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Forensic Analysis of Wild Toxic and Edible Amanita Mushrooms by Gas Chromatography-Mass Spectrometry

التحليل الجنائي لفطر أمانيتا البري السام والصالح للأكل بواسطة كروماتوغرافيا الغاز المقترنة بمطياف الكتلة

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Abstract

For many years, the foraging and consumption of wild mushrooms has been practised in different parts of the world. Despite having various health benefits, few mushroom species are known for causing toxicity as well. In forensic casework conditions, samples from mushroom poisoning cases can be found in dried or powdered form. So, it becomes necessary to characterize mushroom species for identification purposes.

In the present study, volatile fractions of five wild toxic and edible *Amanita* mushroom species (*Amanita muscaria, Amanita pantherina, Amanita caesarea, Amanita subglobosa and Amanita porphyria*) were analyzed so as to identify compounds for the characterization of selected mushroom species.

The obtained volatile fractions were broadly classified into various chemical classes: alcohols, aldehydes, acids, esters, nitrogen-containing compounds, ketones and miscellaneous. The following compounds; octadecanoic acid, 9-octadecenoic acid (z)-, ethane, 1-chloro-1-fluoro- were the most abundant.

The present approach utilizing GC-MS, intends to obtain a fingerprint of each sample for discrimination purposes. Also,

Keywords: Forensic science, Genus *Amanita*, Wild Mushrooms, Toxicity, GC-MS.

المستخلص

لسنوات عديدة، كان يتم البحث عن الفطر البري واستهلاكه في أجزاء مختلفة من العالم. وعلى الرغم من وجود العديد من الفوائد الصحية للفطر،فإن البعض من أنواعه يسبب التسمم. وفي الظروف المحيطة بالقضايا الجنائية، يمكن العثور على عينات من الفطر في حالات التسمم كنباتات مجففة أو على شكل مسحوق. ولذلك، يصبح من الضروري وضع توصيف لأنواع الفطر لأغراض التعرف على أنواعه.

وفي هذه الدراسة، تم تحليل المكونات المتطايرة من خمسة أنواع من فطر أمانيتا البري السام والصالح للأكل (Amanita muscaria, Amanita pantherina, Amanita caesarea, Amanita subglobosa, Amanita porphyria) بهدف التعرف عليها من أجل وضع توصيف لأنواع معينة من الفطر.

وتم تصنيف المكونات المتطايرة التي تم الحصول عليها عمومًا إلى فئات كيميائية مختلفة: الكحوليات والألدهيدات والأحماض والإسترات والمركبات المحتوية على النيتروجين والكيتونات بالإضافة إلى فئة متنوعة. وكانت المركبات التالية هي الأكثر توافراً: octadecanoic acid, 9-oct-adecenoic acid (z)-, ethane, 1-chloro-1-fluoro-.

ويسعى النهج الحالي الذي يستخدم كروماتوغرافيا الغاز المقترنة بمطياف الكتلة للحصول على بصمة لكل عينة بهدف تمييزها. وبالإضافة

الكلمات المفتاحية: علوم الأدلة الجنائية، فطر أمانيتا، الفطر البري، السمية، كروماتوغرافيا الغاز المقترنة بمطياف الكتلة.

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this work is the first study on the forensic analysis of methanol-soluble components of *Amanita* mushroom species from the North-Western Himalaya, India.

إلى ذلك، فإن هذا العمل يُعتبر أول دراسة عن التحليل الجنائي للمركبات القابلة للذوبان في الميثانول لأنواع فطر أمانيتا من شمال غرب الهيمالايا، الهند.

1. Introduction

For many years, wild mushrooms have been very popular in many countries as a food source and are well known for their medicinal and nutritional properties [1]. Another reason for their high popularity is that several mushroom species are composed of unique and pleasant flavors. Additionally, mushrooms have an ample amount of proteins, minerals, vitamins, lipids, carbohydrates and amino acids, in addition to having less calories and fatty substance [2,3]. Some of the mushroom species have gained forensic importance due to the presence of certain toxins and their ingestion may cause severe outcomes, sometimes resulting fatality. Despite warnings, mushroom collectors often get confused with inedible mushroom species due to misidentification, based on morphological characteristics [4]. The majority of such cases are due to accidental consumption, but there is a possibility of their use for homicidal purposes as well [5]. Certain mushroom species are also known for inducing hallucinogenic effects and are often confiscated in illegal drug markets as controlled substances [6]. In forensic scenarios, mushrooms as evidence, are generally recovered in degraded forms such as; dried, powdered, gastric aspirates, vomit etc. Thus, it becomes necessary to characterize mushroom species for identification purposes.

