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Research Article

Antibacterial and Phytochemical Analysis of *Piper nigrum* using Gas Chromatography – Mass Spectrum and Fourier-Transform Infrared Spectroscopy

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ABSTRACT

The aims of the study were to investigate the presence of phytochemical compounds from the fruits of *Piper nigrum*, using methanolic extraction and report the main functional components by using fourier-transform infrared spectroscopy. The phytochemical compound screened by GC-MS method. A total of 55 bioactive phytochemical compounds were identified in the methanolic extract of *P. nigrum*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight, molecular formula, chemical structure, MS Fragment ions and pharmacological actions. GC-MS analysis of *P. nigrum* revealed the existence of the Propanedioic acid , dimethyl ester, Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene-,(1S), 3-Carene, Cyclohexene, 1-methyl-5-(1-methylethenyl)-,(R), 1,6-Octadien-3-ol,3,7-dimethyl, 2-Methyl-1-ethylpyrrolidine, 2-Isopropenyl-5-methylhex-4-enal, L-α-Terpineol, (R)-lavandulyl acetate, Pyrrolizin-1,7-dione-6-carboxylic acid , methyl(ester), 7-epi-cis-sesquisabinene hydrate, Phenol, 2-methoxy-4-(1-propenyl)-,(Z), Eugenol, Alfa.Copaene, Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methyl), Epiglobulol, Caryophyllene, 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-,Z,Z,Z, α- ylangene, ß-copaene, Cedran-diol,8S,13, Isocalamendiol, Cinnami acid , 4-hydroxy -3-methoxy-,{5-hydroxy-2-hydroxymethyl, (-)-Spathulenol, 1-Heptatriacotanol, Desacetylanquidine, 5-Isopropyl-2,8-dimethyl-9-oxatricyclo[4.4.0.0.(2,8)]decan-7-one, Estra-1,3,5(10)-trien-17ß-ol, Trans-1,2-Diaminocyclohexane-N,N,N´,N´-tetraacetic acid, Phytol, Piperidine,1-(1-oxo-3-phenyl-2-propenyl)-, Eicosanoic acid , 2-(acetyloxy0-1-[(acetyloxy)methyl]ethyl ester, 2,5,5,8a-Tetramethyl-6,7,8,8a-tetrahydro-5H-chromen-8-ol, Z-5-methyl-6-heneicosen-11-one, 2H-1,2-Benzoxazine-3-carbonitrile,2- cyclohexyloctahydro-4a,8a-d, Indoxazin-4-one ,4,5,6,7-tetrahydro-3-undecyl, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3ß,5Z,7E), 3-Oxo-10(14)-epoxyguai-11(13)-en-6,12-olide, 7-[2-(Ethoxycarbonyl)-3α,5ß-dimethoxycyclopentyl -1]-heptanoic acid, 2H-Benzo[f]oxireno[2,3-E]benzofuran-8,(9H)-one,9-[[(1,3-benzodio, Nalorphine, 2-Cyclohexen -3-ol-1-one , 2-[1-iminotetradecyl]-, Piperine, Fenretinide, 11-Dehydrocorticosterone, 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,1,1a,1b,4,4a,7a,7b, 17a-Ethyl-3ß-methoxy-17a-aza-D-homoandrost-5-ene-17-one, Bufa-20,22-dienolide m,14,15-epoxy-3,11-dihydroxy-,(3ß,5ß,11α,15, 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol, Retinal ,9-cis-, 6-ß-Naltrexol, Piperine, Ursodeoxycholic acid, 5α-Cholan-24-oic acid , 12α-hydroxy-3,7-dioxo-,methyl ester and Stigmasterol. The FTIR analysis of *P. nigrum* leaves proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxlic acids, Esters and Nitro Compounds. Methanolic extract of bioactive compounds of *P. nigrum* was assayed for *in vitro* antibacterial activity against *Escherichia coli*, *Pseudomonas aerogenosa*, *Proteus mirabilis*, *Staphylococcus aureus* and *Klebsiella pneumonia* by using the diffusion method in agar. The zone of inhibition were compared with different standard antibiotics. The diameters of inhibition zones ranged from 5.00±0.16 to 0.40±0.12 mm for all treatments.

Keywords: FT-IR, GC-MS analysis, *Piper nigrum*.

INTRODUCTION

Piper nigrum L. is a flowering vine in the family of piperaceae, therefore an important medicinal plant is used in traditional medicine in Asia and Pacific islands especially in Indian medicine[1]. Pepper aroma and flavor due to their chemical substances especially the volatile oil. Black pepper oil has medicinal values. It can be used to help in treatment of pain relief, rheumatism, chills, flu, colds exhaustion, muscular aches, physical and emotional coldness, fever as nerve tonic and to increase circulation[2-5]. The main pungent principle in the green berries of pepper *P. nigrum* L. is piperine. Generally the piperine content of black or white peppercorns lies within the range of 3-8 g/100 g, whereas the content of minor alkaloids piperyline and piperettine have been estimated as 0.2-0.3 and 0.2-1.6 g/100g respectively. The bioavailabilityenhancing property of piperine indicates its potential to be used as an adjuvant with therapeutic drugs in chronic ailments, to reduce the effective dose of the drug and, hence,

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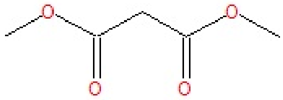
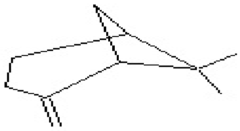
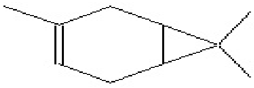
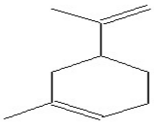
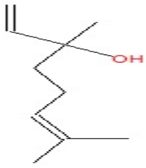
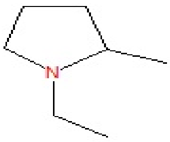
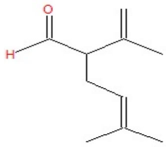
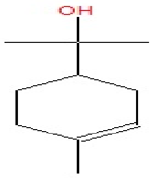
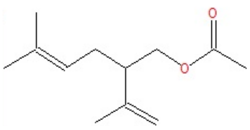
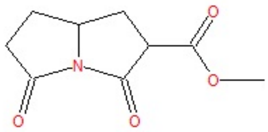
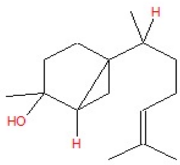
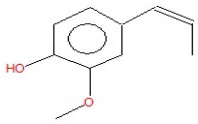
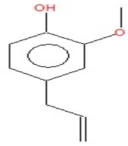
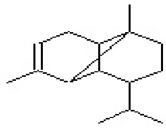
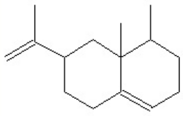
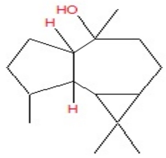
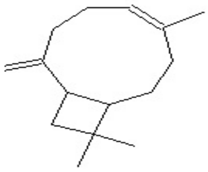
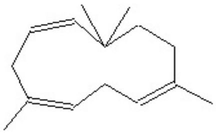
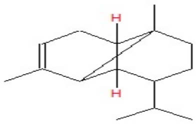
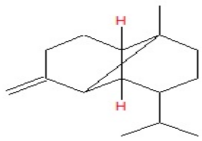
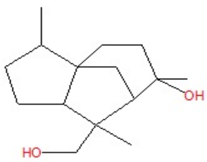
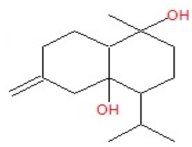
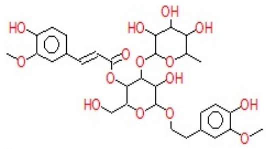
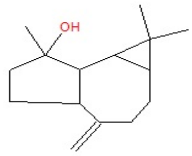
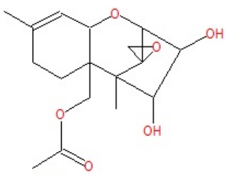
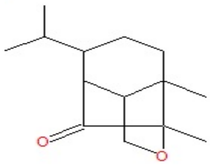
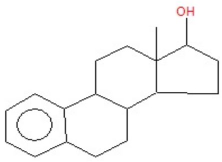
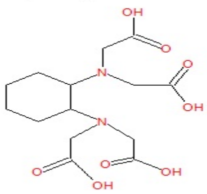
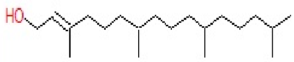
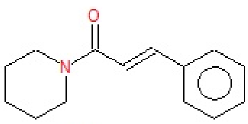
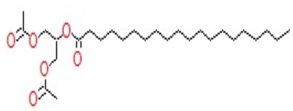
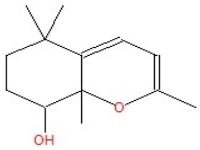
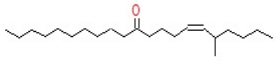
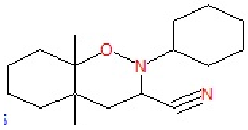
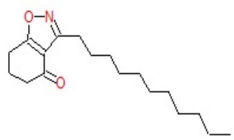
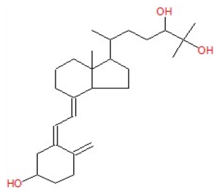
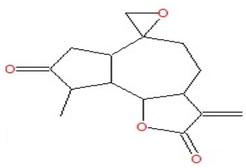
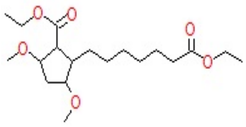
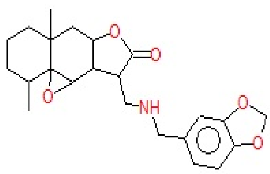
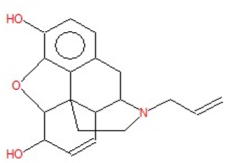
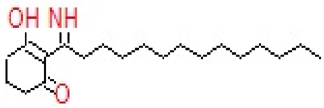
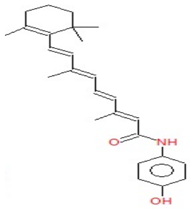
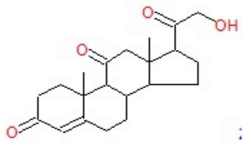
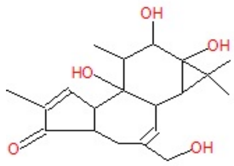
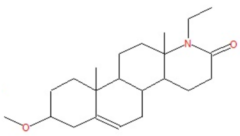
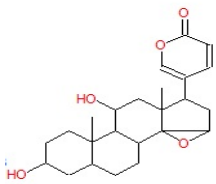
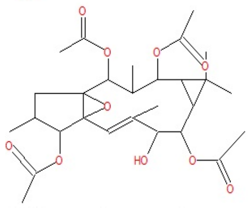
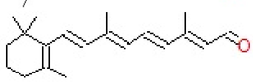
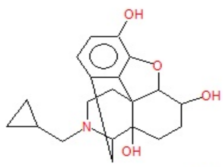
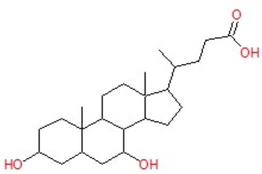
Table 1: Major phytochemical compounds identified in methanolic extract of *Piper nigrum*.

