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CHARACTERIZATION AND EVALUATION OF ANTIOXIDANT ACTIVITY OF *OCIMUM CANUM* LEAVES AND ITS EFFECIENCY ON *SCHISTOSOMA MANSONI* LARVAL STAGE

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ABSTRACT

Schistosomiasis is the most endemic disease caused by the genus *Schistosoma*. Its control is dependent on killing the worm itself or one of its larval stages. The objective of this study is to determine the total phenolic and flavonoid contents, evaluating the antioxidant activity of *Ocimum canum* leaf methanol extract by DPPH and ABTS as well as investigating the efficiency of plant extract against *Schistosoma mansoni* larval stages. Also, GC-MS analysis of plant extract was carried out. The results revealed that *Ocimum canum* leaf methanol extract showed high total phenolic content (321.78±0.69 mg GAE/g extract) and flavonoid content (71.64±10.66 mg RE/g extract). Furthermore, there is a significant correlation between the concentration of the extract and the inhibition percentage of the free radicals. The higher inhibition percentage of plant extract was recorded by DPPH than ABTS at 500µg/ml (84% and 62%) respectively. Also, IC_{50} of DPPH and ABTS (26.41±1.39 and 194.45±2.03) respectively. Regarding to ceracricidal and miracidicidal activity, the plant extract showed larvicidal activity as it killed 82% of cercariae while, it was completely lethal to miracidia at 500ppm within 2 hours. On the other hand, 36 chemical compounds were identified from plant extract. The main components are Phytol (23%), Hexadecanoic acid, methyl ester (15.48%), 9,12,15-Octadecatrienoic acid methyl ester (14.99%), n-Hexadecanoic acid (7.62%), 9,12,15-Octadecatrienoic acid (5.04%), Ethyl linoleate (3.58%) and 2-Propenoic acid, 3-phenyl-, methyl ester (3.17%). This study suggested that *Ocimum canum* can be used as promising plant to control schistosomiasis disease as well as its potential antioxidant activity.

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INTRODUCTION

Schistosomiasis is the most fatal disease of mammals especially human beings. The cause of it is a blood fluke of the genus *Schistosoma* [1, 2, 3]. It has been reported as the second parasitic disease causes death and mortality after malaria [4, 5, 6].

It is a generic health problem in many developing countries. It was estimated that about "200 million people are infected with schistosoma, 600 million people are at the risk of infection" [7, 8, 9, 10]. In Africa, about 80% of people are infected with it [11, 12]. Furthermore, it is a major problem in some Arab countries as, Sudan, Yemen, Saudi Arabia and Iraq [2, 13].

Although, there are several species of *schistosoma*, but all of them have the same life cycle; adult male and female schistosomes still in mesenteric vein where they live there, eggs are excreted through faces into the fresh water environment then hatch to miracidia that penetrate the intermediate host (snail) appropriate to *Schistosoma* sp., after three or four weeks cercariae (the infected stage) developed from miracidium inside the snail and swim freely in water to penetrate skin and repeat the life cycle [3, 14]. As reported by Guo *et* al. [2] that more than 98% of cercariae float on the surface of water. Controlling of Schistosomiasis is urgent so, interruption of its life cycle which involves snails, miracidia and cercariae is the most promising method to prevent transmission of this disease [2, 11].

Since, several chemical compounds are lethal to miracidia and cercariae but they are toxic and have side effects on the environment [2, 6, 15]. So, alternative miracidicidal and cercaricidal from natural sources as plants are safer, cheaper and less dangerous to the environment [2, 16, 17].

Ocimum canum belongs to family Lamiaceae, commonly known as (Kala Tulsi). It is medicinal plant its branches out from its base. It has angle stems and open foliage. The plant has pungent and aromatic flavor. *Ocimum canum* treats various kinds of diseases as it used as traditional medicines. It lowers blood glucose, treats cold and fever [18] (Ngassoum et al. 2004). Also, it has anti-parasitic, anti-inflammation, insecticidal properties and anti-malarial activity [19, 20, 21, 22].

Bioactive compounds from essential oils of *Ocimum canum* leaves showed larvicidal activity against *Aedes aegyptia* and *Anopheles gambiae* mosquitoes larvae [20]. From this connection, our survey has been planned to investigate the efficacy of methanol extract of *Ocimum canum* leaves on cercariaea and miracidia. Therefore, studying the relationship between the antioxidant activity of the plant and its larvicidal activity on *Schistosoma mansoni* larval stages (cercariae and miracidia). Also, its bioactive compounds were identified by GC-MS analysis.

MATERIALS AND METHODS

Materials:

Chemical Materials:

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical, gallic acid, rutin, ascorbic acid and Folin-Ciocalteau reagent were obtained from Sigma-USA. sodium hydrogen phosphate and Potassium persulphate, sodium nitrite, sodium hydroxide, sodium bicarbonate and aluminum chloride were purchased from Merck (Germany).

Biological Materials:

Leaves of *Ocimum canum* were collected from gardens in Giza government, Egypt and identified by Professor El-Sayed Hassan Hussien Shaban, Head of Aromatic and Medicinal Plant Research Department, Agriculture Research Centre. The leaves shade dried and ground into fine powder then stored for extraction. Cercareae and miracidia were purchased from Schistosome Biological Supply Center, Theodor Bilharz Research Institute, Giza, Egypt.

Methods:

Extraction of plant materials:

300 gm of dried *Ocimum canum* leaves were soaked in 90% methanol for one week then, the extract was filtered several times through Whatman No.1 filter paper. After that, it was concentrated through Buchi Rotatory evaporator at 40^0C to remove methanol completely. Finally, the concentrated dried methanolic extract was kept in Medicinal Chemistry department, Theodor Bilharz Research Institute for more studies.

GC-MS Analysis:

The methanol extract of *Ocimum canum* was subjected to GC-MS analysis using Thermo Scientific TRACE 1310 Series Gas Chromatograph on Helium as a carrier gas in TG-SQC column. Analysis of extract was held on temperature program as follows: initial temperature 50^oC for 1min then increased to 250^oC for 5 min, finally increased to 290^oC for 2min. The sample was injected in split mode constant flow 1.5ml/min. Mass spectral range was set as 40-1000Hz also, mass transfer line temperature was 300^oC and ion source temperature was 300^oC then identification of components was carried out by using their MS data compared to the NIST mass spectral library.

Total Phenolic Content:

The phenolic content of the methanolic extract of *Ocimum canum* leaves was determined using a spectrophotometric method described by [23]. 0.5ml of the extract (250 µg/mL); 2.5ml of Folin- Ciocalteus reagent (10 %) dissolved in water and 2.5ml NaHCO3 (7.5%). Blank sample contains 0.5ml MeOH, 2.5ml of Folin-Ciocalteus reagent (10 %) dissolved in water and 2.5ml NaHCO3 (7.5%). Gallic acid was used as the standard contains 2.5ml gallic acid (200 µg/ml), 2.5ml of Folin-Ciocalteus reagent (10 %) and 2.5ml of NaHCO3 (7.5%). All mixtures were shaken and incubated at 45°C for 45 min. the absorbance was recorded at 765 nm against a blank sample. The experiment was carried out in triplicate. The total phenolic content was expressed in mg gallic acid equivalent (GAE) per gram dry weight of the extract (mg GAE/gm dry extract).

