

Study of Chemical Content and Antioxidant Activity of *Palmaria palmata*, *Chondrus crispus* Algae and *Raphanus sativus* Leaves Extracts

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ABSTRACT

This study's objective was to evaluate the bioactive compound and antioxidant capacity of *Palmaria palmata*, *Chondrus crispus*, and *Raphanus sativus* leaves extracts. The assay known as 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to measure antioxidant activity. Methanolic extracts of red algae and radish leaves were examined for their phytochemical and bioactive algal component content as well as their antioxidant capacity. Bioactive substances with a range of characteristics, such as hypocholesterolemic, antibacterial, antiviral, antidiabetic, anticancer, anti-inflammatory, antioxidant, antiobesity, and antimalarial activities, were discovered using gas chromatography-mass spectrometry (GC-MS) investigation. Radish leaf extract had the highest DPPH radical scavenging activity of all the tested samples, with an inhibition rate of roughly 90.67%. According to these findings, the extracts under investigation, particularly those derived from radish leaves and algae, are viable natural sources of bioactive compounds with potential therapeutic uses.

INTRODUCTION

Natural remedies have been shown to be a safe and effective option, offering a sustainable alternative for managing weight, without significant side effects. Numerous studies have shown that natural products from plants and algae are rich in bioactive compounds that help regulate metabolism, suppress appetite, and burn fat (Murray *et al.*, 2018; Mir *et al.*, 2019). According to a number of scientific research, medicinal herbs and marine microalgae are rich sources of biological components that are active in most conventional medications used to treat a wide range of illnesses (Manlusoc *et al.*, 2019). The discovery and isolation of bioactive chemicals from marine sources, including algae, that have demonstrated their medicinal efficacy has been emphasized by several researchers in recent years (Cotas *et al.*, 2020). Recent studies have shown that seaweeds such as two red algae, *Rhododendron palmatum* and *Chondrus crispus*, contain antioxidant compounds that can reduce inflammation and improve metabolism. These characteristics make them promising agents in the fight against obesity. Similarly, extracts from radish leaves (*Raphanus sativus*) have shown effectiveness in lowering

blood lipid levels and improving liver and digestive system function, thereby reinforcing their anti-obesity potential (Lee *et al.*, 2021; Al-Badri *et al.*, 2024). The Arab world possesses a rich diversity of medicinal and aromatic herbs that have traditionally been used in herbal formulations to treat various diseases. As a result, there is growing interest in the development, cultivation, and extraction of active ingredients from medicinal plants for pharmaceutical use, particularly as alternatives to synthetic compounds that may cause adverse side effects. This interest is largely due to the strong antioxidant properties of many medicinal plants (Adebayo *et al.*, 2010; Halima *et al.*, 2019). Current research estimates that over 5,000 species of medicinal plants have been used for therapeutic purposes or as raw materials in drug production.

MATERIALS AND METHODS

1. Sample collection

Radish leaf samples (*Raphanus sativus*) were collected from local markets in the city of Nasiriyah, Dhi Qar Governorate, Iraq, during October 2024. The plant materials were identified and taxonomically classified at the Plant Taxonomy Laboratory, Natural History Research Center, Iraq. The collected radish leaves were thoroughly cleaned and air-dried using a hot air oven. After drying, the samples were ground into a fine powder using an electric grinder and stored at 4°C until further use. Red algae powders (*Chondrus crispus* and *Palmaria palmata*) were obtained in finely ground form from Amazon (USA) and stored appropriately until utilized in the experimental procedures.

2. Preparation of crude alcoholic extracts from algae and *Raphanus* leaves

Ten milliliters of ethanol were combined with one gram of the powdered material. To guarantee effective extraction, each combination was put on a heated magnetic stirrer and shaken continuously for two hours. The solutions were mixed and then let to settle. Pipettes were used to collect the supernatant, which was then moved to sterile tubes. After that, the samples were centrifuged for 15 minutes at 3,000 rpm. The resultant supernatants were gathered and preserved for use in Gas Chromatography–Mass Spectrometry (GC-MS) analysis later on.

