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GC–MS analysis of bioactive compounds present in different extracts of rhizome of *Curcuma aeruginosa* Roxb.

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ABSTRACT

To analyze and characterize the chemical composition of the different crude extracts from the rhizome of *Curcuma aeruginosa* Roxb a medicinal plant. The air-dried rhizomes were powdered and subjected to Soxhlet extraction using solvent n-hexane and Supercritical fluid extraction. Then, each of the extracts was further subjected to gas chromatography-mass spectrometry. Qualitative determination of the different biologically active compounds from crude extracts of *C aeruginosa* Roxb using gas chromatography–mass spectrometry revealed different types of high and low molecular weight chemical entities with varying quantities present in each of the extracts. These chemical compounds are considered biologically and pharmacologically important. Furthermore, the two different extracts SCF and n-hexane possess unique physicochemical characteristics. The two extracts possess major bioactive compounds that were identified and characterized spectroscopically. Thus, identification of different biologically active compounds in the extracts of *C aeruginosa* Roxb warrants further biological and pharmacological studies.

Keywords: Supercritical Fluid, Soxhlet extraction, GC-MS

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INTRODUCTION

Plants play a significant role in the prevention and treatment of diseases and can even prevent and reduce the adverse effects of conventional treatments [1]. They can be a source of chemical compounds of biological and pharmacological importance. History reveals that plants are sources of successful drugs, and will continuously be important for screening of new lead compounds [2]. An essential part in the investigation of a plant is the identification of the biologically active compounds present in plant leading to further biological and pharmacological studies [3–5]. The search for new plant-derived chemicals should thus be a priority in current and future efforts toward sustainable conservation and rational utilization of biodiversity [6]. The family Zingiberaceae comprises advanced monocot plants and is characterized by aromatic, non-tuberous and tuberous rhizomes, which have tremendous ethno medicinal properties.[7,8] *Curcuma aeruginosa* was commonly known as 'Black turmeric' is a perennial underutilized herb of the family Zingiberaceae. It usually grows well in moist deciduous forests.[9] The plant has a characteristic rhizome flesh of bluish black color with a pungent smell and hot bitter taste, because of the presence of essential oil rich in camphor and starch. The rhizome is traditionally used in the treatment of hemorrhoids, leprosy, asthma, cancer, fever, wounds, vomiting, menstrual disorder, anthelmintic, aphrodisiac, gonorrheal discharges, and inflammation.[10,11]. The rhizome of *C. aeruginosa* is a promising source of potential anti-oxidants.[12] It is employed for making various cosmetic items and for sprains and bruises.[13] Anti-androgenic effect of sesquiterpenes isolated from the rhizomes of *C. aeruginosa* had been reported.[14] Due to its high medicinal value and indiscriminate harvest from the wild, the natural population has come down and according to International Union for Conservation of Nature (IUCN) report, the plant is in the critically endangered category.[15] This paper mostly highlighted on the analysis and identification of bioactive compounds present in the plant extracts through GC-MS.

MATERIAL AND METHODS

Plant material: Plant material was collected in February 2015 from Waynad district of Kerala, India. Taxonomic identification of the plants was carried out by Dr. S John Britto, Director at the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamilnadu, India. Voucher specimens (RHT 68570) are submitted at the Rapinat Herbarium. Fresh rhizomes of Black turmeric (*Curcuma aeruginosa*) was collected and cultivated in Vadakkencherry Holy Family garden.

Preparation of Plant Extract

The collected rhizomes were dried under the room temperature and powdered with a mechanical grinder and stored in air tight container.

Super Critical Fluid Extraction

SFE was carried out on the extraction system (model HA220-50-06, Nantong, China) which consists of a 5 L volume extractor, an HA220-50-06 controller, two syringe pumps (model 100 DX, Jiangsu, China), and a CO_2 cylinder. The operation pressure and temperature could reach up to 80 MPa and 45ºC. The extraction pressure of the system was adjusted by a pressure regulator and the temperature was controlled by a thermostatic water bath. The parameters, namely, extraction pressure (P), temperature (T), dynamic extraction time (t), and flow rate of CO2 (F), were studied and optimized.

For each extraction, about 1.0 kg of the prepared plant material was loaded into the extractor. Dense liquid [16] CO_2 is then pumped through a cylinder containing the material to be extracted. The extract-laden liquid is then pumped into a separation chamber where the extract is separated from the gas and the gas is recovered for re-use. The extracted substances were collected at the bottom of the separators.