S. No.	Phytochemical compound	RT (min)	Formula	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions	P'cological actions
1.	Propanedioic acid , dimethyl ester	3.150	$C_5H_8O_4$	132	132.042258		59,74,101,132	Anti-tumor and antioxidant activity
2.	Bicyclo[3.1.1] heptane,6,6-dimethyl-2-methylene-,(1S)-	3.339	$C_{10}H_{16}$	136	136.1252		53,69,79,93,107,121,136	Anti-Helicobacter pylori activity and Anti-Candida activity
3.	3-Carene	3.693	$C_{10}H_{16}$	136	136.1252		53,67,79,93,105,121,136	Several biological activities such as anti-inflammatory
4.	Cyclohexene , 1-methyl-5-(1-methylethenyl)-,(R)-	3.997	$C_{10}H_{16}$	136	136.1252		53,68,79,93,107,121,136	Anti-microbial agents
5.	1,6-Octadien-3-ol,3,7-dimethyl-	5.067	$C_{10}H_{18}O$	154	154.135765		55,71,80,83,93,107,121,127,136,154	Pharmacological effects such as: anti-inflammation
6.	2-Methyl-1-ethylpyrrolidine	5.696	$C_7H_{15}N$	113	113.1204495		56,69,84,98,113	Anti-tumor activity.
7.	2-Isopropenyl-5-methylhex-4-enal	6.108	$C_{10}H_{16}O$	152	152.120115		69,84,95,109,123,137	Antihyperglycemic and anticariogenic
8.	L-α-Terpineol	6.303	$C_{10}H_{18}O$	154	154.135765		55,59,67,81,93,107,121,136	Anti-inflammatory
9.	(R)-lavandulyl acetate	6.629	$C_{12}H_{20}O_2$	196	196.14633		53,69,80,93,107,121,136,154	Anti-inflammatory
10.	Pyrrolizin-1,7-dione-6-carboxylic acid , methyl(ester)	7.562	$C_9H_{11}NO_4$	197	197.068808		55,69,84,98,110,142,197	Anti-Viral and Anti-Tumor Activity
11.	7-epi-cis-sesquisabinene hydrate	8.357	$C_{15}H_{26}O$	222	222.198365		55,69,82,93,105,119,133,147,161,175,189,204,222	Antioxidant effects and anti-mutagenic action

12.	Phenol , 2-methoxy-4-(1-propenyl)-,(Z)-	8.494	$C_{10}H_{12}O_2$	164	164.08373		51,55,65,77,91,103,115,121,131,149,164	Anti-inflammatory effects
13.	Eugenol	8.523	$C_{10}H_{12}O_2$	164	164.08373		51,55,65,77,91,103,115,121,131,137,149,164	Anti-Inflammatory agents and antioxidants
14.	Alfa.Copaene	8.740	$C_{15}H_{24}$	204	204.1878		55,69,77,91,105,119,133,147,161,175,189,204	Analgesic and anti-inflammatory activities
15.	Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methyl	8.958	$C_{15}H_{24}$	204	204.1878		55,67,79,107,119,133,161,175,189,204	Anti-bacterial
16.	Epiglobulol	9.181	$C_{15}H_{26}O$	222	222.198365		55,69,82,93,109,121,147,161,204,222	Antioxidant activity, antimicrobial, anti-inflammatory
17.	Caryophyllene	9.456	$C_{15}H_{24}$	204	2041878		55,69,79,93,105,120,133,147,161,175,189,204	Several biological activities are attributed to beta-caryophyllene, such as anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and local anaesthetic
18.	1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-,Z,Z,Z-	9.810	$C_{15}H_{24}$	204	204.1878		55,67,80,93,107,121,136,147,161,175,189,204	Anti-aging, anti-hyperlipidemia and antimicrobial activities
19.	α- ylangene	10.005	$C_{15}H_{24}$	204	204.1878		55,67,77,93,105,119,133,147,161,189,204	Anti-inflammatory properties
20.	ß-copaene	10.085	$C_{15}H_{24}$	204	204.1878		55,79,105,161,204	Anti-neuroinflammatory and neuroprotective
21.	Cedran-diol,8S,13-	10.468	$C_{15}H_{26}O_2$	238	238.19328		55,74,93,107,119,149,161,190,238	Anti-fungal activity

22.	Isocalamendiol	10.852	$C_{15}H_{26}O_2$	238	238.19328		55,81,111,159,202,223,238	Anti- fertility activity and anti-bacterial activity
23.	Cinnami acid , 4-hydroxy -3-methoxy-,{5-hydroxy-2-hydroxymethyl-	12.385	$C_{31}H_{40}O_{15}$	652	652.23672		55,77,91,105,121,151,168,194,215,296,330,354,386,418	Anti-inflammatory properties
24.	(-)-Spathulenol	13.570	$C_{15}H_{24}O$	220	220.182715		55,69,91,119,159,187,205,220	Antioxidant and anti-inflammatory activities
25.	1-Heptatriacotanol	13.924	$C_{37}H_{76}O$	536	536.58962		55,81,95,147,161,190,229,244,257	Antioxidant, anticancer, anti inflammatory and sex hormone activity
26.	Desacetylanquidine	14.474	$C_{17}H_{24}O_6$	324	324.157288		55,67,91,105,124,145,159,187,205,264,281	New chemical compound
27.	5-Isopropyl-2,8-dimethyl-9-oxatricyclo[4.4.0.0.(2,8)]decan-7-one	15.240	$C_{14}H_{23}O_2$	222	222.16198		55,69,81,95,109,123,151,162,194,222	Anti-termitic Activity
28.	Estra-1,3,5(10)-trien-17ß-ol	15.818	$C_{18}H_{24}O$	256	256.182714		57,73,85,97,129,157,185,213,241,256	Anti-osteoporosis activity
29.	Trans-1,2-Diaminocyclohexane-N,N,N´,N´-tetraacetic acid,	16.253	$C_{14}H_{22}N_2O_8$	346	346.137615		55,81,96,110,153,181,197,225,241,270	Unknown
30.	Phytol	16.665	$C_{20}H_{40}O$	296	296.307917		57,71,81,95,111,123,137,196,221,249,278	Antioxidant activity
31.	Piperidine,1-(1-oxo-3-phenyl-2-propenyl)-	16.883	$C_{14}H_{17}NO$	215	215.131014		51,84,103,131,149,172,198,215	Antibacterial, analgesic and anti-inflammatory activity
32.	Eicosanoic acid , 2-(acetyloxy0-1-[(acetyloxy)methyl]ethyl ester	18.147	$C_{27}H_{50}O_6$	470	470.360739		57,71,84,98,117,137,159,171,227,269,295	Anti-inflammatory, and anti-melasma properties

33.	2,5,5,8a-Tetramethyl-6,7,8,8a-tetrahydro-5H-chromen-8-ol	18.531	$C_{13}H_{20}O_2$	208	208.14633		57,91,106,134,175,190,208	Anticancer and antioxidant activity
34.	Z-5-methyl-6-heneicosen-11-one	18.559	$C_{22}H_{42}O$	322	322.323566		55,71,83,97,139,169,197,241,307,322	Anti-Mycobacterium tuberculosis Activity, anti-inflammatory, analgesic and antimicrobial activities
35.	2H-1,2-Benzoxazine-3-carbonitrile,2-cyclohexyloctahydro-4a,8a-d	18.748	$C_{17}H_{28}N_2O$	276	276.220163		55,67,83,96,108,134,150,165,178,191,206,216,234,249,276	Anti-inflammatory
36.	Indoxazin-4-one ,4,5,6,7-tetrahydro-3-undecyl-	18.943	$C_{18}H_{29}NO_2$	291	291.21983		55,69,83,123,138,151,164,178,206,234,262,291	New chemical compound
37.	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3ß,5Z,7E)-	19.246	$C_{27}H_{44}O_3$	416	416.329044		55,118,136,158,176,207,253,383,416	Biocides and anti-corrosion agents
38.	3-Oxo-10(14)-epoxyguai-11(13)-en-6,12-olide	19.377	$C_{15}H_{18}O_4$	262	262.120508		53,81,91,105,135,174,193,244,262	Anti fungal activity
39.	9-Octadecenamide , n-butyl-	20.751	$C_{22}H_{43}NO$	337	337.334465		55,69,100,128,184,210,252,280,294,308,337	Antimicrobial, anti-inflammatory and antioxidant
40.	7-[2-(Ethoxycarbonyl)-3α,5ß-dimethoxycyclopentyl-1]-heptanoic acid	20.968	$C_{19}H_{34}O_6$	358	358.235538		265,280,297,311,326	New chemical compound
41.	2H-Benzo[f]oxireno[2,3-E]benzofuran-8,(9H)-one,9-[[(1,3-benzodio	21.243	$C_{23}H_{29}NO_5$	399	399.204573		68,77,91,105,135,150,183,202,236,264	Antimicrobial and antifungal activities
42.	Nalorphine	21.271	$C_{19}H_{21}NO_3$	311	311.152143		81,91,115,128,152,174,188,200,226,241,311	Anti-pruritic agent against morphine

43.	2-Cyclohexen -3-ol-1-one , 2-[1-iminotetradecyl]-,	21.734	$C_{20}H_{35}NO_2$	321	321.266779		55,83,110,125,153,166,180,194,208,236,278,304,321	Antiulcer, hypolipidemic, antiatherosclerotic and anti-HIV
44.	Piperine	22.313	$C_{17}H_{19}NO_3$	285	285.136494		63,84,115,143,159,173,201,229,256,285	Anti-pyretic and analgesic activities
45.	Fenretinide	22.782	$C_{26}H_{33}NO_2$	391	391.25113		58,69,81,95,109,135,148,161,202,213,255,268	Anti-proliferative activity
46.	11-Dehydrocorticosterone	24.670	$C_{21}H_{28}O_4$	344	344.19876		55,67,79,91,105,121,147,189,227,267,285,313,344	Anti-proliferative activity
47.	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,1,1a,1b,4,4a,7a,7b	24.836	$C_{20}H_{28}O_5$	348	348.193674		55,83,173,199,217,312,330,348	Biocides, anti-corrosion agents, drying agents, and coating
48.	17a-Ethyl-3ß-methoxy-17a-aza-D-homoandrost-5-ene-17-one	24.916	$C_{22}H_{35}NO_2$	345	345.266779		55,71,91,105,138,193,209,241,296,330,345	Anti- cancer activity
49.	Bufa-20,22-dienolide m,14,15-epoxy-3,11-dihydroxy-,(3ß,5ß,11α,15	25.111	$C_{42}H_{32}O_5$	400	400.224974		55,67,79,91,107,135,187,213,231,249,278,349,364,382,400	Cell growth inhibitory activity, and antitumor
50.	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	25.523	$C_{28}H_{40}O_{10}$	536	536.262146		55,69,122,207,236,297,357,417,477	Biocide and anti-fungal
51.	Retinal ,9-cis-	25.963	$C_{20}H_{28}O$	284	284.214016		55,69,79,95,119,133,173,199,213,241,255	Anti-oxidant role
52.	6-ß-Naltrexol	27.194	$C_{20}H_{25}NO_4$	343	343.178358		55,84,98,110,128,161,202,228,246,288,302,324,343	Potential anti-angiogenic activity
53.	Ursodeoxycholic acid	28.344	$C_{24}H_{40}O_4$	392	392.29266		55,67,81,93,145,213,255,302,356,374,392	Antiinflammatory effect and anti-inflammatory activity

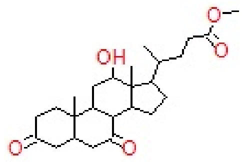
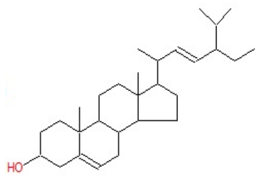
54.	5α-Cholan-24-oic acid , 12α-hydroxy-3,7-dioxo-,methyl ester	28.510	$C_{25}H_{38}O_5$	418	418.271925		55,95,219,245,285,345,382,400,418	Having biological activity (insect moulting hormones)
55.	Stigmasterol	29.168	$C_{29}H_{48}O$	412	412.370516		69,83,133,213,255,300,351,369,412	Anti-inflammatory and anti-parasitic effects

Table 2: FT-IR peak values of methanolic seeds extract of *Piper nigrum*.