Total Flavonoid Content:

The content of flavonoids of methanolic extract of *Ocimum canum* leaves was determined using a colorimetric assay reported by [24]. 0.5 ml of the extract was mixed with 2ml distilled water and 150µl of NaNO2 (5%) for 6 min, then 150µl of AlCl3 (10%) was added and allow to stand for 5 min then added of 2ml of NaOH (4%) and adjusted to 5ml with 200µl distilled water. Blank sample contains 0.5ml MeOH instead of extract and rutin was used as standard. The mixture was incubated at room temperature for 15 min. the absorbance was measured at 510 nm against a blank sample. The experiment was carried out in triplicate. The total flavonoid content was estimated as mg rutin equivalents (RE) per gram extract (mg RE/g of extract).

Antioxidant Activity:

DPPH Radical Scavenging Activity:

The antioxidant activity of *Ocimum canum* methanolic extract was evaluated using DPPH free radical scavenging method according to [25]. Various concentrations of the extract from 5-500 µg/ml were prepared. 2 ml of each extract concentration was mixed with 2ml of DPPH in MeOH (0.1 mM/l). The control contained solvent and DPPH without extract. The mixtures were shaken well and kept in dark for 30 min at 37˚C.The absorbance was measured at 517nm. Ascorbic acid was used as standard. The experiment was carried out in triplicate. The DPPH scavenging % of the plant extract was calculated from this equation.

$$\textbf{Scavenging activity \%} = [(A_{control} - A_{sample}) / (A_{control})] \times 100$$

Where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of sample. Data were expressed as IC_{50}. The lower IC_{50} value is an indication of more powerful antioxidant activity.

ABTS Scavenging Activity:

The plant extracts can able to quench $ABTS^{\cdot+}$ (2-2`azinobis (3-ethylbenzthiazoline-6-sulphonic acid) in comparison to Trolox® [26]. ABTS assay was evaluated according to [27] with some modifications. The concentrated reagent solution was prepared by dissolving 9.6 mg ABTS in 2.5ml water(final concentration 7mM) and then adding 110µl of a solution made by dissolving 37.5 mg of potassium persulphate (K_2S_2O8) in 1ml of water (final concentration 2.45mM) to produce $ABTS^{\cdot+}$ radical cation. The stock solution was kept in the dark room for 12-16 hours before use; for study the $ABTS^{\cdot+}$ solution was diluted to an absorbance value 0.700±0.020 at wavelength 734 nm. Subsequently, 100µl of aqueous or alcoholic plant extract (according to solubility) with various concentrations from 5-500 µg/ml was added to 1ml of work solution, and it was measured exactly after 2.5 min. Also, an appropriate solvent blank was measured. $ABTS^{\cdot+}$ + methanol are used as control. The experiment was carried out in triplicates. Results were expressed in terms of mm Trolox® equivalent per 100 g dry weight of plant extract. The ABTS scavenging % was calculated from the following equation.

$$\textbf{Scavenging activity \%} = [(A_{control} - A_{sample}) / (A_{control})] \times 100$$

Where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of sample. Data were expressed as IC_{50}. The lower IC_{50} value is an indication of more powerful antioxidant activity.

Miracidicidal Assay:

Schistosoma mansoni ova were diluted with dechlorinated tap water and exposed to direct light for about 15 minutes to allow it to hatch into miracidia. Miracidia test was carried out in laboratory Petri dishes. 100µl of miracidia were placed in 1 ml of dechlorinated water in petri dish. Three concentrations (100, 300, 500ppm) were dissolved in dechlorinated water and were added, giving total volume 2 ml in each experimental dish. The experiment was carried out in three replicates for each concentration. 100 µl of miracidia were kept in 2ml of dechlorinated water as control. Mortality of miracidia was recorded by the absence of movement at 30, 60, 90, 120min respectively and then, a fixative (bouin) was added and the total number of miracidia in each dish was determined.

Cercaricidal Activity:

Schistosoma mansoni cercariae test was carried out in laboratory petri dishes. 100µl of cercariae were placed in 1ml of dechlorinated water in Petri dish. Three concentrations (100, 300, 500, 600ppm) were dissolved in dechlorinated water and were added, giving total volume 2ml in each experimental dish. The experiment was carried out in three replicates for each concentration. 100µl of cercariae were kept in 2ml of dechlorinated water as control. Movement and mortality of cercariae were recorded at 30, 60, 90, 120min respectively and then, a fixative (bouin) was added and the total number of cercariae in each dish was determined.

RESULTS AND DISCUSSION

GC-MS Analysis:

Medicinal plants are known as sources of active compounds that are widely sought after worldwide for their natural properties. They have been used since ancient times. Plants secondary metabolites have recently been referred to as phytochemicals. Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities [28].

Table 1: phytochemical compounds identified in methanolic extract of *Ocimum canum* leaves:

NO.	RT	Compound Name	Area %	Molecular weight.	Molecular Formula	Pharmacological actions
1	10.32	4-Hydroxy-4-methyl-4H-naphth alen-1-one	0.4	174	$C_{11}H_{10}O_2$	----------
2	11.5	2-Propenoic acid, 3-phenyl-, methyl ester	1.45	162	$C_{10}H_{10}O_2$	Anti-cancer agents
3	13.97	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-c yclohexen-1-yl)	0.37	206	$C_{14}H_{22}O$	Antimicrobial properties
4	14.26	(+) spathulenol	1.2	220	$C_{15}H_{24}O$	Sedative &anesthetic activities
5	14.49	Cubedol	0.49	222	$C_{15}H_{26}O$	----------
6	14.84	7-epi-cis-sesquisabinene hydrate	0.28	222	$C_{15}H_{26}O$	----------
7	15.29	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6 -dimethyl-4-(1 methylethyl)	3.06	222	$C_{15}H_{26}O$	Antimicrobial &antioxidant
8	15.43	Methyl arachidonate	0.39	318	$C_{21}H_{34}O_2$	control of vascular reactivity and systemic blood pressures.
9	16.81	13,16-Octadecadiynoic acid, methyl ester	0.53	290	$C_{19}H_{30}O_2$	----------
10	17.98	Tetradecanoic acid	0.28	228	$C_{14}H_{28}O_2$	Larvicidal and repellent Activity
11	18.95	Neophytadiene	1.87	278	$C_{20}H_{38}$	antipyretic, analgesic, and anti-inflammatory, antimicrobial, antioxidant
12	19.13	2-Pentadecanone, 6,10,14-trimethyl	1.77	268	$C_{18}H_{36}O$	allelopathic activity
13	19.43	3,7,11,15-Tetramethyl-2-hexade cen-1-ol	0.71	296	$C_{20}H_{40}O$	Antimicrobial Antiinflammatory
14	19.77	17-Octadecynoic acid	1.12	280	$C_{18}H_{32}O_2$	antibacterial, antimalarial and antifungal activity
15	20.74	Hexadecanoic acid, methyl ester	15.48	270	$C_{17}H_{34}O_2$	Antibacterial and Antifungal
16	21.42	Isochiapin	0.24	346	$C_{19}H_{22}O_6$	Treat arthritis, tonsillitis in Chinese medicine
17	21.97	n-Hexadecanoic acid	7.62	256	$C_{16}H_{32}O_2$	Antioxidant,Hypocholesterolemic Nematicide, Pesticide,Lubricant, Antiandrogenic, Flavor, Hemolytic, inflammatory,
18	23.07	Cholestan-3-ol, 2-methylene	0.59	400	$C_{28}H_{48}O$	Anti-inflammatory, Anti-inflammatory and cytotoxic activities
19	23.24	7-Methyl-Z-tetradecen-1-ol acetate	0.28	268	$C_{17}H_{32}O_2$	Anti cancer, antiinflammatory, hepatoprotective
20	23.76	Ethyl linoleate	3.58	308	$C_{20}H_{36}O_2$	Antioxidant
21	23.92	9,12,15-Octadecatrienoic acid, methyl ester	14.99	292	$C_{19}H_{32}O_2$	Anti-inflammatory, antatherogenic catalase activator Antiin-flammatory,Hypocholesterolemic, Cancer preventive,Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic,
22	24.03	Hexadecadienoic acid, methyl ester	0.11	266	$C_{17}H_{30}O_2$	Anti-oxidant, decrease blood cholesterol, anti-inflammatory Antioxidant, Hypocholesterolemic