The toxicology of most poisonous mushrooms remains unexplored, but new poisoning syndromes continue to emerge [7]. Based on toxin composition, poisonous mushrooms are generally grouped into: cyclopeptides (amatoxins, phallotoxins and virotoxins), gyromitrin, muscarine, isoxaxoles, psilocybin, orellanine and gastrointestinal irritants. Among these, mushrooms comprising cyclopeptides of genus *Amanita* are responsible for the highest number of cases of mushroom poisoning throughout the world [4,8–10]. The genus *Amanita* consists of approximately 600 species, among which few are known to cause toxicity that contains amatoxins and the rest are non-toxic. Based on syndromes produced, the toxins from *Amanita* mushrooms are generally divided into three classes: neurotoxic, nephrotoxic and hepatotoxic. Neurotoxic *Amanita* species comprises *Amanita muscaria, Amanita pantherina* which contains ibotenic acid and muscimol, which causes hallucinogenic effects after consumption. Nephtotoxic *Amanita* mushroom species cause acute renal failure, which includes *A. smithiana, A. proxima and A. pseudoporphyria*. Hepatotoxic mushroom species contain cyclopeptides that cause severe liver damage and sometimes result in death. These include A. phalloides, A. virosa, A. verna, A. fuliginea and A. subjunquillea [11,12]. Amatoxins inhibit the activity of RNA polymerase II which is an important enzyme for the synthesis of RNA, leading to cell death. The lethal dose of amatoxin is 0.1 mg/kg. These toxins are heat-resistant and are not affected by any food preparation methods like frying, grilling, boiling and steaming [10,13–15].

Numerous identification techniques have been developed to determine amatoxins, both qualitatively and quantitatively. Methods previously reported include enzyme-linked immunosorbent assay (ELISA) [16,17], thin-layer chromatography (TLC) [18], liquid chromatography (LC) [10,19], high-performance liquid chromatography (HPLC) [20–24], gas-chromatography mass spectrometry (GC-MS) [25,26], infrared (IR) [27] spectroscopy and capil-

lary zone electrophoresis (CE) [28,29]. However, to the best of our knowledge no previous studies have exploited the use of GC-MS for the qualitative and quantitative examination of amatoxins (α-, β-, and γ-amanitin), because these toxins are non-volatile [30]. Few studies have explored the utilization of LC-MS and GC-MS for the analysis of amatoxins, muscimol and ibotenic acid [31,32]. Few others have explored the potential of GC-MS for the discrimination of wild mushroom, by identifying volatile biomarkers. Carvalho et al., [2] performed the targeted and untargeted analysis of twenty-two mushroom species (twelve edible, three toxic and seven potentially toxic) by GC-MS. Targeted analysis could analyze amino acids, fatty acids and sterols, and untargeted analysis allowed the identification of six molecules that were species or genus specific. Malheiro et al., [33] studied the volatile fractions in six wild mushrooms *(Clitocybe odora, Clitocybe fragrans, Hebeloma crustuliniforme, Lepista nuda, Tricholoma fracticum and Tricholoma terreum*) to discriminate mushroom species. Forty-six volatile components obtained, were grouped into various chemical classes, such as; alcohols, aldehydes, ketones and compounds like sesquiterpene and terpenes.

The present study is the first to incorporate the examination of inedible and edible species from the north-western Himalaya by GC-MS, for the purpose of species identification. The methanol-soluble fraction obtained was chemically classified into alcohols, aldehydes, ketones, acids, nitrogen-containing compounds for the differentiation of wild mushroom species.