S. No.	Peak (Wave number cm-l)	Intensity	Bond	Functional group assignment	Group frequency
1.	650.1	57.535	-	Unknown	-
2.	688.59	59.347	C-H	Alkenes	675-995
3.	704.02	60.135	C-H	Alkenes	675-995
4.	759.95	64.754	C-H	Alkenes	675-995
5.	827.46	67.689	C-H	Alkenes	675-995
6.	852.54	65.098	C-H	Alkenes	675-995
7.	894.97	68.412	C-H	Alkenes	675-995
8.	927.76	60.301	C-H	Alkenes	675-995
9.	999.13	38.637	-	Unknown	-
10.	1014.56	38.696	C-F stretch	Aliphatic fluoro compounds	1000-10150
11.	1076.28	54.627	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
12.	1134.14	61.967	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
13.	1149.57	63.780	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
14.	1195.87	71.015	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
15.	1249.87	55.761	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
16.	1292.87	72.884	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
17.	1361.74	68.851	NO2	Nitro Compounds	1300-1370
18.	1417.68	64.723	C-H	Alkanes	1340-1470
19.	1436.97	59.046	C-H	Alkanes	1340-1470
20.	1489.05	65.725	-	Unknown	-
21.	1506.41	66.871	-	Unknown	-
22.	1593.20	66.461	-	Unknown	-
23.	1616.35	64.515	-	Unknown	-
24.	1635.64	61.408	-	Unknown	-
25.	2665.62	88.750	-	Unknown	-
26.	2854.65	79.246	C-H	Alkanes	2850-2970
27.	2926.01	75.034	C-H	Alkanes	2850-2970

Table 3: Zone of inhibition (mm) of test bacterial strains to Piper nigrum bioactive compounds and standard antibiotics.

/ *Piper nigrum* Antibiotics	*Bacteria*				
	Staphylococcus aureus	*Escherichia coli*	*Proteus mirabilis*	*Klebsiella pneumonia*	*Pseudomonas eurogenosa*
Piper nigrum	4.00±0.31	4.90±0.13	5.00±0.16	4.63±0.41	*4.12±0.11*
Rifambin	1.01±0.10	0.77±0.41	0.98±0.11	1.00±0.30	*1.05±0.42*
Streptomycin	0.91±0.27	1.60±0.29	1.90±0.10	0.96±0.47	*0.87±0.20*
Kanamycin	0.42±0.18	1.12±0.46	0.40±0.12	1.00±0.10	*0.90±0.47*
Cefotoxime	0.87±0.95	0.96±0.27	0.93±0.25	0.92±0.18	*0.71±0.13*

subsequent adverse effects[6-12] Recently, biochemical activities of some important medicinal plants including Piper species and their metabolites have been described[13-20]. Pharmacological and clinical studies have revealed that piperine has CNS depressant, antipyretic, analgesic, anti-inflammatory (Ratner et al., 1991), antioxidant, and hepatoprotective activities[21,22]. Studies have revealed anticonvulsant and bioavailability-enhancing properties of the drug. The fruits contain 1.0–2.5% volatile oil, 5–9% alkaloids, of which the major ones are piperine, chavicine, piperidine, and piperetine, and a resin[23]. The present study involves an assessment using GC-MS and FT-IR

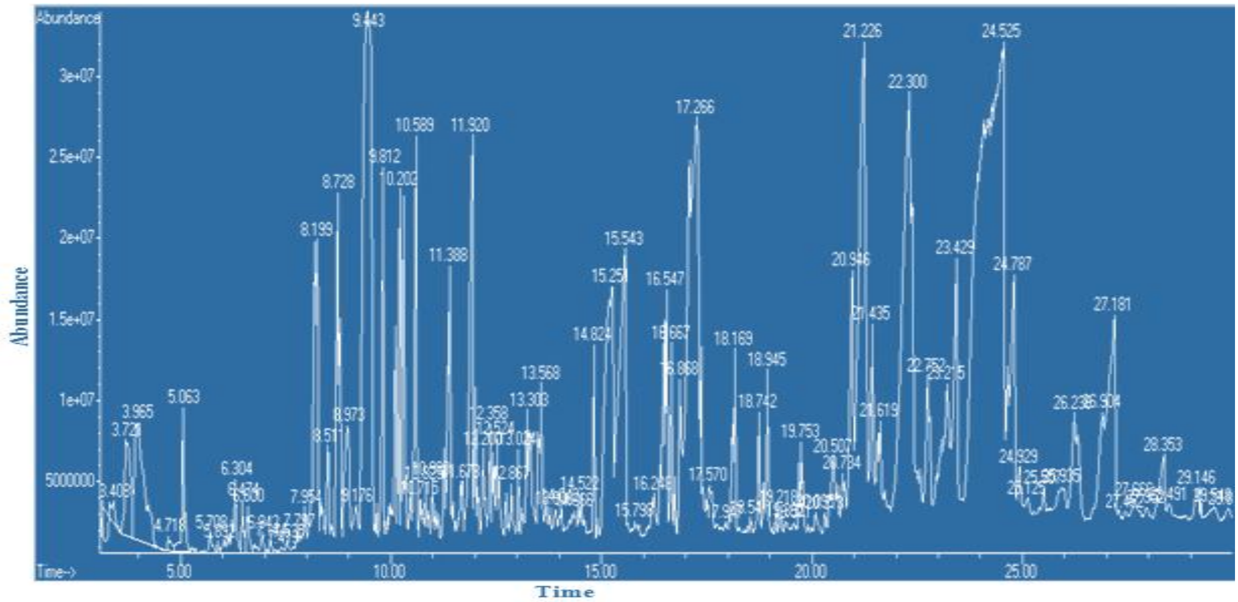


Figure 1: GC-MS chromatogram of methanolic extract of *Piper nigrum*.

spectroscopic techniques to investigate the chemical composition methanolic fruit extract of *P. nigrum*.

MATERIALS AND METHODS

Plant Material and Preparation of Extracts

P. nigrum dried fruits were purchased from local market in Hilla city, middle of Iraq. after thorough cleaning and removal foreign materials, the fruits were stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use. About 30 g of the plant sample powdered were soaked in 100 mL of methanol for 16 h in a rotatory shaker[24,25]. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture.

Identification of component by gas chromatography – mass spectrum analysis

The physicochemical properties of the essential oil of *P. nigrum L.* (Black Pepper) are presented in Table1. Interpretation of mass spectroscopy (GC-MS) was conducted using data base of the National Institute Standard and Technology (NIST) having more than 62000 patterns. The spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST library. The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures[26,27]. The GC-MS analysis of the plant extract was made in a (Agilent 789 A) instrument under computer control at 70 eV. About 1μL of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (initial time) to when elution occurred is referred to as the retention time (RT). While the instrument was run, the computer generated a graph from the signal called chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the gas chromatography column into the detector. The x-axis showed the RT and the y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass[28]. The M/Z (mass / charge) ratio obtained was calibrated from the graph obtained, which was called as the Mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using gas chromatography and mass spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1mL per min[29-33]. The electron gun of mass detector liberated electrons having energy of about 70eV.The column employed here for the separation of components was Elite 1(100% dimethyl poly siloxane).

Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of *P. nigrum* specimen was treated for FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). The sample was run at infrared region between 400 nm and 4000 nm[31].

Determination of antibacterial activity of crude bioactive compounds of Piper nigrum.

The test pathogens (*Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli,* and *Staphylococcus aureus)* were swabbed in Müller-Hinton agar plates. 60 μL of plant extract was loaded on the bored wells. The wells were

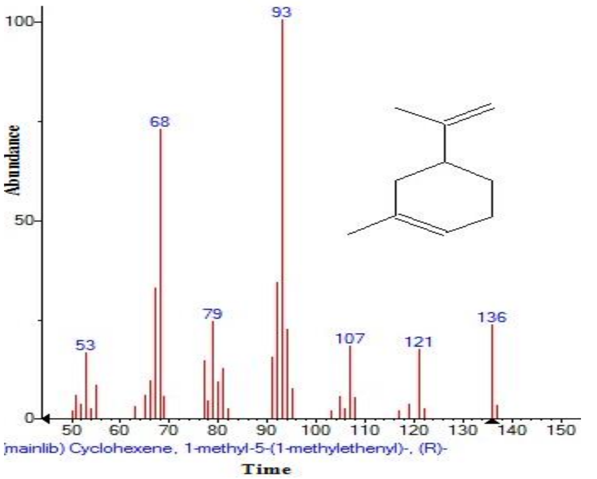


Figure 2: Structure of Cyclohexene, 1-methyl-5-(1-methylethenyl)-,(R) present in the methanolic seeds extract of Piper nigrum by using GC-MS analysis.

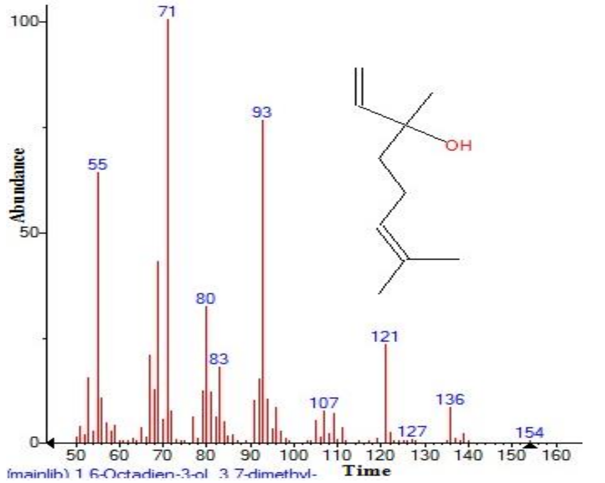


Figure 3: Structure of 1,6-Octadien-3-ol,3,7-dimethyl present in the methanolic seeds extract of Piper nigrum by using GC-MS analysis.

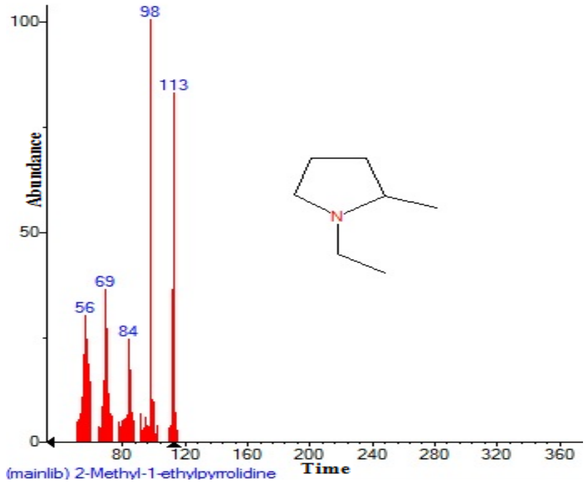


Figure 4: Structure of 2-Methyl-1-ethylpyrrolidine present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

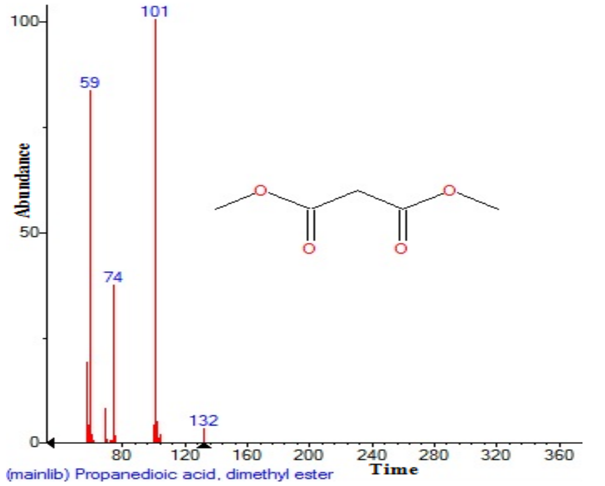


Figure 5: Structure of Propanedioic acid , dimethyl ester present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