3. GC-MS analysis

Using a Shimadzu QP2010 Plus GC-MS system, a GC-MS study of the extracts of *Palmaria palmata*, *Chondrus crispus*, and *Raphanus sativus* leaves was performed. Using the procedure outlined by Ayal (2023), the apparatus was fitted with a capillary column (30m in length, 0.25mm internal diameter, and 0.25µm film thickness). In splitless mode, a sufficient injection was carried out with a purging duration of two minutes. The carrier gas, helium, was employed at a flow rate of 1mL/ min. Initially, the oven was set at 50°C and maintained there for three minutes, then set to rise to 80°C at a rate of 5°C per minute, and then to 340°C at a rate of 10°C per minute. The solvent delay was set to four minutes, and the injector temperature was kept at 250°C. Using automatic matching software and manual comparison to reference data, the mass spectra were

compared to the NIST 08s and NIST 08 mass spectral libraries in order to identify the compound.

4. Extraction of antioxidants

In a 25ml universal container, sample extracts were made with 1g of dried sample powder and 10ml of 50% aqueous acetone. A magnetic stirrer was used to agitate the mixture for one hour at 1000rpm. The samples were then centrifuged for five minutes at 3000rpm, and the supernatants were used for additional analysis.

5. Free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, as outlined in **Brands-Williams *et al.* (1995)**, was used to measure the antioxidant activity. 40mg of DPPH was dissolved in 100mL of methanol to create a standard DPPH solution, which was then kept at -20°C until it was needed. Using a UV-visible spectrophotometer, the absorbance was adjusted by mixing 350µL of the standard solution with 350µL of methanol, resulting in an absorbance of 0.70 ± 0.01 at 517nm. % Antioxidant Activity = $\frac{\text{DPPH}_{\text{blank}} - \text{DPPH}_{\text{sample}}}{\text{DPPH}_{\text{blank}}} \times 100$

6. Statistical analysis

Using SPSS software (SPSS ver.25), the samples were assessed using the Duncan triplicates range test and one-way ANOVA. Statistical significance was defined as *P* values less than 0.05.

RESULTS AND DISCUSSION

1. GC-MS analysis of samples extract

The GC-MS analysis of *Palmaria palmata* extract revealed a wide range of biologically active compounds, as listed in Table (1). Among the detected compounds, a diverse range of chemical constituents included sterols, fatty acid esters, alcohols, hydrocarbons, and heterocyclic compounds. Notable compounds identified include Desmosterol; 1,4-Methanonaphthalen-9-ol, 1,2,3,4-tetrahydro-, acetate, syn-; 2H-Benzopyran-3-carbotriazanyde, 2-oxo-; 9-Octadecenoic acid ethyl ester; Linoleic acid ethyl ester; 14-Pentadecenoic acid; cis-3-Methyl-endo-tricyclo[5.2.1.0(2.6)]decane; (R)-(-)-14-Methyl-8-hexadecyn-1-ol; 2-Pentadecanone, 6,10,14-trimethyl-; Nonane, 2,2,4,4,6,8,8-heptamethyl-; Tetradecanoic acid; Nonane, 3-methyl-5-propyl-; Fumaric acid, ethyl 2-methylallyl ester; 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-; 3,3,5,5-Tetramethylcyclohexanol; β-D-Glucopyranose, 1,6-anhydro-; 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-o-; 1,4:3,6-Dianhydro-α-D-glucopyranose; 1-Methyl-5-fluorouracil; 1,2-Ethanediamine, N,N,N',N'-tetramethyl-; Isobutyl acrylate; Silanediol, dimethyl-; Phencyclidine diacetate; Dimethyl sulfoxide; Carbonyl sulfide; Dodecane; Tetradecane; Tetramethylammonium tetrafluoroborate; n-Hexadecanoic acid; and Hydroxyurea, N,N',O-trimethyl-, along with various methoxy-phenolic derivatives (**Hossain & Rahman, 2011; Kati *et al.*, 2023; Abbas & Yazea, 2025**).

