ANALYSIS OF BIOACTIVE COMPONENTS FROM CHLOROFORM EXTRACT OF LYCOPERDON SP. (APIOPERDON)

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ABSTRACT
Objective: The present work was done to find out various bioactive compounds present in the chloroform extract of Lycoperdon pyriforme using gas chromatography-mass spectrometry (GC-MS).

Methods: L. pyriforme was collected cleaned to remove any dirt and oven dried at 60°C. The sample was then finely powdered and extracted with chloroform using Soxhlet apparatus. The chemical composition of chloroform extract was then analyzed using QP2010S-Shimadzu GC-MS instrument.

Results: Chloroform extract resulted in the presence of 33 compounds with 1-Heneicosanol (11.17%) and E-15-Heptadecenal (11.08%) forming major compounds and 1-Tetradecanol (0.16%), Dichloroacetic acid, and decyl ester (0.15%) forming least.

Conclusion: The results indicated the presence of a variety of compounds thus providing the information about various bioactive compounds present in Lycoperdon sp. and its further application in the field of pharmacology.

Keywords: Lycoperdon pyriforme, Chloroform extract, Gas chromatography-mass spectrometry.

INTRODUCTION
Fungi besides being pathogens are also known to possess medicinal values. Since ancient times mushrooms, visually distinctive compared with other class of fungi with naked fruiting bodies, have been used in traditional medicines as “the ultimate health food” [1]. Their medicinal characteristics have led them to be used in the field of drug development. A number of mushrooms have been screened for their antimicrobial potential for their use in pharmacology [2-5].

Lycoperdon otherwise “puffballs” a saprobe belongs to the family Agaricaceae. They are widely distributed in tropics growing on various habitats, on tree stumps, on decaying logs, and on ground among fallen leaves [6]. A number Lycoperdon species have been tested for their antimicrobial potentiality [7]. The present work has been carried out to understand various bioactive compounds present in Lycoperdon pyriforme. In the present study, chloroform extract has been used and various compounds are detected using gas chromatography-mass spectrometry (GC-MS) technique.

MATERIALS AND METHODS
Fungal specimen and extraction
L. pyriforme (Schaeff.) Vizzini (Apioperdon pyriforme) (Fig. 1) was collected from in and around the Kerala Forest Research Institute campus. The specimen was identified and confirmed by referring standard manual [8]. The specimen was cleaned to remove any dirt and was then oven dried at 60°C. The dried specimen was then pulverized to fine powder in a mechanical grinder. The powder was weighed and then subjected to Soxhlet extraction using chloroform. The extract was concentrated using rotary evaporator. Thus obtained final residue was subjected to GC-MS analysis.

GC-MS analysis
GC-MS analysis was carried out using QP2010S-Shimadzu GC-MS instrument (30 m × 0.25 mm × 0.25 μm, Rxi-5Sil MS). One microliter of the chloroform extract was injected into the GC-MS instrument. Initially, the column temperature was maintained at 80°C for 2 min, followed by a temperature gradient from 80°C to 280°C and held constant for 10 min and finally raised temperature to 280°C and held constant for 6 min. The instrument operated in a split mode and libraries used for analysis was NIST 11 and WILEY 8.

RESULTS AND DISCUSSION
GC-MS analysis of chloroform extract of L. pyriforme showed the presence of 33 different compounds. The active compounds with their retention time, area, area percentage, height, height percentage, base m/z, and their names are presented in Table 1. The GC-MS chromatogram of the chloroform extract is shown in Fig. 2. About 33 compounds have been found in which 1-Heneicosanol (11.17%) and E-15-Heptadecenal (11.08%) are the major compounds and 1-Tetradecanol (0.16%), Dichloroacetic acid, and decyl ester (0.15%) forming least.

Conclusion: The results indicated the presence of a variety of compounds thus providing the information about various bioactive compounds present in Lycoperdon sp. and its further application in the field of pharmacology.
potentiality. Chemicals compounds such as sterol derivatives ((S)-23-hydroxylanostrol, ergo-sterol α-endoperoxide, ergosterol 9,11-dehydroendoperoxide and ((2E)-lanosta-8,23-dien-3β,25-diol), volatile compounds (3-octanone, 1-octen-3-ol and ((Z))-3-octen-1-ol), and an unusual amino acid such as lycoperdic acid have been identified from Lycoperdon perlatum fruit bodies [9,10]. The study represents a primary account of various bioactive compounds in the chloroform extract and a detailed study can be carried out for the isolation and purification of specific compounds for further application in the chemical industry.

