Research Article

iMedPub Journals
www.imedpub.com

Der Chemica Sinica

2019
Vol.10 No.1:1

Solvent Based Variations in Yield of Bioactive Extracts from the Sclerotium of *Pleurotus tuber-regium*

Reginald C Ohiri*, Mercy O Ifeanachor and Keku Preye

Department of Biochemistry, Faculty of Science, University of Port Harcourt, Nigeria

***Corresponding author:** Ohiri RC

reginald.ohiri@uniport.edu.ng

National Root Crops Research Institute, Umudike P.M.B 7006, Umuahia, Abia State, Nigeria.

Tel: +234 803880807

Citation: Ohiri RC, Ifeanachor MO, Preye K (2019) Solvent Based Variations in Yield of Bioactive Extracts from the Sclerotium of *Pleurotus tuber-regium*. Chem Sin. Vol.10 No.1:1

Abstract

Background: The therapeutic effectiveness of herbs and fungi used for medicinal purposes is not only a factor of their bioactive constituents but also a factor of both the extraction solvent and extraction method.

Objective: The objective of this study is to extract and analyze the bioactive components in the sclerotum of Pleurotus tuber-regium using different solvents, as to ascertain the solvent that gives a better yield.

Method: A quantity of 10.0 kg of fresh sclerotia of P. tuber-regium purchased at Zarama Market in Southern Nigeria was washed, peeled and the white inner parts were sliced using a sterilized knife. The sliced samples were dried at room temperature for fourteen days. After grinding, the bioactive components were extracted by weighing 10 g of sample into three well stopper bottles and each was extracted in 20 mL of specific extraction solvent (methanol, hexane and dichloromethane), while that of soxleth extraction was done in a soxleth apparatus, using ethanol as the solvent. The process was repeated twice and the combined aliquot obtained were concentrated to 5.0 mL and purified. Two milliliters of the extracts were used for gas chromatographic and mass spectroscopy analysis.

Result: The highest peak on the chromatogram for the methanol extract was observed at 32.644 min., while hexane, dichloromethane and soxhlet extracts had their highest peaks at 31.459 min., 14.254 min. and 18.060 min. respectively. The highest bioactive component in methanol extract was (3aR,4R,7R)-1,4,9,9-Tetramethyl-3, 4,5,6,7,8-hexahydro-2H-3a,7-methane with a value of 62.856 %, while hexane, dichloromethane and soxhlet extracts had Hexasiloxane, tetradecamethyl- , Bis(2-ethylhexyl) phthalate and Phthalic acid, 3-chlorobenzyl butyl ester with values of 29.170 %, 5.092 % and 25.490 % respectively.

Conclusion: Hexane and dichloromethane extracts yielded more bioactive components with better nutriceutical and medicinal properties and may be regarded as better solvents for mushroom and fungi extractions.

Keywords: Mushroom, Bioactive components, Extraction methods, Solvents, extracts

Received: February 12, 2019; **Accepted:** February 28, 2019; **Published:** April 05, 2019

Introduction

Different plants and fungal materials have been used in traditional medicine to solve varieties of health problems. The effectiveness of various combinations of herbs and other ingredients used by traditional medical practitioners depends on the constituents of the selected plant or fungi materials. The availability of the constituents is a factor of the adopted extraction method. The extraction of a given compound from a substrate depends on both the solubility of the compound and the polarity of the solvent. Modern extraction techniques such as accelerated solvent extraction, supercritical fluid extraction, microwave-assisted extraction and ultrasound-assisted extraction are presently explored in nutraceuticals extractions from plants.

These modern techniques are mainly targeted at decreasing extraction time, while increasing yield and enhancing extract's quality with a reduced solvent consumption. Dadi et al. [1] improve the total phenolic content, total flavonoid content, and antioxidant activity assessed from *Moringa stenopetala* leaves by optimizing ultrasonic-assisted extraction using response surface methodology. They recorded an extraction time of 26 min, 68 % ethanol concentration, and solvent-to-sample ratio of 42 mL/g.

Oil extraction is mainly a diffusion process in which the solvent penetrates into the lipid containing cells of a raw sample material, thereby forming a solution of the oil in the solvent. Though some of these plants and fungal materials are still very relevant in traditional medicine for treatment of diseases mainly of microbial related infections [2], most of their isolates have shown potential anti-inflammatory and antidiabetic properties in preliminary studies [3].

Pleurotus tuberregium is a notable saprotroph that produces a food storage sclerotium upon its consumption of decaying wood [4]. It is one mushroom that both its basidiocarp and sclerotium are of economic importance due to their nutritive and medicinal properties [4]. The extract of this notable fungus has been used in traditional combinations for the treatment and management of diseases ranging from skin infections, inflammation, childhood malnutrition, headache, stomach problem, cold, asthma, fever, high blood pressure, diabetes and small pox [5]. Isikhuehmen and LeBauer [6] reported the use of this mushroom and its extract in Eastern Nigeria as a thicker and flavoring agent for soups and for the treatment of heart related ailments, while it is used for the treatment and management of asthma, cough and obesity in the Southern part of Nigeria [6]. Aside Nigeria and Africa, *P. tuber-regium* also grows in Asia and Australia [7].

Other therapeutic activities such as antitumour, immunomodulatory, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, antihyperglycaemic, antimicrobial and antiviral activities *Pleurotus* spp. has also been reported [8]. Though these therapeutic activities are exhibited by the extracts or isolated compounds, fermentation broth, mycelia and fruiting bodies of *Pleurotus* spp. [9], the biochemical mechanisms are still elusive due poor characterization and identification of the bioactive components. The aim of this present study is to extract the bioactive components in the sclerotum of *P. tuber-regium* using different solvents, to analyze the extracts obtained for identification of the bioactive components and to ascertain the solvent that gives a better yield.

MATERIALS AND METHODS

Sample collection, preparation and extraction

A quantity of 10.0 kg of fresh sclerotia of *Pleurotus tuberregium* purchased at Zarama Market in Southern Nigeria was washed, peeled and the white inner parts were sliced using a sterilized knife. The sliced samples were allowed to dry at room temperature in a dust free environment for a period of fourteen days. The samples were ground in a warring blender into a fine powder. Using an analytical weighing balance, 10 g of the ground sample was weighed into three well stopper bottles. A volume of 20 mL of a specific extraction solvent (methanol, hexane and dichloromethane) was added to each stopper bottle. The mixtures were vigorously agitated and were left to stand for 5 days, while that of soxleth extraction was done in a soxleth apparatus, using ethanol as the solvent. The crude extract from each process was collected by filtering into a quartz beaker in a fume hood. The process was repeated twice and the combined aliquot collected from each extraction solvent were separately concentrated on a steam berth to 5.0 mL and purified by passing through a pasture pipette packed with silica gel and anhydrous sodium sulphate. The purified samples were air dried to 2.0 mL for gas chromatographic analysis.

2.2 GC-MS analysis of extracts

The extracts were analysed using a combined gas chromatograph model HP 6890 and mass spectrometer model 5973 (Agilent Tech.) fitted with a capillary column HP-5 MS (5% phenylmethylsiloxane) 30.0 m × 250 µm × 0.25 µm, using Helium as a carrier gas at initial column temperature 120°C for 5 minutes. Thereafter, the column temperature was increased at 5°C per minutes to 320°C and held for 5 minutes. Electron impact ionization for mass spectroscopy was done at ionization energy of 70 eV. The oil was diluted with 98% hexane and 2 µL of the diluted sample was automatically injected into Agilent Tech. model 5973 mass spectrometer. The constituent compounds were identified using the Chem-Office software attached tothe MS library. The names and structures of the component oils were confirmed using the database of National Institute of Standard and Technology (NIST).

Results

The Chromatogram of bioactive components of methanol, hexane, dichloromethane and soxhlet extracts of the sclerotia of *P. tuberregium* are shown in **Figures 1-4**, respectively. The highest peak for the methanol extract was observe at 32.644 min. Hexane extract has its highest peak at 31.459 min., while dichloromethane and soxhlet extracts have their highest peaks at 14.254 min. and 18.060 min. respectively.