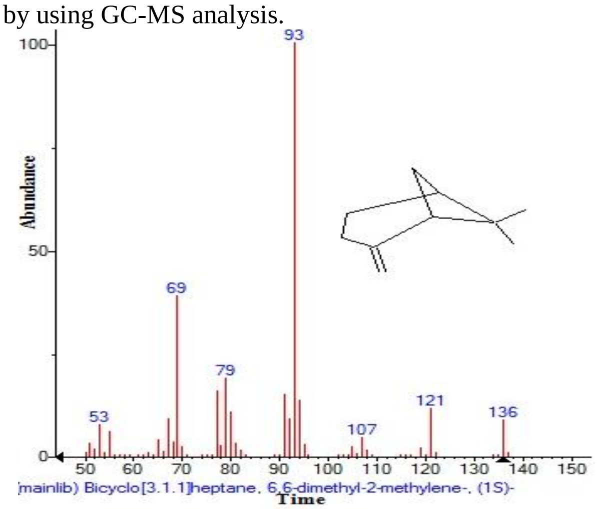


Figure 6: Structure of Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-,(1S) present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

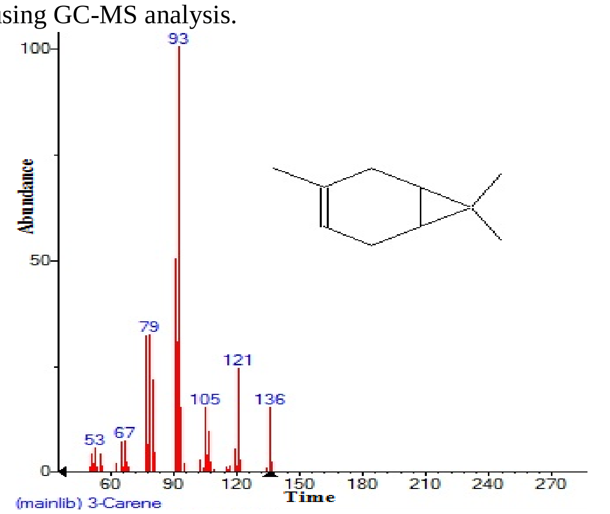


Figure 7: Structure of 3-Carene present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

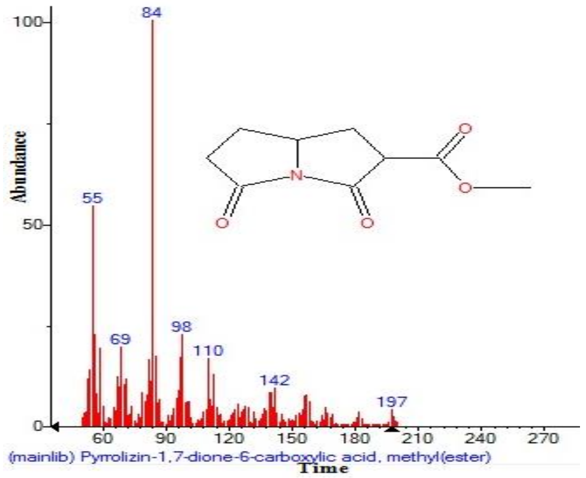


Figure 8: Structure of Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester) present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

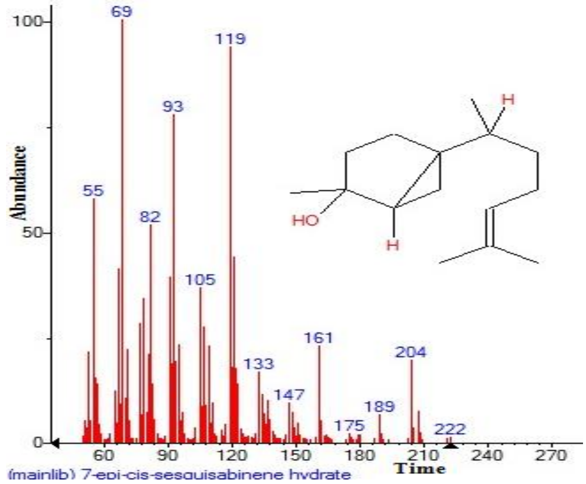


Figure 9: Structure of 7-epi-cis-sesquisabinene hydrate present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

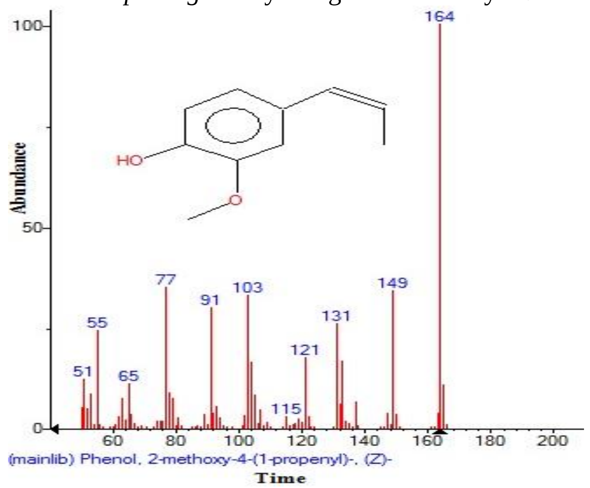


Figure 10: Structure of Phenol, 2-methoxy-4-(1-propenyl)-,(Z) present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

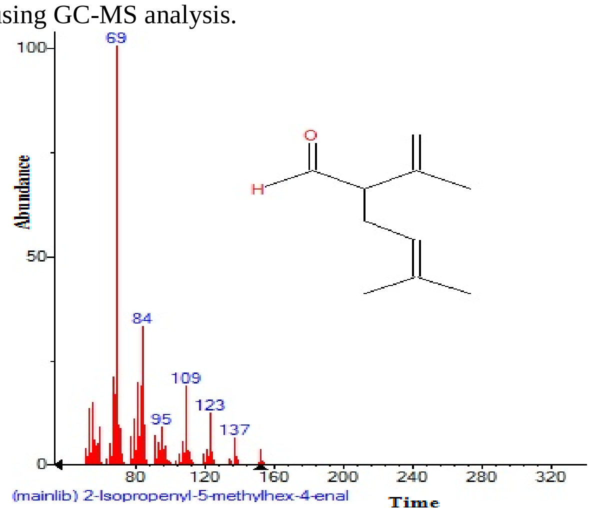


Figure 11: Structure of 2-Isopropenyl-5-methylhex-4-enal present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

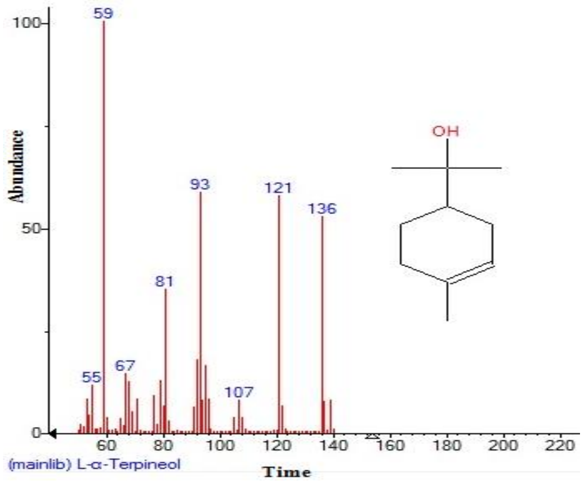


Figure 12: Structure of L-α-Terpineol present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

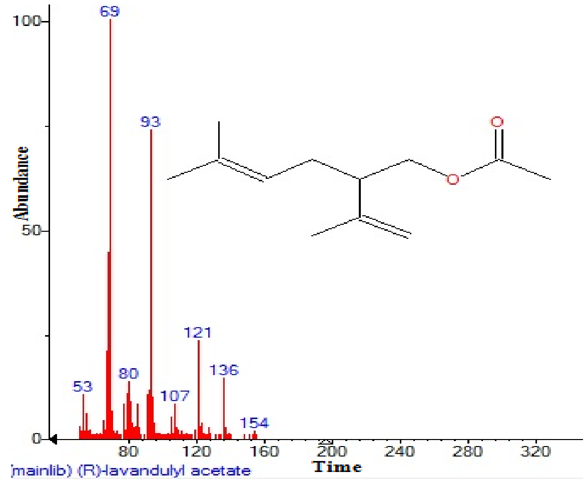


Figure 13: Structure of (R)-lavandulyl acetate present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

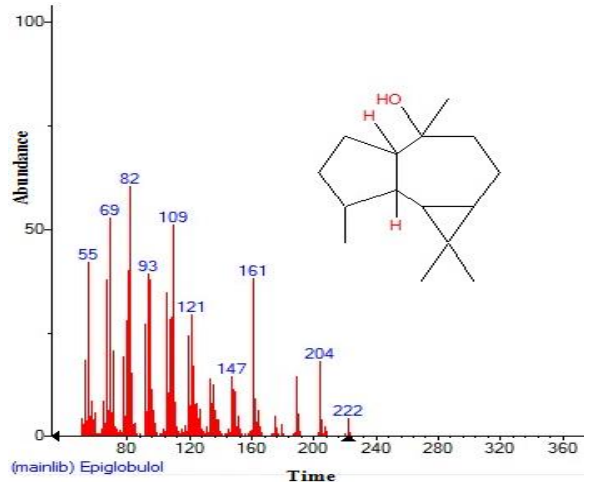


Figure 14: Structure of Epiglobulol present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

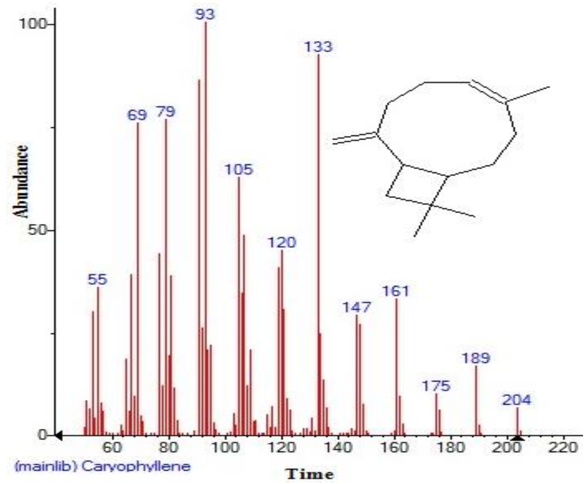


Figure 15: Structure of Caryophyllene present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

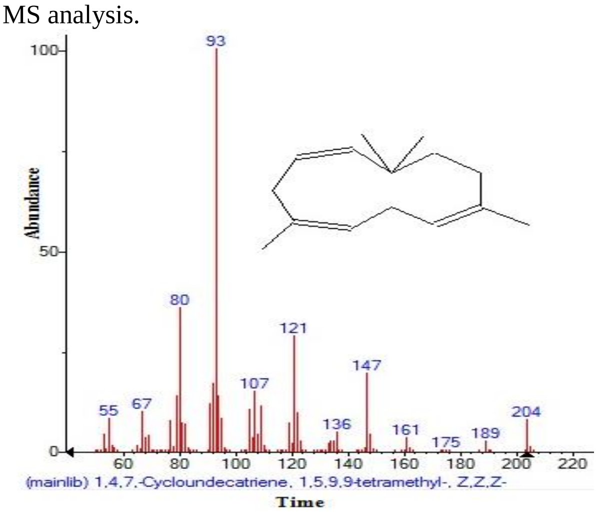


Figure 16: Structure of 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-,Z,Z,Z present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

