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Research Article

# GAS CHROMTOGRAPHY MASS SPECTROMETRY ANALYSIS OF VOLATILE COMPOUNDS FROM TERMITOMYCESHEIMII

**SanthiKumari.G, Ratna Kumar. P.K and Geetha. S***

Department of Botany, Andhra University, Visakhapatnam, A.P., India

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**ABSTRACT**

Termitomycesheimiiis wild edible mushroom used asfood by the Trible communities in Agency area of Visakhapatnam. The aim of this study was to investigate the volatile components present in Termitomycesheimiiusing gas chromatography mass spectrometry (GCMS). In this analysis revealed that the volatile compounds were 4-hydroxy-3-methoxybenzyl alcohol(0.008%), Flavone(0.035%), Estra-1,3,5(10)-trien–trien-17a-ol(0.241%), Phytol(0.107%), Octadec-9-enoic acid(0.483%), 2,6-Bis(1,1-dimethylethyl)-4-phenylmethylenecyclohexa-2,5-din-1-one(0.025%), Octadecanoic acid,3-oxo- methyl ester(0.024%), 1H-Pyrrol(2,3-b)quinoxalin-2-imine,2,3,3a,4,9,9a-hexahydro-1,N-diphenyl-(0.048%), Benzoic acid,2,4-dimethoxy-6-methyl-,(8,8-dimethoxy-2-octyl)ester(0.010%), 1,2-Proopanediol,bis(N-phenylcarbamate)(0.014%). Methanol extracted the greatest number of volatile compounds from T. heimii.Our study is the first to report on the volatile constituents of T. heimii, which is found to be Oleic acid (octadecadienoic acid), Flavone and Phytol.



## INTRODUCTION

Mushrooms are known to produce a wide range of volatile and flavour compounds with distinct profiles that may vary according to the species, variety and sometimes due to cultural conditions (Rapior *et al.,*1997). Mushrooms contain a vast amount of compounds among which are polysaccharides and triterpenes. Polysaccharides are regarded for their anti-tumour and immune stimulating activities and are majorly composed of glucans. The glucan linkages confer anti-tumour properties which is dependent on their molecular weight composition. Polysaccharides of mushroom origin prevent oncogenesis, tumour metastasis, and angiogenesis (Kao *et al*., 2013).

The gas chromatography (GC-MS) method provides both significantly better separation of substances in a mixture than TLC or HPLC and better reproducibility. The gas chromatography-mass spectrometry (GC-MS) analyses are quite sensitive, and extremely small amounts of material can be analyzed. The logical step forward was the development of GC- MS analysis. The use of MS in connection with GC-MS for detection provides a unique capacity to identify unknown substances, or to verify the presence of target molecules in complex mixtures. GC-MS is a core analytical technique with a broad range of applications, including the analysis of pharmaceuticals, pesticides, environmental pollutants, xenobiotics and toxins.

Many pharmaceutical substances with potent and unique health-enhancing properties have been isolated from Mushrooms and distributed worldwide. Mushroom based products either from the mycelia or fruiting is consumed in the forms of capsules, tablets or extracts (**Johnsy *et al*., 2015**). But, there is no much reports on the detailed analysis of GCMS and bioactive constituents of wild edible mushroom material, hence, it was planned to take up detailed investigation on ***Termitomycesheimii***for isolation of active biomolecules and its pharmacological activities from the potent constituent.

## MATERIALS AND METHODS

### *Preparations of extract*

The mushroom material was dried in the shade at 30± 2°C. The dried material were ground into a powder using mortar and pestle and passed through a sieve of 0.3 mm mesh size. The powder obtained was extracted with methanol by using the soxhlet method.

### *Extraction of Mushroom Powder by Soxhlet Method*

The shade dried, powdered 100gm mushroom material was soxhleted with methanol in a soxhlet extractor for 48 hours.

**Corresponding author:* **Geetha. S**
Department of Botany, Andhra University, Visakhapatnam, A.P., India

The extract was concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (50-60°C) to obtain the crude extract. Remaining trace of the solvent if any was further removed by placing the crude extract in vacuum overnight. Brown coloured extract was obtained and was stored in refrigerator at 4°C until used for experiment. 2µl of the methanol extract of two edible mushroom samples wereemployed for GC-MS analysis.