2. Materials and Methods

2.1 Sample Collection

In this study, five wild toxic and edible *Amanita* mushroom species (*Amanita muscaria, Amanita pantherina, Amanita caesarea, Amanita subglobosa* and *Amanita porphyria*) were collected from the North-Western Himalaya (Table-1 and Figure-1). For sample collection, various localities were visited in the months from July-September (Table-2). The samples were then morphologically identified, preserved and stored under optimum conditions as per the key given by Rai et al., 2005 [34].

Table 1- *The details of the collected mushroom samples examined in the present study.*

Sample Code	Species (*n*=5)	Edibility
S1	*Amanita muscaria*	Toxic
S2	*Amanita pantherina*	Toxic
S3	*Amanita caesarea*	Edible
S4	*Amanita subglobosa*	Edible
S5	*Amanita porphyria*	Edible

Figure 1- Map representing the study area.

2.2 Sample Preparation

The Collected samples were hot air dried in a specially designed wooden drier to avoid any further contamination. During drying, 40-45°C temperatures was maintained inside the chamber overnight. For analysis, one gram of dried mushroom sample was powdered using a pestle and mortar. Fifteen mL of methanol was then added, and it was kept undisturbed overnight. The supernatant was filtered

Table 2- *Details of localities visited for sample collection.*

Sr. No	Locality	Latitude (N)	Longitude (E)	Altitudinal range (meters)
1	Palampur	N 32.1109°	E 76.5363°	2250 - 1472
2	Dharamshala	N 32.2190°	E 76.3234°	2082 - 1457
3	Chamba	N 32.5534°	E 76.1258°	900-1260
4	Dalhousie	N 32.5387°	E 75.9710°	2768 - 1185
5	Shimla	N 31.1048°	E 77.1734°	3400 - 2276
6	Dehradun	N 30.3165°	E 78.0322°	700 – 410

through 0.22 µm nylon filter and 10 µL extract was injected to GC-MS.

2.3 GC-MS Conditions

The selected samples were analysed with Schimadzu GC-MS-QP2010 Ultra. Analysis was done using the following parameters: carrier gas - helium, column- Rxi-5Sil ms (30 m, 0.25 mm internal diameter, 0.25 mm thickness), column oven temperature elevated from 60 to 250°C with column flow of 1 ml/min in splitless mode. The total run time was 33 minutes. An Auto sampler (AOC-20i) was used for sample injection and 10 µL sample was injected. The ion source and interface temperatures were 230°C. The Shimadzu software (Real-time analysis) provided along with the instrument was used to obtain spectra, and Post-run analysis software was used for the spectral interpretation. The obtained chromatogram was identified by comparing the peaks with the National Institute of Standard and Technology (NIST) standard reference library 1A.

3. Results and Discussion

GC-MS is a powerful tool for the identification of volatile and soluble compounds present in wild mushrooms. In the present study, two wild toxic (*Amanita muscaria, Amanita pantherina*) and three edible Amanita mushroom species (*Amanita caesarea, Amanita subglobosa and Amanita porphyria*) were examined through GC-MS. The prepared methanol fraction was analyzed based on the National Institute of Standards and Technology (NIST) GC-MS library. The total number of compounds identified in all the samples were 250. Mushrooms are well-known for their differences in the composition of constituents. The components obtained were broadly classified into six major groups namely alcohols, acids, aldehydes, esters, ketones and nitrogen-containing compounds based on their parent chain. The obtained compounds which were not classified in any of the aforementioned groups were added to the miscellaneous category. The reliability of our results was based on the 85-95 percent matching of the identified compounds in the NIST library.