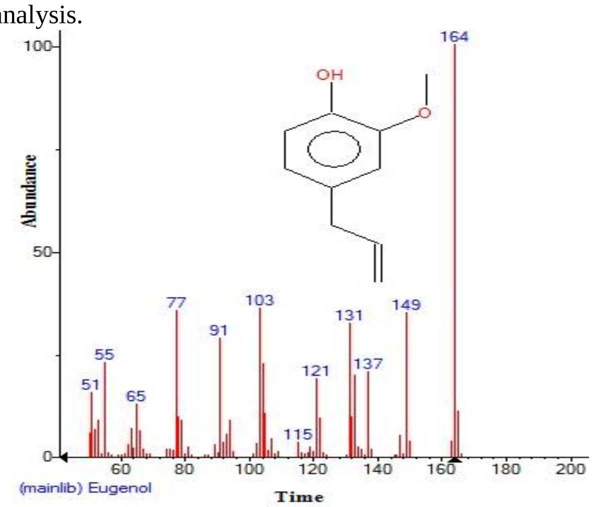


Figure 17: Structure of Eugenol present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

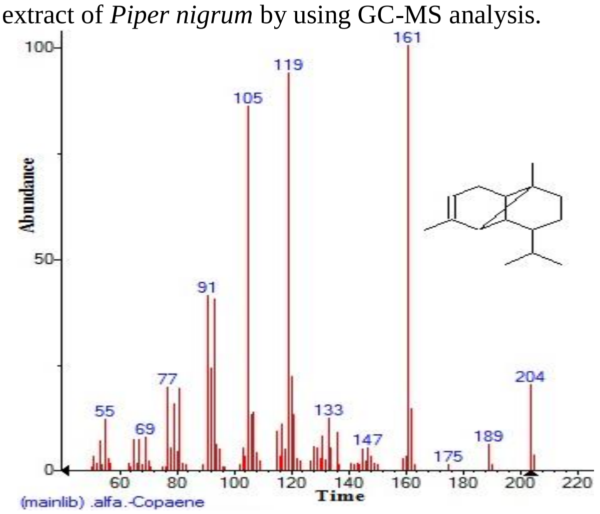


Figure 18: Structure of Alfa.Copaene present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

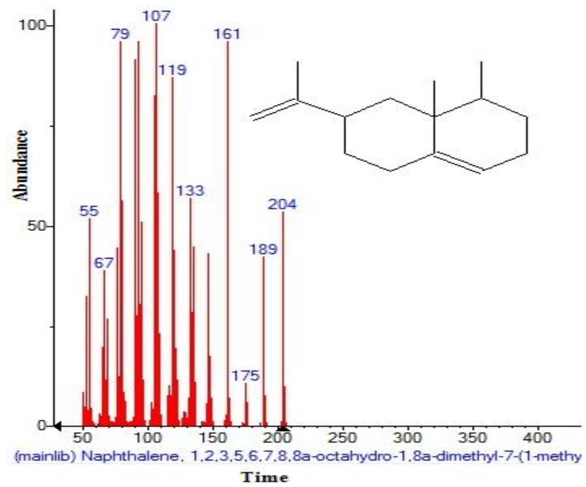


Figure 19: Structure of Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methyl present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

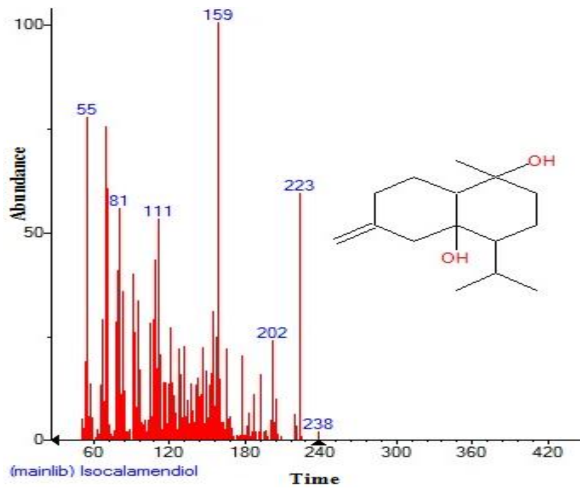


Figure 20: Structure of Isocalamendiol present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

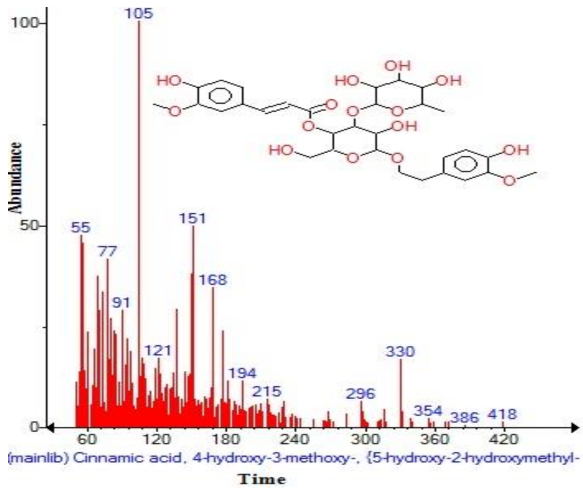


Figure 21: Structure of Cinnami acid , 4-hydroxy -3-methoxy-,{5-hydroxy-2-hydroxymethyl present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

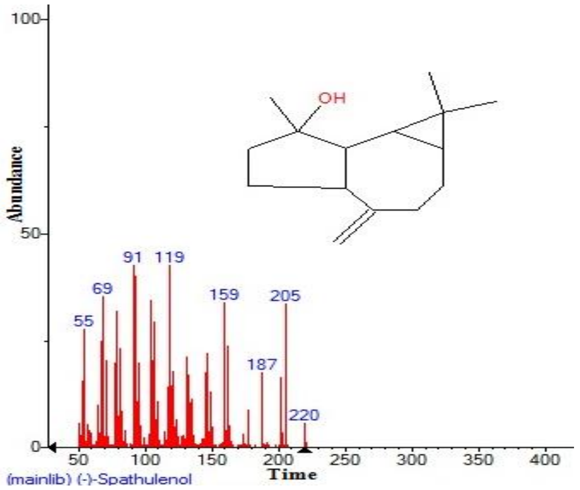


Figure 22: Structure of (-)-Spathulenol present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

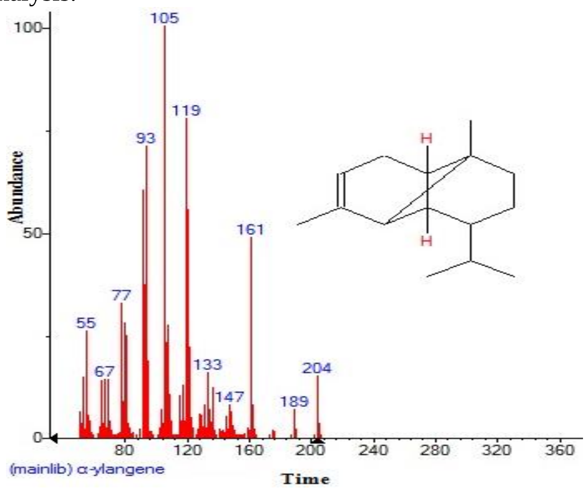


Figure 23: Structure of α- ylangene present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

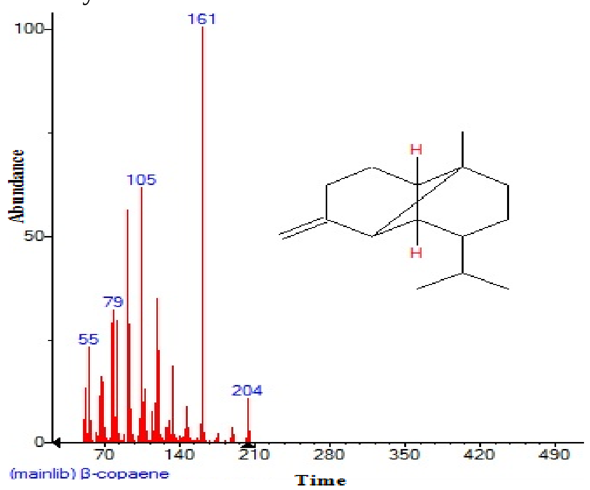


Figure 24: Structure of ß-copaene present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

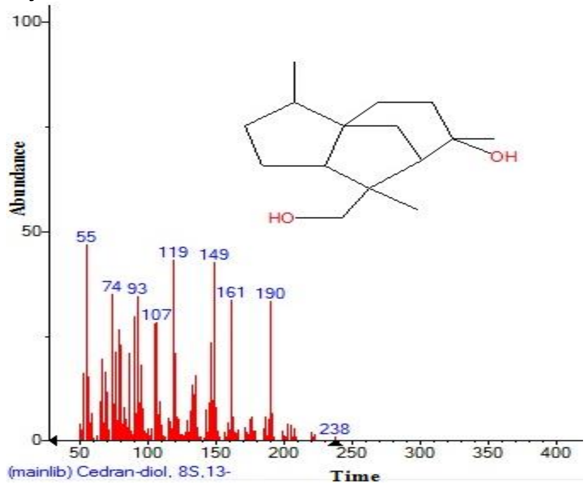


Figure 25: Structure of Cedran-diol,8S,13present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

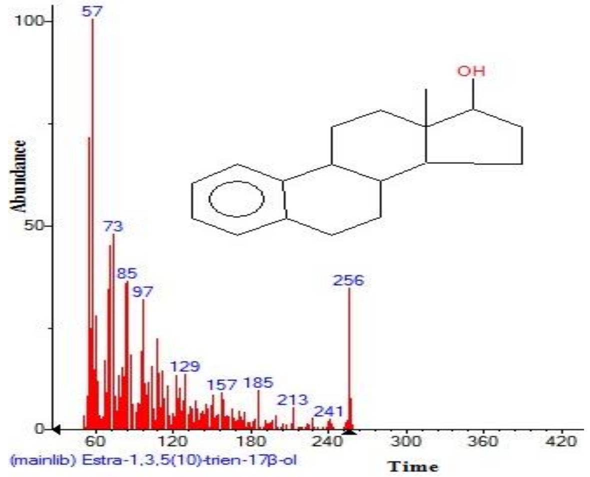


Figure 26: Structure of Estra-1,3,5(10)-trien-17ß-ol present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

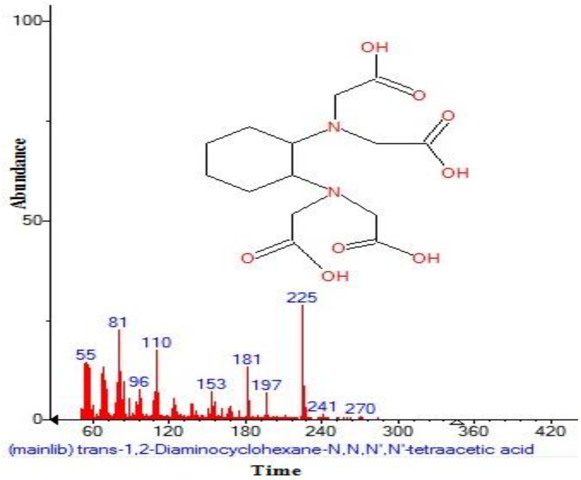


Figure 27: Structure of Trans-1,2-Diaminocyclohexane-N,N,N´,N´-tetraacetic acid present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

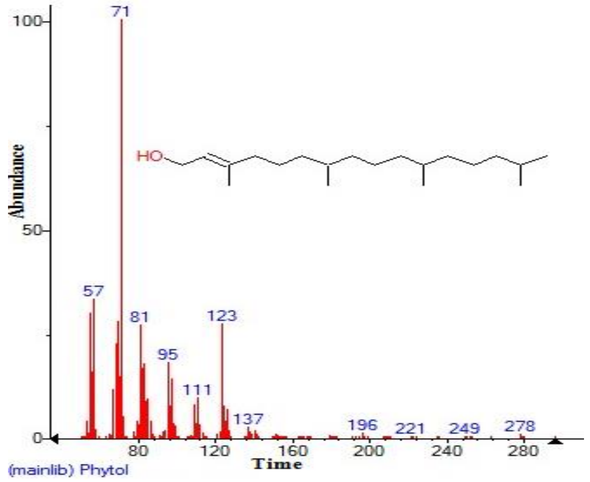


Figure 28: Structure of Phytol present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