Soxhlet method of hexane extraction

The 20 g of dried powder was subjected to Soxhlet extraction using hexane. The solvent (150 ml of hexane) is added to a round bottom flask (250ml volume), which is attached to a Soxhlet extractor and condenser on an isomantle. The rhizome powder is loaded into the thimble, which is placed inside the Soxhlet extractor. The sidearm is lagged with glass wool. The solvent is heated using the isomantle and will begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of the solvent reaches the siphon it pours back into the flask and the cycle begins again. The process runs for two days at 60ºC.

GC–MS analysis

GC-MS analysis was performed on a Shimadzu (Tokyo, Japan) Make GCMS-TQ8030 with nonpolar Rxi 5Sil MS capillary column, full scan mode, injector mode-splitless, quadra pole mass selective detector(MSD), injection temperature 250ºC, GC-MS interface temperature 250ºC, the injection volume was 1µl. Helium was employed as the carrier gas, at a pressure of 57.5KPa; flow rate was 1ml/min. Mass spectra were detected at 70eV. Temperature programming was set as follows: column temperature was started from 60ºC (held for2 min) and linearly increased by 5ºC/min to 200ºC (held for 2min); after that, it was increased by 3ºC/min to 220ºC (held for 1 min); further, it was increased by 6ºC/min. to 250ºC (held for 7min). Total GC running time was 51.67 min. The components of the oil were identified by comparison of their mass spectra with those of the spectrometer database using NIST library (Shimadzu). The identifications were confirmed by comparison of the fragmentation pattern and their retention indices with those reported in the literature.

RESULTS AND DISCUSSION

Bioactive compounds present in the SFE and Hexane extracts, since various parameters potentially affect the extraction process, the optimization of the experimental conditions is a critical step in developing an SFE method. Based on the previous knowledge of SFE, the extraction pressure, temperature, the dynamic extraction time and flow rate of CO_2 are usually considered as the most important factors of SFE. The optimal conditions for extraction of *C aeruginosa* by SFE were 40 MPa of pressure, 50ºC of temperature, 1.5 h dynamic extraction time and 40 L/Hour (20Hz) flow rate of CO_2. Figure 1 and 2 depicts a GC-MS chromatograph of *C aeruginosa* obtained by SFE and Soxhlet method**.** The identification of the compounds was done by NIST as well as of compound, quality, retention times, molecular weight (MW) and relative content with those from literature data [17]. The GC–MS analysis of compounds from *C aeruginosa* extracts was performed using a Rxi 5 Sil MS capillary column. In Table 1 (Fig:1)was reported the GC–MS data and the compounds identified in the extracts of *C aeruginosa* by SFE at the set of 40 MPa of pressure, 50ºC of temperature, 1.5 h dynamic extraction time and 40 L/Hour (20Hz) flow rate of CO_2. Based on abundance, the major compounds present in the SCF extract were 5 .beta.- Guaia-7(11),10(14)-dien-8.alpha.-ol,5,8-epoxy-,(+)-(27.09%),7H-2,4a-Methanonaphthalen-7one, 1,2,3,4,5,6-hexahydro-1,1,5,5-tetramethyl-,(2S,4aR)-(-)-(6.31%), Boldenone(5.89%), (2E)-2-(4Methoxybenzylidene) Cyclo hexanone (4.2%), 17.alfa.,21.beta.-28,30 Bisnorhopane (3.81%), 5.beta.-Guaia-7(11),10(14)-dien-8.α-ol,5,8-epoxy-,(+)-(3.28%), Nootkaton-11,12-epoxide(3%), Bis(2-ethylhexyl) phthalate(2.85%), (+)-2Bornanone (2.08%),2-(1-(Beta-d-glucopyranosyloxy)-1-methylethyl)-2,3-dihydro-7-oxo-7H furo(3,2-g)chromene, (R)-(2.6%), Tetracyclo [5.4.3.0(7,11)]tetradeca-2,5,10-trione, 1,4,6,14-tetramethyl-4-vinyl-(1.84%), Naphthalene, deca hydro-1,4a-dimethyl-2-methylene-(1.81%), 1,5,9-Cyclo dodecatriene, 1,5,9-trimethyl-(1.81%),2H-Cyclohepta [b]furan-2-one,6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene-(1.81%),as-Indacen-4(1H)-one,decahydro-,(3a.alpha.,5a. beta., 8a.beta.,8b.beta.)-(1.42%),2,4-DIISO PROPENYL-1-METHYL-1-VINYLCYCLO HEXANE (1.35%), 1,2-Dimethyl-5-nitroadamantane(1.31%), Caryophyllene (1.29%), 5-Isoborneol(1.03%).