NO.	RT	Compound Name	Area %	Molecular weight.	Molecular Formula	Pharmacological actions
						Nematicide, Pesticide,Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
23	24.18	Phytol	23.48	296	$C_{20}H_{40}O$	Antimicrobial, anticancer, anti-inflammatory, anti-diuretic, immunostimulatory and anti-diabetic Antioxidant Diuretic
24	24.38	Methyl stearate	1.32	298	$C_{19}H_{38}O_2$	Anti-diarrheal, cytotoxic and antiproliferative activity
25	24.62	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	0.49	250	$C_{16}H_{26}O_2$	----------
26	25.03	9,12,15-Octadecatrienoic acid	5.04	278	$C_{18}H_{30}O_2$	Antialgesic, antipyretic ,Anticonvulsant & Antiseptic
27	25.34	Oleic Acid Oleic Acid	0.77	282	$C_{18}H_{34}O_2$	Cancer preventive, Anemiagenic, Insectifuge, Antiandrogenic, Dermatitigenic.
28	25.94	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	1.17	312	$C_{20}H_{40}O_2$	--------
29	26.70	1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl	1.23	173	$C_5H_8ClN_5$	antitumor properties to treat lung breast and ovarian cancer, respectively
30	27.73	2-Propenoic acid, 3-phenyl-, methyl ester	3.17	162	$C_{10}H_{10}O_2$	Antifungal
31	27.86	Alanine, N-acetyl-3-phenyl-N-(trifluoro acetyl)-, methyl ester	2.55	317	$C_{14}H_{14}F_3N_{O4}$	Antifungal
32	28.24	Aspartame	0.17	294	$C_{14}H_{18}N_2O_5$	Artificial low Calorie sweetener
33	29.19	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	1.51	340	$C_{23}H_{32}O_2$	retroviral infections
34	31.13	1,2Benzenedicarboxylic acid, dioctyl ester	1.3	390	$C_{24}H_{38}O_4$	Antibacterial Activity Antimicrobial, antifungal, anti-malarial
35	38.94	Dotriacontane	0.38	450	$C_{32}H_{66}$	----------
36	43.01	Quercetin 7,3',4'-Trimethoxy	0.62	344	$C_{18}H_{16}O_7$	promoted for prevention and treatment of cancer

The components present in the methanol extract of leaves of *Ocimum canum* were identified by GC-MS analysis (Fig. 1). Compounds with their retention time (RT), molecular formula, molecular weight (MW), concentration (%) and their biological activities in the methanol extract of leaves of *Ocimum canum* are listed in Table 1.

In the present study, 36 compounds have been identified from methanol extract of *Ocimum canum* leaves by GC-MS analysis. The results revealed that the major components are: Phytol (23%) which is an acyclic terpenoid that has a promising effect against *Schistosoma mansoni* even in its different classes [29, 30], Hexadecanoic acid, methyl ester (15.48%), 9,12,15-Octadecatrienoic acid methyl ester(14.99%), n-Hexadecanoic acid (7.62%),9,12,15-Octadecatrienoic acid(5.04%), Ethyl linoleate (3.58%) and 2-Propenoic acid, 3-phenyl-, methyl ester (3.17%).

These compounds are sited in literature with their antimicrobial, antifungal, antioxidant, anticancer, anti-inflammatory, hypo-cholesterolemic properties, anti-diuretic, antibacterial, antifungal, nematicide, pesticide, lubricant, anti-androgenic, hemolytic and larvicidal activity [31, 32, 33, 34].

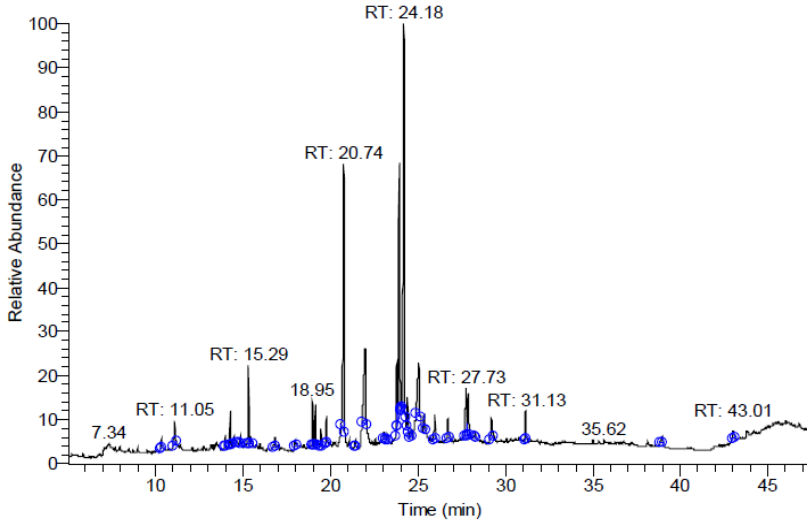


Fig (1): Total GC-MS analysis of *Ocimum canum* leaves.

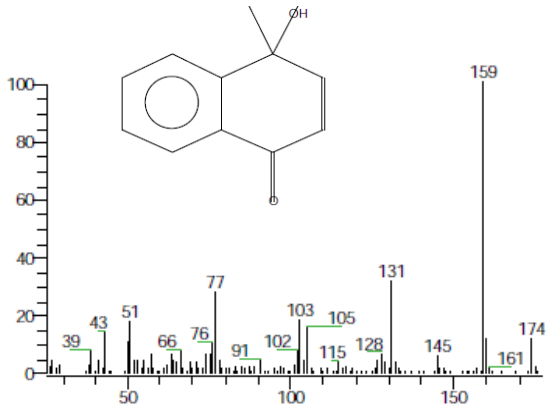


Fig. 2: Mass spectrum of 4-Hydroxy-4-methyl-4H-naphth alen-1-one.

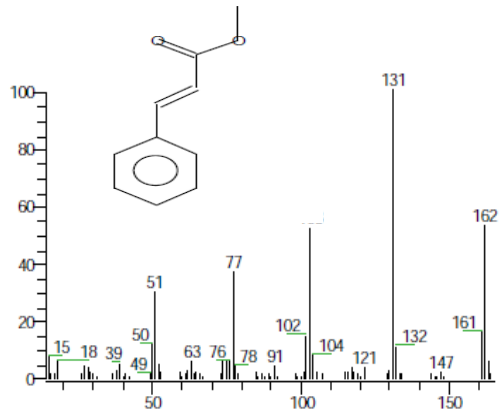


Fig3: Mass spectrum of 2-Propenoic acid, 3-phenyl-, methyl ester.

