_2$	Nonadecanoic acid, methyl ester ($C_{19:0}$)
17	19.99	0.96	294	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid, methyl ester ($C_{18:2}$)
18	20.36	0.56	292	$C_{19}H_{32}O_2$	6,9,12-Octadecatrienoic acid, methyl ester ($C_{18:3}$)
19	20.48	0.83	296	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester ($C_{18:1}$)
20	20.89	1.60	292	$C_{19}H_{32}O_2$	γ-Linolenic acid, methyl ester ($C_{18:3}$)
21	21.27	4.46	324	$C_{21}H_{40}O_2$	cis-11-Eicosenoic acid, methyl ester ($C_{20:1}$)
22	21.38	0.42	294	$C_{19}H_{34}O_2$	9,15-Octadecadienoic acid, methyl ester ($C_{18:2}$)
23	21.81	15.51	326	$C_{21}H_{42}O_2$	Arachidic acid methyl ester ($C_{20:0}$)
24	22.00	0.57	296	$C_{19}H_{36}O_2$	12-Octadecenoic acid, methyl ester ($C_{18:1}$)
25	22.64	0.14	320	$C_{21}H_{36}O_2$	7,10,13-Eicosatrienoic acid, methyl ester ($C_{20:3}$)
26	23.68	0.54	340	$C_{22}H_{44}O_2$	Heneicosanoic acid, methyl ester ($C_{21:0}$)
27	24.04	0.35	396	$C_{26}H_{52}O_2$	Lauric acid, tetradecyl ester ($C_{25:0}$)
28	24.87	0.26	354	$C_{23}H_{46}O_2$	Behenic acid, methyl ester ($C_{22:0}$)
29	27.39	0.49	368	$C_{24}H_{48}O_2$	Tricosanoic acid, methyl ester ($C_{23:0}$)
30	29.17	1.62	382	$C_{25}H_{50}O_2$	Lignoceric acid methyl ester ($C_{24:0}$)
31	32.52	0.17	410	$C_{27}H_{54}O_2$	Cerotic acid methyl ester ($C_{26:0}$)
32	35.67	0.12	438	$C_{29}H_{58}O_2$	Octacosanoic acid, methyl ester ($C_{28:0}$)

3.4. High-performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a multifunctional and extensively employed analytical method that offers exceptional precision and sensitivity in the separation and analysis of intricate mixtures. It is essential in many scientific and industrial applications due to its precision in component identification and quantification (24).

The external standard approach was used to quantify phenolic compounds by injecting known amounts of standard chemicals to create calibration curves. The peak area or height of each phenolic component in the sample was then compared to that of the matching standard to ascertain its quantity. The quantification results yielded useful information on the concentration of phenolic chemicals in the sample solution, and were expressed as µg/mL and µg/g. In order to evaluate the phenolic content of the *C. ambrosioides* aerial parts extract and determine its potency and bioactivity, quantitative data is necessary.

Overall, the presented HPLC analysis process shows a methodical and reliable technique for identifying phenolic compounds in the aerial parts extract of *C. ambrosioides*, enabling precise quantification and comparison with reference compounds. The methanolic extract made from the aerial portions of *C. ambrosioides* was subjected to a quantitative and qualitative assessment of its flavonoid and other phenolic contents using high-performance liquid chromatography (HPLC). Five different flavonoid compounds; quercetin, catechin, rutin, naringenin, and hesperetin were found and measured during HPLC analysis. Kaempferol and daidzein were lacking.

The conspicuously quantification results, which are presented in Fig. 5 and Table 6, indicate that the most common flavonoids were found to be rutin and catechin. Rutin had the highest concentration, 14.30µg/mL, followed by catechin 6.91µg/mL, while quercetin had the lowest concentration, 0.42 µg/mL. Since many plant species contain large concentrations of phenolic compounds, which are mostly recognized for their antioxidant qualities resulting from their redox characteristics, it is critical to clarify the precise roles played by these compounds.

For example, rutin exhibits a remarkable affinity for building complexes with a broad range of metals, which is crucial for scavenging free radicals. Catechin, which is well known for its ability to chelate and for its antioxidant qualities, has also been found to work better as an antioxidant when attached to metals, making it a good option for uses like the production of sensors (28, 29). A similar project involved the identification and measurement of ten phenolic compounds found in the aerial parts extract of *C. ambrosioides* using HPLC analysis. Interestingly, the extract was noticeably devoid of coumaric acid. The main phenolic compounds are shown in Table 6, where vanillin, gallic acid, and chlorogenic acid stand out as the main ingredients. Conversely, cinnamic acid had the lowest concentration in the extract.