Table 2 (Fig; 2) depicts that biologically active chemical compound of n-hexane extract from C aeruginosa Roxb. rhizome the major compounds found in this were 5.beta.-Guaia-7(11),10(14)-dien-8.alpha.-ol, 5,8-epoxy-, (+)-(13.34%), 17-HYDROXYANDROSTA-1,4-DIEN-3-ONE(8.5%), 5.beta.-Guaia-7(11),10(14)-dien-8.alpha.-ol, 5,8-epoxy-, (+)-(8.47%), Naphthalene, decahydro-1,4a-dimethyl-2-methylene-(7.37%),(2E)-2-(4-Methoxybenzylidene) Cyclo hexanone(6.94%), Cycloprop[e]indene-1a,2(1H)-dicarbox aldehyde, 3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl-,(1a.alpha.,3a.beta.,6a.beta.,6b.alpha(5.72%), 5,8-Dihydroxy-4a-methyl-4,4a,4b,5,6,7,8,8a,9,10-decahydro-2(3H)-phenanthrenone(4.77%), n-Hexadecanoic acid (4.02%), Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde, 3a,4,5,6, 6a,6b-hexahydro-5,5,6b-trimethyl-, (1a.alpha.,3a. beta.,6a. beta.,6b.alpha(3.86%), Valerenal (3.56%), 5-Isopropenyl-3,6-Dimethyl-6-Vinyl-4,5,6,7-Tetrahydro-1-Benzofuran (2.72%), 5.beta.-Guaia-7(11),10(14)-dien-8.alpha.-ol, 5,8-epoxy-, (+)-(1.93%), (3aRS,4SR,6aRS,2'Z)-Hexahydro-3-methylidene-4-(pent-2'-enyl)-2H-cyclopenta[b]furan-2-one(1.91%), Cyclopropane, 1,1-Dimethyl-2-(2-Methyl -3-Buten-2-YL)- (1.83%), Thunbergol(1.83%), (-)-Isolongifolol,

pentafluoropropionate(1.68%), 7-Tetradecenal, (Z)-(1.59%), Bufa-20,22-dienolide, 14,15-epoxy-3,5,16-trihydroxy-, (3.beta.,5.beta.,15.beta.,16.beta.)-(1.44%), 1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, (+)-(1.41%), 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol(1.22%), 17-Hydroxyandrosta-1,4-DIEN-3-ONE(1.18%), 3,7-Cyclodeca dien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E)-(1.16%), 1,1,7-TRIMETHYL-4-METHYLENEDECAHYDRO-1H-CYCLOPROPA[E]AZULEN-7-OL(1.07%), Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde, 3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl (1a.alpha.3a.beta.6a. beta., 6b. apha (1.3%) , Isospathulenol(1.1%).

DISCUSSION

The usage of plants or herbs for medicinal purposes relies on their phytochemical composition that exhibits some interesting and specific biological activities. Different phytochemicals identified in the present study have been found to possess a wide range of biological activities. It has long been reported that Zingiberaceae families contained a number of phytochemicals such as terpenoids, flavonoids, phenypropanoids and sesquiterpenes which exhibited anti-tumor activities [18, 19]. GC–MS analysis of the SCF and Hexane extract revealed the presence of various bioactive compounds. Direct solvent extraction using SCF revealed the occurrence of 68 compounds whereas Hexane allowed the extraction of 52 compounds respectively. All the major compounds from different extracts are biologically active molecules. They are considered to be a part of plants' defense systems, and as such have been included in a large group of protective molecules found in plants named 'phytoanticipins' or 'phytoprotectants'[20-22]. There are several reports in the chemical composition of the essential oil from the rhizome of Curcuma species [23-25], Monoterpene and sesquiterpene hydrocarbons represented the most common chemical groups found in the essential oils from the rhizomes of the investigated Curcuma species [26]. Several phytochemicals identified in the rhizome of *C. aeruginosa* in the present study have previously been reported by other researchers. These included compounds such as germacrone[27,28], cycloisolongifolene 8,9-dehydro-9-formyl, velleral and camphor[29], 1,8-cineole, pinene[30], curzerene and elemene[31]. However, most of the above mentioned compounds were detected in the essential oil of *C. aeruginosa* rhizome. While comparing the GCMS results of two extracts three compounds present in both extracts namely,5.beta.-Guaia-7(11),10(14)-dien-8.alpha.-ol, 5,8-epoxy, (+)- (30.37% SCF and 23.74% Hexane) (2E)-2-(4METHOXYBENZYLIDENE) CYCLOHEXANONE (4.20% and 6.94%) Naphthalene, decahydro-1,4a-dimethyl-2-methylene-(1.81% and7.37%) respectively in different concentrations. These major compounds have cytotoxic, antioxidant, antitumor antiangiogenic[32,33] cytotoxicity[34,35] cholesterol-lowering activity[36] use in agrochemicals, pharmaceuticals and perfumes[37]. It has been reported that terpenes have antibacterial activity because of their bacteriostatic and bactericidal effects [38].