***Gas Chromatography Mass Spectroscopy Analysis***

***Instruments and Chromatographic Conditions***

GCMS analysis was carried out on a GC-MS Clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GCMS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. The plant extract was dissolved in methanol and filtered with polymeric solid phase extraction (SPE) column andanalysed in GC-MS for different constituents. Using computer searches on a NIST REFPROP Version 9.1 database and comparing the spectrum obtained through GC-MS compounds present in the plants sample were identified.

***Identification of Bioactive Constituents***

Interpretation on Mass-Spectrum GC-MS was carried out by using the database of National institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular formula, weight and chemical structure of the Components of the test materials were ascertained.

## RESULTS

***GC-MS Analysis***

The compounds present in the methanolic extract of two wild mushrooms were identified by GC-MS analysis presented in table no 1 and 2. The active principle Retention Time (RT), Molecular Weight (MW), Molecular Formula (MF),concentration (Peak Area (%)) and the chemical structures were analyzed.

***Termitomycesheimii*Sample:**

GCMS chromatogram of the methanol extract of***Termitomycesheimii*** showed 10 peaks indicating the presence of ten bioactive constituents. The identified compounds can be mainly divided into five groups according to the diverse functional groups. They are carbohydrates, flavonoids, steroids, saturated fatty acids and other organic compounds. The most abundant compound that was present in dry ***T. heimii*** mushroom extract was Octadec-9-enoic acid with highest peak area of 0.483% Shown in Table 1.

The prevailing compounds were 4-hydroxy-3-methoxybenzyl alcohol(0.008%)Flavone(0.035%),Estra-1,3,5(10)-trien–trien-17a-ol(0.241%), Phytol(0.107%), Octadec-9-enoic acid(0.483%), 2,6-Bis(1,1-dimethylethyl)-4-phenylmethylenecyclohexa-2,5-din-1-one(0.025%), Octadecanoic acid,3-oxo- methyl ester(0.024%), 1H-Pyrrol(2,3-b)quinoxalin-2-imine,2,3,3a,4,9,9a-hexahydro-1,N-diphenyl-(0.048%), Benzoic acid,2,4-dimethoxy-6-methyl-,(8,8-dimethoxy-2-octyl)ester(0.010%), 1,2-Proopanediol,bis(N-phenylcarbamate)(0.014%).

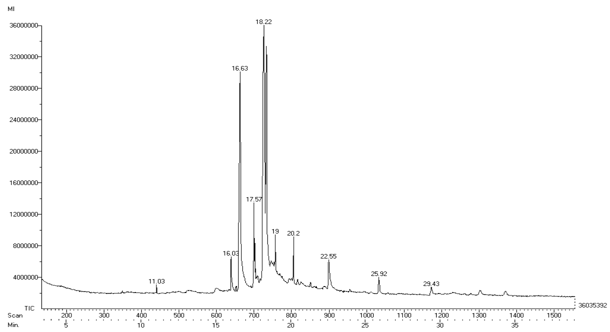
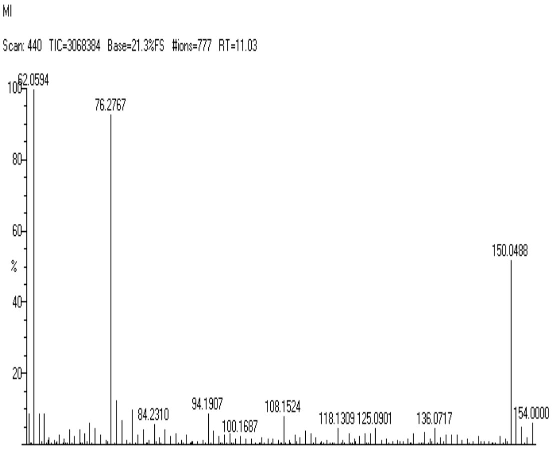
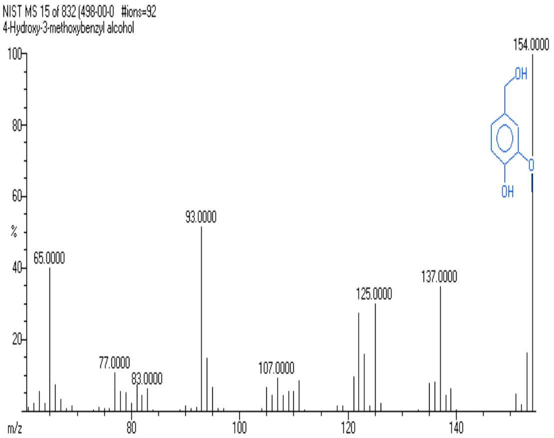
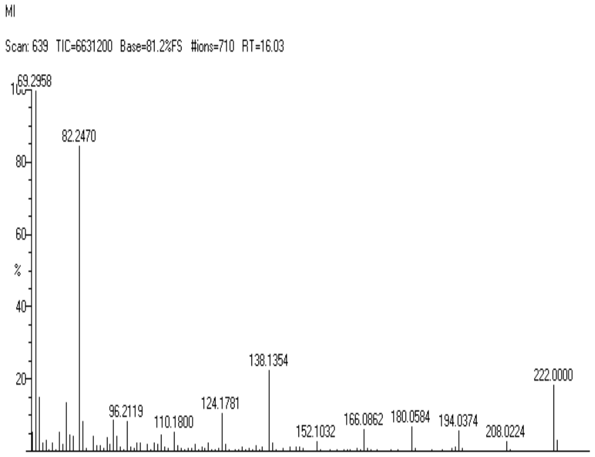


