Biological Activity Assessment and GC–MS Screening of Phytoconstituents in Dentella repens Leaf Methanolic Extract

Faiyaz K. Shaikh[1], Shamal D. Talole[2], Sarwan W. Bradosty[3], Raghavendra L.S Hallur[4]*

[1, 2, 4] College of Biosciences and Technology, Pravara Institute of Medical Sciences (Deemed to be University), Loni - 413736, Rahata Taluka, Ahmednagar District, Maharashtra, India

[3] Department of Community Health, College of Health Technology, Cihan University-Erbil, Kurdistan Region, Iraq

Abstract—**Context: Dentella repens (L.) J.R. Forst. & G. Forst., a member of the Rubiaceae family, is renowned for its therapeutic properties.**

Aim: This research pursued to examine the phytoconstituents and assess the biological activities of the *Dentella repens* Leaf Methanolic Extract (DLME).

Material and Methods: In vitro experiments were carried to evaluate its anti-inflammatory, anthelmintic, and antioxidant potential. Qualitative and quantitative analyses of phytochemicals, along with fingerprint analysis using gas chromatography and mass spectroscopy (GC-MS), were performed.

Results: Our findings demonstrated the occurrence of phenolics, glycosides, tannins, steroids, terpenoids, and alkaloids in the methanolic leaf extract of *Dentella repens*. The total phenolic content (TPC) was found to be 0.152 ± 0.012 mg GAE/g dry sample, while the total flavonoid content (TFC) 2.9 ± 0.42 mg rutin equivalent/g dry sample. GC-MS analysis identified sixty-four therapeutic compounds, including 1-amino-2,6-dimethylpiperidine, phytol, and omega-3 arachidonic acid methyl ester. The extract displayed significant anti-inflammatory action (IC50 61.94 g/mL) by inhibiting protein denaturation and remarkable antioxidant action evaluated through DPPH and ABTS assays (IC50 481.82 µg/mL and 46.15 µg/ml, respectively). Regarding antihelmintic activity, earthworms exhibited paralysis after 70–90 minutes and mortality after 110–120 minutes when exposed to the extract.

Conclusions:These outcomes demonstrate the prospective of the DLME in possessing phytoconstituents with anti-inflammatory, antioxidant, and antihelmintic activities. Supplementary research is warranted to elucidate the phytoconstituents of D. repens for potential inclusion in drug formulations targeting various human diseases.

Key-words: Dentella repens, methanolic leaf extract, phytoconstituents, biological activity, GC-MS analysis

Key Messages: DLME exhibits significant anti-inflammatory, antioxidant, and antihelmintic potential, highlighting its therapeutic value and warranting further investigation for drug development.

INTRODUCTION

The majority of pharmaceutical ingredients used to treat numerous human ailments today are derived from plants [1, 2]. Most of these bioactive ingredients are known as natural products or secondary metabolites and include phenolic compounds, flavonoids and alkaloids. Due to the unprecedented abundance of chemical diversity, these natural plant products, while as pure molecules or as standard extracts, offer unlimited prospects for new therapeutic possible leads [3]. These bioactive compounds are effective in treating various human ailments due to their individual, additive, or synergistic health-improving effects [2, 4]. They are safe and have fewer side effects. Numerous plant-derived bioactive components have been demonstrated to have anticancer, antidiabetic, antioxidant, antibacterial, anti-inflammatory, anti-diarrheal, and analgesic properties [5, 6, 7]. The discovery of bioactive ingredients is the first step in the development of novel drugs [2].

Dentella repens (L.) J.R. Forst. & G. Forst. is a terrestrial perennial or annual plant with a worldwide distribution covering the Indian continent, China, Malesia, Australia and America [8]. *D. repens* possesses big leaves, root nodes, a quadrangular stem, and stem stipules. *D. repens* is used in modern medicine, tribal, homeopathic, and veterinary medicine [9]. It is used to treat wound wounds, restricted movement in newborns, blood pressure, blood purification, improving vision and constipation [10, 11]. The molecular composition of the phytoconstituents of D. repens and their effectiveness are poorly understood.

This study aimed to identify phytoconstituents and assess the anti-inflammatory, antioxidant, and antihelmintic properties of the DLME. The molecular composition was determined using GC-MS, a widely utilized analytical technique for conducting chemotaxonomic studies and phytochemical analyses of medicinal plants containing biologically active constituents [12].

DOI: http://doi.org/10.24086/biohs2024/paper.1249

SUBJECTS AND METHODS

Chemicals And Reagents

The study utilized chemical reagent that were all of analytical grade quality.

Plant Leaves Assortment and Identification

The fresh leaves were collected in March 2022 from Konandur village, Tirthahalli taluka in Shivamogga district of Karnataka, India. The latitude of Konandur is 13.81271 and longitude 75.24441 with GPS coordinates 13 48 45.75 N and 75 14 39.87 E. The leaves were identified and authenticated at Shivamogga Karnataka, India (S. R. N. M. N. College of Applied Sciences).

DLME preparation

D. repens leaves were rinse thoroughly with tap water, air-dried at atmospheric temperature, and pulverized using a mixer mill. The solvent extract of the plant was prepared through the maceration process. A quantity of 10 grams of the powder was subjected to maceration in 40-50 mL of methanol for a duration of 24-72 hr. at 28°C. Subsequently, the mixture was filtered using Whatman filter no. 1 paper. The filtrate obtained was then concentrated at atmospheric temperature, and the resulting concentrate was utilized for subsequent investigations.

Qualitative Analysis of Phytochemicals

The DLME was qualitatively tested for the presence of primary and secondary metabolites using the following protocols [13].

1*) Protein*

To ascertain the presence of proteins, a small quantity of the DLME was added to 2 mL of 0.2% ninhydrin solution. The attendance of a purple coloration in the sample aided as an indicator of the existence of proteins.

2*) Carbohydrates*

To assess the presence of carbohydrates, equal volumes of Fehling reagents (A & B) were combined with a small quantity of the DLME. The development of a red precipitate specified the carbohydrates.

3) *Phenol And Tannins*

To check the presence of phenol and tannins, 1% lead acetate solution was added to a small amount of DLME. The yellow & red precipitate indicated the existence of phenols and tannins.