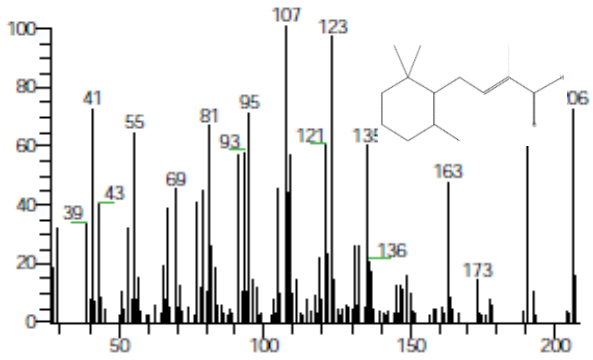


Fig 4: Mass spectrum of 2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-c yclohexen-1-yl).

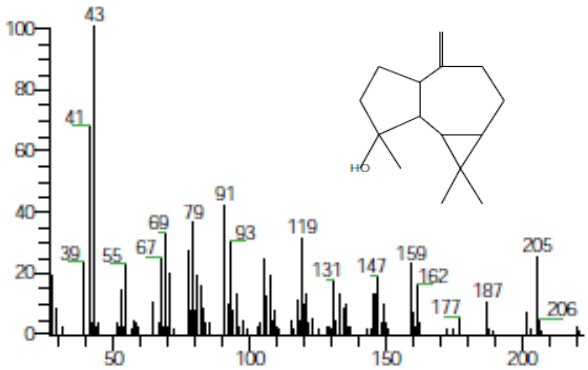


Fig 5: Mass spectrum of spathulenol.

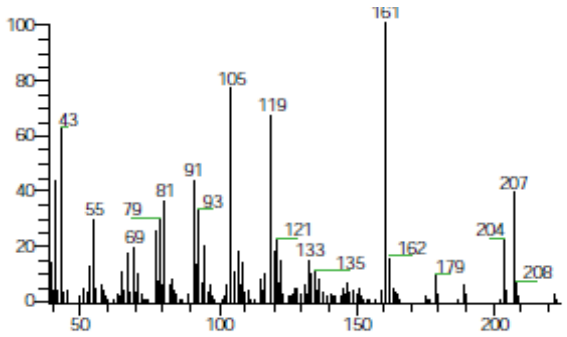


Fig 6: Mass spectrum of Cubedol

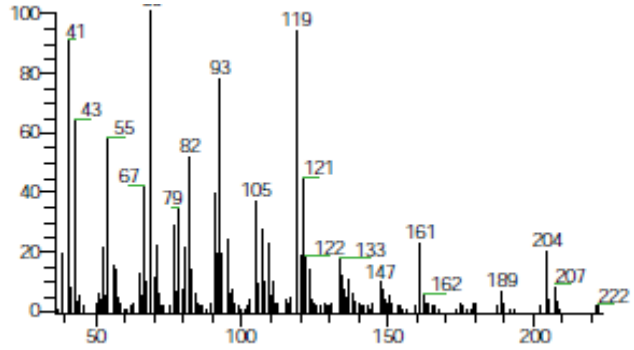


Fig 7: Mass spectrum of 7-epi-cis-sesquisabinene hydrate.

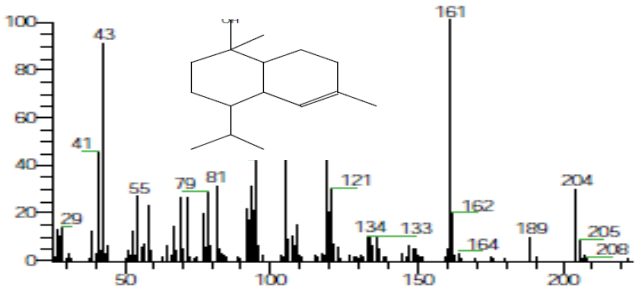


Fig 8: Mass spectrum of 1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6 -dimethyl-4-(1-methylethyl) 1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6 -dimethyl-4-(1-methylethyl).

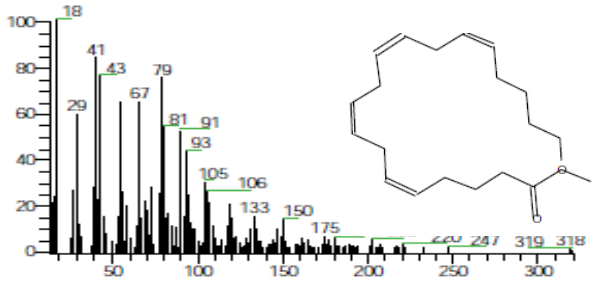


Fig 9: Mass spectrum of Methyl arachidonate.

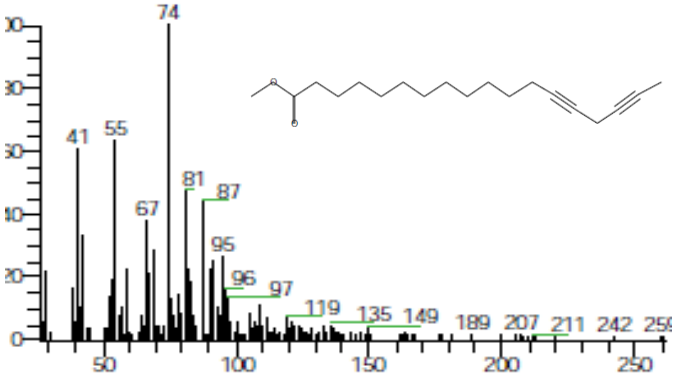


Fig 10: Mass spectrum of 13,16-Octadecadiynoic acid, methyl ester.

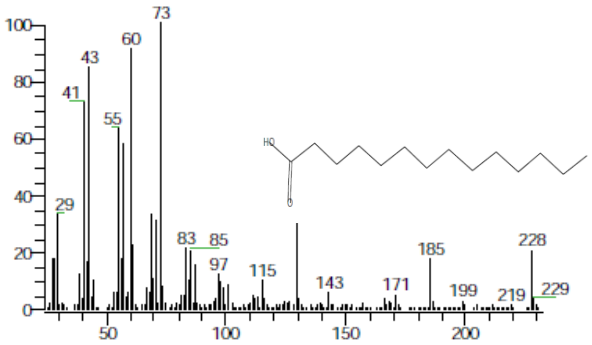


Fig 11: Mass spectrum of Tetradecanoic acid.

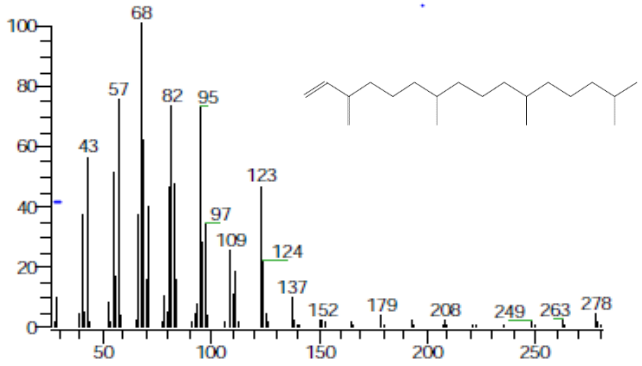


Fig 12: Mass spectrum of Neophytadiene.