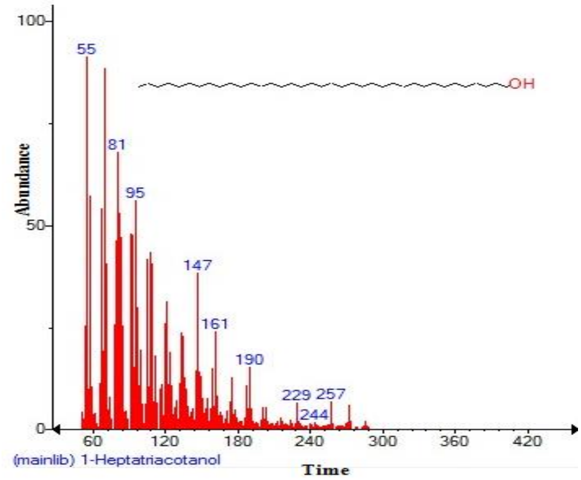


Figure 29: Structure of 1-Heptatriacotanol present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

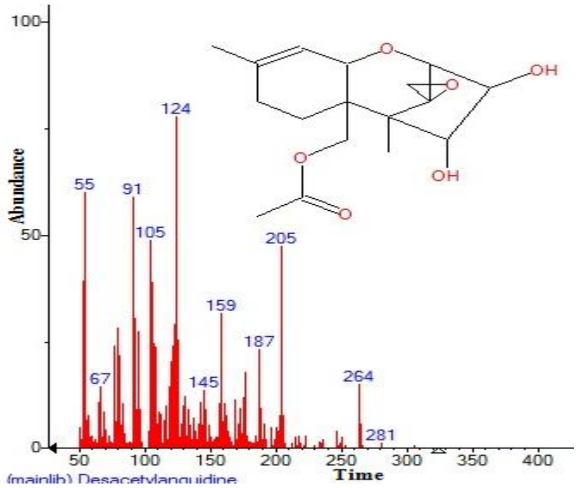


Figure 30: Structure of Desacetylanquidine present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

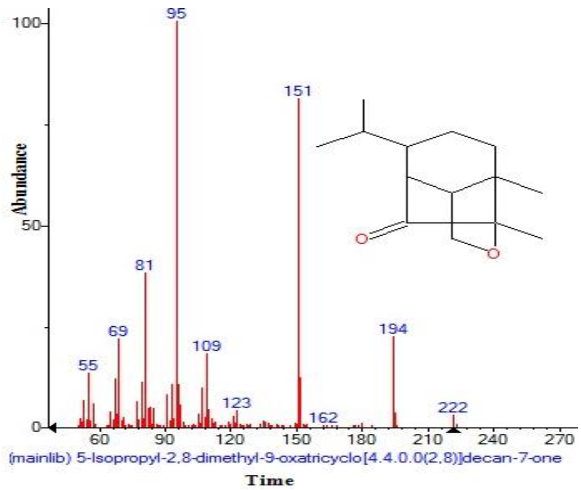


Figure 31: Structure of 5-Isopropyl-2,8-dimethyl-9-oxatricyclo[4.4.0.0.(2,8)]decan-7-one present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

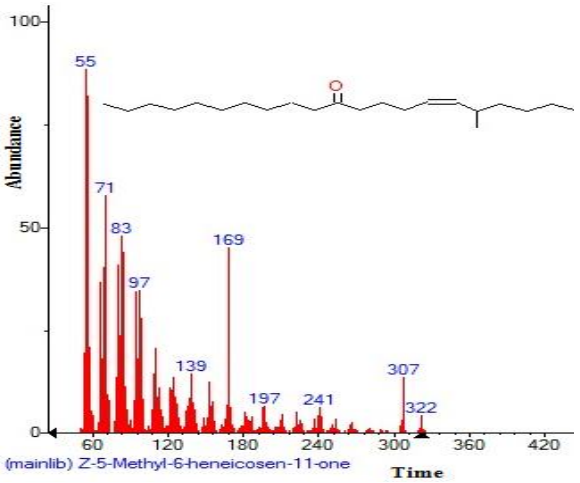


Figure 32: Structure of Z-5-methyl-6-heneicosen-11-one present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

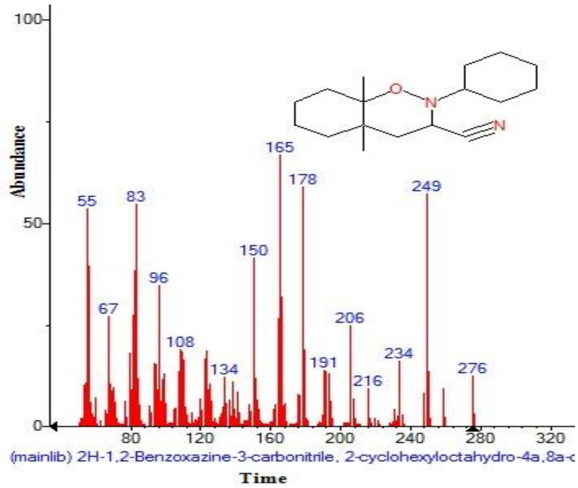


Figure 33: Structure of 2H-1,2-Benzoxazine-3-carbonitrile,2- cyclohexyloctahydro-4a,8a-d present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

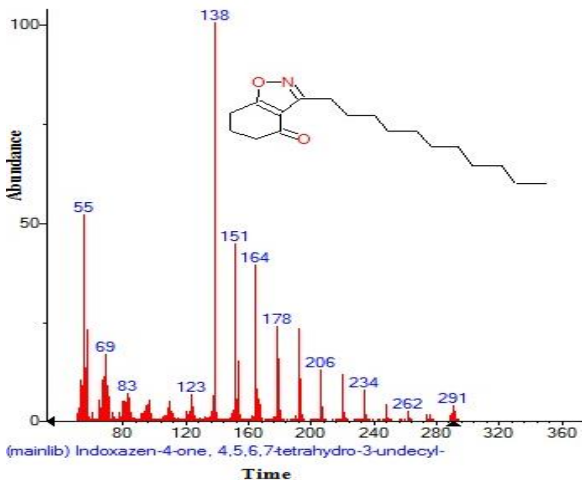


Figure 34: Structure of Indoxazin-4-one ,4,5,6,7-tetrahydro-3-undecyl present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

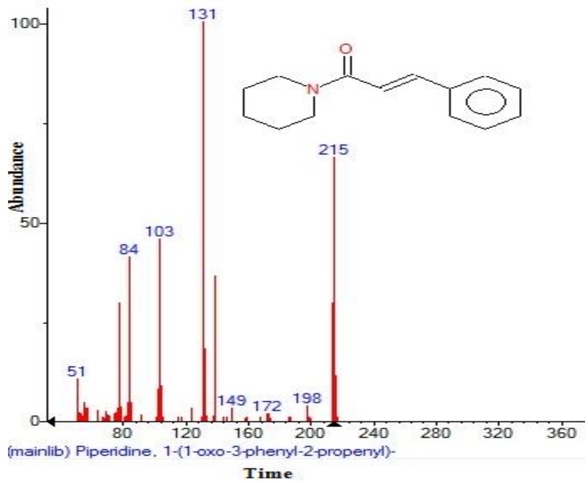


Figure 35: Structure of Piperidine,1-(1-oxo-3-phenyl-2-propenyl) present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

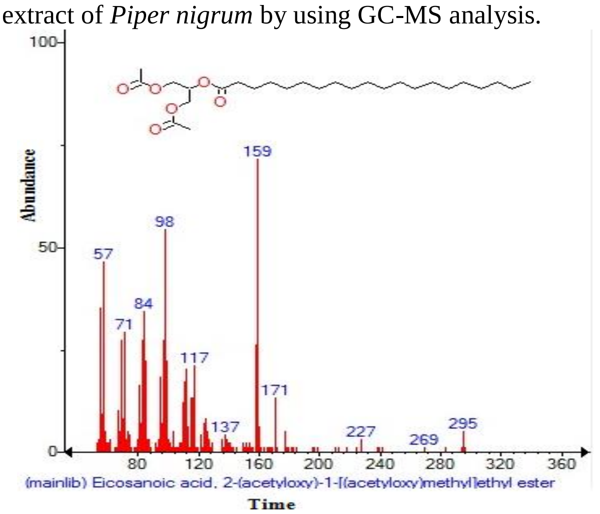


Figure 36: Structure of Eicosanoic acid , 2-(acetyloxy0-1-[(acetyloxy)methyl]ethyl ester present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

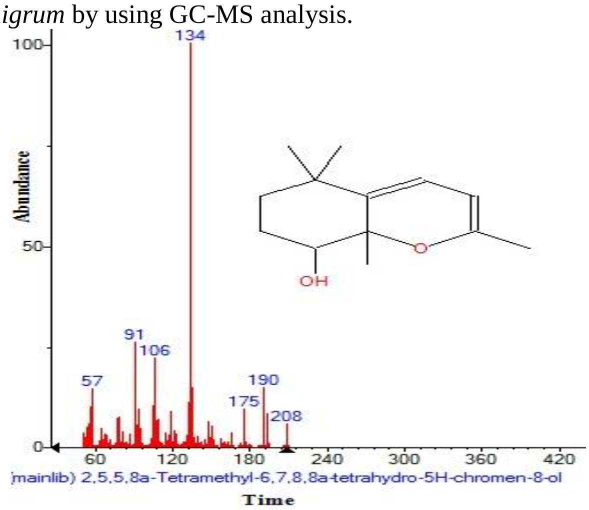


Figure 37: Structure of 2,5,5,8a-Tetramethyl-6,7,8,8a-tetrahydro-5H-chromen-8-ol present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

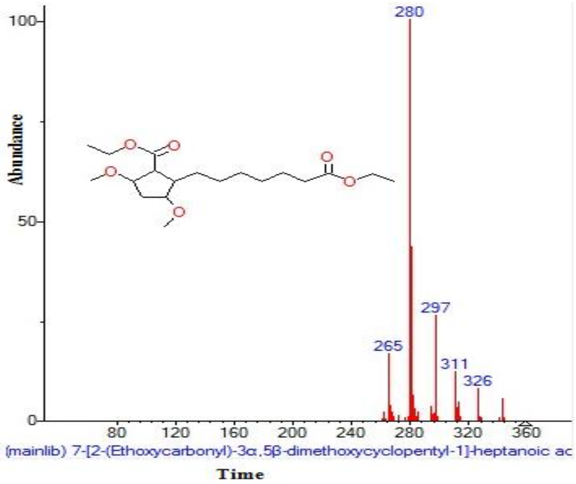


Figure 38: Structure of 7-[2-(Ethoxycarbonyl)-3α,5ß-dimethoxycyclopentyl-1]-heptanoic acid present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

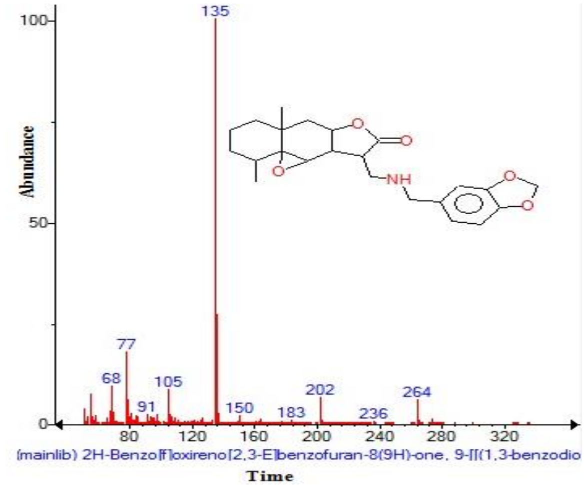


Figure 39: Structure of 2H-Benzo[f]oxireno[2,3-E]benzofuran-8,(9H)-one,9-[[(1,3-benzodio present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