4*) Flavonoids*

To determine the presence of flavonoids, a small amount of the DLME was subjected to the addition of 1–5 drops of concentrated hydrochloric acid (HCl). The formation of a red coloration served as an signals of the presence of flavonoids.

5) *Saponin*

To verify the presence of saponin, a small quantity of the DLME was combined with distilled water (5 ml) and robustly shaken. The occurance of foam (1 cm) suggests existance of saponin.

6) *Alkaloids*

To confirm the presence of alkaloids, a small amount of the DLME was treated with 2 mL of 1% HCl and heated. Following this, a small amount of Mayer's reagent was added to mixture. The resulting precipitate suggests alkaloids in the sample.

7) *Terpenoids*

To determine the presence of terpenoids, a small amount of the DLME was mixed with chloroform (3 mL) and sulfuric acid (2 mL). The appearance of a reddish-brown coloration advocates presence of terpenoids.

Estimation of TPC

The TPC in the DLME was determined using a previously described method with minor modifications [14]. 2mL sodium carbonate (20%), and 0.1 mL DLME (100 g/mL w/v in H2O) were added into the reaction tube containing 2.5 mL Folin-Ciocalteu reagent (1:10 diluted with distilled water). This reaction tube was then incubated for 3 minutes at 28°C and for an additional 1 minute in a boiling water bath. The optical density was recorded at 650 nm. The estimation of TPC was carried out using gallic acid standard curve and expressed as gallic acid equivalent (GAE) per gram of extract.

Estimation of TFC

The TFC of the DLME was assessed colorimetrically using rutin as a standard [15]. Various rutin solutions (0.25–1.5 mg/mL) in methanol were prepared in dissimilar test tubes. Subsequently, 0.3 mL of the DLME (300 µg/mL) was added to each test tube. To each of these test tubes, 75 µL of sodium nitrate (5%), 1.5 mL of methanol, 150 µL of aluminum chloride (10% w/v), 0.5 mL of sodium hydroxide (1 M), and 275 µL of distilled water were mixed separately. All test tubes were then incubated at at 28°C for 30 minutes. The optical density was recorded at 510 nm. The rutin was used as standard and expressed as rutin equivalent (RE), mg/g extract.

Antioxidant Assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay

This assay of DLME was conducted according to a previously described method [16]. Various concentrations of the DLME (with increased phenol concentrations) were incubated with 2 mL of a 4 mg/100 mL DPPH solution (prepared in methanol). The test tubes were incubated for 15 minutes in the dark, and The optical density was recorded at 517 nm. Ascorbic acid was employed as the standard reference antioxidant. The test was performed in triplicate. The following formula was used to calculate percentage (%) of DPPH scavenger inhibition.

$$\text{DPPH scavenging (\%)} = \frac{O.D.Control - O.D.Sample}{O.D\ Control\ (517\ nm)} X\ 100$$

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay.

This assay was conducted following a previously described method with minor modifications in the procedure [17]. The stock solution was prepared by mixing ABTS (7 mM) and potassium persulfate (2.4 mM) in equal parts, followed by

DOI: http://doi.org/10.24086/biohs2024/paper.1249

incubation in the dark at 28°C for 14 hours to allow the reaction. The resulting ABTS working solution was then diluted with distilled water to achieve an optical density 0.706 ± 0.01 at 734 nm. For the assay, 1 mL of the DLME was incubated with 1 mL of the diluted ABTS solution, and the optical density at 734 nm after 7 minutes was recorded. The ABTS scavenging capacity of the DLME was evaluated by comparing with ascorbic acid. Using following formula percentage (%) ABTS radical scavenging was calculated.

$$\text{ABTS radical scavenging (\%)} = \frac{O.D.Control - O.D.Sample}{O.D\ Control\ (734\ nm)} X\ 100$$

Reducing Power Assay

This assay was done using method described previously [18]. The reaction mixture was prepared by combining 0.1 mL of the DLME in phosphate buffer (0.25 mL, 0.2 M, pH 6.6) and potassium ferricyanide (0.25 mL, 1%). The reaction mixture was incubated at 50°C for 20 min. After incubation, trichloroacetic acid (0.25 mL, 10%) was added and reaction mixture were centrifuged for 10 min. at 3000 rpm. The top layer of the reaction mixture (0.25 mL) was recoverd and 0.25 mL of distilled water and 0.05 mL of ferric chloride (0.1%) were mixed. The conversion of Fe^{3+} to Fe^{2+} in the presence of the DLME or standards was observed, and the optical density was recorded at 700 nm.

$$\text{Reducing Power (\%)} = (A\text{s} / A\text{c}) \times 100$$

Where,

*Ac is the absorbance (*optical density*) of the control (L-ascorbic acid), and*

*As is the absorbance(*optical density*) of samples (extracts) or standard.*

Anti-Inflammatory Activity

The in *vitro* anti-inflammatory bioassay was conducted following a previously published protocol [19]. The reaction mixture comprised 500 µl of bovine serum albumin, phosphate-buffered saline (2.8 ml, pH 6.4), and 2 ml of variable concentrations of the DLME (20–100 µg/ml). After incubation for 10 minutes at 28°C, the mixture was heated to 51°C for 15–20 minutes and cooled. The optical density was measured at 660 nm. Using following formula percentage (%) of inhibition of protein denaturation was calculated.

$$\text{\% Inhibition} = \frac{O.D.Control - O.D.Sample}{O.D\ Control\ (660\ nm)} X\ 100$$

Anthelmintic Activity

The anthelmintic activity was carried out on Indian earthworms with an average size of 5 to 6 cm. The weight of an earthworm ranged from 1 to 1.07 g. Albendazole (standard anthelmintic) was used as a standard. The DLME was added at different concentrations (5, 10 and 15 mg/mL in saline). Subsequently, 5-6 worms were introduced into the solution, and the time required for paralysis and death was recorded. The maximum time earlier recorded was 90-110 min. [20].