Table 1. List of compounds identified in the ethanolic extract of *Palmaria palmata* by gas chromatography–mass spectrometry (GC-MS) along with their retention times and reported biological activities

No.	Compound name	R.T.	Area	Biological activity
1	Desmosterol	32.821	655044	Strol isan intermediate in cholesterol synthessis
2	1,4-Methanonaphthalen-9-ol, 1,2,3,4-tetrahydro-, acetate, syn-	26.466	5701269	Antioxidant and anticancer
3	2H-Benzopyrane-3-carbotriazanyde, 2-oxo-	26.421	983694	antiparasitic
4	9-Octadecenoic acid, ethyl ester	24.421	766894	Cholesterol bonud fatty acid
5	Linoleic acid ethyl ester	24.706	184969	Lioleic acid ester bound cholesterol
6	14-Pentadecenoic acid	24.504	4108718	Saturated fatty acid
7	cis-3-Methyl-endo-tricyclo [5.2.1.0(2.6)] decane	24.474	2541769	Antibacterial and antifungal
8	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	21.67	1740905	antimicrobial
9	2-Pentadecanone, 6,10,14-trimethyl	21.67	731509	Reduces LDL cholesterol
10	Nonane, 2,2,4,4,6,8,8-heptamethyl	21.159	514012	Antibacterial and antifungal
11	Tetradecanoic acid	20.801	2582070	Cholesterol bonud fatty acid
12	Nonane, 3-methyl-5-propyl-	20.087	6345190	Saturated hydrocarbon
13	Fumaric acid, ethyl 2-methylallyl ester	18.831	920092	Intermediatecompound Plastic hndustry
14	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	18.427	676355	Aromatic organic compound
15	3,3,5,5-Tetramethylcyclohexanol	17.757	1244477	Organic componud
16	beta. -D-Glucopyranose, 1,6-anhydro .	1639	1611703	Regulates sugar levels
17	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	14.293	774483	It is used in the synthesis of polymers
18	1,4:3,6-Dianhydro-. alpha. -d-glucopyranose	13.986	612017	Creates sugars
19	1-Methyl-5-fluorouracil	12.98	642848	Anticancer
20	1,2-Ethanediamine, N, N, N', N'-tetramethyl	11.821	789173	Complex factor in chemistry
21	Isobutyl acrylate	10.197	1068135	Monomer compound
22	Silanediol, dimethyl-	4.696	590613	Silicone organosolvent
23	Phenacylidene diacetate	5.24	1348365	Intermediate componnd in the synthesis of pharmaceutical
24	Dimethyl Sulfoxide	7.673	1223276	Strong polar solvent
25	Carbonyl sulfide	4.32	3542425	Colorless poisonous gas
26	Dodecane	13.667	3239655	Atimicrobial and insecticidal
27	Tetradecane-	16.488	3621711	Antibacterial and antifungal
28	Octan,3,5-dimethyl	10.179	1864663	Saturated hydrocarbon
29	Tetramethylammonium tetra fluoroborate	11.744	6238882	Ionic salt
30	n-Hexadecanoic acid	22.835	6085253	Palmitic acid is a major component of vegetable oils
31	Hydroxyurea, N, N', O-trim ethyl-	11.71	48935468	Potential anti – cancer drug componud