It was concluded that SCF and hexane extract of rhizome of *Curcuma aeruginosa* possess various potent bioactive compounds and is recommended as a plant of phytopharmaceutical importance. Further studies are needed to explore the potential compounds responsible for the biological activity from *Curcuma aeruginosa* for application in drug delivery, nutritional or pharmaceutical fields.

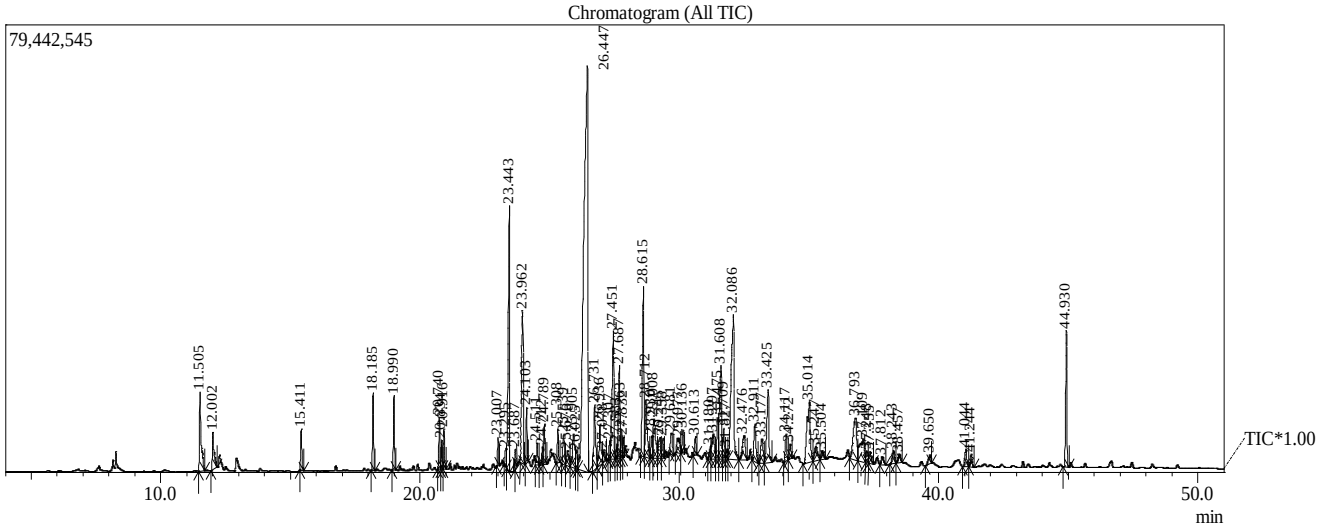

Figure 1: A typical chromatogram of the bioactive compounds present in SCF extract.

Table 1: Biologically active chemical compounds of SCF extract from *C aeruginosa* Roxb.

Sl. No.	RT	Peak area (%)	Name of the compound	Molecular formula	MW (g/mol)
1	7.601	0.07	3-Carene	$C_{10}H_{16}$	136.24
2	8.15	0.23	D-Limonene	$C_{10}H_{16}$	136.24
3	8.261	0.25	Eucalyptol	$C_{10}H_{18O}$	154.25
4	11.505	2.08	(+)-2-Bornanone	$C_{10}H_{16}O$	152.24
5	12.002	1.03	Isoborneol	$C_{10}H_{18}O$	154.25
6	12.251	0.5	1,7,7-TRIMETHYLBICYCLO[2.2.1]HEPTAN-2-OL	$C_{10}H_{17}ClO$	188.70
7	12.902	0.52	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-	$C_{12}H_{20}O_2$	196.29