GC-MS analysis of phytoconstituents

Approximately 20 mg of the DLME was mixed with 10–15 mg of graphitized carbon black to eliminate excess pigments. The mixture was then reconstituted with 1 mL of a suitable solvent (methanol extract in methanol) and vortexed for proper dissolution. Subsequently, the mixture underwent centrifugation at 8000 rpm for 5 minutes, and the supernatant was sieved using a membrane filter (AllPureTM syringe filter, 25 mm diameter, 0.45 µm pore size). The resulting filtrate was stored in amber vials at 4°C for further analysis using GC-MS. For the GC/MS analysis of the DLME, a Shimadzu GCMS-TQ8040 instrument equipped with a RESTEK Rxi-5ms column and an auto-injector (AOC-20i+s) was utilized. Plant extracts (1 µL) were injected in splitless mode at an injection temperature of 280°C and analyzed in scanning mode. The carrier gas flow (He) was set at 1.2 mL/min with a linear velocity of 39.5 cm/s. The column temperature was initially maintained at 40°C for 1 minute, then increased at a rate of 5°C/min to 270°C, and finally held at 270°C for 5 minutes. The transfer line and ion source temperatures were set at 270°C and 220°C, respectively. Ionization was carried out using electron ionization (EI) at 0.94 kV+0.2 kV, and EI mass spectra were documented at 10,000 u/s over the mass range m/z 45–550. The total sample run time was 52 minutes. The system control and data acquisition were managed using Shimadzu's GC-MS solution software. Data processing, including peak selection, peak identification, and peak integration, was performed within the GCMS software application for post-run analysis. Compounds were identified using the NIST library.

STATISTICAL ANALYSIS

The experimental procedures were conducted and analyzed in triplicate to ensure reliability and accuracy of the results. Mean values and standard deviations were calculated for each set of data. Statistical comparisons were done using Microsoft Excel 2013 to assess the significance of the results.

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Results and Discussion

A variety of chemical components with biological and pharmacological effects can be found in medicinal plants. Phytochemical isolation is a common practice in phytochemistry. *D. repens* belongs to the Rubiaceae family and is used medicinally. The scientific literature contains very little information about the bioactive components of this plant that have biological and pharmacological activity.

Qualitative Analysis Of Phytoconstituents

The therapeutic potential of the plant extract can be assessed through preliminary qualitative screening to confirm the presence of bioactive agents. Table 1 presents the results indicating the presence of phytochemicals, including phenols, tannins, flavonoids, and terpenoids, in the DLME.

TABLE 1 PHYTOCONSTITUENTS OF DLME.

Sr. No.	Phytoconstituents	DLME
1	Proteins	-
2	Carbohydrates	+
3	Phenol and Tannins	+
4	Flavonoids	+
5	Saponins	-
6	Alkaloids	+
7	Terpenoids	+

TPC And TFC Content Of DLME

Quantitative spectrophotometric analysis of TPC and TFC revealed a significant amount of phenolics and flavonoids in the DLME. Using the standard curve for gallic acid ($R^2 = 0.9913$) as depicted in Figure 1, the equivalents of the standards for TPC in the DLME were calculated, resulting in a TPC value of 0.152 ± 0.012 mg gallic acid equivalent per gram of dry sample (mg GAE/g dry sample).

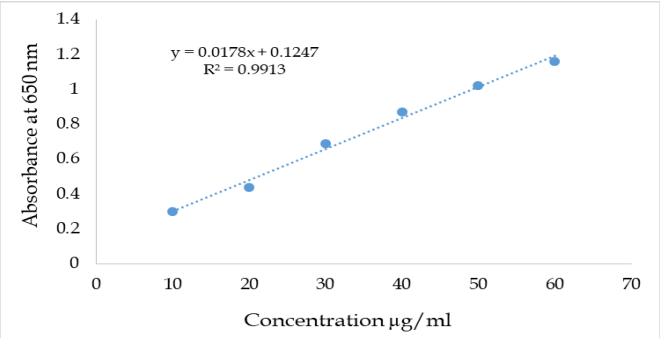

Fig. 1. Regression line for gallic acid.

Based on the standard curve for rutin ($R^2 = 0.9938$) as illustrated in Figure 2, the equivalents of the standards for Total Flavonoid Content (TFC) were calculated, resulting in a TFC value of 2.9 ± 0.42 mg rutin equivalent per gram of dry sample (mg RE/g dry sample).

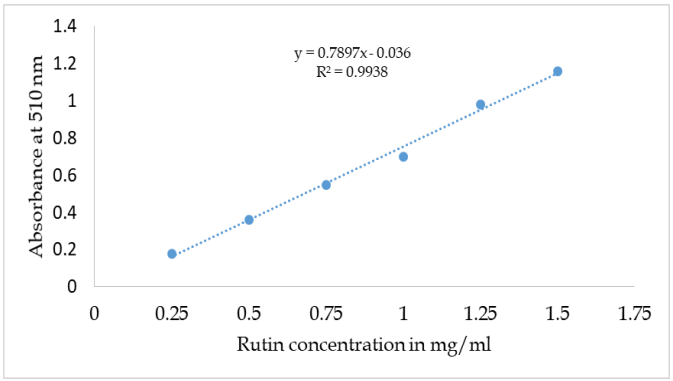

Fig. 2. Regression line for rutin.

Antioxidant Activity

To evaluate the antioxidant abilities of plants, various antioxidant assays with different mechanisms and kinetics have been developed. These tests assess plants in different ways before confirming their antioxidant properties. One such assay is the in vitro DPPH assay, which measures the hydrogen donation or radical scavenging activity of plant extracts. This assay is sensitive and provides a rapid estimation of the antioxidant potential of a specific extract or sample. DPPH, a stable nitrogen-centered dark purple compound, undergoes a color change from purple to yellow upon reduction. The extent of color change depends on the scavenging capabilities of the crude antioxidant extract or an isolated pure compound as it reduces the DPPH radical by releasing hydrogen. Table 2 presents the DPPH scavenging efficacy of the DLME.

TABLE 2 ANTIOXIDANT ACTIVITY (DPPH) OF DLME.

Sr.no.	Concentration µg/ml	% Scavenging activity Mean ± S.D.
1	0	0
2	250	41.30 ± 0.71
3	500	52.80 ± 5.66
4	750	57.85 ± 0.35
5	1000	61.85 ± 5.30
6	1250	65.80 ± 4.25

The IC50 value for the DLME was determined to be 481.82 µg/mL. When compared to a well-known antioxidant, ascorbic acid (IC50 4.42 µg/mL) (Table 3), the antioxidant potential was considered statistically insignificant. The scavenging capabilities of the plant are attributed to the presence of phenolics and flavonoids. [16].