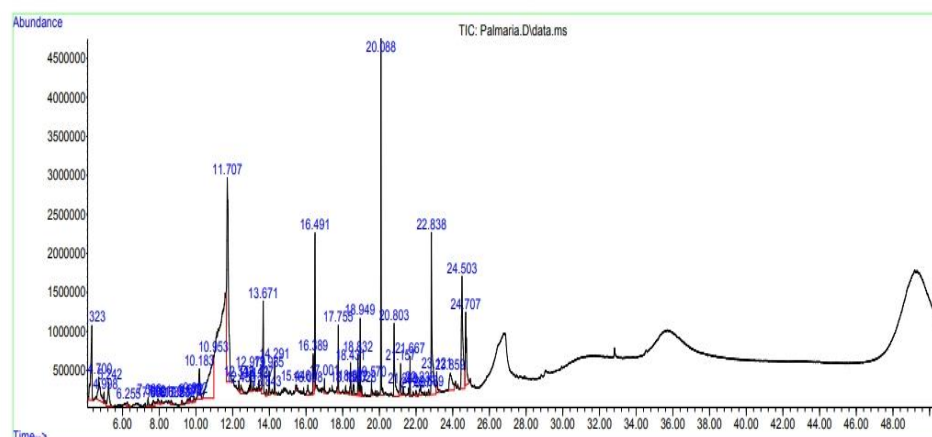


Fig. 1. Gas chromatogram of ethanol *Palmaria palmata* extract

2. GC-MS analysis *Chondrus crispus* extract

Gas chromatography–mass spectrometry (GC-MS) analysis of *Chondrus crispus* extract revealed the presence of various groups of bioactive compounds (Table 2). Identified compounds included: 2-Propenoic acid; 1,3-Dioxane; Betazole; Thiocyanic acid, ethyl ester; Thiazole; Dimethylsulfoxonium formylmethylide; Propanoic acid; Oxirane, [(methylthio)methyl]-; Dideoxy-dimethylene-iditol; Cyclohexylmethylsilane; Levoglucosenone; Hexanoic acid; *D*-Limonene; Tetraethyl silicate; Ethosuximide; Inositol; 2-Furancarboxaldehyde, 5-methyl-; Fumaric acid, ethyl 2-methylallyl ester; Cyclotetrasiloxane, octamethyl-; Diethyl phthalate; *n*-Hexadecanoic acid; Hexadecanoic acid, ethyl ester; Neophytadiene; Linoleic acid ethyl ester; Carbonic acid, eicosyl vinyl ester; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Cholesterol; 5-Iodo-2,3-dimethylbenzene-1,4-diol; 1-*O*- β -methyl-2-butenate ester; and Tetracosamethyl-cyclododecasiloxane.

Several of these compounds, also found in lemon fruit extracts, have been reported in the literature to exhibit a wide range of therapeutic properties, including antimicrobial, antiviral, antioxidant, antitumor, antihistaminic, anti-inflammatory, and anticancer activities (Addai *et al.*, 2022; Sheikhi *et al.*, 2022; Ayal *et al.*, 2023)

Table 2. List of compounds from ethanol *Chondrus crispus* extract observed in GC-MS with their retention time and biological activity

No.	Compound name	R.T.	Area	Biological activity
1	2-Propenoic acid	4.449	169229	Antibacterial and anti-fungal
2	1,3-Dioxane	4.246	133599	Not known for its bioavailability
3	Betazole	12.493	221667	Antacid
	Thiocyanic acid, ethyl ester	9.773	194522	Antioxidant and antibacterial
5	Thiazole	11.557	172789	Antibacterial and antifungal
6	Dimethylsulfoxonium	8.435	213487	Antioxidant and antibacterial
7	Propanoic acid	4.43	1074140	Antioxidant and antibacterial
8	Oxirane, [(methylthio)methyl]-	10.74	2141638	Anti- cancer
9	Dideoxy-dimethylene-iditol	12.053	271672	It play a role in the metabolism
10	Cyclohexylmethylsilane	13.245	221136	Not known for its bioavailability
11	Levogluconone	12.4	1543040	Used in organic chemistry
12	Hexanoic acid	10.131	870596	Antioxidant and antibacterial
13	D-Limonene	10.851	618587	Antioxidant and antibacterial
14	Tetraethyl silicate	10.097	232463	Used in chemical in dustries
15	Ethosuximide	14.6	808283	Antiepileptic
16	Inositol	12.452	243398	Vitamin that reduces harmful fats
17	2-Furancarboxaldehyde, 5-	9.649	763799	Antioxidant and antibacterial
18	Fumaric acid	18.791	394231	Antioxidant and antibacterial
19	Cyclotetrasiloxane, octamethyl-	10.221	249717	Topical medication holder
20	Diethyl Phthalate	19.039	176739	Used in chemical in dustries
21	n-Hexadecanoic acid	22.838	1282191	Anti-oxidant
22	Hexadecanoic acid, ethyl ester	23.121	412721	Improves fat metabolism
23	Neophytadiene	21.587	202704	Anti- cancer
24	Linoleic acid ethyl ester	24.703	1533295	Reduces LDL cholesterol levels
25	Carbonic acid, eicosyl vinyl	23.356	159037	Not known for its bioavailability
26	3,7,11,15-Tetramethyl-2-	22.029	149869	Anti- anxiety, anti -mood
27	Cholesterol	32.421	2439847	Enters into formation of cell membranes.
28	5-Iodo-2,3-dimethylbenzene-1,4-diol, 1-O- [. beta. -methyl-2-	39.241	153105	Antioxidant and antibacterial
29	Tetracosamethyl-cyclododecasiloxane	28.855	149792	Not known for its bioavailability