The retention time, percentage concentration, molecular formula, molecular weight and structures of the bioactive components of methanol, hexane, dichloromethane and soxhlet extracts of the sclerotia of *P. tuberregium* are shown in **Tables 1-4**. (3aR, 4R, 7R)-1,4,9,9-Tetramethyl-3, 4,5,6,7,8-hexahydro-2H-3a,7-methane was the highest bioactive component in the methanol extract with a value of 62.856 % followed by Urs-12-en-28-oic acid, 3-hydroxy-,methyl ester, (3.beta.)- With a value of 23.151 %. Hexane extract has it highest bioactive component as Hexasiloxane, tetradecamethyl- with a value of 29.170 % followed by Hexacosane, 13-dodecyl- with a value of 28.339 %, while Bis(2-ethylhexyl) phthalate and Eicosane were the highest bioactive components observed in dichloromethane extract with values of 5.092% and 4.721% respectively. The highest bioactive component observed in the sohxlet extract was Phthalic acid, 3-chlorobenzyl butyl ester

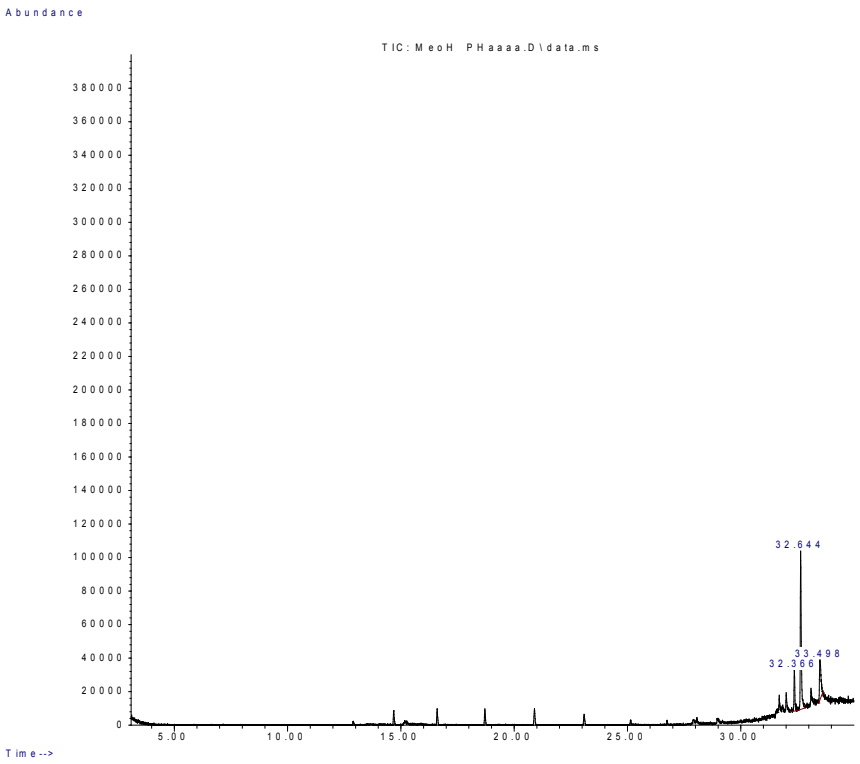


Figure 1 Chromatogram of bioactive components of methanol extracts of the sclerotia of *P. tuberregium*.

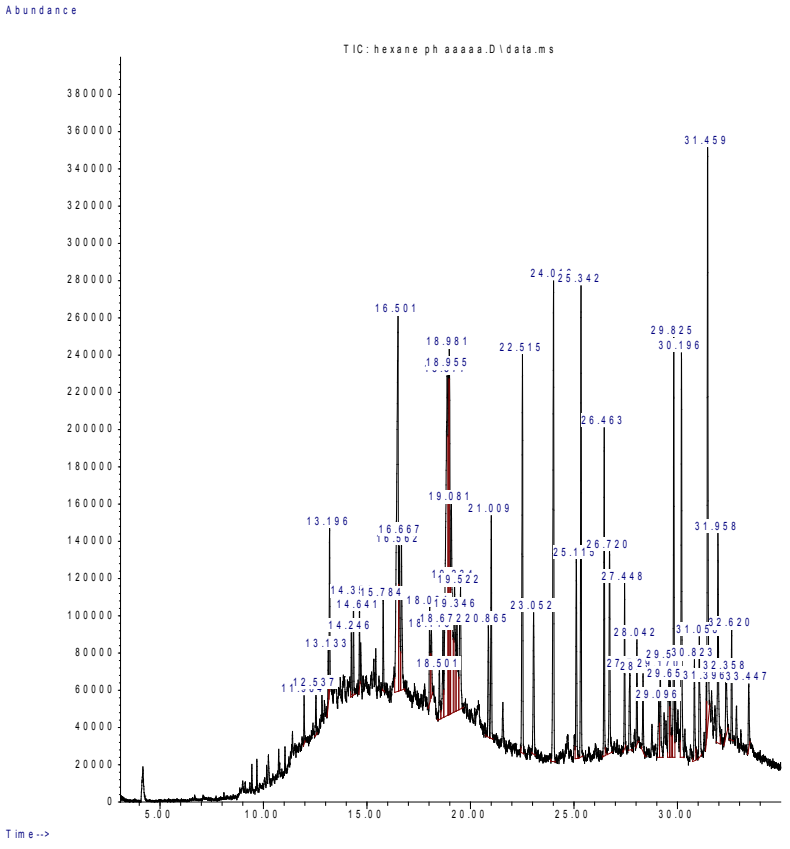


Figure 2 Chromatogram of bioactive components of hexane extract of the sclerotia of *P. tuberregium.*

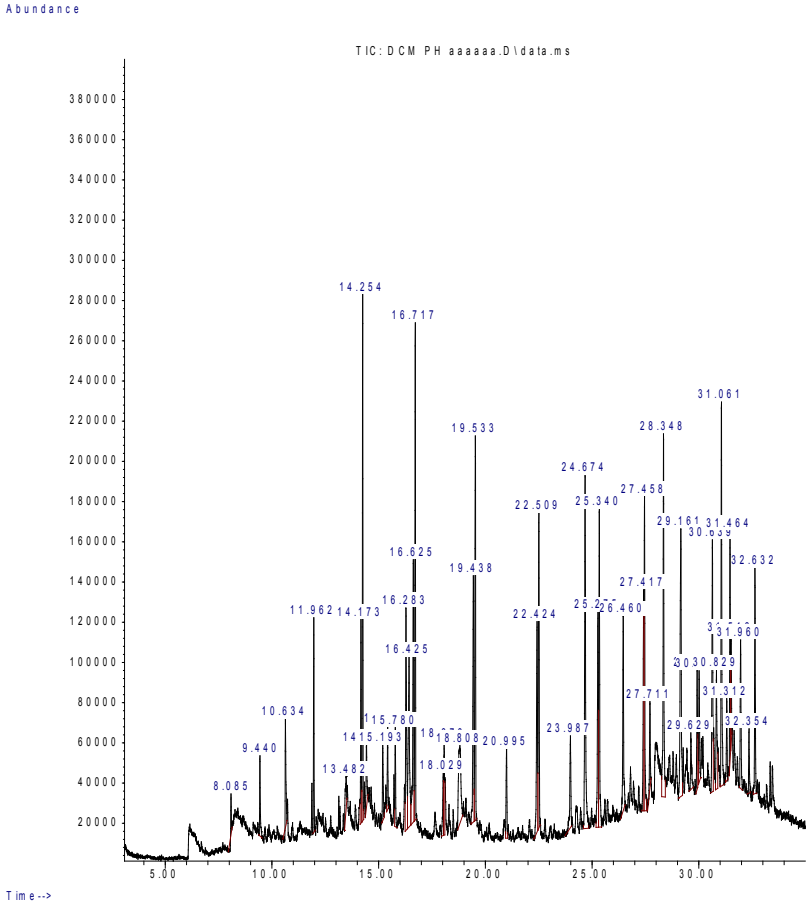


Figure 3 Chromatogram of bioactive components of dichloromathane extract of the sclerotia of *P. tuberregium.*

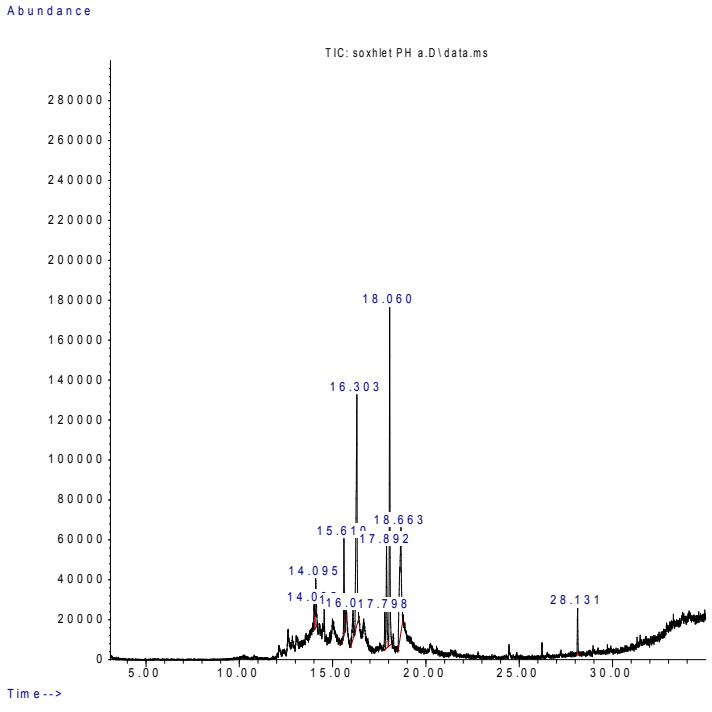


Figure 4 Chromatogram of bioactive components of sohxlet extract of the sclerotia of *P. tuberregium.*