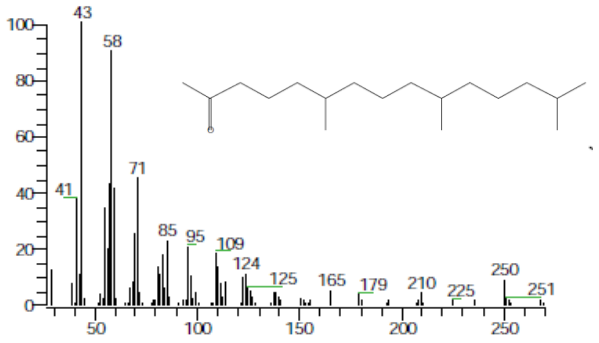


Fig 13: Mass spectrum of 2-Pentadecanone, 6,10,14-trimethyl.

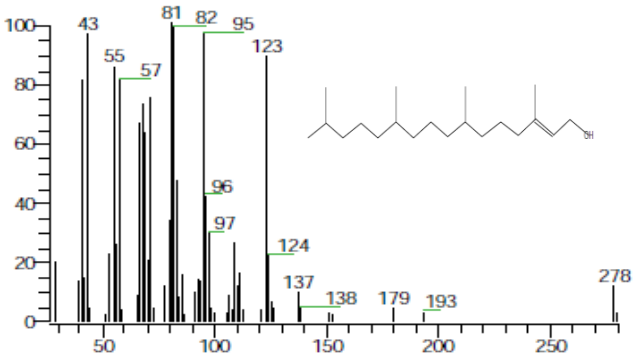


Fig 14: Mass spectrum of 3,7,11,15-Tetramethyl-2-hexade cen-1-ol.

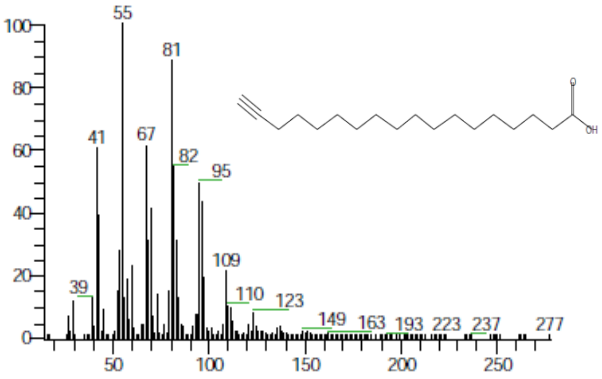


Fig 15: Mass spectrum of 17-Octadecynoic acid.

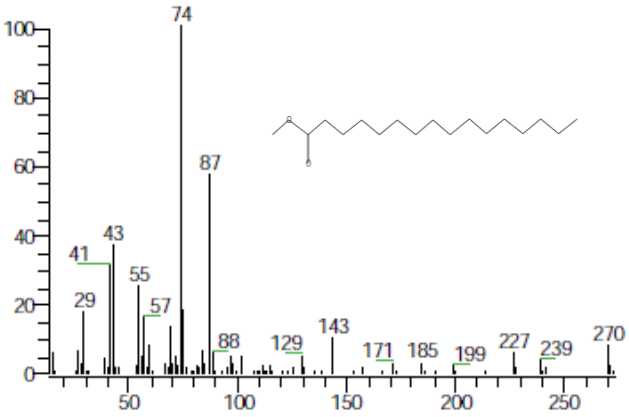


Fig 16: Mass spectrum of Hexadecanoic acid, methyl ester.

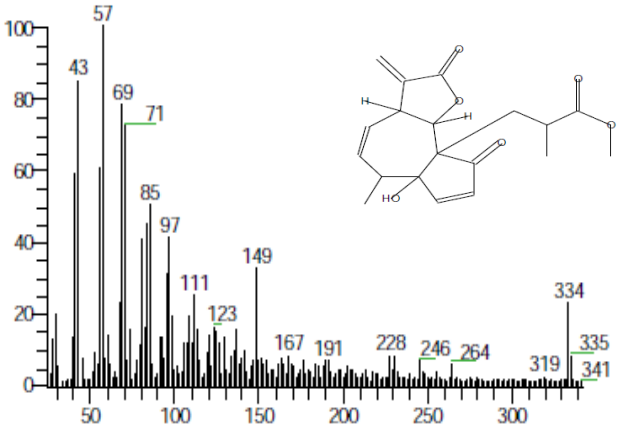


Fig 17: Mass spectrum of Isochiapin.

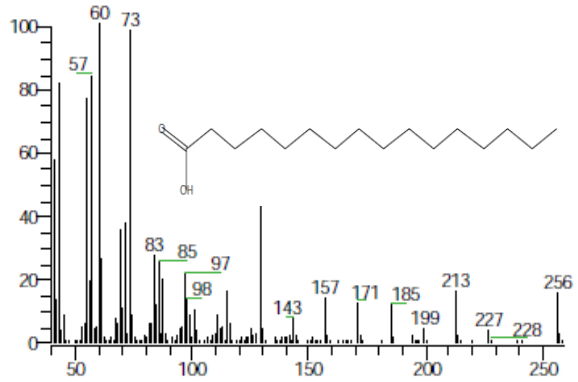


Fig 18: Mass spectrum of n-Hexadecanoic acid.

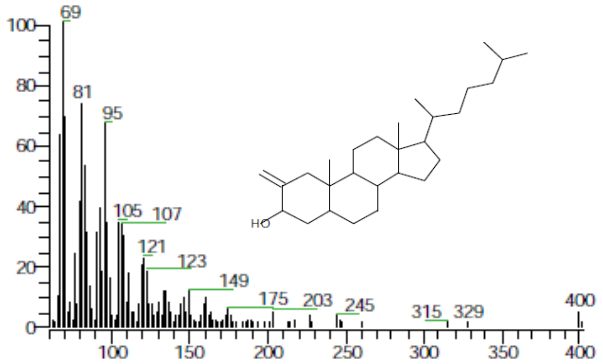


Fig 19: Mass spectrum of Cholestan-3-ol, 2-methylene.

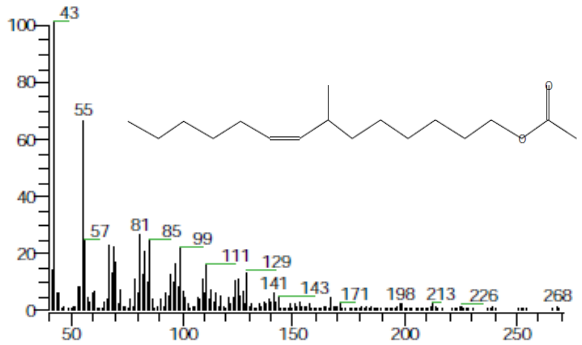


Fig 20: Mass spectrum of 7-Methyl-Z-tetradecen-1-ol acetate

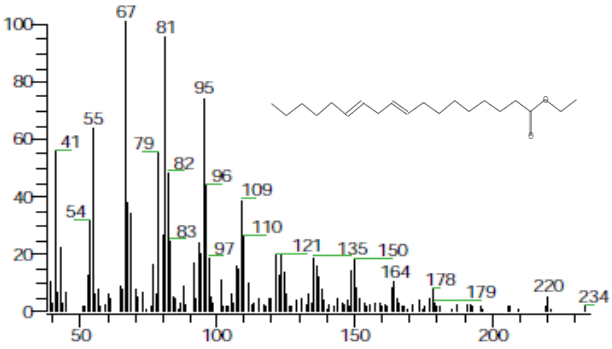


Fig 21: Mass spectrum of Ethyl linoleate

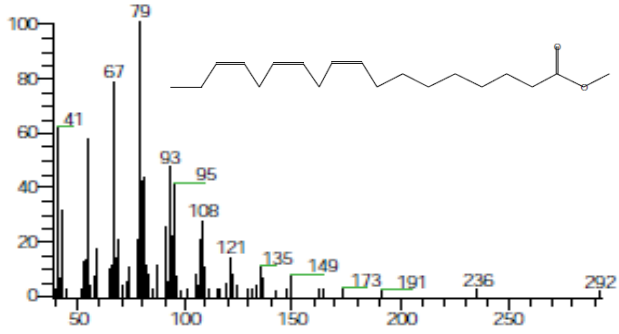


Fig 22: Mass spectrum of 9,12,15-Octadecatrienoic acid, methyl ester.