Table 3 Antioxidant activity of ascorbic acid

Sr.no.	Concentration µg/ml	% Scavenging activity Mean ± S.D.
1	0	0
2	2	35.24 ± 3.48
3	4	47.96 ± 7.55
4	6	59.2 ± 5.29
5	8	71.36 ± 5.86
6	10	79.34 ± 0.93

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The DLME exhibited a concentration-dependent ability to scavenge ABTS radicals, as demonstrated by the results presented in Table 4.

TABLE 4 ABTS RADICAL SCAVENGING ACTIVITY OF DLME.

Sr.no.	Concentration µg/ml	% ABTS radical scavenging activity Mean ± S.D.
1	10	35.01 ± 2. 01
2	25	48.01 ± 2.14
3	50	74.27 ± 2.75
4	75	75.73 ± 2.75
5	100	77.02 ± 2.73

The IC_{50} value for ABTS scavenging was determined to be 46.15 µg/ml, while ascorbic acid exhibited a lower IC_{50} value of 29.64 µg/ml, indicating a higher potential for scavenging ABTS radicals (Table 5).

TABLE 5 ABTS RADICAL SCAVENGING ACTIVITY OF ASCORBIC ACID.

Sr.no.	Concentration µg/ml	% ABTS radical scavenging activity Mean ± S.D.
1	20	46 ± 3.48
2	40	54 ± 2.75
3	60	64 ± 2.14
4	80	73 ± 3.40
5	100	77 ± 3.48

The evaluation of antioxidant potential in plant-derived compounds frequently includes the utilization of in vitro reducing power assays. This assay detects the presence of reductants that can exhibit antioxidant activity by disrupting free radical chains complete the provision of a hydrogen atom. In the current study, the DLME exhibited notable reducing power, with an IC50 value of 46.15 µg/mL (Table 6).

TABLE 6 REDUCING POWER POTENTIAL OF DLME.

Concentration (µg/ml)	Optical density 700 nm
Control	1.880
50	1.890
100	1.898
150	1.974
200	2.130
250	2.351

ANTI-INFLAMMATORY AND ANTHELMINTIC ACTIVITY

Previously, a multitude of medicinal plants were discovered to demonstrate anti-inflammatory and antihelmintic properties [20]. The anti-inflammatory activity of the DLME was evaluated using the denaturation of egg albumin. The results presented in Table 7 and Table 8 indicate that the DLME exhibited a noteworthy rate of inhibiting protein denaturation, surpassing the effectiveness of aspirin, which served as a positive control.

TABLE 7 THE PERCENTAGE OF INHIBITION RATE OF PROTEIN DENATURATION USING DLME.

Sr.no.	Concentration µg/ml	Rate of inhibition (%) Mean ± S.D.
1	Control	0
2	20	43.14 ± 6.12
3	40	55.98 ± 5.66
4	60	73.85 ± 5.54
5	80	78.88 ± 1.77
6	100	81.15 ± 2.39

TABLE 8 THE PERCENTAGE OF INHIBITION RATE OF PROTEIN DENATURATION USING ASPIRIN.

Sr.no.	Concentration µg/ml	Rate of inhibition (%) Mean ± S.D.
1	Control	0
2	20	68 ± 5.12
3	40	73 ± 4.16
4	60	80 ± 3.14
5	80	89 ± 5.11
6	100	90 ± 6.90

Furthermore, the anthelmintic activity of the DLME was evaluated and documented in Table 9.

TABLE 9 ANTHELMINTIC ACTIVITY OF DLME

Conc.mg/ml	Paralyzing time (min.)	Death time(min.)
5	70.40 ± 0.67	85.30 ± 0.33
10	59.33 ± 0.55	68.18 ± 0.36
15	50.56 ± 0.23	61.40 ± 0.80

Upon exposure to the DLME, the earthworms experienced a loss of motility. The DLME induced paralysis in a dosage-dependent manner, ranging from a decrease in motility to an absence of response to external stimuli, ultimately leading to death. Additionally, externally visible haemorrhagic and necrotic spots were observed on the worms at higher concentrations (15 mg/ml).

GC-MS analysis of DLME

The GC-MS, a method of analysis, is employed to ascertain and detect active components existing in extracts derived from plants. In today's era, the GC-MS technique assumes a vital role In the analysis of phytoconstituents found in medicinal plants for the purpose of identifying compounds possessing biological activity [2].

According to the mass spectra analysis conducted by GC-MS, it was found that the DLME contained 64 bioactive principles exhibiting diverse phytochemical activities. The chromatogram is depicted in Fig. 3, and the chemical constituents along with their retention time (RT), CAS number, compound name, molecular formula, molecular weight (MW), and area percentage are presented in Table 10.

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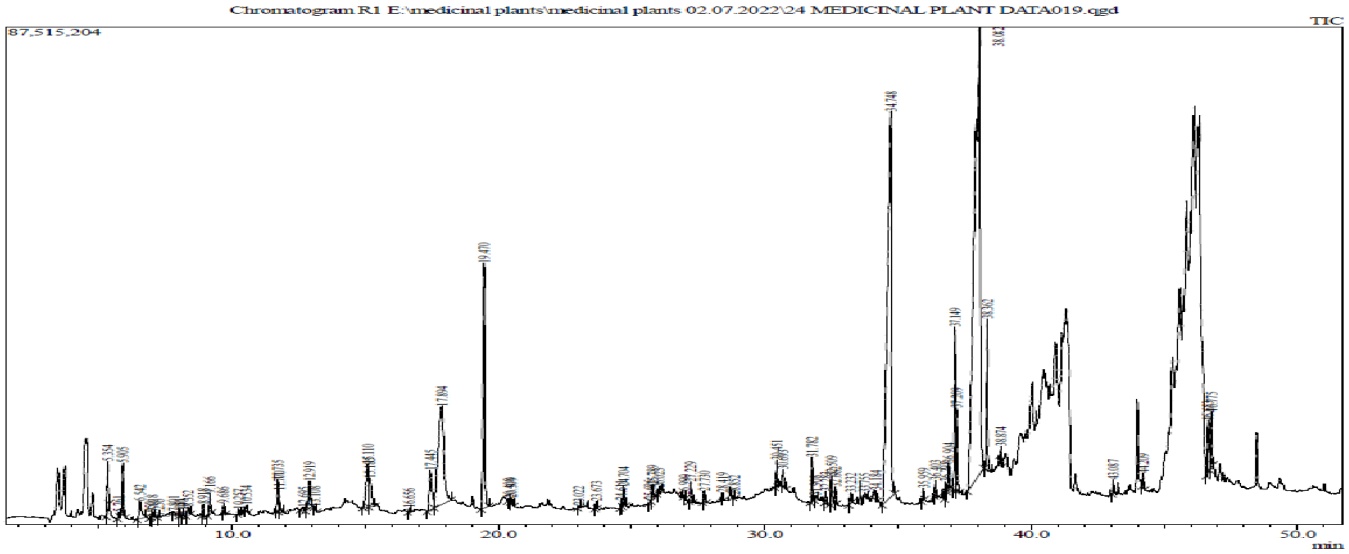