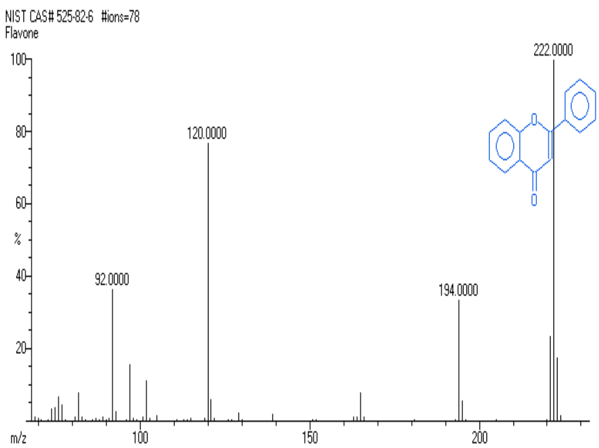

**Table 1** List of Volatile Compounds in Methanol Extract of *Termitomycesheimii*by GCMS Analysis

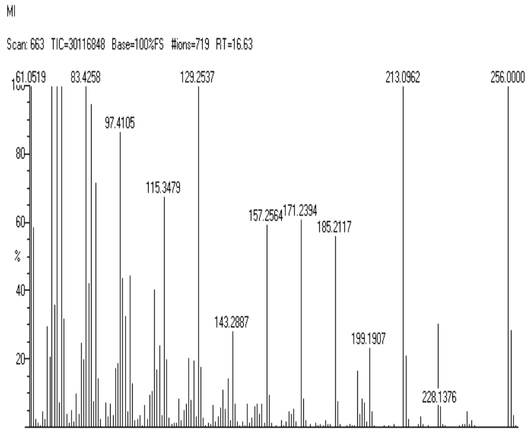
| S.NO | RT | Nameofthe Compound | Molecular Formula | Molecular weight(g/mol) | Peak Area% | Compound Nature |
|---|---|---|---|---|---|---|
| 1. | 11.03 | 4-hydroxy-3-methoxybenzyl alcohol | $HOC_6H_3(OCH_3)CH_2OH$ | 154.16 | 0.008 | Carbohydrate |
| 2. | 16.03 | Flavone | $C_{15}H_{10}O_2$ | 222.243 | 0.035 | Flavonoids |
| 3. | 16.63 | Estra-1,3,5(10)-trien –trien-17a-ol | $C_{18}H_{24}O$ | 256.3826 | 0.241 | Steroids |
| 4. | 17.57 | Phytol | $C_{20}H_{40}O$ | 296.539 | 0.107 | Diterpene |
| 5. | 18.22 | Octadec-9-enoic acid | $C_{18}H_{34}O_2$ | 282.46 | 0.483 | Mono saturated Omega 9fatty acids |
| 6. | 19 | 2,6-Bis(1,1-dimethylethyl)-4-phenylmethylenecyclohexa-2,5-dien-1-one | ---- | ---- | 0.025 | ---- |
| 7. | 20.2 | Octadecanoic acid, 3-oxo- methyl ester | $C_{19}H_{36}O_3$ | 312.49 | 0.024 | Saturated fatty acids |
| 8. | 22.55 | 1H-Pyrrol(2,3-b)quinoxalin-2-imine,2,3,3a,4,9,9a-hexahydro-1,N-diphenyl- | ---- | ---- | 0.048 | ---- |
| 9. | 25.92 | Benzoic acid,2,4-dimethoxy-6-methyl-,(8,8-dimethoxy-2-octyl)ester | ---- | ---- | 0.010 | ---- |
| 10. | 29.43 | 1,2 Proopanediol,bis(N-phenylcarbamate) | ---- | ---- | 0.014 | ---- |