8	15.411	0.82	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, exo-	$C_{19}H_{24}O_2$	284.40
9	16.728	0.09	.delta.-Elemene	$C_{15}H_{24}$	204.36
10	18.185	1.35	2,4-DIISOPROPENYL-1-METHYL-1-VINYLCYCLOHEXANE	$C_{15}H_{24}$	204.36
11	18.99	1.29	Caryophyllene	$C_{15}H_{24}$	204.36
12	19.204	0.08	.gamma.-Elemene	$C_{15}H_{24}$	204.36
13	19.715	0.09	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, (1aR,4aS,7R,7aR,7bS)-(-)-	$C_{15}H_{24}$	204.36
14	19.89	0.12	.alpha.-Humulene	$C_{15}H_{24}$	204.36
15	20.332	0.17	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	$C_{15}H_{24}$	204.36
16	20.74	0.86	.beta.-Selinene	$C_{15}H_{24}$	204.36
17	20.834	0.48	5-ISOPROPENYL-3,6-DIMETHYL-6-VINYL-4,5,6,7-TETRAHYDRO-1-BENZOFURAN	$C_{15}H_{20}O$	216.32
18	20.916	0.73	2-ISOPROPENYL-4A,8-DIMETHYL-1,2,3,4,4A,5,6,8A-OCTAHYDRONAPHTHALENE	$C_{15}H_{24}$	204.36
19	21.146	0.13	.beta.-Bisabolene	$C_{15}H_{24}$	204.36
20	21.314	0.07	1-ISOPROPYL-7-METHYL-4-METHYLENE-1,2,3,4,4A,5,6,8A-OCTAHYDRONAPHTHALENE	$C_{15}H_{24}$	204.36
21	21.413	0.11	4-epi-cubedol	$C_{15}H_{26}O$	222.37
22	22.803	0.19	Spiro[4H-cycloprop[e]azulene-4,2'-oxirane], decahydro-1,1,7-trimethyl-, [1aR-(1a.alpha.,4.beta.,4a.alpha.,7.alpha.,7a.beta.,7b.		
23	23.007	0.49	(-)-5-Oxatricyclo[8.2.0.0(4,6)]dodecane,,12-trimethyl-9-methylene-, [1R-(1R*,4R*,6R*,10S*)]-	$C_{15}H_{24}O$	220.36
24	23.163	0.11	1,1,4,7-TETRAMETHYLDECAHYDRO-1H-CYCLOPROPA[E]AZULEN-4-OL	$C_{15}H_{26}O$	222.37
25	23.295	0.41	2-(4a,8-Dimethyl-2,3,4,4a,5,6,7,8-octahydro-2-naphthalenyl)-2-propanol	$C_{15}H_{26}O$	222.37
26	23.443	5.89	Boldenone	$C_{19}H_{26}O_2$	286.42
27	23.62	0.05	Hexagermane	$Ge_6H_{16}Si$	479.99
28	23.64	0.04	ALLO AROMADENDRENOXID-(1)	$C_{15}H_{24}$	204.36
29	23.687	0.23	N-(4-HYDROXYPHENYL)-N,N',N'-TRIMETHYLSULFAMIDE	$C_9H_{14}N_2O_3S$	230.28
30	23.962	3.28	5.beta.-Guaia-7(11),10(14)-dien-8.alpha.-ol, 5,8-epoxy-, (+)-	$C_{15}H_{22}O_2$	234.34
31	24.103	0.94	isospathulenol	$C_{15}H_{24}O$	220.36
32	24.511	0.4	5.beta.-Guaia-7(11),10(14)-dien-8.alpha.-ol, 5,8-epoxy-, (+)-	$C_{15}H_{22}O_2$	234.34
33	24.789	0.77	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1.alpha.,4a.beta.,8a.alpha.)]-	$C_{15}H_{26}O$	222.37
34	25.029	0.88	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1a (1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	$C_{15}H_{24}O$	220.36
35	25.308	0.61	(3E)-5-Isopropyliden-6-methyl-3,6,9-decatrien-2-one	$C_{14}H_{20}O$	204.31
36	25.539	0.77	3,7-Cyclodecadien-1-one, 10-(1-methylethenyl)-, (E,E)-	$C_{13}H_{18}O$	190.29
37	25.905	0.45	valerenol	$C_{15}H_{24}O$	220.36
38	26.447	27.09	5.beta.-Guaia-7(11),10(14)-dien-8.alpha.-ol, 5,8-epoxy-, (+)-	$C_{15}H_{22}O_2$	234.15
39	26.731	1.81	Naphthalene, decahydro-1,4a-dimethyl-2-methylene-	$C_{13}H_{22}$	178.32
40	26.936	0.92	5,8-Dihydroxy-4a-methyl-4,4a,4b,5,6,7,8,8a,9,10-decahydro-2(3H)-phenanthrenone	$C_{15}H_{22}O_3$	250.34
41	27.295	0.46	Murolan-3,9(11)-diene-10-peroxy	$C_{15}H_{24}O_2$	236.36
42	27.451	3.81	17.alfa.,21.beta.-28,30-Bisnorhopane	$C_{28}H_{48}$	384.69
43	27.687	2.16	5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahydro-.alpha.,.alpha.,3,8-tetramethyl-, [3S-(3.alpha.,5.alpha.,8.alpha.)]-	$C_{15}H_{26}O$	222.37
44	27.763	0.49	1,2,3,4-TETRAKIS(1-METHYLETHYLIDENE)CYCLOBUTANE	$C_{16}H_{24}$	216.37
45	27.832	0.44	Norethynodrel	$C_{20}H_{26}O_2$	298.43
46	28.615	4.2	(2E)-2-(4-METHOXYBENZYLIDENE)CYCLOHEXANONE	$C_{14}H_{16}O_2$	216.28
47	28.712	1.81	1,5,9-Cyclododecatriene, 1,5,9-trimethyl-	$C_{15}H_{24}$	204.36
48	28.925	0.49	4-Isopropyl-7,11-dimethyl-3,7,11-cyclotetradecatrienone	$C_{19}H_{30}O$	274.45
49	29.008	0.87	7-ISOPROPENYL-1,4-DIMETHYL-1,2,3,4,5,6,7,8-OCTAHYDROAZULENE	$C_{15}H_{24}$	204.36
50	29.358	0.36	3-Oxatricyclo[20.8.0.0(7,16)]triaconta-1(22),7(16),9,13,23,29-hexaene	$C_{29}H_{42}O$	406.65
51	29.681	0.66	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	324.51
52	30.136	0.45	.gamma.-Elemene	$C_{15}H_{24}$	204.36
53	30.613	0.37	1,6-Dimethyl-9-(1-methylethylidene)-5,12-dioxatricyclo[9.1.0.0(4,6)]dodecan-8-one	$C_{15}H_{22}O_3$	250.34
54	31.47	0.94	2,10-Dimethyl-4H,8H-benzo[1,2-b : 3,4-b']dipyran-4-one		
55	31.608	3	Nootkaton-11,12-epoxide	$C_{15}H_{22}O_2$	234.34
56	31.709	0.61	2(3H)-Benzofuranone, 6-ethenylhexahydro-6-methyl-3-methylene-7-(1-methylethenyl)-, [3aS-	$C_{15}H_{20}O_2$	232.32