Fig. 3. GC-MS chromatogram of DLME.

The GC-MS analysis revealed the occurrence of various bioactive compounds in the DLME. Some of these compounds include methane, trimethoxy-; furfural; 4-cyclopentene-1,3-dione; 2(1H)-pyridinone, 3-hydroxy-; 2,4-diamino-6-methyl-1,3,5-triazine; 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; 5-hydroxymethylfurfural; pentadecanoic acid; 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien-2, 8-dione; omega-3 arachidonic acid methyl ester, and others. The structures of these components are illustrated in Fig. 4.

TABLE 10 BIOACTIVE COMPOUNDS FOUND IN DLME

R.Time	CAS NO	Name	Molecular Formula	Molecular Weight	Area%	Biological Activity
5.354	149-73-5	Methane, trimethoxy-	C4H10O3	106	0.85	Oxidative and nitrosative stress responses [21]
5.761	822-67-3	2-Cyclohexen-1-ol	C6H10O	98	0.07	Cleaning product,control odours (https://pubchem.ncbi.nlm.nih.gov)
5.905	98-01-1	Furfural	C5H4O2	96	0.79	Antimicrobial [22], insectide and pesticide [23]
6.542	98-00-0	2-Furanmethanol	C5H6O2	98	0.75	Antibacterial activity [24]
6.950	542-92-7	1,3-Cyclopentadiene	C5H6	66	0.01	Manuacture of resins (https://m.chemicalbook.com)
7.018	930-60-9	4-Cyclopentene-1,3-dione	C5H4O2	96	0.11	Antifungal [25]
7.256	696-59-3	Furan, tetrahydro-2,5-dimethoxy-	C6H12O3	132	0.03	Antibacterial [26]
7.801	4164-37-8	Piperazine, 1,4-dinitro-	C4H8N4O4	176	0.11	--
8.051	74646-14-3	1,3,2-Dioxaborolan-4-one, 2-ethyl-5-methyl-	C5H9BO3	128	0.02	--
8.140	59303-05-8	2-Furanmethanethiol, 5-methyl-	C6H8OS	128	0.01	Coffee aroma, coffee flavoring agent [27]
8.352	3008-40-0	1,2-Cyclopentanedione	C5H6O2	98	0.27	Antioxidant activity [28]
8.918	16867-04-2	2(1H)-Pyridinone, 3-hydroxy-	C5H5NO2	111	0.16	--
9.166	620-02-0	2-Furancarboxaldehyde, 5-methyl-	C6H6O2	110	0.41	Preservative, antimicrobial [29]
9.686	10230-62-3	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-on	C6H8O4	144	0.16	Aroma compound, antioxidant, anticancer [30]
10.257	84823-59-6	4S,6R-Dimethyl-7R-hydroxynonan-3-one	C11H22O2	186	0.11	Antineuoplastic activity, Treatment of cancer (https://pubchem.ncbi.nlm.nih.gov)
11.735	39135-39-2	1-Amino-2,6-dimethylpiperidine	C7H16N2	128	0.64	Anti-inflammatory, antimicrobial, local anaesthetic
11.770	542-02-9	2,4-Diamino-6-methyl-1,3,5-triazine	C4H7N5	125	0.37	