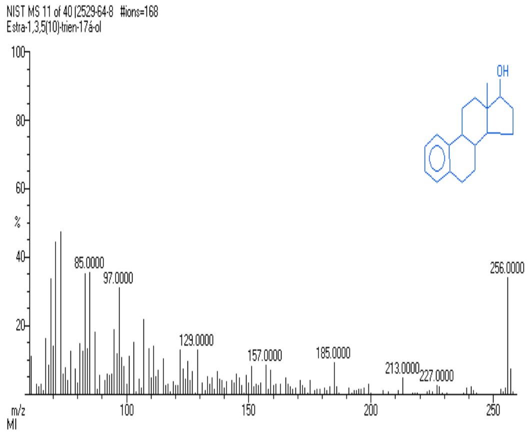

MI
Scan: 440 TIC=3068384 Base=21.3%FS #ions=777 RT=11.03
62.0594
76.2767
150.0488
84.2310
94.1907
100.1687
108.1524
118.1309 125.0901
136.0717
154.0000

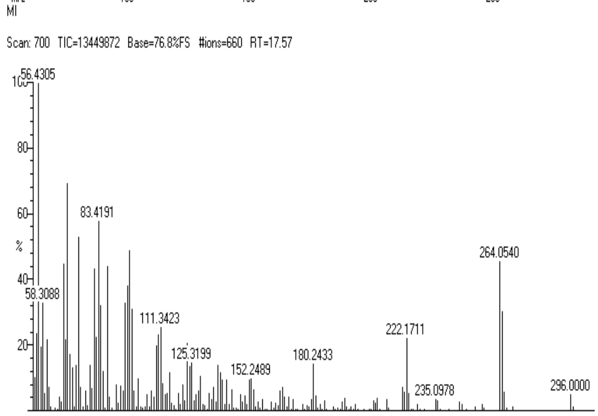

NIST MS 15 of 832 (498-00-0 #ions=92
4-Hydroxy-3-methoxybenzyl alcohol
154.0000
93.0000
65.0000
137.0000
125.0000
77.0000
83.0000
107.0000
OH
O
OH
m/z

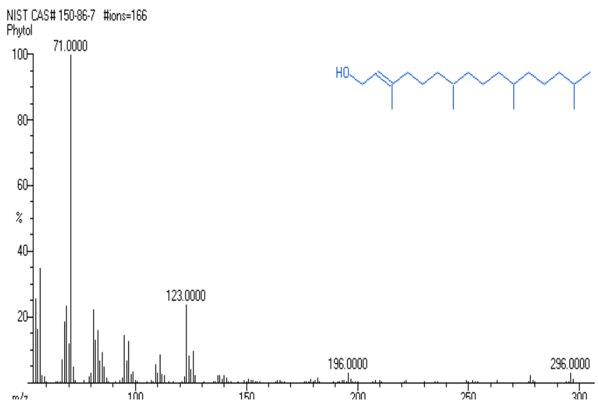

MI
Scan: 639 TIC=6631200 Base=81.2%FS #ions=710 RT=16.03
69.2958
82.2470
138.1354
222.0000
124.1781
96.2119
110.1800
152.1032
166.0862
180.0584
194.0374
208.0224