Table 1: Bioactive components of methanol extract of sclerotia of *P. tuberregium*

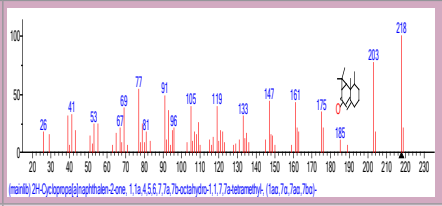
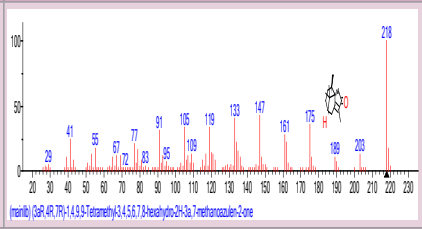
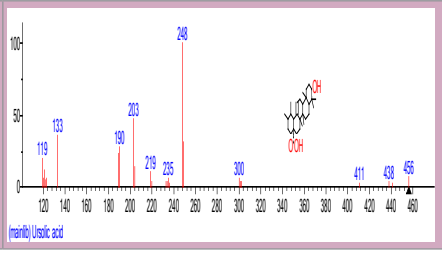
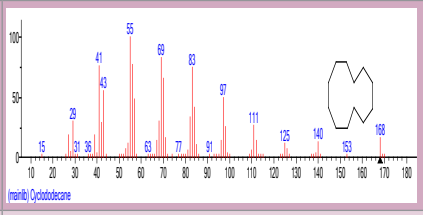
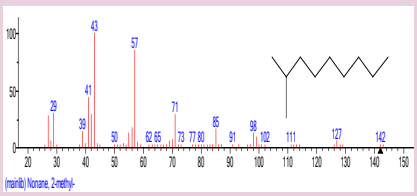
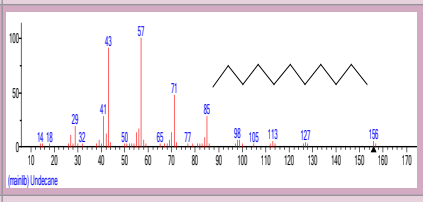
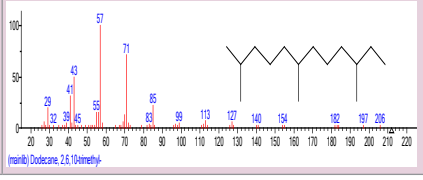
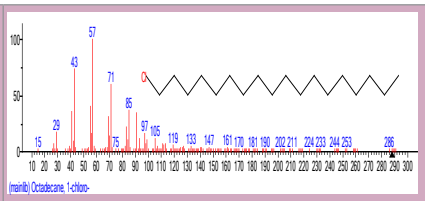
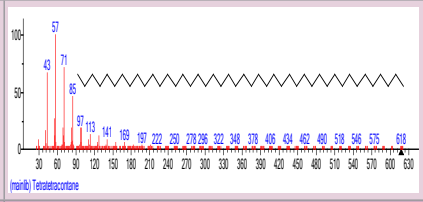
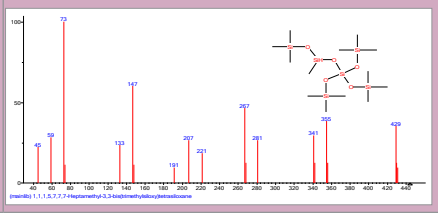
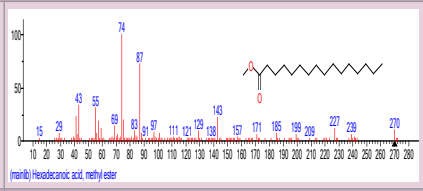
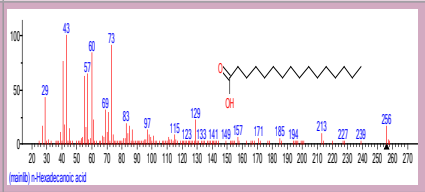
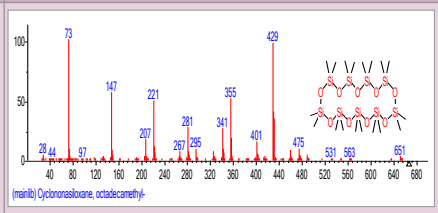
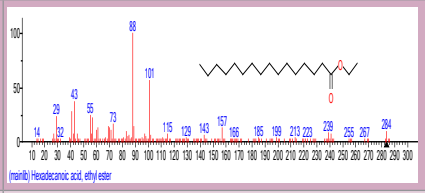
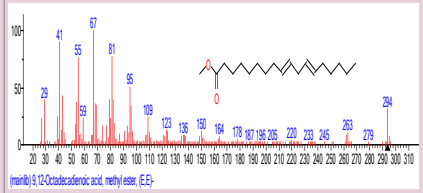
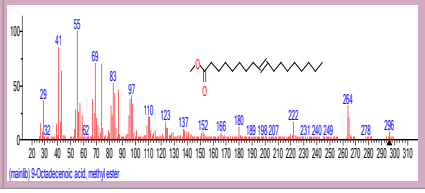
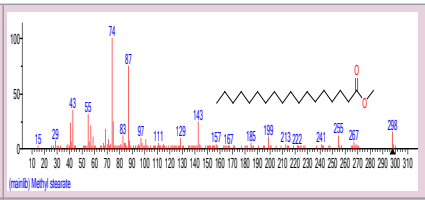
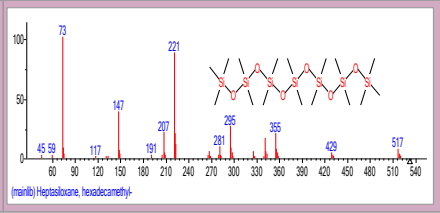
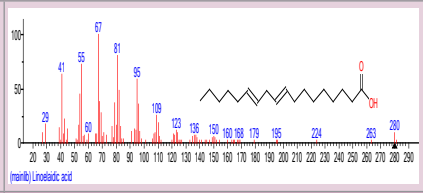
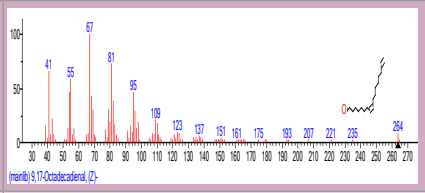
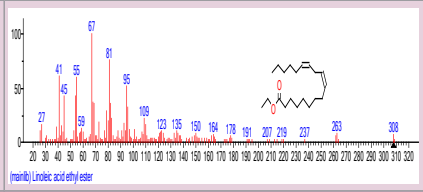
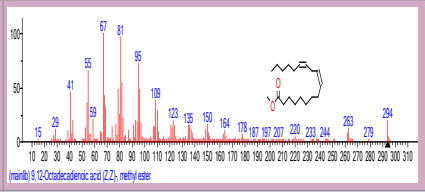
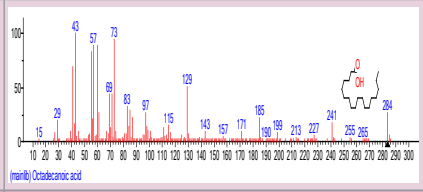
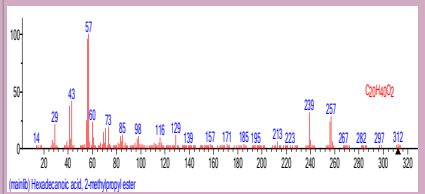
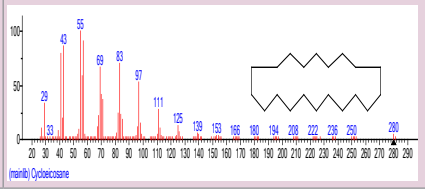
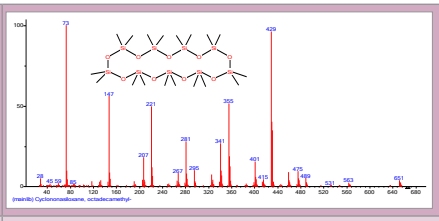
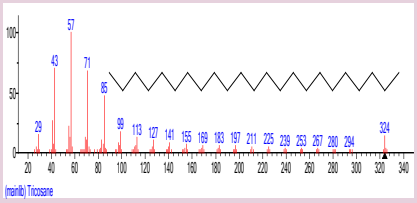
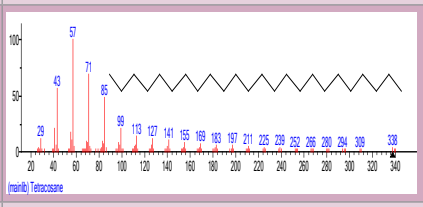
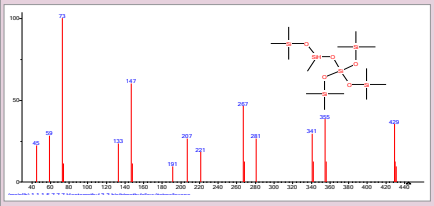
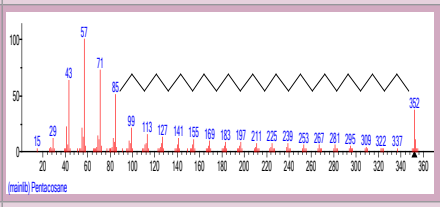
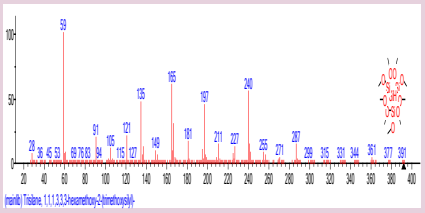
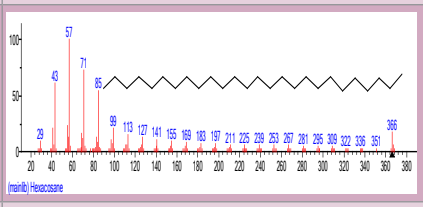
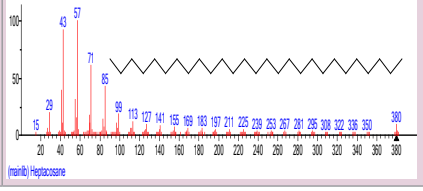
S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight	Structure
1	2H-Cyclopropa[a]naphthalen-2-one	32.366	13.992	$C_{15}H_{24}$	204.3511	
2	(3aR,4R,7R)-1,4,9,9-Tetramethyl-3,4,5,6,7,8-hexahydro-2H-3a,7-methane	32.644	62.856	$C_8H_{15}N_3$	153.22	
3	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)-	33.498	23.151	$C_{30}H_{48}O_3$	456.700	