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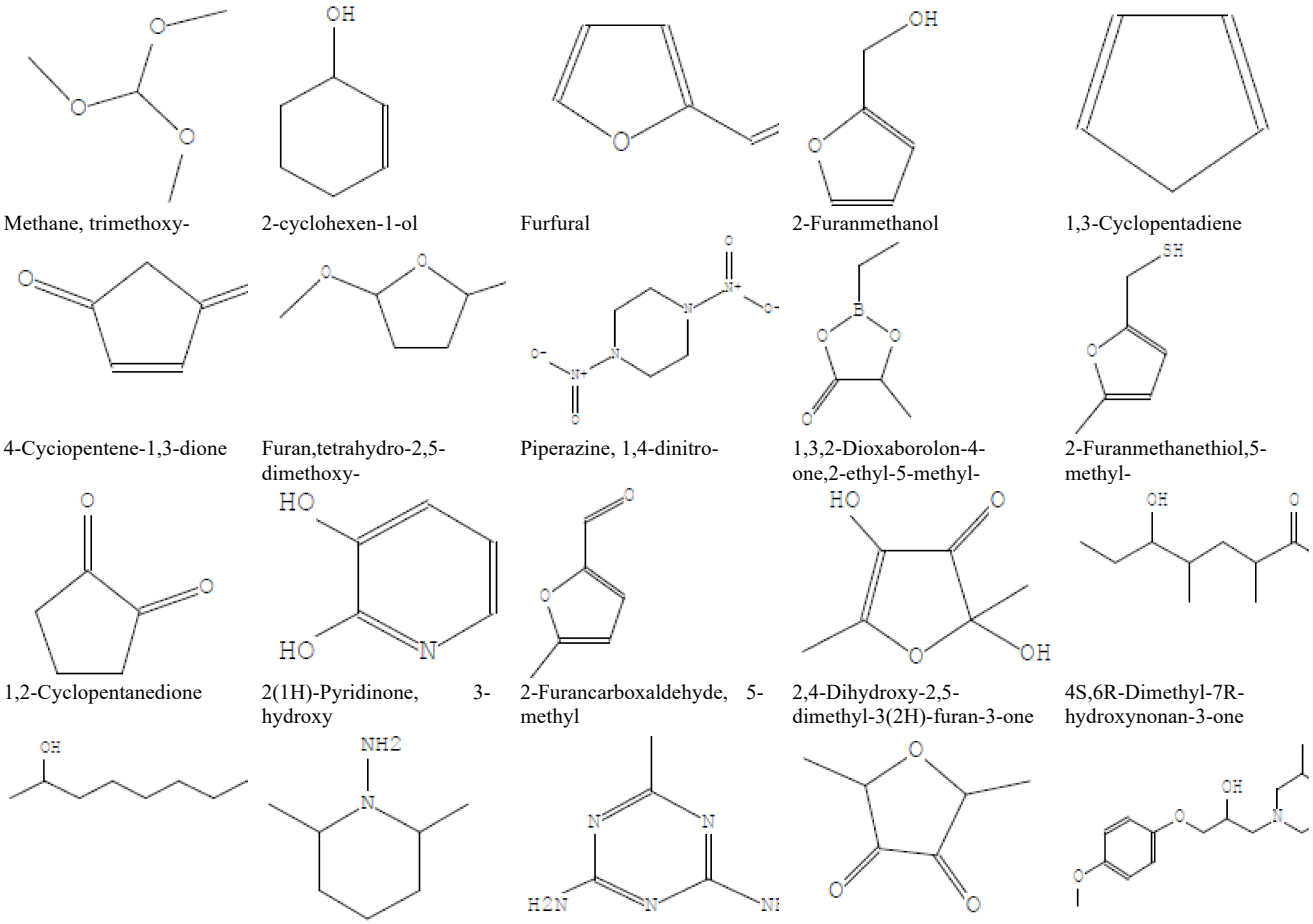
12.695	68755-49-7	2,5-Dimethylfuran-3,4(2H,5H)-dione	C6H8O3	128	0.11	Flavouring for foods, beverages and cosmetics
13.108	93-58-3	Benzoic acid, methyl ester	C8H8O2	136	0.04	Antiplatelet aggregating, antiviral, antioxidant [22]
15.110		2,2-Diisopropyl-1,3-dioxolane	C9H18O2	158	1.54	Anticancer drug screen (https://pubchem.ncbi.nlm.nih.gov)
15.185	28564-83-2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C6H8O4	144	1.10	Antimicrobial, antiinflammatory [29]
16.656	101-48-4	Benzene, (2,2-dimethoxyethyl)-	C10H14O2	166	0.06	Air freshener, flavoring agent (https://pubchem.ncbi.nlm.nih.gov)
17.445	496-16-2	Benzofuran, 2,3-dihydro-	C8H8O	120	1.64	Nuroinflammation
17.894	67-47-0	5-Hydroxymethylfurfural	C6H6O3	126	8.00	Antioxidant, antiproliferative [22]
19.470	7786-61-0	2-Methoxy-4-vinylphenol	C9H10O2	150	5.74	Antimicrobial, antioxidant, Analgesic [29]
20.408	91-10-1	Phenol, 2,6-dimethoxy-	C8H10O3	154	0.05	Antimicrobial and production of mecralptans [26]
20.509		Naphthalene, 1,2-dihydro-1,1,6-trimethyl-			0.09	--
23.022	5932-68-3	trans-Isoeugenol	C10H12O2	164	0.03	As an Allergent, plant metabolite (https://www.ebi.ac.uk)
23.673	1203-08-3	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-	C13H18O	190	0.10	Antibacterial
24.611	96-76-4	2,4-Di-tert-butylphenol	C14H22O	206	0.10	Anti-inflammatory, antimicrobial, antioxidant
24.704	18256-48-9	2,4'-Dihydroxy-3'-methoxyacetophenone	C9H10O4	182	0.37	Anticancer agent, antioxidant (https://pubchem.ncbi.nlm.nih.gov)
25.686	38818-55-2	Megastigmatrienone	C13H18O	190	0.03	Aroma
25.789	39151-19-4	3',5'-Dimethoxyacetophenone	C10H12O3	180	0.21	Antioxidant (https://www.medchemexpress.com)
26.023	638-53-9	Tridecanoic acid	C13H26O2	214	0.09	Antimicrobial, antioxidant, antiinflammatory
26.990	102488-09-5	3-Hydroxy-.beta.-damascone	C13H20O2	208	0.04	Anti-inflammatory, dietary sources in animal model
27.730	34318-21-3	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-	C13H20O2	208	0.24	Antifungal agent
28.419	39900-12-4	1,3-Butanedione, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)	C13H20O2	208	0.08	--
28.852	7320-37-8	Oxirane, tetradecyl-	C16H32O	240	0.04	Antimicrobial [25]
30.451	544-63-8	Tetradecanoic acid	C14H28O2	228	0.44	Cancer preventive, antioxidant
30.693		4-(2,4-Dimethylcyclohex-3-enyl)but-3-en-2-on			0.38	--
31.782	61886-62-2	3-Hexadecyne	C16H30	222	0.63	--
31.908		1-Dodecanol, 3,7,11-trimethyl-			0.13	--
32.283	0-00-0	Isophthalic acid, 3,7-dimethyloct-6-enyl tridecyl ester	C31H5PO4	486	0.16	Anti-cancer
32.509	1002-84-2	Pentadecanoic acid	C15H3PO2	242	0.33	Milk fat, fatty acid [30]
32.662	71899-38-2	9-Eicosyne	C20H38	278	0.22	Antimicrobial and cytotoxic properties
33.232	82304-66-3	7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-dien-2, 8-dione	C17H24O3	276	0.17	Antioxidant
34.184	84-74-2	Dibutyl phthalate	C16H22O4	278	0.08	Antifungal, antimicrobial, antimalarial agent
34.748	1002-84-2	Pentadecanoic acid	C15H30O2	242	21.35	Milk fat, fatty acid [30]
35.959	7561-64-0	7,10,13-Hexadecatrienoic acid, (Z,Z,Z)-	C16H26O2	250	0.23	Antioxidant, anti inflammatory, antimicrobial
36.798	13038-47-6	9,11-Octadecadienoic acid, methyl ester, (E,E)	C19H34O2	294	0.17	Antineoplastic agent, anti-inflammatory, antiatherogenic