			(3a.alpha.,6.alpha.,7.beta.,7a.beta.)]-		
57	32.086	6.31	7H-2,4a-Methanonaphthalen-7-one, 1,2,3,4,5,6-hexahydro-1,1,5,5-tetramethyl-, (2S,4aR)-(-)-	$C_{15}H_{22}O$	218.34
58	32.476	1.11	1-Cyclohexene-1-crotonaldehyde, .alpha.,2,6,6-tetramethyl-		
59	32.911	1.31	1,2-Dimethyl-5-nitroadamantane	$C_{12}H_{19}NO_2$	209.29
60	33.177	1.04	1,6-Methanonaphthalene, decahydro-1,4,8a-trimethyl-9-methylene-, (1S,4S,4aS,6R,8aS)-(-)-	$C_{15}H_{24}$	204.36
61	33.425	2.6	2-(1-(Beta-d-glucopyranosyloxy)-1-methylethyl)-2,3-dihydro-7-oxo-7H-furo(3,2-g)chromene, (R)-	$C_{20}H_{24}O_9$	408.40
62	34.117	0.77	5aH-3a,12-Methano-1H-cyclopropa[5',6']cyclodeca[1',2':1,5]cyclopenta[1,2-d][1,3]dioxol-13-one, 1a,2,3,9,12,12a-hexahydro-9-hydr	$C_{23}H_{32}O_5$	388.50
63	34.272	0.38	1,2-Naphthalenedione, 6-hydroxy-3,8-dimethyl-5-(1-methylethyl)-	$C_{15}H_{16}O_3$	244.29
64	34.925	1.42	as-Indacen-4(1H)-one, decahydro-, (3a.alpha.,5a.beta.,8a.beta.,8b.beta.)-	$C_{12}H_{18}O$	178.28
65	35.014	1.84	Tetracyclo[5.4.3.0(7,11)]tetradeca-2,5,10-trione, 1,4,6,14-tetramethyl-4-vinyl-	$C_{20}H_{28}O_3$	316.44
66	36.793	1.81	2H-Cyclohepta[b]furan-2-one, 6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene-	$C_{17}H_{22}O_5$	306.36
67	38.243	0.47	4,7-Methanofuro[3,2-c]oxacycloundecin-6(4H)-one, 7,8,9,12-tetrahydro-3,11-dimethyl-	$C_{15}H_{18}O_3$	246.31
68	44.93	2.85	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.56