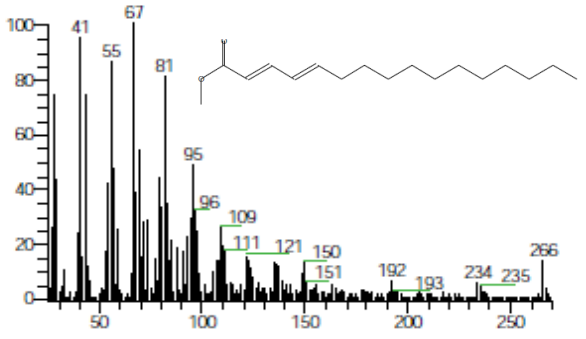


Fig 23: Mass spectrum of Hexadecadienoic acid, methyl ester.

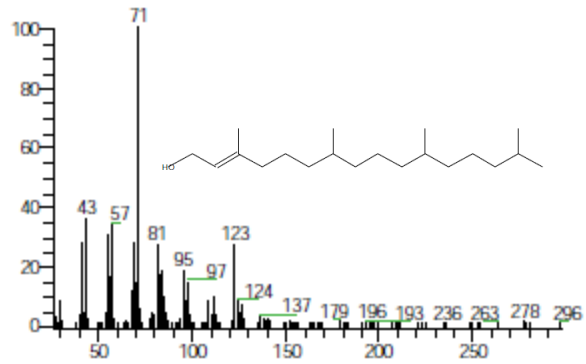


Fig 24: Mass spectrum of Phytol.

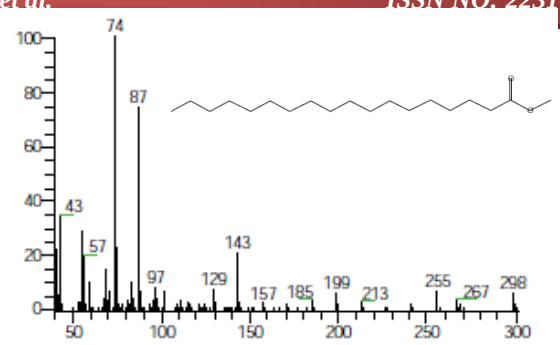


Fig 25: Mass spectrum of Methyl stearate.

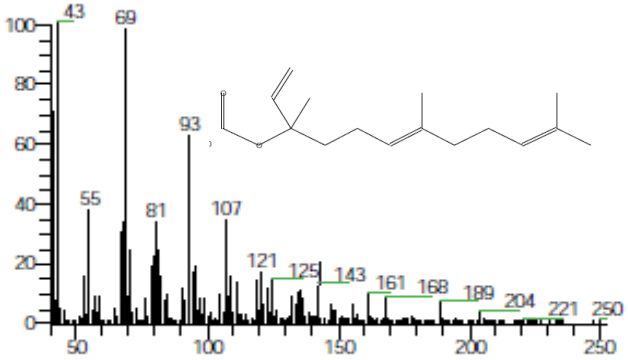


Fig 26: Mass spectrum of Formic acid, 3,7,11-trimethyl-1,6,10-dodecat rien-3-yl ester.

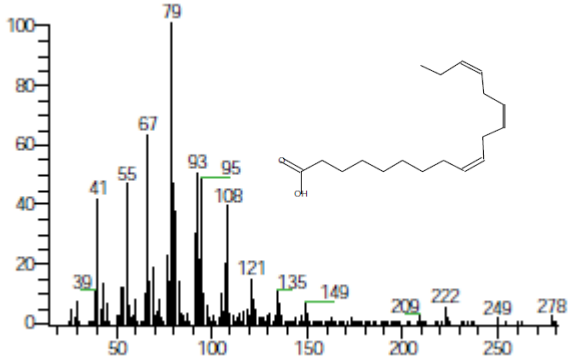


Fig 27: Mass spectrum of 9,12,15-Octadecatrienoic acid.

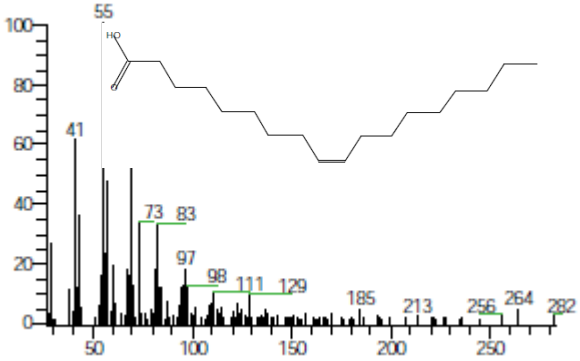


Fig 28: Mass spectrum of Oleic Acid Oleic Acid.

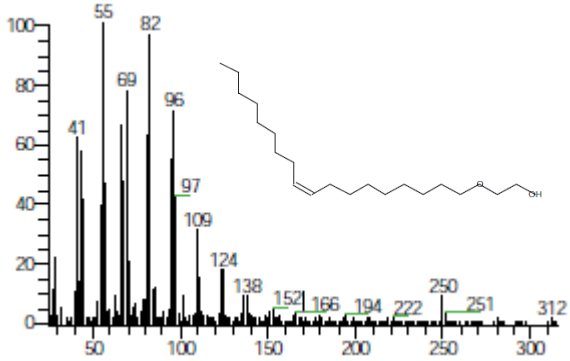
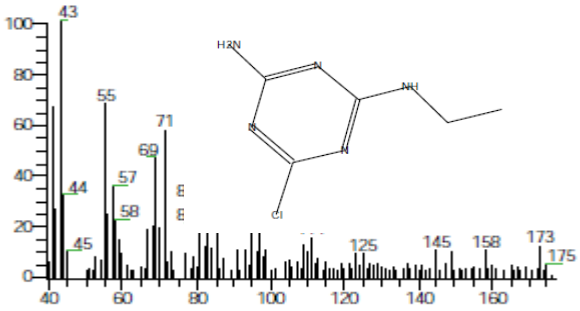


Fig 29: Mass spectrum of Ethanol, 2-(9-octadecenyloxy)-, (Z).



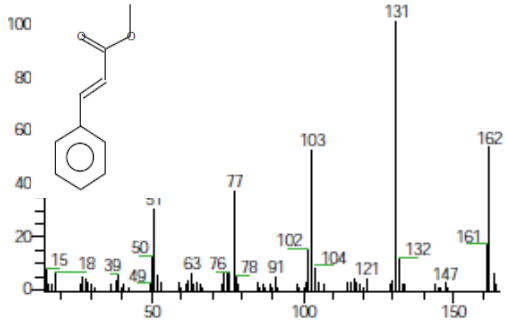


Fig 30: Mass spectrum of 1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl.