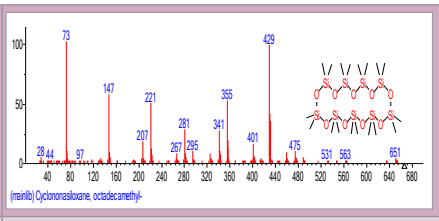
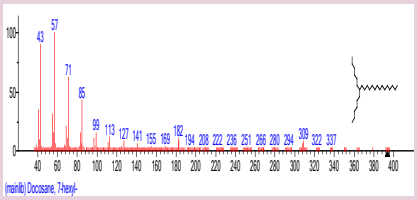
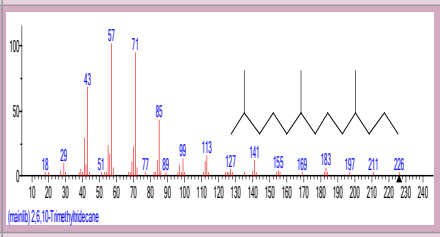
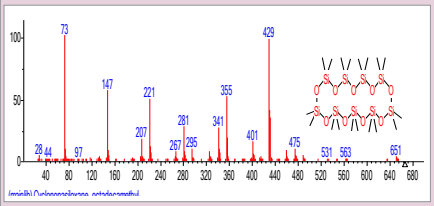
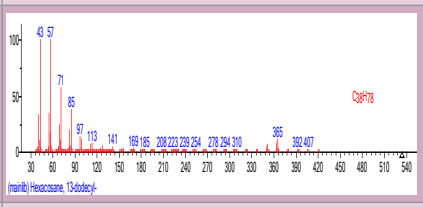
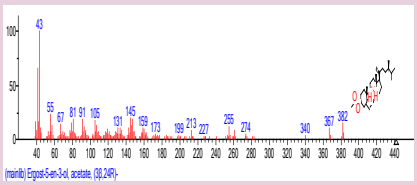
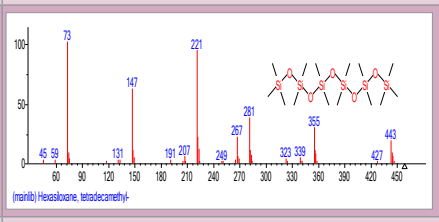
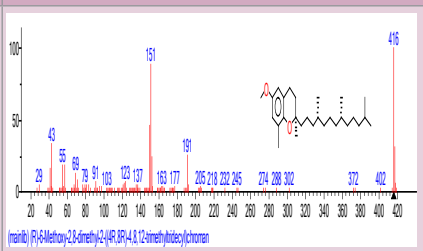
Table 2: Bioactive components of hexane extract of sclerotia of P. tuberregium

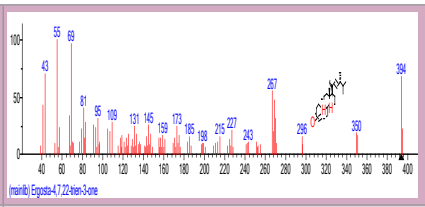
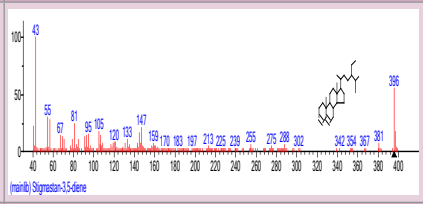
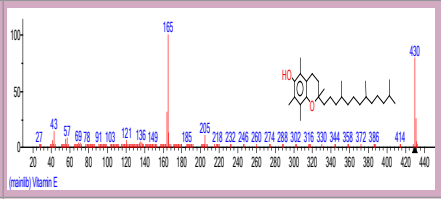
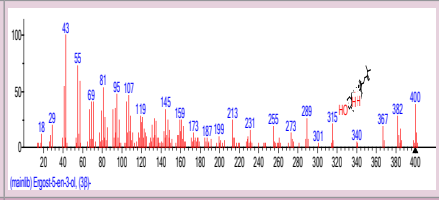
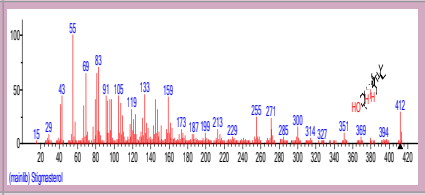
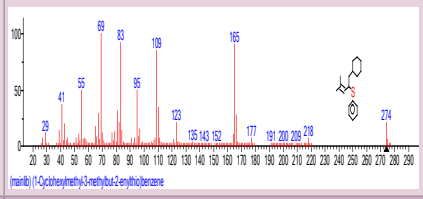
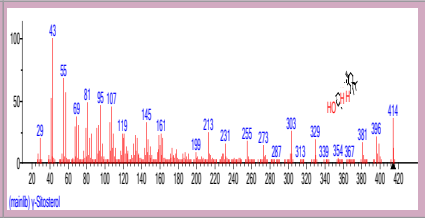
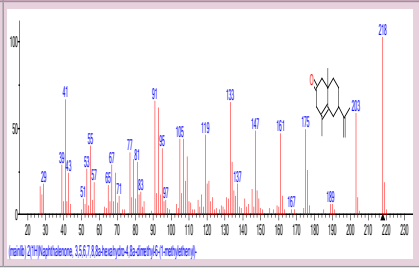
S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight	Structure
1	Cyclododecane	11.964	0.300	$C_{12}H_2$	168.3190	
2	Nonane, 2-methyl-	12.537	0.401	$C_{10}H_{22}$	142.2817	
3	Undecane	13.133	0.363	$C_{11}H_{24}$	156.3083	
4	Dodecane, 2,6,10-trimethyl-	13.196	1.234	$C_{15}H_{32}$	212.4146	

5	Octadecane, 1-chloro-	14.246	0.435	$C_{18}H_{37}Cl$	288.939	
6	Tetratetracontane	14.351	0.764	$C_{44}H_{90}$	619.1854	
7	1,1,1,5,7,7,7-Hepta methyl-3,3-bis(trimet hylsiloxy)tetrasiloxane	14.641	0.549	$C_{13}H_{40}O_5Si_6$	444.967	
8	Hexadecanoic acid, methyl ester	15.784	0.688	$C_{17}H_{34}O_2$	270.4507	
9	n-Hexadecanoic acid	16.501	7.223	$C_{16}H_{32}O_2$	256.4241	
10	Cycloheptasiloxane, tetradeca methyl-	16.562	1.909	$C_{14}H_{42}O_7Si_7$	519.0776	
11	Hexadecanoic acid, ethyl ester	16.667	1.913	$C_{18}H_{36}O_2$	284.4772	
12	9,12-Octadecadienoic acid,methyl ester, (E,E)-	18.031	0.765	$C_{19}H_{34}O_2$	294.4721	
13	9-Octadecenoic acid, methyl ester (E)-	18.116	0.643	$C_{19}H_{36}O_2$	296.4879	