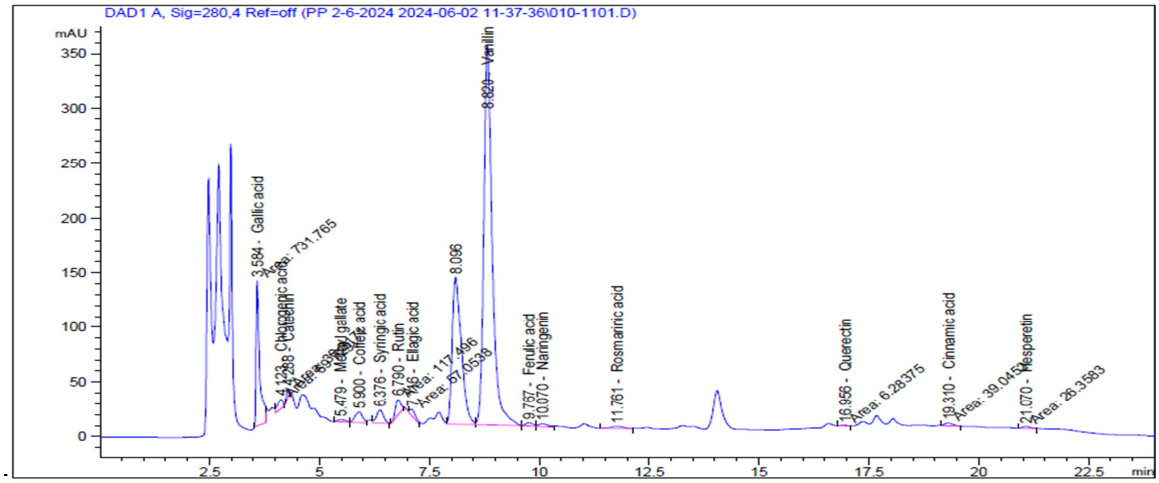

Figure 5: HPLC chromatogram revealing identified compounds in *C. ambrosioides* aerial parts methanol extract.

Table 6: Quantification of identified compounds in *C. ambrosioides* aerial parts methanol extract via HPLC

No.	R_t (min.)	Compounds	Area	Conc. (µg/mL)	Conc. (µg/g)
1	3.58	Gallic acid	731.77	58.94	2946.77
2	4.12	Chlorogenic acid	69.73	8.90	445.03
3	4.28	Catechin	30.32	6.91	345.40
4	5.47	Methyl gallate	25.21	1.30	64.85
5	5.90	Caffeic acid	112.89	9.21	460.50
6	6.37	Syringic acid	133.85	7.95	397.35
7	6.79	Rutin	117.50	14.30	714.95
8	7.11	Ellagic acid	57.05	7.03	351.37
9	8.54	Coumaric acid	0.00	0.00	0.00
10	8.82	Vanillin	4620.55	170.28	8514.14
11	9.76	Ferulic acid	32.48	1.82	90.90
12	10.07	Naringenin	37.13	3.33	166.58
13	11.76	Rosmarinic acid	45.06	4.49	224.75
14	15.79	Daidzein	0.00	0.00	0.00
15	16.95	Quercetin	6.28	0.42	20.92
16	19.31	Cinnamic acid	39.05	0.67	33.42
17	20.50	Kaempferol	0.00	0.00	0.00
18	21.07	Hesperetin	26.36	1.26	62.96

3.5. Antioxidant activity

The investigation on antioxidant activity (Fig. 6) shown that the methanolic extract had the maximum activity (inhibition%= 92.22), with the ethyl acetate extract coming in second (inhibition%= 90.77). The butanol extract and chloroform extract demonstrated moderate action (inhibition% = 87.11, 73.26) respectively.

These findings make sense in light of the fact that various flavonoids' structures influence how effective they are as antioxidants, with flavonoid structure being the primary factor influencing radical scavenging (28, 29). According to the total amount of OH groups (30), the OH groups on ring-B provide hydrogen and an electron to OH and peroxyl radicals, increasing the stability of flavonoidal radicals and causing the activity to rise linearly.