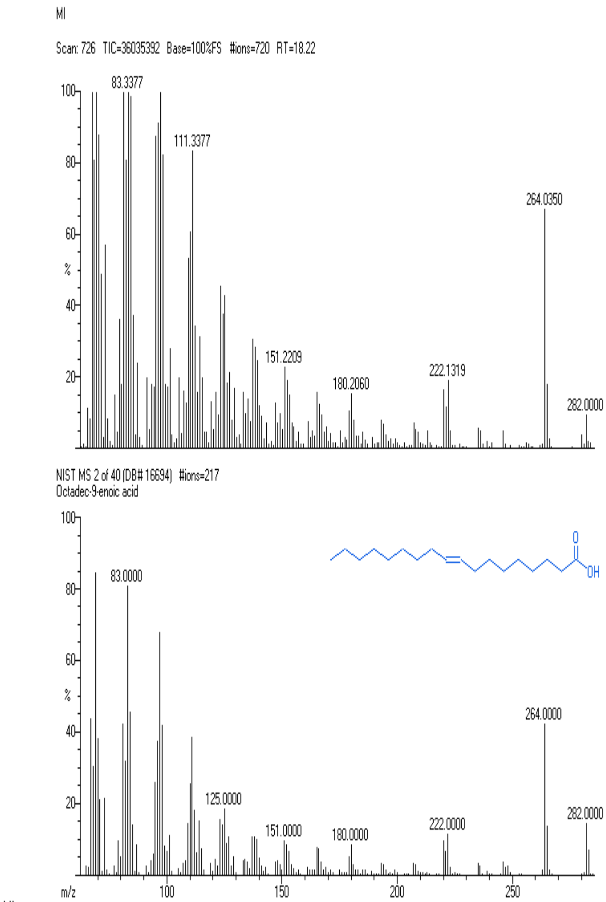

NIST CAS# 525-82-6 #ions=78
Flavone
222.0000
120.0000
92.0000
194.0000
O
O
m/z

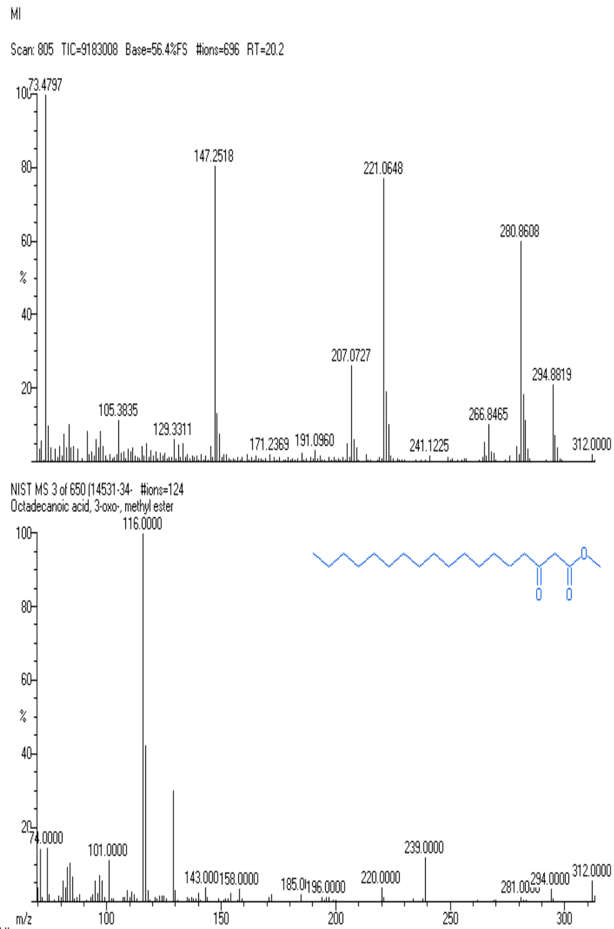

MI
Scan: 663 TIC=30116848 Base=100%FS #ions=719 RT=16.63
61.0519
83.4258
129.2537
213.0962
256.0000
97.4105
115.3479
157.2564
171.2394
185.2117
143.2887
199.1907
228.1376

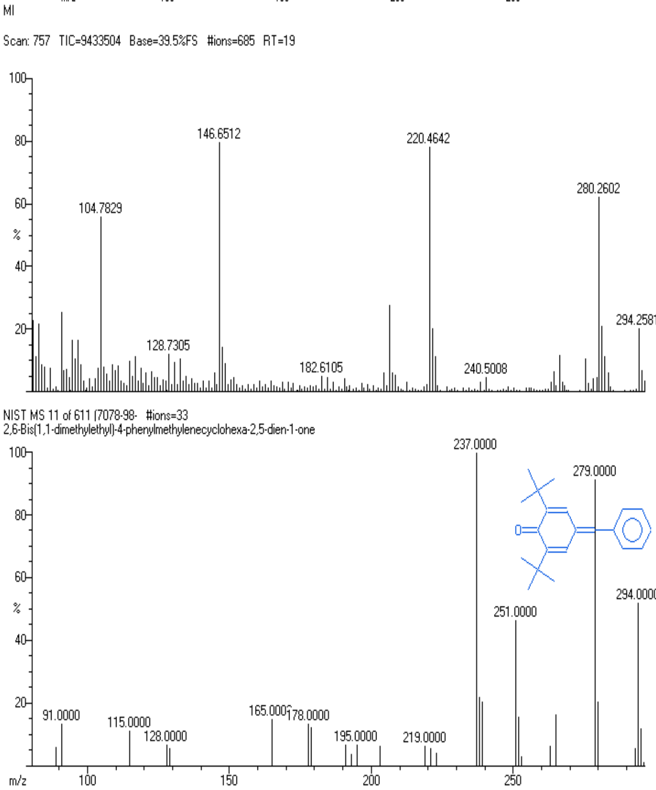

NIST MS 11 of 40 (2529-64-8 #ions=168
Estra-1,3,5(10)-trien-17â-ol
OH
85.0000
97.0000
256.0000
129.0000
157.0000
185.0000
213.0000
227.0000
m/z