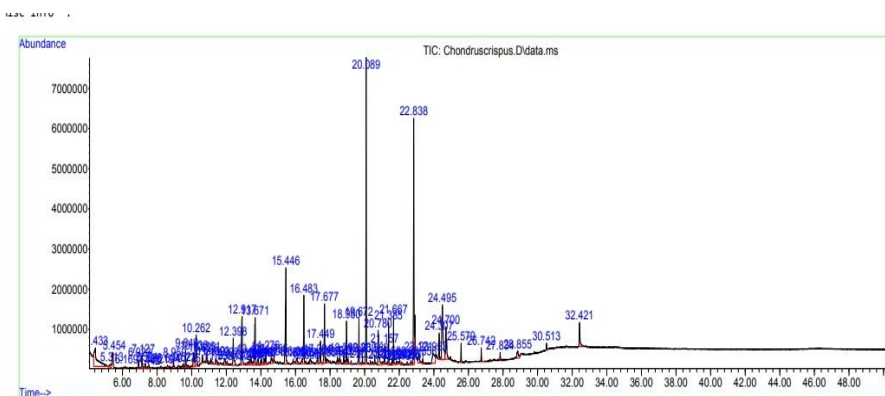


Fig. 2. Gas chromatogram of ethanol *Chondrus crispus* extract

3. GC-MS analysis of *Raphanus sativus* extract

GC-MS analysis of *Raphanus sativus* extract revealed the presence of several groups of bioactive compounds (Table 3). Identified constituents included: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; 1,2-Benzenediol, O-(2-furoyl)-O'-(pentafluoropropionyl)-; 2-Methoxy-4-vinylphenol; 5-Methylene-4,5,6,6a-tetrahydro-3aH-pentalen-1-one; Naphthalene, 1,2-dihydro-1,5,8-trimethyl-; 3-Furoic acid, benzyldimethylsilyl ester; Dimethyl 2,5-thiophenedicarboxylate; Benzofuran, 2,3-dihydro-; Hexanoic acid; 2-Ethyl propionaldehyde; Diethylhydrazone; Dimethyl sulfoxide; Thiazide, 2,4,5-trimethyl-; 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-; 3-Furancarboxylic acid; 2-Furancarboxylic acid, hydrazide; 1(2H)-Naphthalene, 3,4,5,6,7,8-hexahydro-; 1H-Indole-3-acetonitrile; 2H-Azepine-2-thione, hexahydro-1-methyl-; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; Thiazole, 2,4,5-trimethyl-; Glycine, N-(ethoxycarbonyl)-, ethyl ester; 2-Butenedioic acid (Z)-, dimethyl ester; Octadecanoic acid; 9-Octadecenoic acid, (E)-; cis-7,cis-11-Hexadecadien-1-yl acetate; Maltol; *n*-Hexadecanoic acid; Hydrazine, 1,1-diethyl-2-(1-methylpropyl)-; 1-Pentanone, 1-(2-furanyl)-; and 5-Hydroxymethylfurfural.

Several of these compounds have also been identified in apple cider extracts and are known for their broad therapeutic properties, including antioxidant, anti-ulcer, hypocholesterolemic, antimicrobial, anti-inflammatory, anticancer, antidiabetic, antitumor, antiviral, and anti-obesity effects (Lee *et al.*, 2018; Seo *et al.*, 2021; Ali & Ayal, 2024).