14	Methyl stearate	18.501	0.600	$C_{19}H_{38}O_2$	298.511	
15	Heptasiloxane, hexadeca methyl-	18.672	1.149	$C_{16}H_{48}O_6Si_7$	533.1472	
16	Linoelaidic acid	18.877	8.730	$C_{18}H_{32}O_2$	280.4455	
17	9,17-Octadecadienal, (Z)-	18.955	3.233	$C_{18}H_{32}O$	264.4461	
18	Linoleic acid ethyl ester	18.981	4.337	$C_{20}H_{36}O_2$	308.4986	
19	9,12-Octadecadienoic acid (Z,Z)-	19.081	3.668	$C_{18}H_{32}O_2$	280.4455	
20	Octadecanoic acid	19.234	1.839	$C_{18}H_{36}O_2$	284.4772	
21	Hexadecanoic acid, 2-methyl propyl ester	19.346	1.622	$C_{20}H_{40}O_2$	312.5304	
22	Cycloeicosane	19.522	2.099	$C_{20}H_{40}$	280.5316	

23	Cyclononasiloxane, octadeca methyl-	20.865	1.227	$C_{18}H_{54}O_9Si_9$	667.3855	
24	Tricosane	21.009	2.206	$C_{23}H_{48}$	324.6272	
25	Tetracosane	22.515	4.205	$C_{24}H_{50}$	338.6538	
26	3-Isopropoxy-1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimet hylsiloxy)tetrasiloxane	23.052	1.645	$C_{18}H_{52}O_7Si_7$	577.200	
27	Pentacosane	24.016	5.347	$C_{25}H_{52}$	352.6804	
28	2,5-Dihydroxy benzoic acid, 3TMS derivative	25.115	2.085	$C_{16}H_{30}O_4Si_3$	370.6635	
29	Hexacosane	25.342	4.261	$C_{26}H_{54}$	366.7070	
30	Heptacosane	26.463	2.728	$C_{27}H_{56}$	380.7335	

31	Cyclononasiloxane, octadeca methyl-	26.720	2.297	$C_{18}H_{54}O_9Si_9$	667.3855	
32	Docosane, 7-hexyl-	27.448	1.261	$C_{28}H_{58}$	394.7601	
33	2,6,10-Trimethyltridecane	27.700	1.004	$C_{16}H_{26}$	218.3776	
34	Cyclononasiloxane, octadeca methyl-	28.042	0.904	$C_{18}H_{54}O_9Si_9$	667.3855	
35	Hexacosane, 13-dodecyl-	0.737	28.339	$C_{38}H_{78}$	535.0259	
36	Ergost-5-en-3-ol, acetate, (3.beta.,24R)-	29.096	0.718	$C_{30}H_{50}O_2$	442.7168	
37	Hexasiloxane, tetradecamethyl-	1.095	29.170	$C_{14}H_{42}O_5Si_6$	458.9933	
38	(R)-6-Methoxy-2,8-dimethyl-2-((4R, 8R)-4,8, 12-trimethyltri decyl)chroman	29.594	1.316	$C_{28}H_{48}O_2$	416.6795	

39	Ergosta-4,7,22-trien-3-one	29.652	0.885	$C_{28}H_{42}O$	394.633	
40	Stigmastan-3,5-diene	29.825	3.742	$C_{29}H_{48}$	396.6914	
41	Vitamin E	30.196	4.094	$C_{29}H_{50}O_2$	430.71	
42	Ergost-5-en-3-ol, (3.beta.)-	30.823	1.070	$C_{28}H_{48}O$	400.6801	
43	Stigmasterol	31.058	1.514	$C_{29}H_{48}O$	412.6908	
44	(1-Cyclohexyl methyl-3-methylbut-2-enylthio) benzene	31.396	0.374	$C_{13}H_{18}$	174.282	
45	gamma.-Sitosterol	31.459	5.677	$C_{29}H_{50}O$	414.7067	
46	2(1H)Naphthalenone, 3,5,6,7, 8,8a-hexa hydro-4,8a-dimethyl-6-(1-methyl ethenyl)-	31.958	2.504	$C_{15}H_{22}O$	218.3346	