A free OH group located at C-3 in the flavonoid nucleus is essential for flavonoids to scavenge free radicals (31). It has been observed that flavonoids containing 3',4' dihydroxy and C-3-OH are ten times more powerful against peroxynitrite. Quercetin's free 3-OH substituent is partly responsible for its superiority in preventing oxidative damage caused by metals and non-metals, as it is believed to enhance the stability of the flavonoid radical (32, 33).

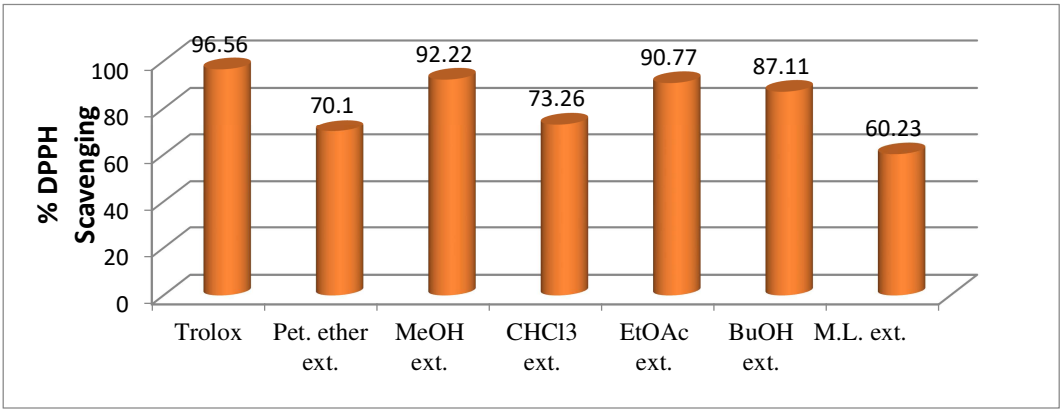

Figure 6: Antioxidant activity of different extracts from *D. ambrosioides* aerial parts.

3.6. Antimicrobial activity

Six different *C. ambrosioides* extracts were tested for their antimicrobial efficacy using eight different microorganisms, including three Gram-negative, three Gram-positive, and two fungal species. Table 7 presents the diameters of zone inhibition that were measured. The outcomes demonstrated that the various *C. ambrosioides* extracts exhibited varying levels of antimicrobial activity against each of the isolates that were tested. In comparison to Gram-negative bacteria *Escherichia coli, Neisseria gonorrhoeae* and *P. aeruginosa*, the pet. ether extract demonstrated a clearer inhibition zone diameter of 13-14 mm and a higher activity, with a larger inhibition zone diameter of 14 mm against Gram-positive bacteria *Staphylococcus aureus, Bacillus subtilis* and *Streptococcus faecalis*.

These results are consistent with those of Harraza, et al. (34), who showed that *C. ambrosioides* oil has antibacterial action against *Escherichia coli* and *Bacillus subtilis*. The pet. ether extract showed negligible inhibitory action against *A. flavus* and moderate activity against *Candida albicans*, with an inhibition zone diameter of 12 mm. These findings concur with those of Kumar et al. and Jardim et al., who reported that the *C. ambrosioides* displayed antifungal activity (7,13).

In comparison to petroleum ether, the ethyl acetate extract of *C. ambrosioides* exhibited less antibacterial activity against Gram-positive bacteria. The clear zone diameter of the extract varied between 12 and 14 mm against Gram-positive bacteria and between 13 and 14 mm against Gram-negative bacteria.

With a clear zone diameter ranging from 12 to 13 mm against Gram-positive bacteria and from 12 to 13 mm against Gram-negative bacteria, the n-butanol extract demonstrated lesser antibacterial efficacy than the petroleum ether and ethyl acetate extracts.

The methanol extract of *C. ambrosioides* shown minimal inhibitory effect against fungal species and moderate antimicrobial activity against Gram-positive bacteria, with a clear zone diameter ranging from 10 to 12 mm against Gram-positive bacteria and from 10 to 12 mm against Gram-negative bacteria.
The *C. ambrosioides* chloroform extract demonstrated no inhibitory activity against fungal species and a weak antimicrobial activity against Gram-negative bacteria with a clear zone diameter ranging from 9 to 10 mm. It also demonstrated a moderate antimicrobial activity against Gram-positive bacteria having clear zone diameter ranged from 10 to 11 mm.