4. DPPH radical scavenging activity

Fig. (4) illustrates the antioxidant potential of the plant and algal extracts evaluated in this study. Statistical analysis revealed significant differences in DPPH free radical scavenging activity among the extracts. The ethanolic extract of *Raphanus sativus* leaves exhibited the highest antioxidant activity, with a scavenging efficiency of 90.67%, significantly outperforming *Palmaria palmata* (86.34%) and *Chondrus crispus* (81.22%). The superior antioxidant activity observed in *Raphanus sativus* may be attributed to its high content of antioxidant compounds such as octanal, decanal, and 1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-. For *Palmaria palmata*, this activity is likely due to the presence of desmosterol, 9-octadecenoic acid ethyl ester, 14-pentadecenoic acid, and 2-pentadecanone, 6,10,14-trimethyl-. Similarly, the antioxidant efficacy of *Chondrus crispus* extract may be linked to bioactive constituents including thiazole, 2-propenoic acid, cholesterol, and linoleic acid ethyl ester, which have previously been reported to possess strong antioxidant properties (Addai *et al.*, 2022; Abbas & Yazea, 2025).

Table 3. List of compounds from ethanol *Raphanus sativus* extract observed in GC-MS with their retention time and biological activity

No.	Compound name	R.T	Area	Biological activity
1	2,4-Dihydroxy-2,5-dimethyl-3(2H)-	10.019	1159065	Antioxidant and antibacterial
2	1,2-Benzenediol, O-(2-furoyl)-O'	6.755	1070364	Antioxidant and antibacterial
3	2-Methoxy-4-vinylphenol	15.473	8994806	Antioxidant and antibacterial
4	5-Methylene-4,5,6,6a-tetrahydro-3ah-	17.467	1053218	Anti-oxidant
5	Naphthalene, 1,2-dihydro-1,5,8-trimethy	16.058	2128550	Antioxidant cytotoxic
6	3-Furoic acid, benzyldimethylsilyl ester	20.339	2885119	Anti inflammation
7	Dimethyl 2,5-thiophenedicarboxylate	20.075	1611542	Anti –cancer
8	Benzo furan, 2,3-dihydro-	14.095	4103498	Antioxidant and antibacterial
9	Hexanoic acid, 2-ethyl-	12.479	1912380	Anti-fungal
10	Propionaldehyde, diethylhydrazone	11.189	1100592	Antimicrobial
11	Dimethyl Sulfoxide	7.479	1313049	Organic solvent
12	Thiazole, 2,4,5-trimethyl-	14.049	45050026	Antioxidant and antibacterial
13	4H-Pyran-4-one, 3,5-dihydroxy-2-	13.697	1281126	Anti inflammation
15	2-Furancarboxylic acid, hydrazide	11.929	3718663	Anti-microbial
16	1(2H)-Naphthalene, 3,4,5,6,7,8-	19.216	1520948	Anti-oxidant
17	1H-Indole-3-acetonitrile	21.549	1238484	Anti-oxidant
18	2H-Azepine-2-thione, hexahydro-1-	18.075	15710858	Anti-fungal
19	4H-Pyran-4-one, 2,3-dihydro-3,5-	13.154	104528248	Antioxidant and antibacterial
20	Thiazole, 2,4,5-trimethyl-	13.568	10877638	Antimicrobial
21	Glycine, N-(ethoxycarbonyl)-, ethyl	13.028	2684178	Anti-oxidant and
22	2-Butenedioic acid (Z)-, dimethyl ester	17.647	1516767	Catalyst in reactions
23	Octadecenoic acid	24.685	1239928	Anti inflammation
24	9-Octadecenoic acid, (E)	24.5	4743026	Reduces LDL cholesterol
25	cis-7, cis-11-Hexadecadien-1-yl acetate	24.466	7678043	aromatic compound
26	Maltol	12.457	1738674	Antioxidant, flavor enhancer
27	n-Hexadecanoic acid	22.813	2396774	Saturated fatty acid
28	Hydrazine, 1,1-diethyl-2-(1-	15.422	1949256	Anti-microbial
29	1-Pentanone, 1-(2-furanyl)-	8.555	1291207	Anti-oxidant
30	5-Hydroxymethylfurfural	14.338	23499665	Anti –cancer