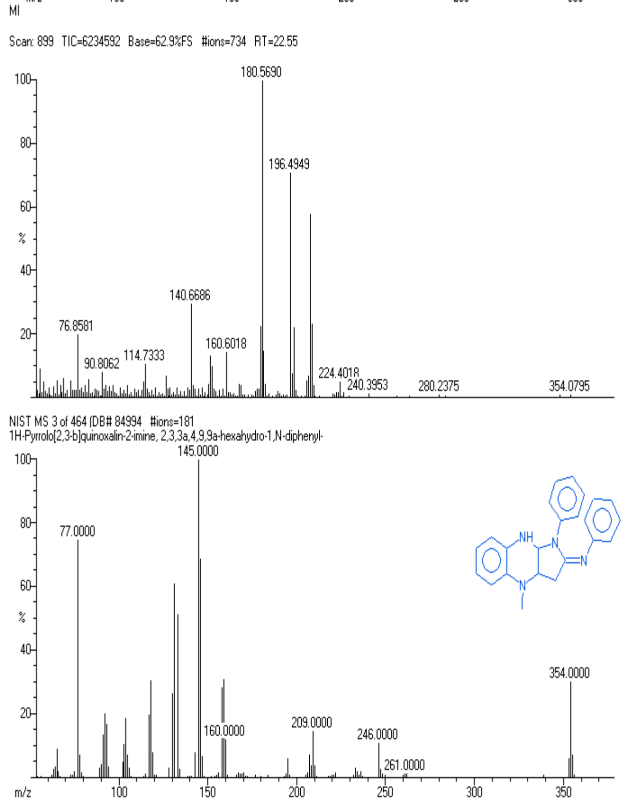

MI
Scan: 700 TIC=13449872 Base=76.8%FS #ions=660 RT=17.57
56.4305
83.4191
58.3088
264.0540
111.3423
222.1711
125.3199
152.2489
180.2433
235.0978
296.0000