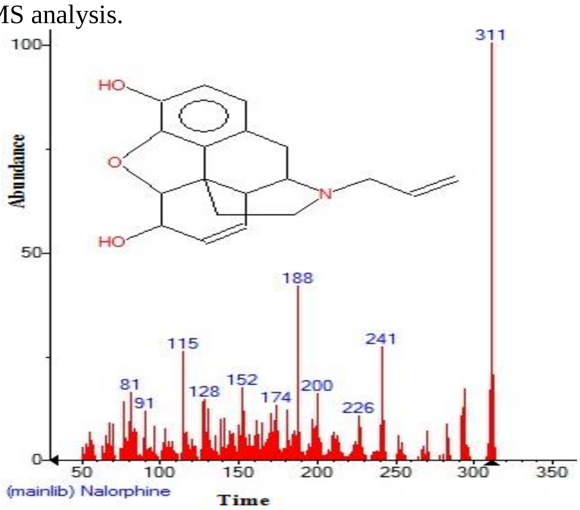


Figure 40: Structure of Nalorphine present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

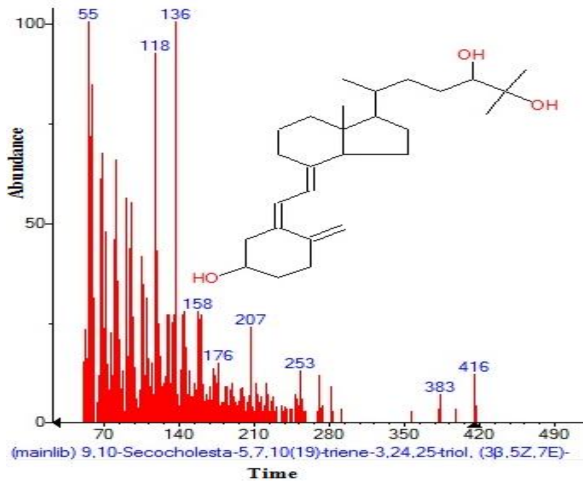


Figure 41: Structure of 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3ß,5Z,7E)present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

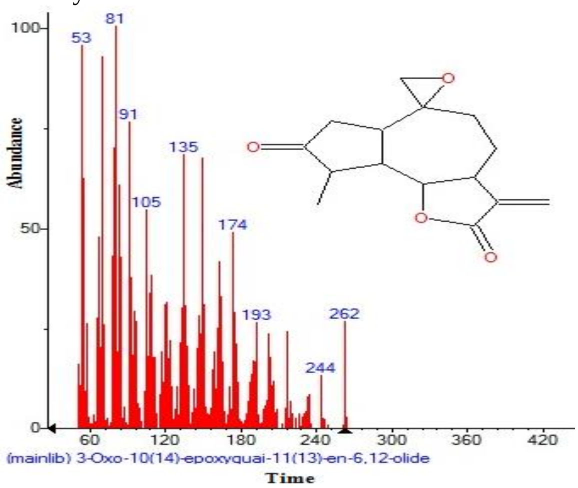


Figure 42: Structure of 3-Oxo-10(14)-epoxyguai-11(13)-en-6,12-olide present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

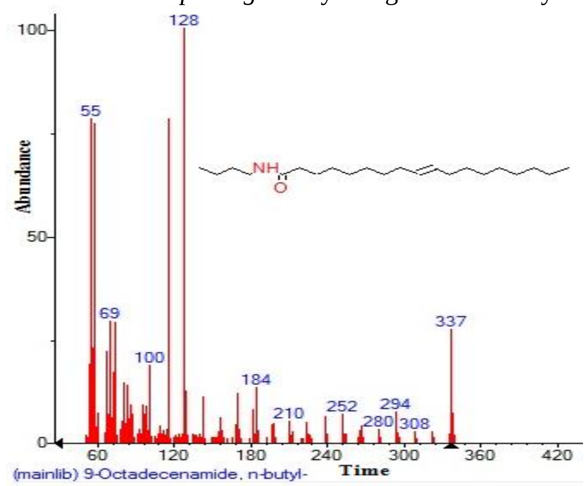


Figure 43: Structure of 9-Octadecenamide , n-butyl present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

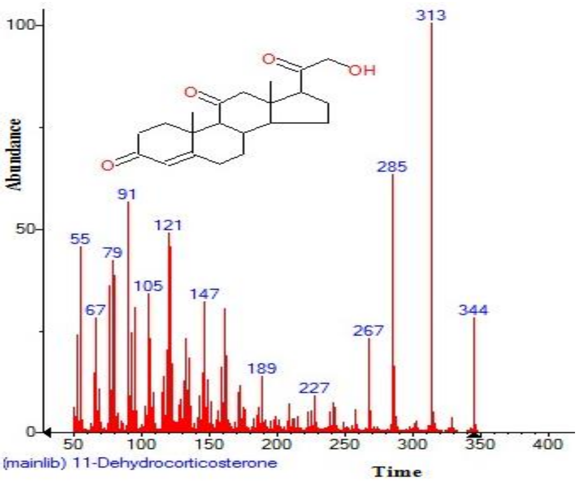


Figure 44: Structure of 11-Dehydrocorticosterone present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

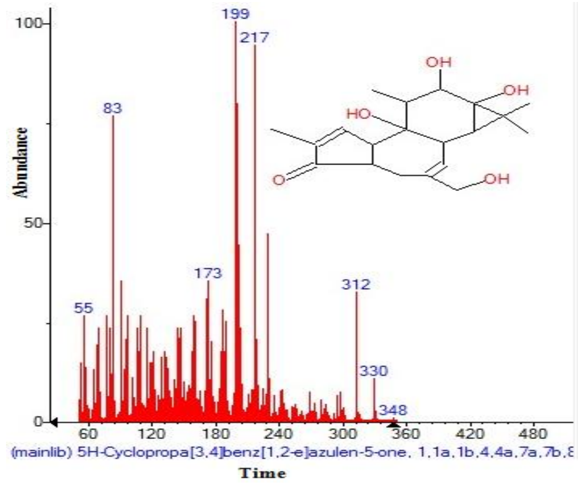


Figure 45: Structure of 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,1,1a,1b,4,4a,7a,7b present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

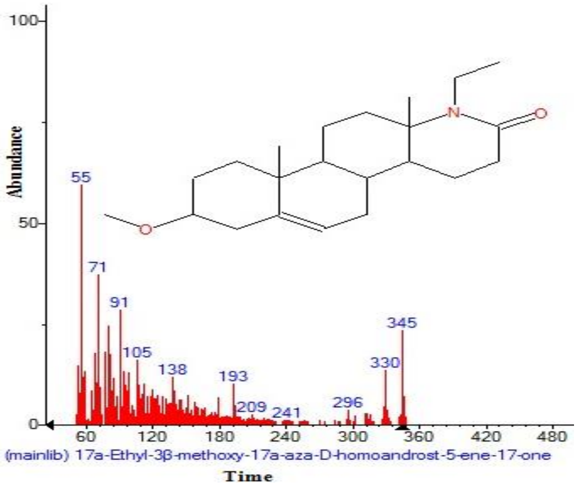


Figure 46: Structure of 17a-Ethyl-3ß-methoxy-17a-aza-D-homoandrost-5-ene-17-one present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

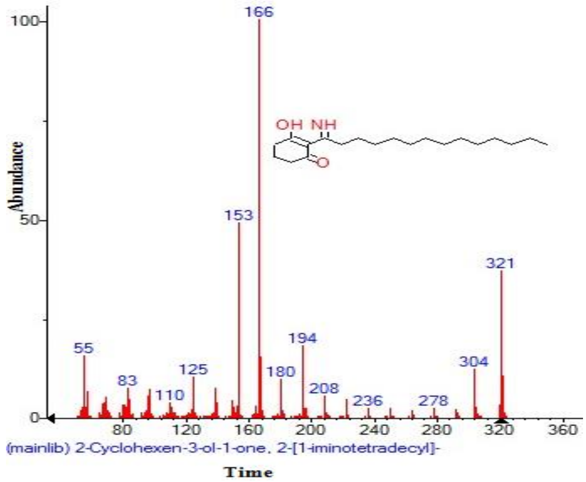


Figure 47: Structure of 2-Cyclohexen -3-ol-1-one , 2-[1-iminotetradecyl] present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

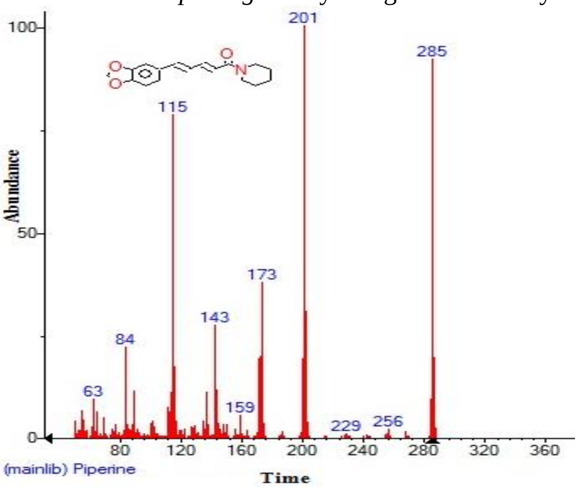


Figure 48: Structure of Piperine present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

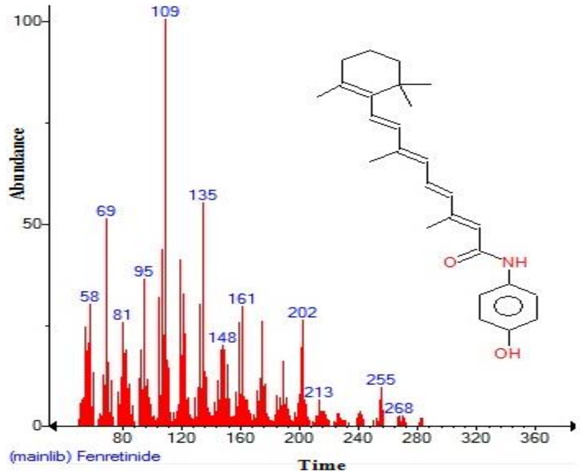


Figure 49: Structure of Fenretinide present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

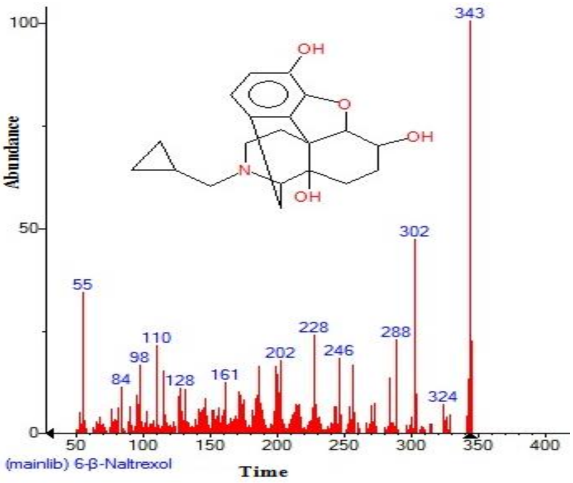


Figure 50: Structure of 6-ß-Naltrexol present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