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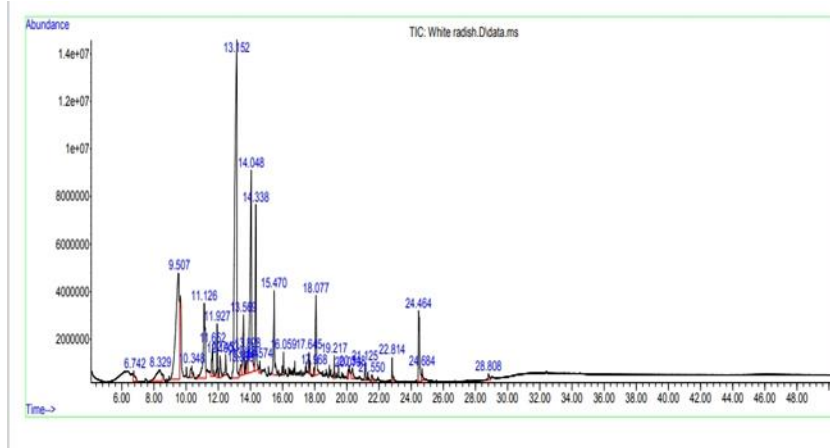


Fig. 3. Gas chromatogram of ethanol *Raphanus sativus* extract

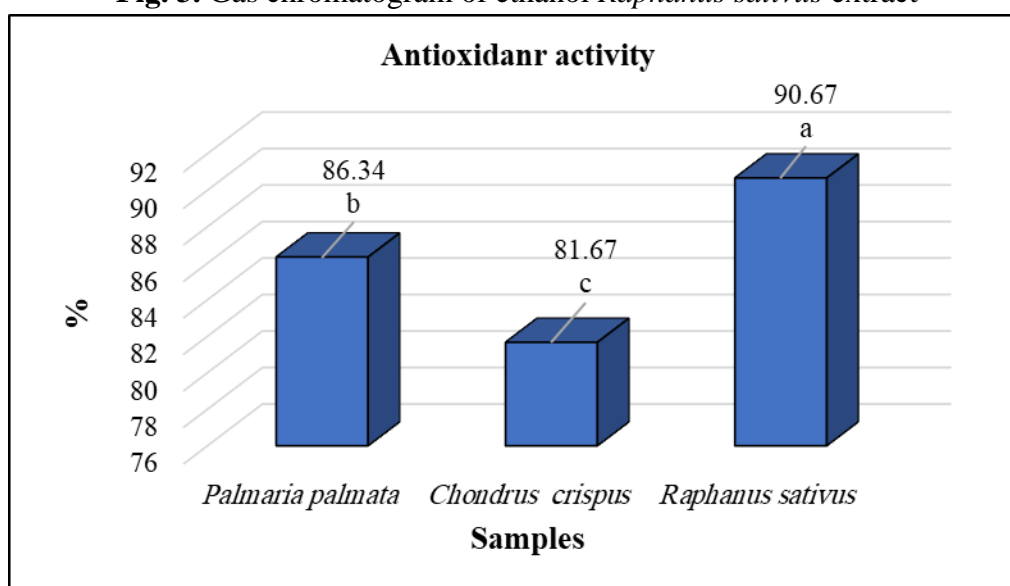


Fig. 4. Antioxidant Activity of *Palmaria palmata*, *Chondrus crispus* and *Raphanus sativus* extracts. ^{a-d} Different letters indicate significant difference ($P < 0.05$)

CONCLUSION

Palmaria, *Chondrus*, and *Raphanus* extracts may possess significant therapeutic potential due to the presence of numerous bioactive medicinal compounds. Identified constituents such as decanal, octanal, benzoic acid, β -myrcene, n-hexadecanoic acid, erythritol, and β -bisabolene have been previously reported to exhibit various pharmacological activities, including antimicrobial, anti-inflammatory, antiviral, antioxidant, anticancer, antidiabetic, hypocholesterolemic, anti-ulcer, and antiparasitic effects. These compounds were identified using the GC-MS technique. Based on the findings of this study, it can be concluded that *Palmaria*, *Chondrus*, and *Raphanus*

extracts contain a variety of bioactive substances and potent antioxidants, supporting their potential use in therapeutic applications.

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