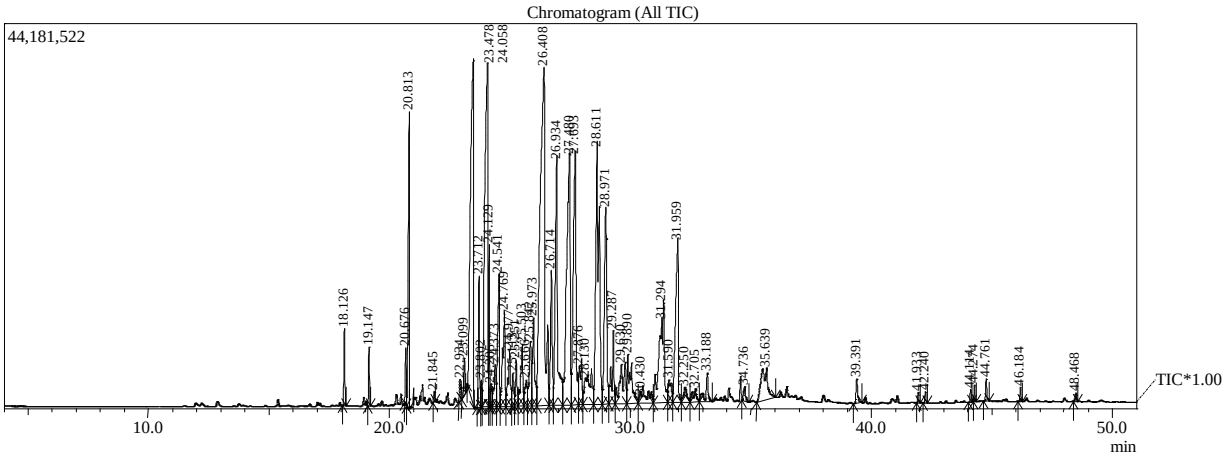

Figure 2: A typical chromatogram of the bioactive compounds present in Hexane extract.

Table 2: Biologically active chemical compounds of Hexane extract from *C aeruginosa* Roxb.

Sl. No.	RT	Peak area (%)	Name of the compound	Molecular formula	MW (g/mol)
1	18.126	0.59	2,4-DIISOPROPENYL-1-METHYL-1-VINYLCYCLOHEXANE	$C_{15}H_{24}$	204.36
2	19.147	0.46	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-	$C_{15}H_{24}$	204.36
3	20.676	0.46	.beta.-Selinene	$C_{15}H_{24}$	204.36
4	20.813	2.72	5-ISOPROPENYL-3,6-DIMETHYL-6-VINYL-4,5,6,7-TETRAHYDRO-1-BENZOFURAN	$C_{15}H_{20}O$	216.32
5	21.845	0.1	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)-	$C_{15}H_{24}$	204.36
6	22.934	0.19	(-)-5-Oxatricyclo[8.2.0.0(4,6)]dodecane,,12-trimethyl-9-methylene-, [1R-(1R*,4R*,6R*,10S*)]-	$C_{15}H_{24}O$	220.36
7	23.099	0.36	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	$C_{15}H_{24}$	204.36
8	23.478	8.5	17-HYDROXYANDROSTA-1,4-DIEN-3-ONE	$C_{19}H_{26}O_2$	286.42
9	23.712	1.18	17-HYDROXYANDROSTA-1,4-DIEN-3-ONE	$C_{19}H_{26}O_3$	286.42
10	23.802	0.24	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	$C_{15}H_{24}O$	220.36
11	24.058	8.47	5.beta.-Guaia-7(11),10(14)-dien-8.alpha.-ol, 5,8-epoxy-, (+)-	$C_{15}H_{22}O_2$	234.34
12	24.129	1.1	isospathulenol	$C_{15}H_{24}O$	220.36