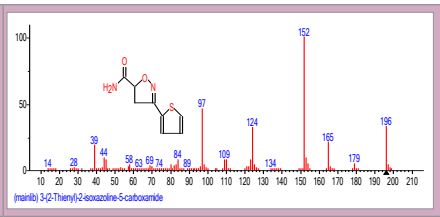
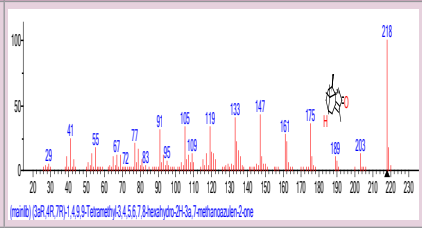
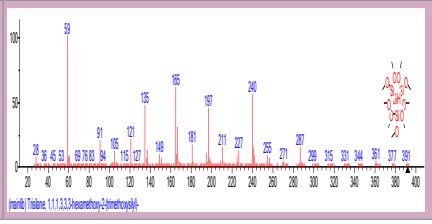
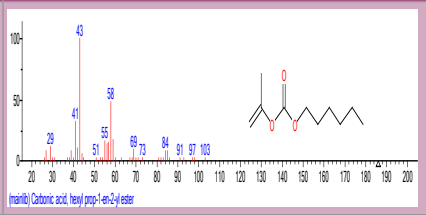
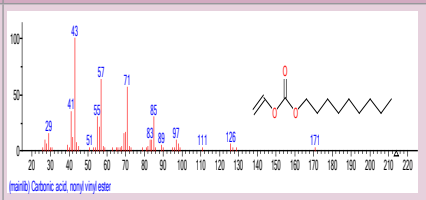
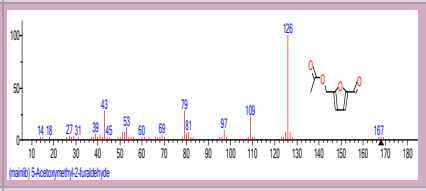
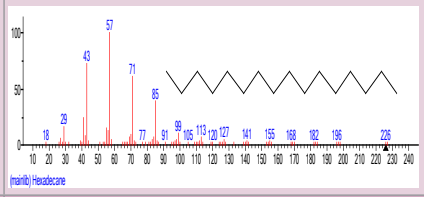
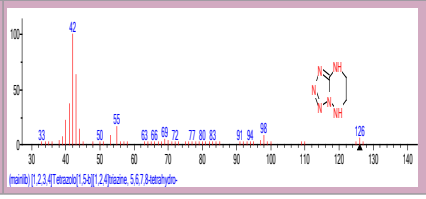
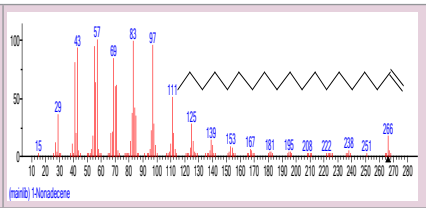
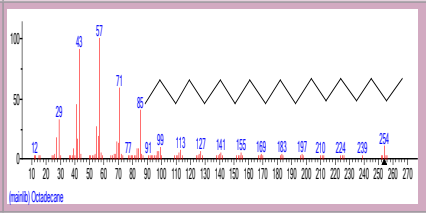
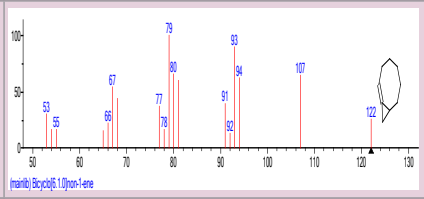
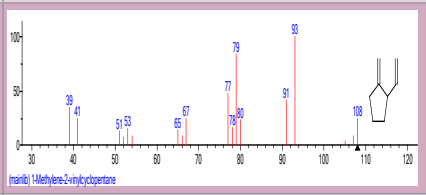
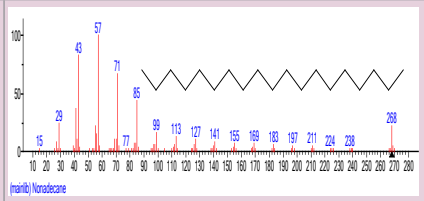
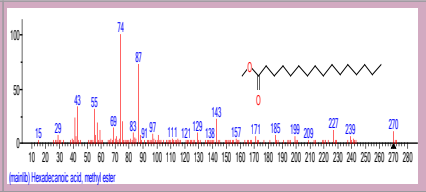
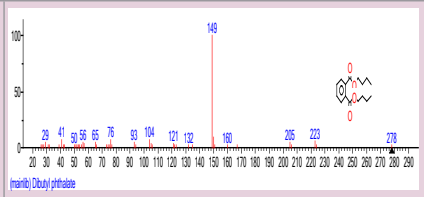
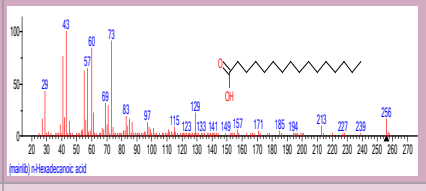
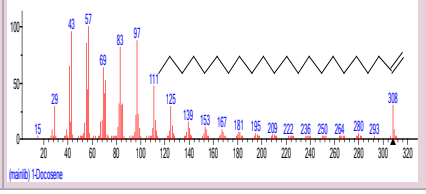
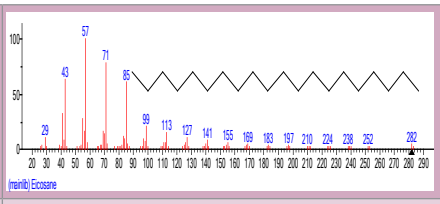
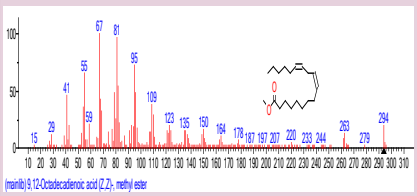
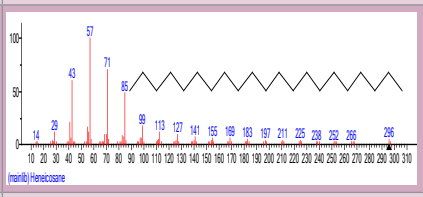
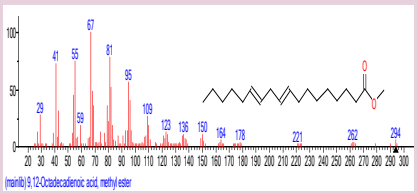
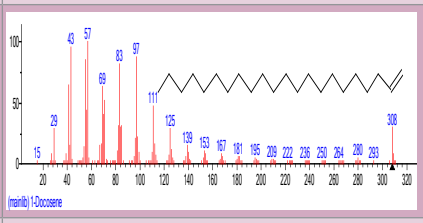
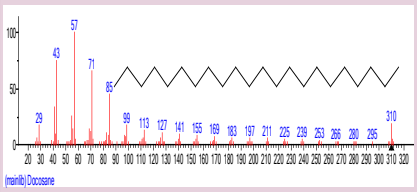
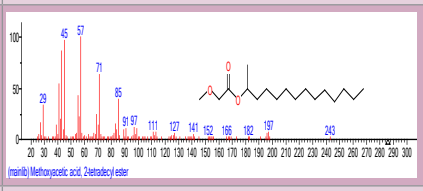
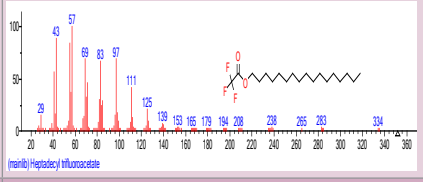
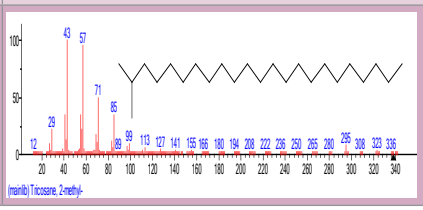
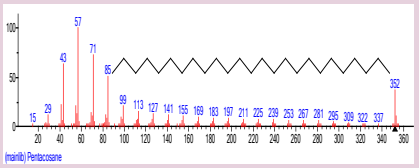
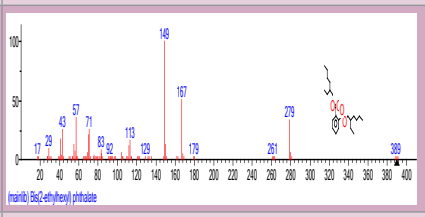
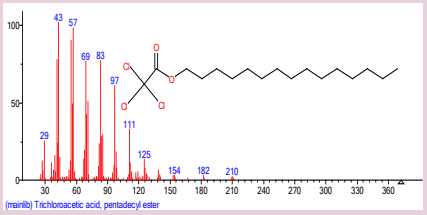
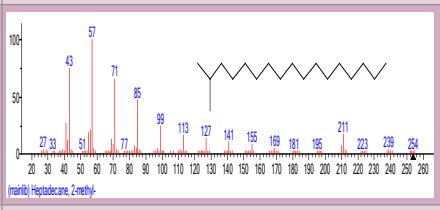
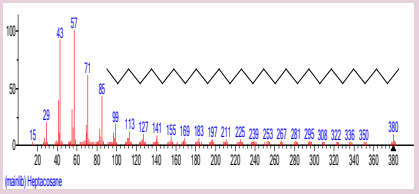
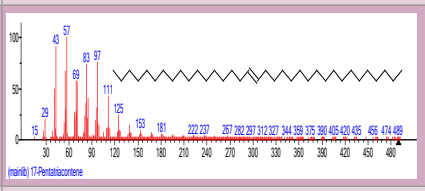
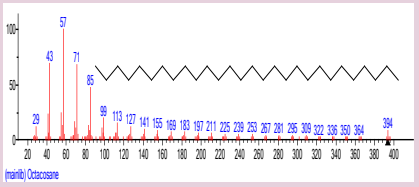
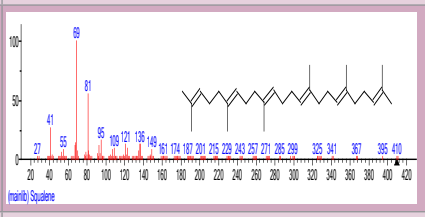
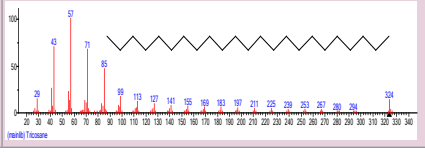
47	3-(2-Thienyl)-4,5,dihydro-5-isoxazo lemethanol	35.955	0.295	$C_8H_9NO_2S$	183.2276	
48	(3aR,4R,7R)-1,4,9,9-Tetra methyl-3, 4,5,6, 7,8-hexahydro -2H-3a,7-meth anoazulen-2-one	32.620	1.010	$C_8H_{15}N_3$	153.22	
49	Tert-Butyl dimethylsilyl 2,3-dimethyl	33.447	0.675	$C_{14}H_{22}O_2Si$	250.4088	

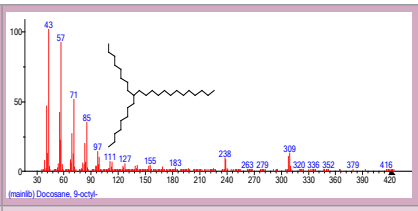
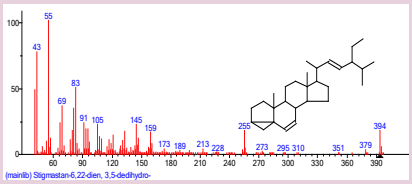
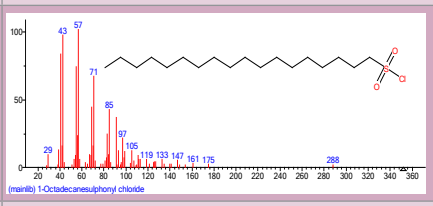
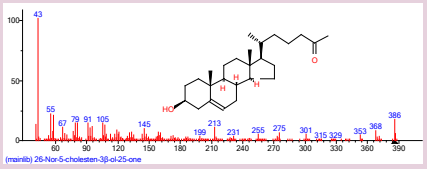
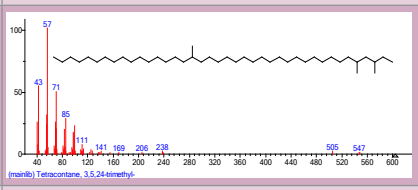
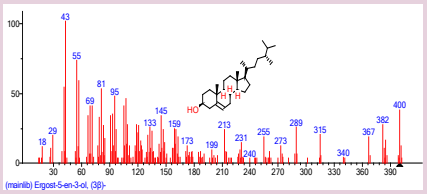
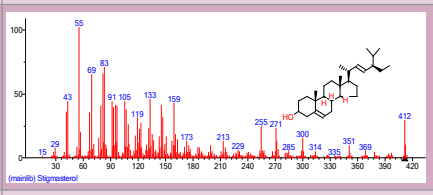
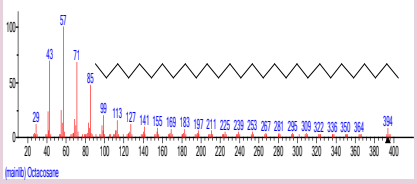
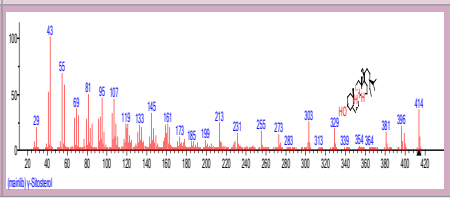
Table 3: Bioactive components of dichloromethane extract of sclerotia of P. tuberregium