Alcohols

In all samples, a total of twenty-one alcohols was identified. Table-3 summarizes the obtained alcohol components with their retention time (Rt). 1,2,3- Propanetriol, a sweetener, was found in abundance and was present in all the mushroom species, except for sample S5. Other alcohols like 1-naphthalenepropanol, ergosta-tetreaen-3-ol, 2-di-

Table 3- *List of alcohol components identified in mushroom samples.*

Species	Retention time (minutes)	Compound name
S1	5.42	1,2,3-propanetriol
	16.198	1-Naphthalenepropanol, .alpha.-ethenyldecahydro-3-hydroxy-.alpha.,5,5,8a-tetramethyl-2-methylene-
S2	5.248	1,2,3-propanetriol
	14.117	(22e,24r)-24-methyl-27-norcholesta-5,7,9(11),22-tetraen-3b-ol
	28.941	Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22e,24s)-
S3	2.429	Ethanol, 2-(dimethylamino)-
	5.401	1,2,3-propanetriol
	14.13	9-Oxa-bicyclo[3.3.1]nonane-1,4-diol
	14.345	Cyclopentadecanone, 2-hydroxy-
	14.43	1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-, [s-(z)]-
	15.876	9,12-octadecadien-1-ol
S4	2.736	2,3-butanediol, [r-(r*,r*)]-
	5.12	1,2,3-propanetriol
	19.63	(6z,9z)-6,9-pentadecadien-1-ol #
S5	1.532	2-propanol, 1-amino-
	1.64	1-hexanol
	1.965	2-butanethiol
	5.225	1,2,3,4-butanetetrol, [s-(r*,r*)]-
	7.495	Dianhydromannitol
	14.075	N-Heptadecanol-1
	14.512	9,12-octadecadien-1-ol
	14.595	Spiro[benzoxazol-2,2'-cyclohexanol]
	15.467	E,E,Z-1,3,12-Nonadecatriene-5,14-diol

methylamino-ethanol, 9,12-octadecadien-1-ol were also observed.

Organic acids

In selected mushroom species, forty-nine organic acid compounds were identified (Table-4). Primary saturated acidic components like formic acid, acetic acid and its derivatives, as well as unsaturated organic acids like tetradecanoic acid, octadecanoic acid, 9-octadecanoic acid, hexadecenoic acid etc. were found to be most common.

Aldehydes and ketones

The relative abundance of the aldehydes and ketones was found to be comparatively less, and in this class eleven and twenty-two compounds were

Table 4- *List of organic acids identified in mushroom samples.*

Species	Retention time (minutes)	Compound name
S1	1.603	Formic acid
	1.707	Acetic acid
	1.966	Acetic acid, [(phenylmethoxy)imino]-, trimethylsilyl ester
	7.755	Benzeneacetic acid
	12.362	Tetradecanoic acid
	13.135	Pentadecanoic acid
	13.2	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester
	13.752	Cis-9-hexadecenoic acid
	13.822	Trans-13-octadecenoic acid
	13.938	Hexadecanoic acid
	14.506	Cis-10-nonadecenoic acid
	14.674	9-octadecenoic acid (z)-
	15.727	Octadecanoic acid
S2	1.686	Acetic acid
	7.872	9-octadecenoic acid (z)-
	12.359	Tetradecanoic acid
	13.133	Pentadecanoic acid
	13.749	Cis-9-hexadecenoic acid
	13.816	Trans-13-octadecenoic acid
	13.929	Hexadecanoic acid
	14.499	Cis-vaccenic acid
	14.671	Octadecanoic acid
	15.698	Hexadecanoic acid
S3	1.682	Acetic acid
	12.369	Tetradecanoic acid
	13.144	Pentadecanoic acid
	13.767	Cis-9-hexadecenoic acid
	13.983	Hexadecanoic acid
	14.517	Cis-10-nonadecenoic acid
	14.687	Octadecanoic acid

Continue Table 4

Species	Retention time (minutes)	Compound name
	16.262	-(9,12-octadecadienoic acid (z,z
	17.615	Cis-11-eicosenoic acid
	25.125	-(9-octadecenoic acid, 1,2,3-propanetriyl ester, (e,e,e
S4	7.874	-(9-octadecenoic acid (z
	9.86	Pidolic acid
	12.361	-(9-octadecenoic acid (z
	13.135	Pentadecanoic acid
	13.759	Cis-9-hexadecenoic acid
	13.952	Hexadecanoic acid
	14.119	Pentadecanoic acid
	14.68	-(9-octadecenoic acid (z
	15.664	Octadecanoic acid
	19.786	Trans-13-octadecenoic acid
S5	1.706	Acetic acid
	2.7	# 2-aminopropanoyl)amino]acetic acid)]
	12.363	-(9-octadecenoic acid (z
	13.138	Pentadecanoic acid
	13.76	Cis-9-hexadecenoic acid
	13.93	Hexadecanoic acid
	14.45	# 4-hydroxyanilino)-4-oxobutanoic acid)4-
	14.676	Octadecanoic acid
	15.609	Octadecanoic acid