DOI: http://doi.org/10.24086/biohs2024/paper.1249

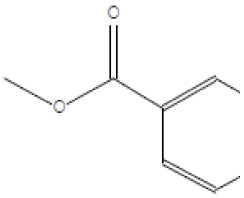
						agent(https://www.ebi.ac.uk/chebi/searchid.do?chebild=cheb1884464)
36.904	463-40-1	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C18H30O2	278	0.44	Antieczemic, anticoronary, antiarthritic
37.149	150-86-7	Phytol	C20H40O	296	2.84	Antimicrobial, anticancer
37.209	954-07-4	9,10-Anthracenedione, 1-methyl-	C15H10O2	222	1.44	Dyes medicinal importance
38.082	1783-84-2	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	C20H34O2	306	38.76	Cardio protective
38.362	57-11-4	Octadecanoic acid	C18H36O2	284	3.01	Antiviral, anti inflammatory, Hypocholesterolemic (Uraku)
38.874	39008-90-7	1-Hydroxy-4-methylanthraquinone	C15H10O3	238	0.20	Colouring agent
43.087	0-00-0	4-Butylbenzoic acid, 2-dimethylaminoethyl ester	C15H23NO2	249	0.20	Antioxidant, alkyl resins (https://www.dsnchem.com/ptbba/china)
44.209	0-00-0	Phthalic acid, octyl 2-propylpentyl ester	C24H38O4	390	0.20	Antioxidant and larvicidal activity [22]
46.653	54766-91-5	Bicyclo[10.1.0]tridec-1-ene	C13H22	178	1.12	Hair regenerative mechanisms ((https://pubchem.ncbi.nlm.nih.gov)
46.775	132712-70-0	omega.-3 Arachidonic Acid methyl ester	C21H34O2	318	1.17	Anti inflammatory, antiallergic activity

Among the identified bioactive principles, those with have highest percent peak area (38.76 %) were 8,11,14-Eicosatrienoic acid, (Z, Z, Z). This compound has cardioprotective activity [30]. Other compounds were 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 5 Hydroxymethylfurfural, 2-methoxy-4-vinylphenol, octadecanoic acid, 1-amino-2,6- dimethylpiperidine, methane, trimethoxy are known to have anti-inflammatory and antioxidant activities [22, 24, 29, 30].

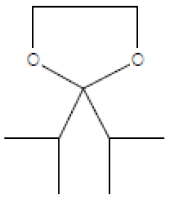


DOI: http://doi.org/10.24086/biohs2024/paper.1249

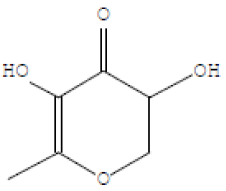
1,7-Octanediol



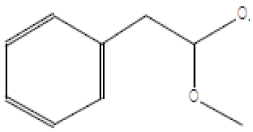
1-Amino-2,6-dimethylpiperidine



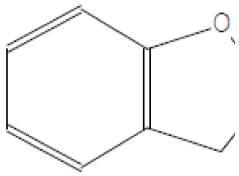
2,4-Diamino-6-methyl-1,3,5-triazine



2,5-Dimethylfuran-3,4(2H,5H)-dione



2-Propanol, 1-(4-methoxyphenoxy)-3-(2,6-dimethyl-4-morpholyl)-



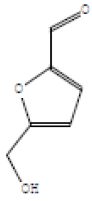
Benzoic acid, methyl ester

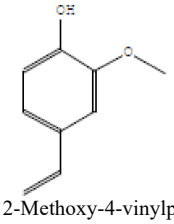
2,2-Diisopropyl-1,3-dioxolane

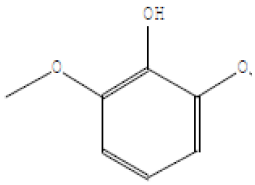
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl

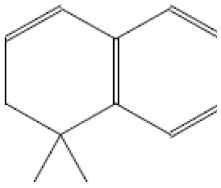
Benzene, (2,2-dimethoxyethyl)-

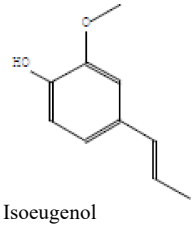
Benzofuran, 2,3-dihydro











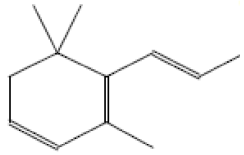
5-Hydroxymethylfurfural

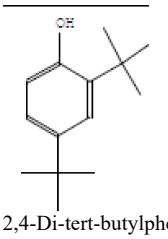
2-Methoxy-4-vinylphenol

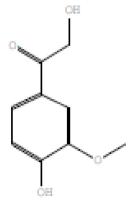
Phenol, 2,6-dimethoxy

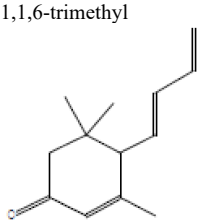
Naphthalene, 1,2-dihydro-1,1,6-trimethyl

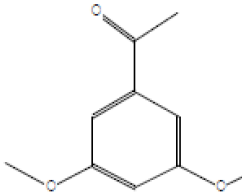
Isoeugenol











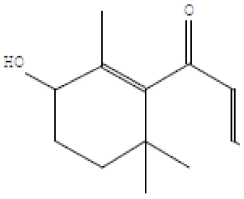
4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one