S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight	Structure
1.	Carbonic acid, hexadecyl prop-1-en -2-yl ester	8.085	0.173	$C_9H_{10}O_3$	166.1739	
2.	Carbonic acid, nonyl vinyl ester	9.440	0.792	$C_{12}H_{22}O_3$	214.3013	
3.	5-Acetoxy methyl-2-furaldehyde	10.634	0.982	$C_8H_8O_4$	168.1467	
4.	Hexadecane	11.962	1.672	$C_{16}H_{34}$	226.4412	
5.	[1,2,3,4] Tetrazolo[1,5-b][1,2, 4]triazine, 5,6, 7,8-tetrahydro-	13.482	0.663	$C_3H_6N_6$	126.12	

6.	1-Nonadecene	14.173	1.811	$C_{19}H_{38}$	266.5050	
7.	Octadecane	14.254	4.162	$C_{18}H_{38}$	254.4943	
8.	Bicyclo[6.1.0]non-1-ene	14.431	0.793	C_9H_{14}	122.211	
9.	1-Methylene-2-vinylcyclo pentane	15.193	0.875	C_8H_{12}	108.181	
10.	Nonadecane	15.423	0.774	$C_{19}H_{40}$	268.5209	
11.	Hexadecanoic acid, methyl ester	15.780	0.950	$C_{17}H_{34}O_2$	270.4507	
12.	Dibutyl phthalate	16.283	3.100	$C_{16}H_{22}O_4$	278.3435	
13.	n-Hexadecanoic acid	16.425	3.167	$C_{16}H_{32}O_2$	256.4241	
14.	1-Docosene	16.625	3.364	$C_{22}H_{44}$	308.5848	

15.	Eicosane	16.717	4.721	$C_{20}H_{42}$	282.5475	
16	9,12-Octadeca dienoic acid (Z,Z)-, methyl ester	18.029	0.689	$C_{19}H_{34}O_2$	294.4721	
17	Heneicosane	18.070	1.175	$C_{21}H_{44}$	296.5741	
18	9,12-Octadeca dienoic acid (Z,Z)-	18.808	2.689	$C_{18}H_{32}O_2$	280.4455	
19	1-Docosene	19.438	3.145	$C_{22}H_{44}$	308.5848	
20	Docosane	19.533	4.304	$C_{22}H_{46}$	310.6006	
21	Methoxyacetic acid, 2-tridecyl est er	20.995	1.043	$C_{16}H_{32}O_3$	272.429	
22	Heptadecyl trifluoroacetate	22.424	3.008	$C_{19}H_{35}F_3O_2$	352.4752	
23	Tricosane, 2-methyl-	22.509	3.745	$C_{24}H_{50}$	338.6538	

24	Pentacosane	23.987	1.344	$C_{25}H_{52}$	352.6804	
25	Bis(2-ethylhexyl) phthalate	24.674	5.092	$C_{24}H_{38}O_4$	390.5561	
26	Trichloroacetic acid, pentade cyl ester	25.275	2.827	$C_{17}H_{31}Cl_3O_2$	373.786	
27	Heptadecane, 2-methyl-	25.340	3.866	$C_{18}H_{38}$	254.4943	
28	Heptacosane	26.460	2.475	$C_{27}H_{56}$	380.7335	
29	17-Pentatria contene	27.417	2.249	$C_{35}H_{72}$	492.9462	
30	Octacosane	27.458	3.463	$C_{28}H_{58}$	394.7601	
31	Squalene	27.711	0.905	$C_{30}H_{50}$	410.718	
32	Tricosane	28.348	4.378	$C_{24}H_{50}$	338.6538	

33	Docosane, 9-octyl-	29.161	4.520	$C_{30}H_{62}$	422.8133	
34	Stigmastan-6, 22-dien, 3,5-de dihydro-	29.629	0.770	$C_{28}H_{46}O$	398.68	
35	1-Octadecane sulphonyl chloride	29.923	1.161	$C_{18}H_{37}ClO_2S$	353.003	
36	26-Nor-5-chole sten-3.beta. -ol-25-one	30.017	1.215	$C_{29}H_{48}O_2$	428.6902	
37	Tetracontane, 3,5,24-trimethyl-	30.639	2.995	$C_{43}H_{88}$	605.1770	
38	Ergost-5-en-3-ol, (3.beta.)-	30.829	1.637	$C_{28}H_{48}O$	400.691	
39	Stigmasterol	31.061	4.407	$C_{29}H_{48}O$	412.6908	
40	Octacosane	31.312	0.834	$C_{28}H_{58}$	394.7601	
41	beta.-Sitosterol	31.464	2.247	$C_{29}H_{50}O$	414.7067	

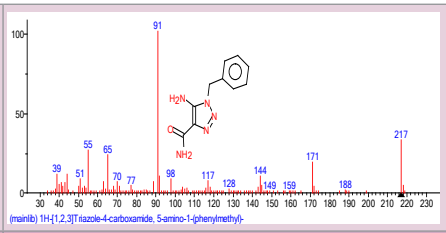
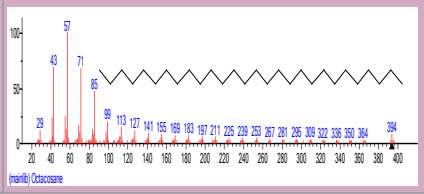
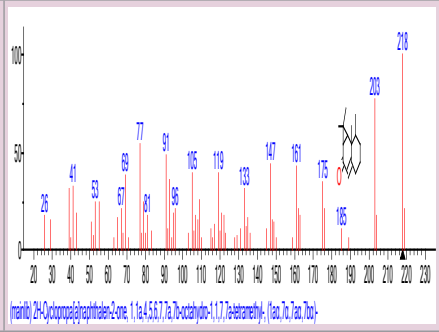
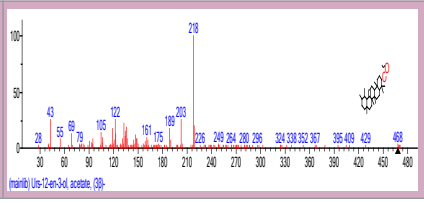
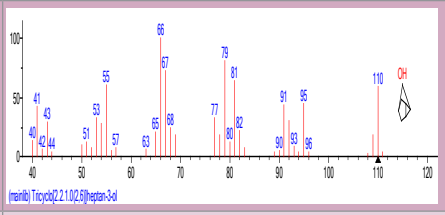
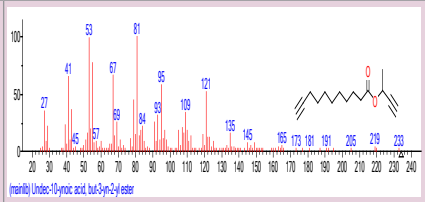
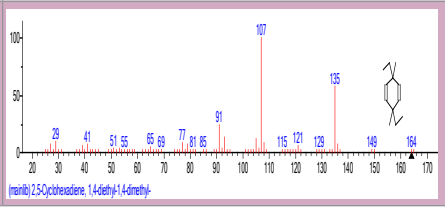
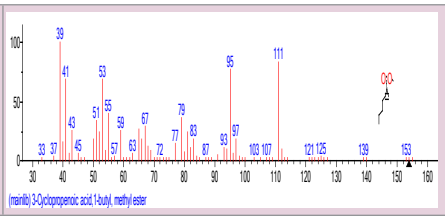
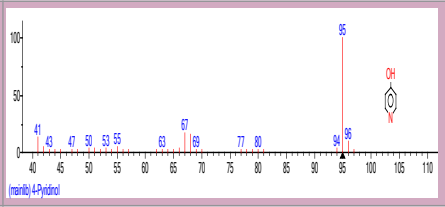
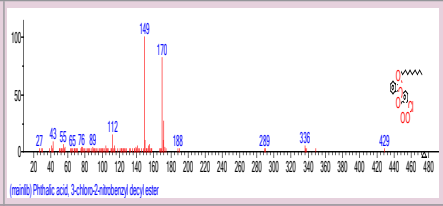
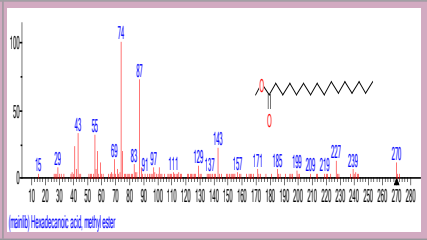
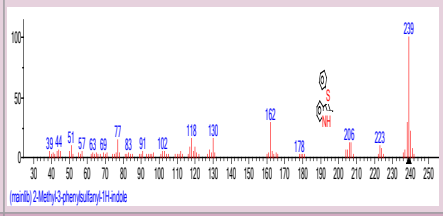
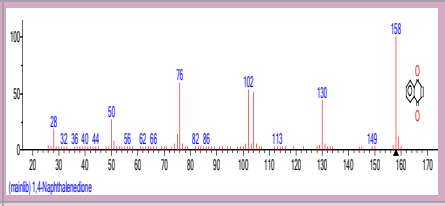
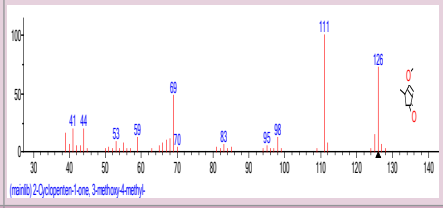
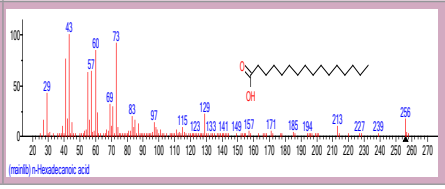
42	1H-1,2,3-Triazole-4-carboxylic acid, 5-amino-1-(phenylmethyl)-, hydrazide	31.516	1.001	$C_{11}H_{11}N_3O_2$	217.22	
43	Octacosane	31.960	1.864	$C_{28}H_{58}$	394.7601	
44	2H-Cyclopropa[a] naphthalen-2-one,1,1a,4,5,6,7,7a,7b-octa hydro-1,1,7,7a-tetramethyl-, (1a. alpha.,7. alpha.,7a.alpha.,7b. alpha.)-	32.354	0.601	$C_{15}H_{24}$	204.3511	
45	Urs-12-en-3-ol, acetate, (3.beta.)	32.632	2.351	$C_{32}H_{52}O_2$		