identified respectively. These compounds are presented in Table-5 and -6. Methylbutanal, which is a flavoring compound in many food materials, as well as a plant metabolite, was found in all the mushroom species evaluated, except for sample S3 [32]. Other compounds like 2-undecanal, 2-methyl undecanal were also identified. In case of ketones, no common compound was identified.

Esters

Compounds containing esters are the most abundant in all the mushroom samples. Esters are responsible for the aroma, as well as other flavoring constituents present in many natural products. In the present analysis, seventy-six ester compounds were identified (Table-7). The percentage matching of ester components ranged from 80-90%.

Table 5- *List of aldehydes identified in mushroom samples.*

Species	Retention time (minutes)	Compound name
S1	1.93	Butanal, 3-methyl-
S2	1.925	Butanal, 3-methyl-
	8.915	2-undecenal
	12.029	Tetradecanal
	17.309	13-octadecenal, (z)-
S3	17.035	8-hexadecenal, 14-methyl-, (z)-
S4	1.64	Propanal, 2-methyl-
	8.917	2-undecenal
	12.225	Undecanal, 2-methyl-
S5	1.931	Butanal, 3-methyl-

Table 6- List of ketones identified in mushroom samples.

Species	Retention time (minutes)	Compound name
S1	3.627	4-cyclopentene-1,3-dione
	3.934	2(3h)-furanone, dihydro-
	4.085	1,2-cyclopentanedione
	7.025	5-methoxypyrrolidin-2-one
	8.686	2-acetamido-2-deoxy-d-mannolactone
	13.462	2-pentadecanone, 6,10,14-trimethyl-
S2	4.087	1,2-cyclooctanedione
S3	3.622	4-cyclopentene-1,3-dione
	4.082	1,2-cyclooctanedione
	4.905	2h-pyran-2,6(3h)-dione
	5.821	2-pyrrolidinone
	12.159	3-methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-dione, n-acetyl-
S4	12.145	3-methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-dione, n-acetyl-
	12.958	3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione #
	13.83	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-
S5	3.934	2(3h)-furanone, dihydro-
	5.175	2(3h)-furanone, 5-methyl-
	7	5-methoxypyrrolidin-2-one
	7.109	2-azacyclooctanone
	11.509	Hexahydro-3h-pyrrolizin-3-one
	17.304	2-dodecylcyclohexanone

Table 7- *List of esters identified in mushroom samples.*

Species	Retention time (minutes)	Compound name
S1	5.315	2-hydroxycyclohexyl acetate
	6.125	Butanedioic acid, monomethyl ester
	12.86	Pentadecanoic acid, methyl ester
	13.612	Hexadecanoic acid, methyl ester
	14.075	Heptafluorobutyric acid, hexadecyl ester
	14.113	Hexadecanoic acid, ethyl ester
	14.975	9,12-octadecadienoic acid (z,z)-, methyl ester
	16.876	Octanoic acid, 2-dimethylaminoethyl ester
	17.386	Methyl 18-methylnonadecanoate
	17.899	Hexadecadienoic acid, methyl ester
	19.861	Octanoic acid, 2-dimethylaminoethyl ester
	25.058	9,12-octadecadienoic acid (z,z)-, 2,3-dihydroxypropyl ester
	25.177	9-octadecenoic acid (z)-, 2,3-dihydroxypropyl ester
	25.892	Octadecanoic acid, 2,3-dihydroxypropyl ester
S2	1.58	Tetramethylammonium acetate
	9.078	Dl-proline, 5-oxo-, methyl ester
	11.007	1,2-benzenedicarboxylic acid, diethyl ester
	12.99	Fumaric acid, decyl 3-oxobut-2-yl ester
	13.195	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester
	13.61	Hexadecanoic acid, methyl ester
	14.235	9(11)-dehydroergosteryl benzoate
	14.962	9,12-octadecadienoic acid (z,z)-, methyl ester
	15.012	9-octadecenoic acid (z)-, methyl ester
	15.218	Methyl stearate
	16.868	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester
	17.381	Methyl 18-methylnonadecanoate
	18.738	9-octadecenoic acid (z)-, 2,3-dihydroxypropyl ester
	19.322	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester
	19.406	Fumaric acid, 2-dimethylaminoethyl nonyl ester
	19.712	2-hydroxy-3-[(9e)-9-octadecenoyloxy]propyl (9e)-9-octadece-noate #
	19.861	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester
	20.71	Docosanoic acid, methyl ester
	25.09	9-octadecenoic acid, 1,2,3-propanetriyl ester, (e,e,e)-
	25.855	Octadecanoic acid, 2,3-dihydroxypropyl ester
S3	9.087	Dl-proline, 5-oxo-, methyl ester
	11.012	1,2-benzenedicarboxylic acid, diethyl ester
	13.475	9-hexadecenoic acid, methyl ester, (z)-
	13.545	13-docosenoic acid, methyl ester, (z)-