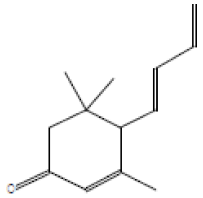
2,4-Di-tert-butylphenol

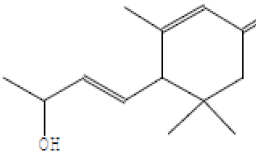
2,4'-Dihydroxy-3'-methoxyacetophenone

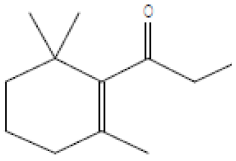
Megastigmatrienone

3',5'-Dimethoxyacetophenone









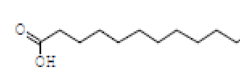
Tridecanoic acid

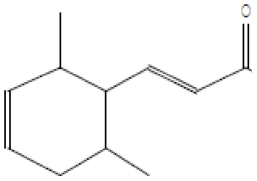
3-Hydroxy-.beta.-damascone

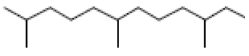
Megastigmatrienone

2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl

1,3-Butanedione, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-







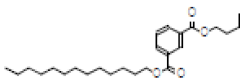
Oxirane, tetradecyl

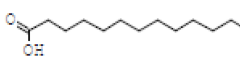
Tetradecanoic acid

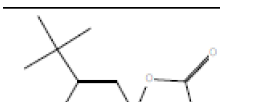
4-(2,4-Dimethylcyclohex-3-enyl)but-3-en-2-one

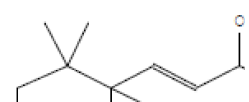
3-Hexadecyne

1-Dodecanol, 3,7,11-trimethyl









Isophthalic acid, 3,7-dimethyloct-6-enyl tridecyl ester

Pentadecanoic acid

9-Eicosyne

7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione

6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-2-ol

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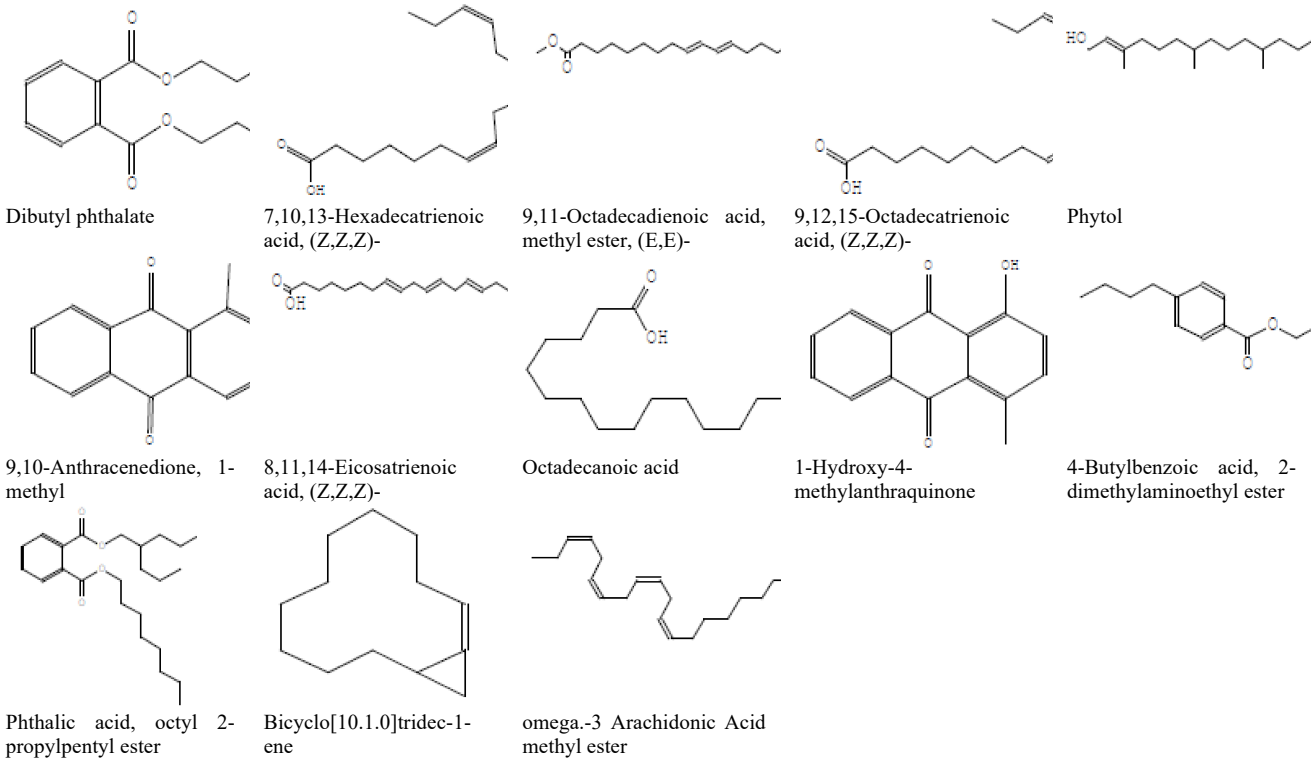

Fig. 4. Structure of Bioactive compounds found in DLME.

Conclusion

In conclusion, our study aimed to explore the bioactive compounds and evaluate the biological properties of the DLME. Our results confirmed the presence of phenols, tannins, flavonoids, glycosides, steroids, and terpenoids. Furthermore, quantitative spectrophotometric analysis revealed a significant amount of phenolics and flavonoids. The antioxidant capacity was validated through in vitro DPPH assay, ABTS assay, and reducing power assay. Additionally, the extract demonstrated anti-inflammatory and antihelmintic activities. GC-MS analysis identified 64 bioactive principles with diverse phytochemical activities, many of which are known for their antioxidant and anti-inflammatory properties. These outcomes suggest that the DLME contains bioactive compounds deserving further investigation for the development of drugs targeting antioxidant, anti-inflammatory, and antihelmintic effects in the treatment of various human ailments.

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Abbreviations

D. repens: *Dentella repens*, DLME: *Dentella repens* Leaf Methanolic Extract. HCl: Hydrochloric acid, TPC: Total phenolic content, GAE: Gallic acid equivalent, TFC: Total flavonoid content, RE: Rutin equivalent, GC-MS: Gas Chromatography/mass spectrometry, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), mM: Millimolar, O.D.: Optical Density, K3Fe(CN)6: Potassium ferricyanide, IC50: Half maximal inhibitory concentration

Acknowledgements

The authors would like to express their gratitude to the authorities of Pravara Institute of Medical Sciences, Loni, Ahmednagar (MS), India, for their financial support.