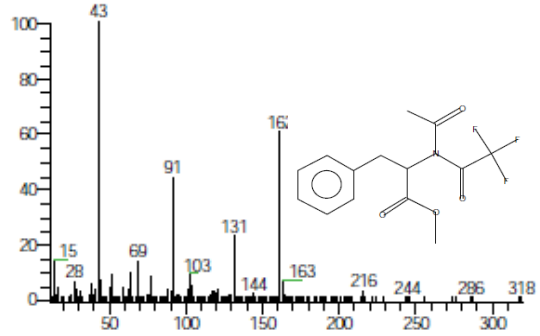


Fig 31: Mass spectrum of 2-Propenoic acid, 3-phenyl-, methyl ester

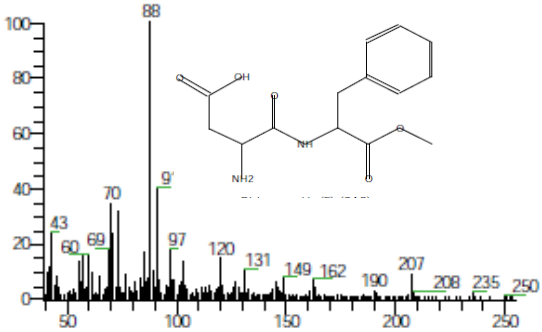
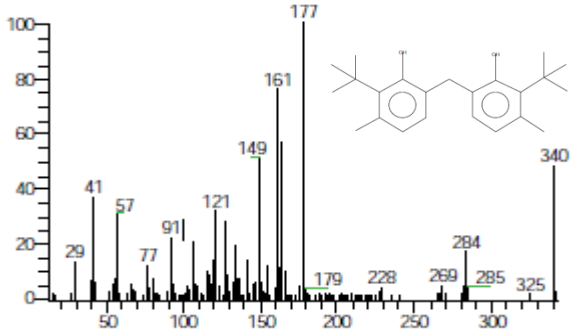


Fig 32: Mass spectrum of Alanine, N-acetyl-3-phenyl-N-(trifluoro acetyl)-, methyl ester.

Fig 33: Mass spectrum of Aspartame.



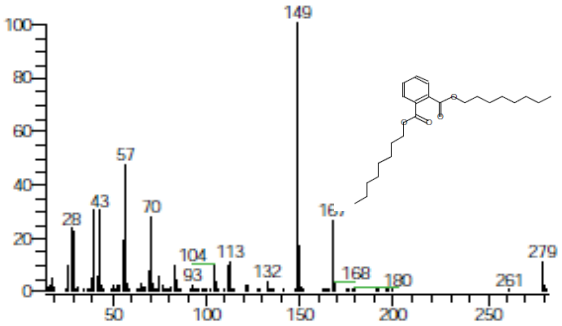
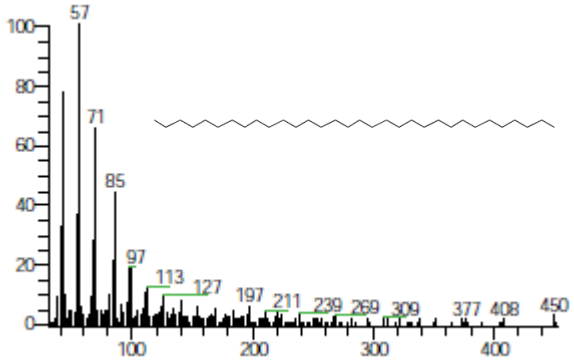


Fig 34: Mass spectrum of Phenol, 2,2'-methylenebis[6-(1,1-dimet hylethyl)-4-methyl.

Fig 35: Mass spectrum of 1,2-Benzenedicarboxylic acid, dioctyl ester.



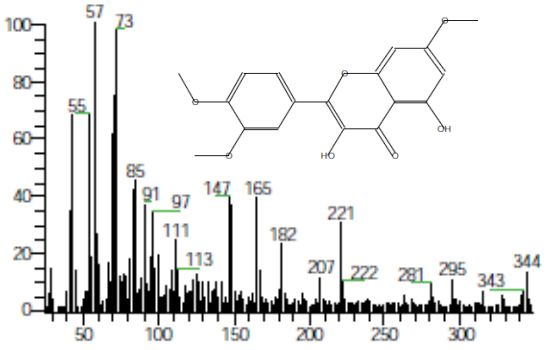


Fig 36: Mass spectrum of Dotriacontane.

Fig 37: Mass spectrum of Quercetin 7,3',4'-Trimethoxy.

Total Phenolic and Flavonoid contents:

Phenolics are the largest group of phytochemical compounds that have been known for their antioxidant activity [35]. Phenols and flavonoids are the major plant secondary metabolite compounds that act as primary antioxidants because of their ability to quench reactive oxygen species [23, 36]. The total phenolic content in the methanol extract of *Ocimum canum* leaves is (321.78±0.69 mg GAE/g extract) as shown in Fig. 38. This agreed with Akinmoladun *et al*. [35] who recorded the total phenolic content of *Ocimum gratissimum* was (5.68±0.06 mg/g GAE) and in *Ocimum americanum* was (240.16±0.17 mg TE/g) [37]. Phenols contain a hydroxyl group responsible for the antioxidant activity. Their antioxidant activity is due to their redox properties which allow them to act as reducing agents, hydrogen donors and hydroxyl radical quenchers [38, 39]. On the other hand, the total flavonoid content of *Ocimum canum* leaves is (71.64±10.66 mg RE/g extract). The flavonoid content in *Ocimum* species was exhibited by other studies [35, 37, 40]. Scientific research showed that generous intake of flavonoids decreases the risk of cardiovascular diseases, cancer and inflammation

due to its radical scavenging ability [26, 41, 42]. However, the promising amount of phenolic and flavonoid compounds in *Ocimum* sp. was reported by several authors [43, 44, 45].

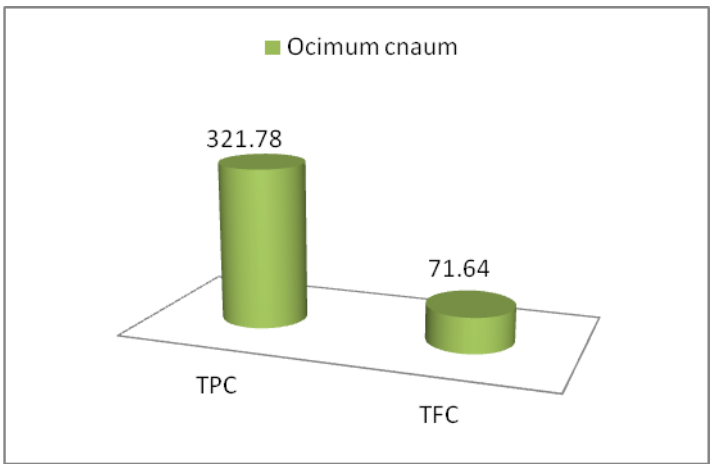


Fig (38): Total Phenolic and flavonoid contents in methanolic extract of *Ocimum canum* leaves.

Antioxidant Assays:

DPPH Radical Scavenging Activity:

DPPH has been widely used to estimate the antioxidant activity of plant extracts in recent years [46]. It is a stable free radical with a spare electron; gives rise to deep violet color in ethanol solution. DPPH method characterized by loss of its violet color when mixed with substance acts as a hydrogen donor to the free radical odd electron which responsible for the free radical's reactivity [42, 47]. In this study, DPPH scavenging ability of *Ocimum canum* leaves extract was reported as results in Fig. 39 showed inhibition percentage of scavenging activity ranged from 9%-84% at concentrations ranged from 5-500µg/ml. Also, IC_{50} which represents the concentration of plant extract required to inhibit 50% of DPPH was 26.41±1.39. This antioxidant activity is due to the hydrogen-donating ability of the plant extract to inhibit the free radical due to phenol and flavonoid contents of the plant. This is in full agreement with [19, 48, 49].