Continue Table 7

Species	Retention time (minutes)	Compound name
	13.616	Hexadecanoic acid, methyl ester
	14.971	9,12-octadecadienoic acid (z,z)-, methyl ester
	15.035	9-octadecenoic acid (z)-, methyl ester
	16.891	Octanoic acid, 2-dimethylaminoethyl ester
	17.329	Docosanoic acid, docosyl ester
	17.4	Eicosanoic acid, methyl ester
	18.758	9-octadecenoic acid (z)-, 2,3-dihydroxypropyl ester
	19.344	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester
	19.429	Fumaric acid, 2-dimethylaminoethyl nonyl ester
	19.73	2-hydroxy-3-[(9e)-9-octadecenoyloxy]propyl (9e)-9-octadece-noate #
	26.25	Tetracosanoic acid, methyl ester
S4	9.072	Dl-proline, 5-oxo-, methyl ester
	11.009	1,2-benzenedicarboxylic acid, diethyl ester
	13.613	Hexadecanoic acid, methyl ester
	14.255	Anthraergostatetraenol benzoate
	14.511	Oleyl alcohol, trifluoroacetate
	14.965	9,12-octadecadienoic acid (z,z)-, methyl ester
	15.015	9-octadecenoic acid (z)-, methyl ester
	16.869	Octanoic acid, 2-dimethylaminoethyl ester
	17.095	Stearic acid, 2-hydroxy-1-methylpropyl ester
	17.885	Ethyl (9z,12z)-9,12-octadecadienoate #
	19.332	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester
	19.414	Fumaric acid, 2-dimethylaminoethyl nonyl ester
	19.717	9-octadecenoic acid, 1,2,3-propanetriyl ester, (e,e,e)-
	20.455	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
	25.126	9-octadecenoic acid, 1,2,3-propanetriyl ester, (e,e,e)-
S5	6.92	Pentanedioic acid, ethyl methyl ester
	9.098	L-proline, 5-oxo-, methyl ester
	9.478	Dl-phenylalanine, methyl ester
	11.008	1,2-benzenedicarboxylic acid, diethyl ester
	13.198	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester
	13.615	Hexadecanoic acid, methyl ester
	14.968	9,12-octadecadienoic acid (z,z)-, methyl ester
	15.016	Cis-13-octadecenoic acid, methyl ester
	17.39	Eicosanoic acid, methyl ester
	17.884	Hexadecadienoic acid, methyl ester
	19.333	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester
	19.425	Fumaric acid, 2-dimethylaminoethyl nonyl ester

Table 8 - *List of nitrogen-containing compounds identified in mushroom samples.*