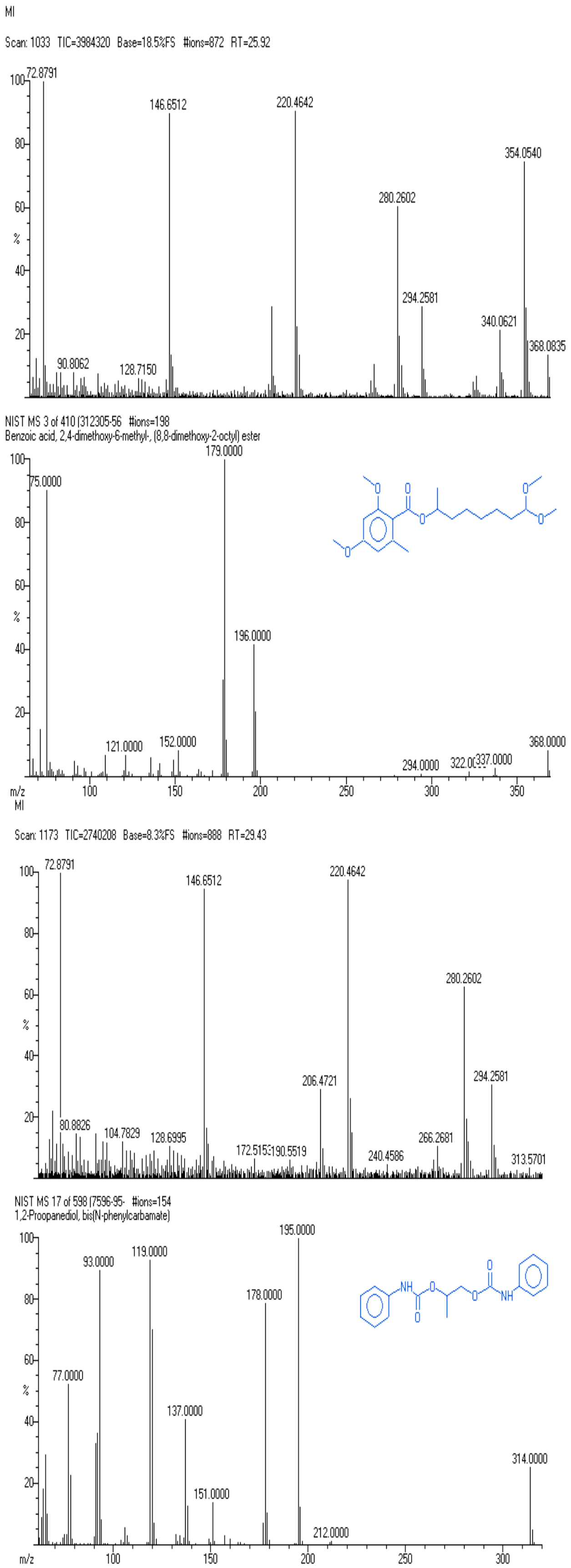

NIST CAS# 150-86-7 #ions=166
Phytol
71.0000
HO
123.0000
196.0000
296.0000
m/z


MI
Scan: 726 TIC=36035392 Base=100%FS #ions=720 RT=18.22
83.3377
111.3377
264.0350
151.2209
180.2060
222.1319
282.0000
NIST MS 2 of 40 (DB# 16694) #ions=217
Octadec-9-enoic acid
83.0000
264.0000
125.0000
151.0000
180.0000
222.0000
282.0000
m/z


MI
Scan: 805 TIC=9183008 Base=56.4%FS #ions=696 RT=20.2
73.4797
147.2518
221.0648
280.8608
207.0727
294.8819
105.3835
129.3311
171.2369
191.0960
241.1225
266.8465
312.0000
NIST MS 3 of 650 (14531-34- #ions=124
Octadecanoic acid, 3-oxo-, methyl ester
116.0000
74.0000
101.0000
143.0000
158.0000
185.0
196.0000
220.0000
239.0000
281.0000
294.0000
312.0000
m/z


MI
Scan: 757 TIC=9433504 Base=39.5%FS #ions=685 RT=19
146.6512
220.4642
280.2602
104.7829
294.2581
128.7305
182.6105
240.5008
NIST MS 11 of 611 (7078-98- #ions=33
2,6-Bis(1,1-dimethylethyl)-4-phenylmethylenecyclohexa-2,5-dien-1-one
237.0000
279.0000
294.0000
251.0000
91.0000
115.0000
128.0000
165.0000
178.0000
195.0000
219.0000
m/z


MI
Scan: 899 TIC=6234592 Base=62.9%FS #ions=734 RT=22.55
180.5690
196.4949
140.6686
76.8581
90.8062
114.7333
160.6018
224.4018
240.3953
280.2375
354.0795
NIST MS 3 of 464 (DB# 84994 #ions=181
1H-Pyrrolo[2,3-b]quinoxalin-2-imine, 2,3,3a,4,9,9a-hexahydro-1,N-diphenyl-
145.0000
77.0000
354.0000
160.0000
209.0000
246.0000
261.0000
m/z




The GC-MS spectrum,the methanol extract from ***T. heimii*** resulted in the identification of 10 major compounds. Out of 10 compounds only 6 were detected that are 4-hydroxy-3-methoxybenzyl alcohol is veratryl alcohol oxidases (VAO) from the lignin degrading compound. Ligninisathree dimensionalpolymerfoundabundantlyinwoodandplanttissue (Sarkanen&Ludwig,1971).Itis composed of phenylpropanoid units inter –connected by stable C-CandC-0bonds.The heterogeneity and complexity of its structure confers resistance to microbial attack. However, lignin canbedegradeds lowly in Nature, mainly by white-rotfungi,and this has considerable impactin forestry and agriculture. Lignin biodegradation by white-rot fungi is an oxidative process in which $H_20_2$ play sanimportantrole. (Crawford & Crawford,1984).

If VAO plays arole in lignin biodegradation, it could be either throught wo-electron redox cycling ofv eratry lalcoholor lignin fragments, with$H_20_2$ productionfrom$0_2$,or, in combination with laccase, a sadehydrogenase of aromatic compounds.