13	24.205	0.29	.gama.-eudesmol	$C_{15}H_{26}O$	222.37
14	24.373	0.69	(+)-epi-Bicyclosesquiphellandrene	$C_{15}H_{24}$	204.36
15	24.541	1.93	5.beta.-Guaia-7(11),10(14)-dien-8.alpha.-ol, 5,8-epoxy-, (+)-		
16	24.769	1.83	Thunbergol	$C_{20}H_{34}O$	290.49
17	24.977	1.07	1,1,7-TRIMETHYL-4-METHYLENEDECAHYDRO-1H-CYCLOPROPA[E]AZULEN-7-OL	$C_{15}H_{24}O$	220.36
18	25.113	0.44	2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{12}H_{20}O$	180.29
19	25.251	0.55	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-, (1S-cis)-	$C_{15}H_{24}$	204.36
20	25.503	1.16	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene)-, (E,E)-	$C_{15}H_{22}O$	218.34
21	25.666	0.5	1,4-Hexadien-3-one, 5-methyl-1-[2,6,6-trimethyl-2,4-cyclohexadien-1-yl]-	$C_{16}H_{22}O$	230.35
22	25.842	1.22	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	$C_{15}H_{24}O$	220.36
23	25.973	1.41	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, (+)-	$C_{15}H_{24}$	204.36
24	26.408	13.34	5.beta.-Guaia-7(11),10(14)-dien-8.alpha.-ol, 5,8-epoxy-, (+)-	$C_{15}H_{22}O_2$	234.34
25	26.714	1.91	(3aRS,4SR,6aRS,2'Z)-Hexahydro-3-methylidene-4-(pent-2'-enyl)-2H-cyclopenta[b]furan-2-one		
26	26.934	4.77	5,8-Dihydroxy-4a-methyl-4,4a,4b,5,6,7,8,8a,9,10-decahydro-2(3H)-phenanthrenone	$C_{15}H_{22}O_3$	250.34
27	27.48	7.37	Naphthalene, decahydro-1,4a-dimethyl-2-methylene-	$C_{13}H_{22}$	178.32
28	27.693	5.72	Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde, 3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl-, (1a.alpha.,3a.beta.,6a.beta.,6b.alpha	$C_{15}H_{20}O_2$	232.32
29	27.876	0.87	1,5-epoxysalvial-4(14)-ene	$C_{15}H_{24}O$	220.36
30	28.13	1.68	(-)-Isolongifolol, pentafluoropropionate	$C_{18}H_{25}F_5O_2$	368.39
31	28.611	6.94	(2E)-2-(4-METHOXYBENZYLIDENE)CYCLOHEXANONE	$C_{14}H_{16}O_2$	216.28
32	28.971	3.86	Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde, 3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl-, (1a.alpha.,3a.beta.,6a.beta.,6b.alpha	$C_{15}H_{20}O_2$	232.32
33	29.287	1.3	Cycloprop[e]indene-1a,2(1H)-dicarboxaldehyde, 3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl-, (1a.alpha.,3a.beta.,6a.beta.,6b.alpha	$C_{15}H_{20}O_2$	232.32
34	29.63	1.83	CYCLOPROPANE, 1,1-DIMETHYL-2-(2-METHYL-3-BUTEN-2-YL)-		
35	29.89	1.44	Bufa-20,22-dienolide, 14,15-epoxy-3,5,16-trihydroxy-, (3.beta.,5.beta.,15.beta.,16.beta.)-	$C_{28}H_{36}O_8$	500.59
36	30.43	0.85	3-METHYL-5-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-YL)-1-PENTYN-3-OL	$C_{15}H_{24}O$	220.36
37	31.294	4.02	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43
38	31.59	0.59	2(3H)-Benzofuranone, 6-ethenylhexahydro-6-methyl-3-methylene-7-(1-methylethenyl)-, [3aS-(3a.alpha.,6.alpha.,7.beta.,7a.beta.)]-	$C_{15}H_{20}O_2$	232.32
39	31.959	3.56	Valerenal	$C_{15}H_{22}O$	218.34
40	32.25	0.4	1-Cyclohexene-1-crotonaldehyde, .alpha.,2,6,6-tetramethyl-	$C_{14}H_{24}O$	208.35
41	32.705	0.43	1,2-Dimethyl-5-nitroadamantane	$C_{12}H_{19}NO_2$	209.29
42	33.188	0.61	2-(1-(Beta-d-glucopyranosyloxy)-1-methylethyl)-2,3-dihydro-7-oxo-7H-furo(3,2-g)chromene, (R)-	$C_{20}H_{24}O_9$	408.40
43	34.736	0.22	1,4-Methanophthalazine, 1,4,4a,5,6,7,8,8a-octahydro-1,4,9,9-tetramethyl-, (1.alpha.,4.alpha.,4a.alpha.,8a.alpha.)-	$C_{13}H_{22}N_2$	206.33
44	35.639	1.59	7-Tetradecenal, (Z)-	$C_{14}H_{26}O$	210.36
45	39.391	0.33	Behenic alcohol	$C_{22}H_{46}O$	326.61
46	41.933	0.1	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370.57
47	42.24	0.11	Heneicosane	$C_{21}H_{44}$	296.58
48	44.114	0.13	1-Heptacosanol	$C_{27}H_{56}O$	396.74
49	44.274	0.13	Hexacosane	$C_{26}H_{54}$	366.72
50	44.761	0.21	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.56
51	46.184	0.13	Hexacosane	$C_{26}H_{54}$	366.72
52	48.468	0.1	Hexacosane	$C_{26}H_{55}$	366.72

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