The mother liquor of *C. ambrosioides*, exhibited no inhibitory activity against *A. flavus* and *Candida albicans*, but it did exhibit a weak antimicrobial activity against Gram-positive bacteria, with a clear zone diameter ranging from 9 to 10 mm and showed a weak antimicrobial activity 9 mm against Gram-negative bacteria.
The antimicrobial activity of six extracts of *C. ambrosioides* showed significant antibacterial activity against *Staphylococcus aureus*. These findings contradicted those of Zohra et al. (17), who reported that no *C. ambrosioides* extract showed any efficacy against *M. luteus* and *Staphylococcus aureus*.

Table 7: Antimicrobial activity of different extracts of *D. ambrosioides*

Samples		Inhibition zone diameter (mm/mg Sample)							
		Bacterial species						Fungal species	
		G^+			G^-				
		Bacillus subtilis	*Staphylococcus aureus*	*Streptococcus faecalis*	*Escherichia coli*	*Neisseria gonorrhoeae*	*Pseudomonas aeruginosa*	*Aspergillus flavus*	*Candida albicans*
Control: DMSO		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Standards	**Ampicillin Antibacterial agent**	26	21	27	25	28	26	-	-
	Amphotericin B Antifungal agent	-	-	-	-	-	-	16	21
Pet. ether ext. (1DA)		14	14	14	14	13	13	0.0	12
MeOH ext. (2DA)		11	12	10	10	11	12	0.0	0.0
Chloroform ext. (3DA)		11	11	10	10	9	10	0.0	0.0
EtOAC ext. (4DA)		12	14	14	14	13	14	0.0	0.0
BuOH ext. (5DA)		13	12	12	12	12	13	0.0	0.0
M.L. ext. (6DA)		9	10	9	9	9	9	0.0	0.0

The ascaridole level in the pet. ether extract of *C. ambrosioides* was 4.43% (Table 1), according to GC/MS analysis. The origin of the plant has an impact on the amount of ascaridole, a component that is linked to several biological actions of the EO (15, 35).

As reported by Ávila-Blanco et al. (19), the essential oil of *D. ambrosioides* demonstrated amoebicidal activity, with ascaridole being the primary constituent that upholds the traditional use. *Chenopodium* oil was previously found to contain ascaridole, a peroxide (14). When any source of protons is present, ascaridole readily rearranges into isoascaridole (12), which then rearranges into 3,4-epoxy-*p*-menthan-2-one (36). Ascaridole is a particularly sensitive molecule. The examined oil did contain the latter metabolite. Notably, there have been reports of two chemotypes of *C. ambrosioides* L.: *var. anthelminticum* and *var. ambrosioides* (37). Compared to the low amounts (0-5%) recorded in the oil of the latter, ascaridole has been discovered to be a substantial component in the essential oil of the former (34, 38, 39).

Nonetheless, it is well known that ascaridole is a heat-sensitive substance that transforms into isoascaridole when heated to 150°C (12).

A soluble iodide peroxidase that was isolated from homogenates of *C. ambrosioides* fruit and leaves accelerated the production of ascaridole from α-terpinene. The product's GC-MS analysis verified the enzymatic production of ascaridole (15).

Ascarisin, or 1, 4-epidioxy-*p*-menth-2-ene, is another name for ascaridole, a bicyclic monoterpene with a unique bridging peroxide functional group. These were separated out and found to contain significant medicinal and insecticidal properties. According to a University of California study, *D. ambrosioides* contains ascaridole, a chemical that prevents neighboring plants from growing. It is advised that more research be done to determine this plant's potential as a pesticidal candidate because its active ingredients may be very important (40).

Maximum antibacterial activity against *Bacillus subtilis* (33±1.5 mm) and maximum antifungal activity against *Aspergillus niger* (16±1.5 mm) were demonstrated by fruit methanol extracts macerated in petroleum ether. No action was demonstrated by aqueous extracts against specific organisms (41).

4. Conclusion

Traditionally, medicinal herbs have been utilized throughout. Consequently, this study aims to investigate the scientific underpinnings of these plants' therapeutic value and to compare the phenolic content of plant extract with the medical applications and chemical activity of these plants. Extracts from *C. ambrosioides* may find application in pharmacological, medical, and nutraceutical goods, as well as an antioxidant supplement. Extracts from *C. ambrosioides* are a great source of nutrients, polyphenols, and bioactive chemicals with antibacterial and antioxidant properties.

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Conflict of interest
The authors declare no conflict of interest.

Data availability statement
All data required to support this study is already included in the manuscript.

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