Flavones are the groups of flavonoids. Flavones are yellow pigments also known as anthoxanthins. Flavones and flavonoids epidemiological evidence shows the beneficial effects of these molecules in cardiovascular and neuropathological diseases. Flavones and flavonds are molecules present in most plants that are an important component of some human diets (Murkovic *et al*., 2003). Flavones are natural antioxidants and might reduce inflammation (Swelling). They might also affect the way the liver processes.

Estra-1,3,5(10)- trien-17 a-olis steroid, present in the essential oil differs from estradiol, a sex hormone, in the absence of the C3-OH. Steroids though similar in basic structure, have extreme specificity, hence this steroid cannot be said to function like estradiol. However, it may be responsible for the observed estrogenic and/or anti-estrogenic activity (Collins *et al*., 1997). A derivative of ascorbic acid, Vit C. Estra-1,3,5(10)-trien –trien-17a-ol compound has anti-androgenic, antitumor, immune stimulant, antioxidant and antiviral activity.

Phytol is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1 (Daines *et al*., 2003).Phytol is used in the fragrance industry and used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents (McGinty *et al*., 2010). Its worldwide use has been estimated to be approximately 0.1–1.0 metric tons per year (IFRA 2004).

Phytol is reported to have antioxidant, antiallergic(Santos *et al*., 2013) antinociceptive and anti-inflammatory activities (Ryu*et al*., 2011). Recent studieshave revealed that phytol is an excellent immunostimulant.It is superior to a number of commercial adjuvants in terms of long –term memory induction and activation of both innate and acquired immunity (Lim *et al*., 2006). Phytol has alsoshown antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus* (Saikia *et al*., 2010). Phytolwas observed to have antibacterial activities against *Staphylococcus aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells (Inoue *et al*., 2005). Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is used along with simple sugar or corn syrup as a hardener in candies.

Octadec-9-enoic acid and Octadecanoic acid, 3-oxo- methyl ester are Mono saturated Omega 9fatty acids other name is oleic acid. Oleic acid is amonounsaturated fatty acid that occurs naturally in the fats and oils of both animals and vegetables. It is naturally odorless and colorless, although commercial products made with it may be yellowish.Researchers indicate that it's the consumption of oleic acid that regulates membrane lipid structure, which controls G protein-mediated signaling and causes a regulation in blood pressure.The monounsaturated fat oleic acid has beneficial effects on insulin sensitivity and type 2 diabetes.This is due to the acid's anti-inflammatory actions and its ability to prevent the action of the insulin signaling pathway. That means oleic acid consumption can help regulate the amount of insulin that is released to promote the uptake of glucose from your bloodstream.

Oleic acid have a heavier consistency, they can be used to seal moisture into our skin. This can be especially helpful for people with dry skin or even dry hair. This acid also works as an emollient and has been used in hair products to make your hair softer and smoother.Oleic acid is an antioxidant that prevents oxidative stress that leads to a number of health conditions, including cancer.Research shows that this acid has beneficial effects in cancer processes because it plays a role in the activation of different intracellular pathways that are involved in the development of cancer cells. According to a scientific review conducted in Spain, oleic acid has been shown to induce apoptosis (cell death) in cancer cells (Carrillo *et al*., 2012andGopalakrishnan *et al*., 2011).

***T. heimii*** contained 55% unsaturated fatty acid. Unsaturated fatty acids are good for human health. Fatty acids (or their salts) do not often occur as in biological systems. Usually, people get unsaturated fatty acids from fish consumption. But recently it was reported that some species fish contain chemical contaminants which are harmful for health (**Zamir *et al*., 2013**).That means foods with this healthy fatty acid may be cancer fighting foods to stave off this disease and other diseases.

## CONCLUSION

In the present study 22 bioactive constituents have been identified from methanol extract of ***T. heimii***by GCMS analysis. From this study, it is concluded that different volatile profiles exhibited by***T.heimii***is a rare and highly sought after edible fungus that grows seasonally, and in symbiosis with termites. It is difficult to cultivate.GCMS analyses indicated the presence of 10 different compounds in the mushroom sample. The results also indicated that mushroom samples are rich polyunsaturated and essential fatty acids. The presence of polyunsaturated fatty acids like n-Hexadecenoic acid and Octadecenoic acid is more related to growth and development of mushroom fungi (**Priyadharshini *et al*., 2017**). Thus, the findings support the therapeutic claims of this mushroom. Further, the consumption of***T. heimii***would be beneficial for health purposes and may have chemopreventive properties of selected diseases of humankind. However, studies to elucidate the mechanisms ofpossible biological activity are needed to validate the claims

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