CONCLUSION

Wild mushrooms growing naturally produce a large number of secondary metabolites which impart lots of medicinal values to them. The present work was carried to understand various bioactive compounds present in the chloroform extract of L. pyriforme. A total of 33 compounds have been identified by GC-MS analysis and most of the compounds obtained were those with antimicrobial potentiality. This suggests their importance and potential application in the field of pharmaceutical research.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Table 1: Compounds detected in the chloroform extract of Lycoperdon pyriforme

| Peak# | R.Time | Area | Area% | Height | Height% | Name                                                                 | Base m/z |
|-------|--------|------|-------|--------|---------|----------------------------------------------------------------------|---------|
| 1     | 5.532  | 392251 | 0.80  | 146463 | 0.57    | 2-Pyrrolidinone                                                       | 85.05   |
| 2     | 7.413  | 109389 | 0.22  | 73947  | 0.57    | 1-Dodecene                                                          | 85.05   |
| 3     | 8.313  | 2183095 | 4.43  | 47712  | 1.86    | Benzeneacetic acid                                                   | 85.05   |
| 4     | 10.276 | 242813  | 4.92  | 1730368 | 6.74    | 1-Tetradecene                                                        | 85.05   |
| 5     | 10.383 | 219369  | 0.44  | 164541  | 0.64    | Tetradecane                                                          | 85.05   |
| 6     | 10.876 | 112748  | 0.23  | 75686   | 0.29    | 4’-(2-Methylpropyl)acetophenone                                      | 85.05   |
| 7     | 11.786 | 3780013 | 7.67  | 241467  | 9.41    | Phenol, 2,4-bis(1,1-Dimethylethyl)-                                   | 85.05   |
| 8     | 12.824 | 478489  | 9.61  | 3279911 | 12.77   | E-14-Hexadecenal                                                     | 85.05   |
| 9     | 12.909 | 304523  | 0.62  | 222487  | 0.87    | Hexadecane                                                          | 85.05   |
| 10    | 14.853 | 72346   | 0.15  | 51891   | 0.20    | Dichloroacetic acid, decylester                                      | 85.05   |
| 11    | 15.042 | 78152   | 0.16  | 55184   | 0.21    | 1-Tetradecanol                                                       | 85.05   |
| 12    | 15.098 | 565560  | 11.08 | 3638303 | 14.17   | E-15-Heptadecanol                                                    | 85.05   |
| 13    | 15.167 | 358390  | 0.73  | 251796  | 0.98    | Nonadecane                                                          | 85.05   |
| 14    | 15.233 | 121518  | 0.25  | 58454   | 0.23    | 1-Dodecene, 2-Ethyl-                                                 | 85.05   |
| 15    | 15.625 | 1774683 | 3.60  | 799656  | 3.11    | Hexadecanoic acid                                                    | 85.05   |
| 16    | 17.154 | 5508171 | 11.17 | 3234728 | 12.60   | 1-Heneicosanol                                                       | 85.05   |
| 17    | 17.211 | 272640  | 0.55  | 188335  | 0.73    | Nonadecane                                                          | 85.05   |
| 18    | 18.490 | 214803  | 4.36  | 999489  | 3.50    | Methyl octadeca-9,12-Dienoate                                       | 85.05   |
| 19    | 18.533 | 639984  | 1.30  | 284065  | 1.11    | 22-Tricosenic acid                                                   | 85.05   |
| 20    | 18.583 | 435018  | 0.89  | 159272  | 0.62    | Tetraethylene glycol, monobutyl ether                                | 85.05   |
| 21    | 18.730 | 274213  | 0.56  | 150768  | 0.62    | Octadecanoic acid                                                   | 85.05   |
| 22    | 19.029 | 463395  | 0.94  | 2745804 | 10.69   | 1-Heneicosanol                                                       | 85.05   |
| 23    | 19.075 | 218168  | 0.44  | 159471  | 0.62    | Hexadecane                                                          | 85.05   |
| 24    | 20.367 | 510895  | 1.03  | 334653  | 1.30    | 0’-0’-Biphenol, 4,4’,6,6’-Tetra-T-Butyl-                             | 85.05   |
| 25    | 20.463 | 199086  | 0.40  | 109773  | 0.43    | Methyl[cylopropyl[methyl]methyl]-, methyl ester                       | 85.05   |
| 26    | 20.812 | 3446566 | 6.99  | 1825058 | 7.11    | n-Tetrasano-1                                                       | 85.05   |
| 27    | 20.858 | 157361  | 0.32  | 113300  | 0.44    | Penta-decane                                                        | 85.05   |
| 28    | 22.053 | 238723  | 0.48  | 106657  | 0.42    | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ster             | 85.05   |
| 29    | 23.117 | 1923043 | 3.90  | 829924  | 3.23    | Eicosyl trifluoroacetate                                             | 85.05   |
| 30    | 23.849 | 265298  | 0.42  | 80125   | 0.31    | Docosyl trifluoroacetate                                             | 85.05   |
| 31    | 35.321 | 941280  | 1.91  | 218643  | 0.85    | Dehydroergosterol, 5,5-dinitrobenzoate                              | 85.05   |
| 32    | 36.267 | 2766552 | 5.61  | 260549  | 1.01    | Ergosterol                                                          | 85.05   |
| 33    | 36.667 | 2650584 | 5.38  | 534458  | 2.08    | Ergosta-7,22-Dien-3-ol, (3.Beta.,22E)-                               | 85.05   |

Fig. 2: Gas chromatography-mass spectrometry chromatogram of chloroform extract of Lycoperdon pyriforme
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