ABTS Scavenging Activity:

$ABTS^{+}$ radical is a stable chromogen compound used to measure the antioxidant activity of the plants [50]. It is a free radical has a characteristic absorbance at 734nm as its color decreases with the scavenging of proton radicals. It is usually reactive against thiols, phenolics and other antioxidants [51, 52]. ABTS radical scavenging activity of *Ocimum canum* leaves extract showed inhibition percentage of scavenging activity ranged from 7%-62% at concentrations ranged from 5-500µg/ml as shown in Fig (39). Also, IC_{50} which represents the concentration of plant extract required to inhibit 50% of ABTS was 194.45±2.03. These results may be attributed to the presence of phenolic and flavonoid compounds in the plant [37, 53]. The antioxidant activity of *Ocimum* sp. was proved by several reports. Javanmardi *et al*. [54] and Lee *et al*. [55] demonstrated the scavenging ability of *Ocimum basilicum* seeds and its volatile oil. Also, Selvi *et al*. [22] exhibited the antioxidant activity of essential oils of *Ocimum canum* leaves.

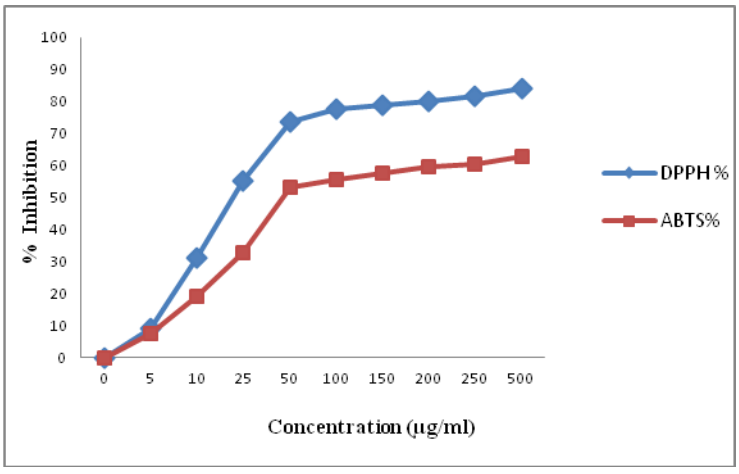


Fig (39): Percentage of inhibition scavenging activity of *Ocimum canum* leaves extract on DPPH and ABTS radicals.

Miracidicidal and Cercaricidal Activity:

Cercariae and miracidia are the larval stages of *Schistosoma*. This study was designed to examine the effect of *Ocimum canum* leaf extract on them. The plant showed good cercaricidal activity from 100 to 600ppm. The mortality rate increased with the time of exposure as after 120min 26%, 62% and 82% for 100, 300 and 500ppm respectively. Increasing the concentration to 600 killed 100% of cercariae (Table 2 and Fig. 40). The plant extract had promising efficiency on miracidia than cercariae as total miracidia were dead at 500ppm within 120min (Table 3 and Fig. 41). All control groups showed highly significant difference than treated groups as recorded 20% and 27% after 120min for cercariae and miracidia respectively. These results showed that *Ocimum canum* extract is effective in killing Schistosoma larval stages especially miracidia which were more sensitive than cercariae. These results are in agreement with Ibrahim *et al.* [56] who proved that miracidicidal mortality was greater than cercariae during application of *Tribulus terrestris* methanol extract at the same time intervals and also, 100% of cercariae dead at 640ppm of the plant. Bagalwa *et al.* [3] proved the cercaricidal activity of two steroid glycoalkaloid compounds from *Solanum syzybrilifolium* while, Abozeid *et al*. [2] proved the miracidicidal activity of tannins extracted from *Punica granatum* as kill 100% of miracidia after 50-150min. The larvicidal activity of the plant related to the secondary metabolites present in it as phenolics, flavonoids, terpenes and other compounds [57].

Table (2): Cercaricidal activity of *Ocimum canum* methanol extract after different exposure time.

Plant concentration	Mortality % in dependent time (min)			
	30	60	90	120
100 ppm	7	11	19	26
300 ppm	19	38	52	62
500 ppm	22	55	71	82
600 ppm	28	75	91	100
Control	4	8	17	20

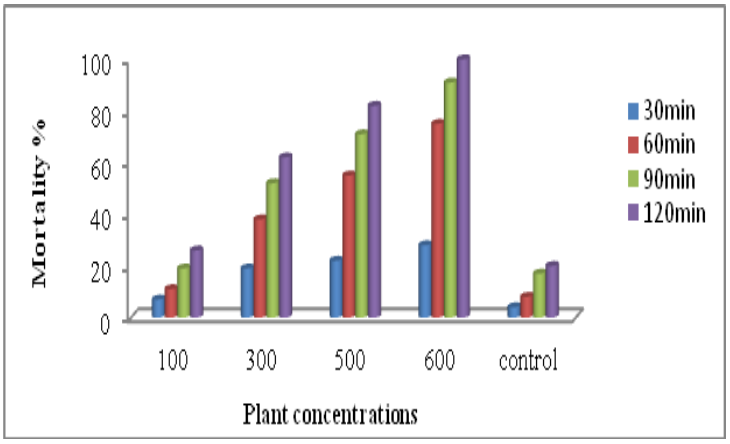


Fig (40): Effect of *Ocimum canum* leaves extract on cercariae after different time intervals.

Table (3): Miracidicidal activity of *Ocimum canum* methanol extract after different exposure time.

Plant concentration	Mortality % in dependent time (min)			
	30	60	90	120
100 ppm	10	14	17	45
300 ppm	15	20	42	70
500 ppm	20	45	80	100
Control	3	7	15	27

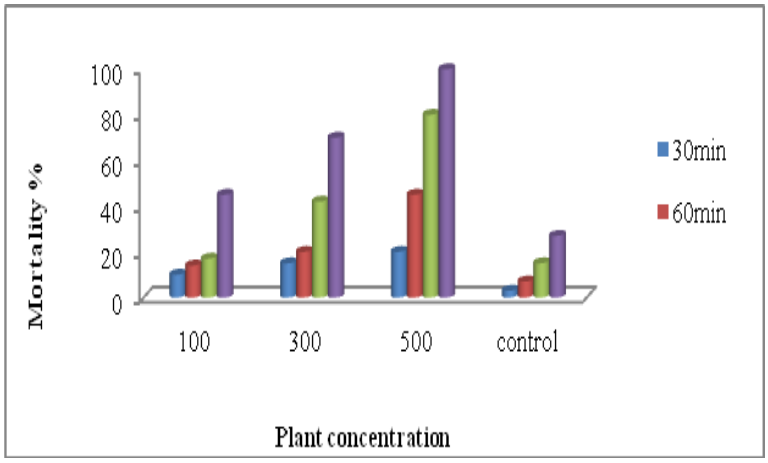


Fig (41): Effect of *Ocimum canum* leaves extract on miracidia after different time intervals.

CONCLUSION

This study throws light, upon the use of *Ocimum canum* leaves extract as cercaricidal and miracidicidal activity. Based on literature survey we believe that components identified by GC-MS analysis of the plant extract showed that it considered as an important medicinal plant.

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