Table 4: Bioactive components of soxhlet extract of sclerotia of *P. tuberregium*

S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight	Structure
1	Tricyclo[2.2.1.0(2,6)] heptan-3-one, oxime	14.025	2.331	C_7H_{10}	94.1543	
2	Undec-10-ynoic acid, but-3-yn-2-yl ester	14.095	5.056	$C_{11}H_{18}O_2$	182.2594	
3	2,5-Cyclohexa diene-1,4-dione, 2,5-dihydroxy-3-methyl-6-(1-methylethyl)-	15.610	6.192	$C_{10}H_{12}O_2$	164.2000	

4	3-Cyclopropen oic acid,1butyl, methyl ester	15.727	0.993	$C_{17}H_{24}O_4$	292.3701	
5	4-Pyridinol	16.096	3.539	C_5H_5NO	95.1000	
6	Phthalic acid, 3-chlorobenzyl butyl ester	16.303	25.490			
7	Hexadecanoic acid, methyl ester	17.798	2.602	$C_{17}H_{34}O_2$	270.4507	
8	2-Methyl-3-(phenylsulfonyl) methyl-2-cyclopenten-1-one	17.892	8.177	C_6H_8SO	96.1290	
9	1,4-Naphtha lenedione, 5,8-dihydroxy-2,7-dimethoxy-	18.060	23.513	$C_{10}H_6O_4$	190.15	
10	2-Cyclopenten-1-one, 3-methyl-	18.663	19.395	$CH_3C_5H_5O$	96.1300	
11	n-Hexadecanoic acid	28.131	2.713	$C_{16}H_{32}O_2$	256.4241	

with a value of 25.490 % followed by 1,4-Naphthalenedione, 5,8-dihydroxy-2,7-dimethoxy- with a value of 23.513 %.

Discussion

The disparity in both compound quantity and concentration observed in the methanol, hexane, dichloromethane and soxhlet extracts of the sclerotia of *P. tuberregium* shows varying capabilities of different solvents to dissolve and liberate different compounds from a substrate. The ability of methanol to extract only three uncommon polycyclic compounds (2H-Cyclopropa[a] naphthalen-2-one, (3aR,4R,7R)-1,4,9,9-Tetramethyl-3,4,5,6,7,8-hexahydro-2H-3a,7-methane and Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)-) from the sclerotia of this mushroom. **Table 1** indicates that the use of methanol as an extraction solvent may favour the extraction of polycyclic compounds especially in a lignin based substrate like mushroom. As a very polar compound, the yield and concentration of extracts in methanol extraction may be lower than in a non-polar solvent. This indicates that methanol can dissolve a larger proportion of polar compounds. However, its solubility may reduce when used as solvent for the extraction non-polar compounds. The result of this study is an indication that methanol may not be a solvent of choice for extraction where both polar and non-polar compounds are needed from a substrate.

Although different solvents has been used for extraction, hexane has been considered ideal for extraction processes because of its non-polar properties, low boiling point (67°C), and its high solubility of lipid compounds. Moreover, Clough and Mulholland [10] also reported that the low reactivity exhibited by hexane also makes it a suitable solvent for the extraction of reactive compounds. The hexane extract of the sclerotia of this mushroom shows the presence of 49 different bioactive components made up of linear, branched, monocyclic and polycyclic compounds **(Table 2)**. The large number of compounds observed in the hexane extract shows the ability of hexane to penetrate, solubilize and release these compounds from the substrate.

The 45 components observed from the dichloromethane extract **(Table 4)** shows that the volatility and ability of dichloromethane to dissolve a wide range of organic compounds makes it a useful solvent for many chemical processes [11]. The presence of both linear and cyclic compounds in the extract is also an indication that dichloromethane has the potentials to penetrate and extract most constituent compounds from a lignin based sample. Though dichloromethane is the least toxic amongst the simple chlorohydrocarbons it low boiling point also makes it a choice solvent for extraction [11]. As an extraction process, soxhlet extraction is mainly used in the extraction of compounds that are either insoluble or sparingly soluble in water. Though soxhlet extraction is a discontinuous extraction process, the repeated application of hot solvent (ethanol in this case) to the mushroom sample allows for a continuous penetration of the solvent into the sample. However, the yield of the extraction process is dependent on the extraction solvent. This may be responsible for the low yield (11 compounds) observed in the soxhlet extract of the sclerotia of this mushroom.

CONCLUSION

Hexane and dichloromethane extracts yielded more bioactive components with better nutriceutical and medicinal properties than methanol and soxhlet extracts. Sequel to these findings, hexane and dichloromethane are better solvents for extraction from a lignin based substrate such as mushrooms and other fungi.

References

1 Dadi DW, Emire SA, Hagos AD, Eun JB **(2018)** Improvement of yield of bioactive compounds and antioxidant activity of moringa stenopetala leaves by optimizing ultrasonic-assisted extraction res & rev. *J Food Sci Technol* 7: 1-11.

2 Borchers AT, Krishnamurthy A, Keen CL, Meyers FJ, Gershwin ME **(2008)** The immunobiology of mushrooms. *Exp Biol Med (Maywood)* 233: 259-276.

3 Lull C, Wichers HJ, Savelkoul HF **(2005)** Antiinflammatory and immunomodulating properties of fungal metabolites. *Mediators Inflamm* 2: 63-80.

4 Ohiri RC, Amadi BA, Isoje FE **(2018)** Phytochemical confinement in sections of pleurotus tuber-regium (king tuber mushroom) basidocarp. *Am J Biomed Sci* 10: 195-201.

5 Chen AW, Huang N **(2004)** Production of tuber-like sclerotia of medicinal value by *Pleurotus tuberregium* (Fr.) Sing (Agaricomycetideae). *Int J Med Mushrooms* 5: 313-319.

6 Isikhuemhen SO, LeBauer DS **(2004)** Growing Pleurotus tuberregium. *Mushworld Pub* 1: 264-274.

7 Oso BA **(1997)** Pleurotus tuber-regium from Nigeria. *Mycologia* 69: 271-279

8 Gregori A, Svagelj M, Pohleven J **(2007)** Cultivation techniques and medicinal properties of pleurotus spp. *Food Tech Biotech* 45: 238-249.

9 Patel Y, Naraian R, Singh VK **(2012)** Medicinal properties of pleurotus species (oyster mushroom) a review. *World J Fungal Plant Biol* 3: 1-12.

10 Clough SR, Mulholland L **(2005)** "Hexane". Encyclopedia of toxicology 2 (2nd edn). Elsevier, USA.

11 Tlili A, Schranck J **(2017)** The application of dichloromethane and chloroform as reagents in organic synthesis; in: solvents as reagents in organic synthesis: reactions and applications. Wiley, USA.