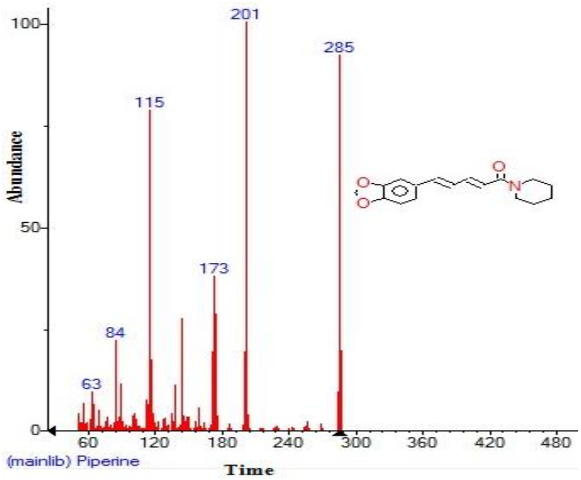


Figure 51: Structure of Piperine present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

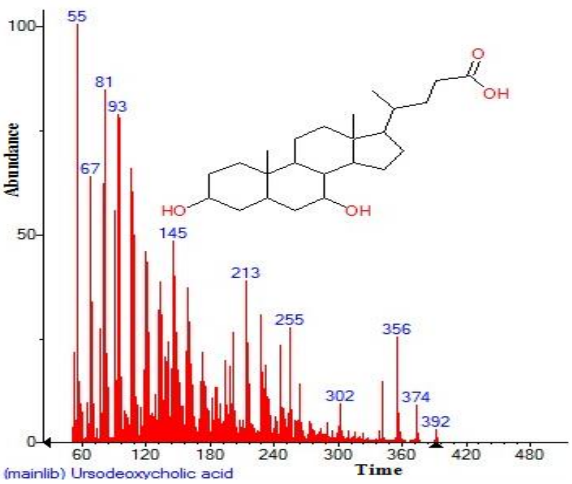


Figure 52: Structure of Ursodeoxycholic acid present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

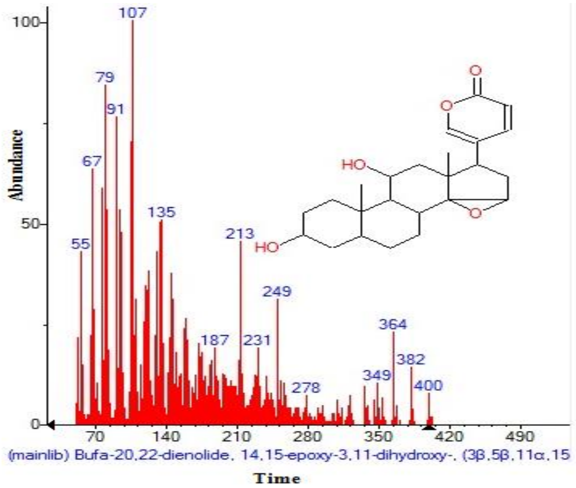


Figure 53: Structure of Bufa-20,22-dienolide m,14,15-epoxy-3,11-dihydroxy-,(3ß,5ß,11α,15present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

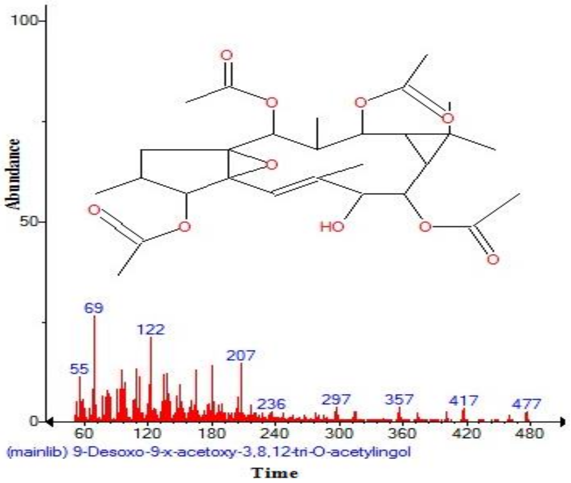


Figure 54: Structure of 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

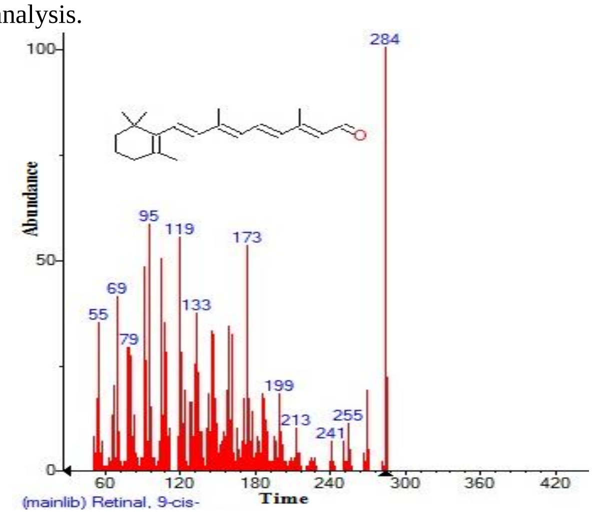


Figure 55: Structure of Retinal ,9-cis present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

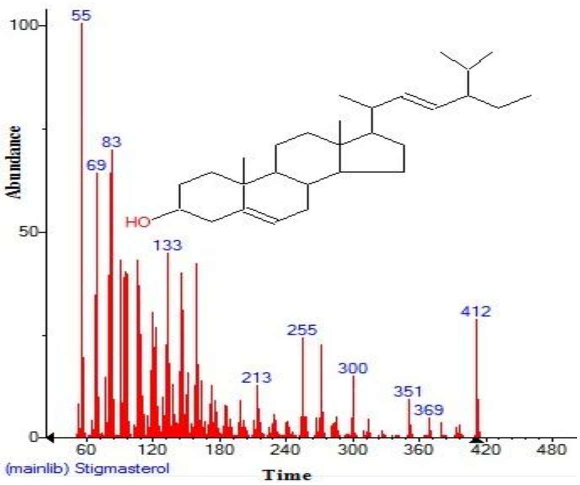


Figure 56: Structure of Stigmasterol present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

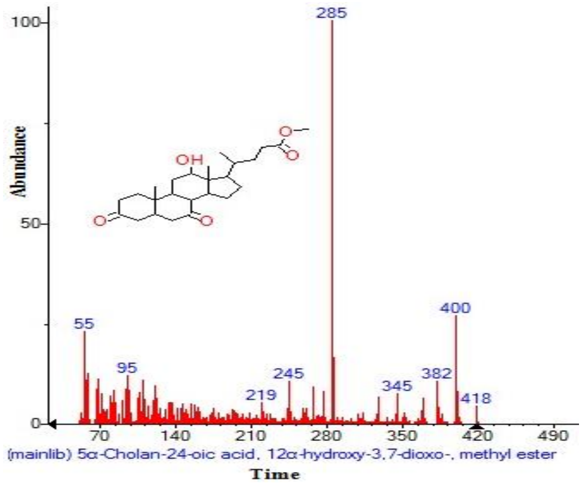


Figure 57: Structure of 5α-Cholan-24-oic acid , 12α-hydroxy-3,7-dioxo-,methyl ester present in the methanolic seeds extract of *Piper nigrum* by using GC-MS analysis.

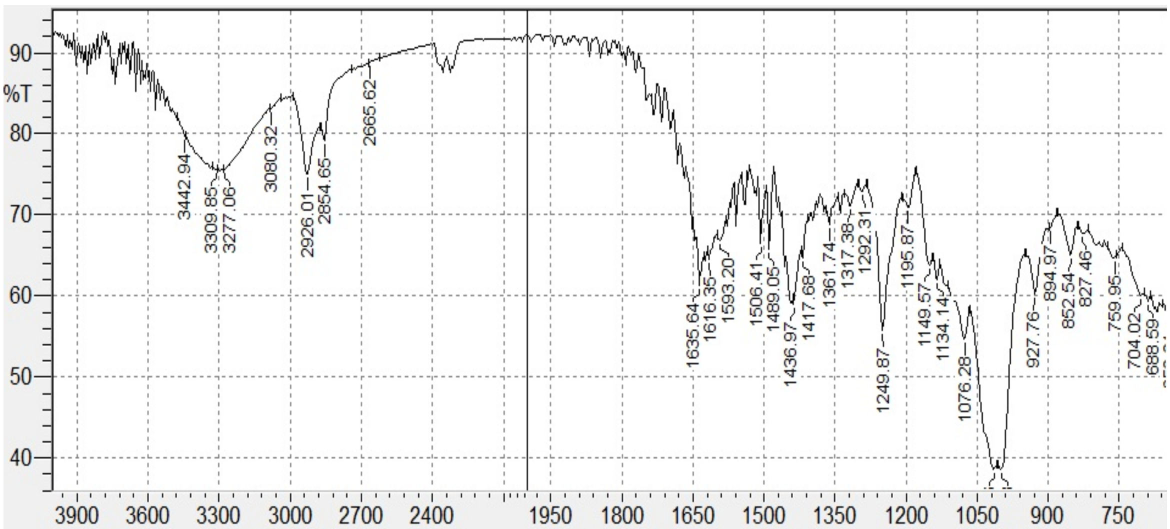


Figure 58: FT-IR profile of *Piper nigrum*.

bored in 0.5cm in diameter. The plates were incubated at 37ºC for 24 h and examined. After the incubation the diameter of inhibition zones around the discs was measured.

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic fruits extract of *P. nigrum*, shown in Table1. The GC-MS chromatogram of the 55 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *P. nigrum* showed the presence of fifty five major peaks and the components corresponding to the peaks were determined as follows. The first set up peak were determined to be α-pinene Figure 2. The second peak indicated to be Camphene Figure 3. The next peaks considered to be Eucalyptol, 2-Methoxy-4-vinylohenol, 1-Oxaspiro [4,5] deca-3,6-diene,2,6,10,10-tetramethyl, 1-Oxaspiro [4,5]deca-3,6-diene,2,6,10,10-tetramethyl, Neocurdione, Isoaromadendrene epoxide, 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9] cycloundec., Cis-Vaccenic acid, Phenanthrenol,4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trimethyl-1, Galanthamine, Dibenz[a,c]cyclohexane,2,4,7-trimethoxy, 2,4a,7-Trihydroxy-1-methyl-8-methyleneqibb-3-ene. 1,10-carboxylic acid, Retinoic acid, 7,8,12-Tri-O-acetyl-3-desoxy-ingol-3-one, 4,6-Androstadien-3β-ol-17-one, acetate. (Figure 4-57). FTIR analysis of dry methanolic extract of *Rosmarinus oficinalis* leaves proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxlic acids, Esters and Nitro Compounds which shows major peaks at 688.59, 827.46, 927.76, 1014.56, 1249.87, 1361.74, 1417.68, 1506.41, 2665.62, 2854.65 and 2926.01 (Table 2; Figure 58). Boutekedjiret *et al.* (2003)[32] studied the constituents of rosemary essential oil from Algeria. They reported 1, 8-cineole, camphor, β-pinene, and α-Pinene as the major constituents in the oil. Viuda-Martos et al. (2007)[33] investigated chemical composition of the essential oil of anther sample of rosemary leaves from Spain. The major constituents identified were α-pinene, camphor, 1,8-cineole and camphene. The main components detected in the oils were: α-pinene, 1, 8-cineole, camphene, camphor, myrcene and broneol[34]. GC and GC-MS analysis of oils from rosemary leave samples from India revealed the presence of camphor, 1,8-cineole and α-pinene as major constituents in the oils[35-37]. In this study five clinical pathogens selected for antibacterial activity namely, (*staphylococcus aeureus, klebsiella pneumoniae, pseudomonas aeroginosa*, *E. coli.* and *Proteus mirabilis*. Maximum zone formation against *Proteus mirabilis,* Table 3.

CONCLUSION

P. nigrum is native plant of Iraq. It contain chemical constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthamatic.

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