Species	Retention time (minutes)	Compound name
S1	1.514	Methanamine, n,n-dimethyl-
	12.961	1h-purin-6-amine
	17.955	9-octadecenamide, (z)-
	19.414	S-[2-[n,n-dimethylamino]ethyl]n,n-dimethylcarbamoyl thiocarbohydroximate
	26.253	1h-purin-6-amine, [(2-fluorophenyl)methyl]-
S2	1.517	N-methylethanamine
	12.89	1h-purin-6-amine
	14.175	Iron, tricarbonyl[n-(phenyl-2-pyridinylmethylene)benzenamine-n,n’]
	17.942	9-octadecenamide
S3	1.506	2-amino-n-methylpropanamide
	1.635	Methanamine, n,n-dimethyl-
	8.685	1-acetyl-2-cyanoacetylhydrazine
	13.007	1h-purin-6-amine
	17.7	Dodecanamide, n-(2-hydroxyethyl)-
	17.973	9-octadecenamide, (z)-
	18.315	Octadecanamide
S4	1.543	Di-isopropyl acetamide
	5.303	1-butanamine, 2-methyl-n-(2-methylbutylidene)-
	5.424	1-butanamine, 3-methyl-n-(3-methylbutylidene)-
	12.681	Iron, tricarbonyl[n-(phenyl-2-pyridinylmethylene)benzenamine-n,n’]
	12.85	1h-purin-6-amine
	15.862	Hexadecanamide
	17.954	9-octadecenamide, (z)-
S5	1.415	Methanamine, n-methyl-
	2.125	(3s)-(+)-4,4-dimethylpent-1-en-3-ylamine hydroxylchlorode
	5.305	1-butanamine, 2-methyl-n-(2-methylbutylidene)-
	12.781	1h-purin-6-amine
	15.825	9-octadecenamide, (z)-

Nitrogen-containing compounds

Twenty-seven compounds containing nitrogen were also identified (Table-8). Most of the components belong to the amine group. Chen et al., [35] reported some nitrogen-containing compounds like 2-pentylpyridine, 1-furfurylpyrrole, ethylpyrazine, methylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-formylpyrrole and 1-methyl-2-pyrrolidinone were obtained by heating mushroom hydrolysate. The compounds identified in the present study comprises methanamine,n,n-dimethy-, 1h-purin-6-amine, 9-octadecanamide,(z)-

Table 9 - List of miscellaneous compounds identified in mushroom samples.

Species	Retention time (minutes)	Compound name
S1	1.442	Ethane, 1-chloro-1-fluoro-
	1.644	Propane, 1-nitro-
	9.087	Methyl 5-oxo-2-pyrrolidinecarboxylate #
	13.53	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-icosamethyl-cyclodecasiloxane #
	14.598	Cyclododecasiloxane, tetracosamethyl-
	15.222	Methyl stearate
	17.319	Silicone oil
	19.337	2-(dimethylamino) ethyl 1-adamantanecarboxylate
	22.2	Cyclononasiloxane, octadecamethyl-
S2	1.434	Ethane, 1-chloro-1-fluoro-
	1.638	Butane, 1-fluoro-
	5.425	1-butoxy-3-methyl-2-butene #
	11.885	Heptadecane
	12.678	Eicosane
	13.433	Nonadecane
	17.027	2-methyltetracosane
	19.627	9,12-octadecadienoyl chloride, (z,z)-
S3	1.434	Ethane, 1-chloro-1-fluoro-
	9.5	1-(1-cyclohexen-1-yl) pyrrolidine
	15.227	Methyl stearate
	17.91	9,12-octadecadienoyl chloride, (z,z)-
	23.729	5,5-dimethyl-1,3-dioxane-2-ethanol, tert-butyldimethylsilyl ether
	27.439	Pyrrolidine, 1-(1-oxo-9-octadecenyl)-, (e)-
S4	1.434	Ethane, 1-chloro-1-fluoro-
	4.99	Oxazolidine, 2-propyl-
	7.941	Formamide, n-methyl-n-phenyl-
	11.889	Heptadecane
	13.435	Heptadecane
	14.18	Hexadecane
	15.222	Methyl stearate
	17.031	2-methyltetracosane
S5	1.443	Ethane, 1-chloro-1-fluoro-
	2.08	Aziridine, 1-ethenyl-
	3.831	Oxazolidine, 2-propyl-
	4.992	Oxazolidine, 2-propyl-
	9.712	4-methoxy-2-methoxymethyl-pyrrolidine-1-carboxaldehyde
	10.836	1,4-anhydrohexitol #
	11.6	.beta.-d-glucofuranosiduronic acid, methyl, .gamma.-lactone
	15.224	Methyl stearate

Continue Table 9

Species	Retention time (minutes)	Compound name
S1	1.442	Ethane, 1-chloro-1-fluoro-
	1.644	Propane, 1-nitro-
	9.087	Methyl 5-oxo-2-pyrrolidinecarboxylate #
	13.53	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-icosamethylcyclodecasiloxane #
	14.598	Cyclododecasiloxane, tetracosamethyl-
	15.222	Methyl stearate
	17.319	Silicone oil
	19.337	2-(dimethylamino)ethyl 1-adamantanecarboxylate
	22.2	Cyclononasiloxane, octadecamethyl-
S2	1.434	Ethane, 1-chloro-1-fluoro-
	1.638	Butane, 1-fluoro-
	5.425	1-butoxy-3-methyl-2-butene #
	11.885	Heptadecane
	12.678	Eicosane
	13.433	Nonadecane
	17.027	2-methyltetracosane
	19.627	9,12-octadecadienoyl chloride, (z,z)-
S3	1.434	Ethane, 1-chloro-1-fluoro-
	9.5	1-(1-cyclohexen-1-yl)pyrrolidine
	15.227	Methyl stearate
	17.91	9,12-octadecadienoyl chloride, (z,z)-
	23.729	5,5-dimethyl-1,3-dioxane-2-ethanol, tert-butyldimethylsilyl ether
	27.439	Pyrrolidine, 1-(1-oxo-9-octadecenyl)-, (e)-
S4	1.434	Ethane, 1-chloro-1-fluoro-
	4.99	Oxazolidine, 2-propyl-
	7.941	Formamide, n-methyl-n-phenyl-
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S5	1.443	Ethane, 1-chloro-1-fluoro-
	2.08	Aziridine, 1-ethenyl-
	3.831	Oxazolidine, 2-propyl-
	4.992	Oxazolidine, 2-propyl-
	9.712	4-methoxy-2-methoxymethyl-pyrrolidine-1-carboxaldehyde
	10.836	1,4-anhydrohexitol #
	11.6	.beta.-d-glucofuranosiduronic acid, methyl, .gamma.-lactone
	15.224	Methyl stearate

Table 10 - List of species-specific compounds.

S1	S2	S3	S4	S5
Formic acid	Tetramethylammonium acetate	9-hexadecenoic acid, methyl ester, (z)-	Di-isopropyl acetamide	Spiro[benzoxazol-2,2'-cyclohexanol]
Propane, 1-nitro-	Butane, 1-fluoro-	5,5-dimethyl-1,3-dioxane-2-ethanol, tert-butyldimethylsilyl ether	Hexadecanamide	2-propanol, 1-amino-
Acetic acid, [(phenylmethoxy) imino]-, trimethylsilyl ester	Tetradecanal	Pyrrolidine, 1-(1-oxo-9-octadecenyl)-, (e)-	Formamide, n-methyl-n-phenyl-	(3s)-(+)-4,4-dimethylpent-1-en-3-ylamine hydroxylchlorode
1,2-cyclopentanedione	Eicosane	2-amino-n-methylpropanamide	2,3-butanediol, [r-(r*,r*)]-	Dl-phenylalanine, methyl ester
2-hydroxycyclohexyl acetate	Fumaric acid, decyl 3-oxobut-2-yl ester	Cis-11-eicosenoic acid	Ethyl (9z,12z)-9,12-octadecadienoate #	.beta.-d-glucofuranosiduronic acid, methyl, .gamma.-lactone
Butanedioic acid, monomethyl ester	9(11)-dehydroergosteryl benzoate	2-pyrrolidinone	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	2-dodecylcyclohexanone
Benzeneacetic acid	Cis-vaccenic acid	1-acetyl-2-cyanoacetylhydrazine	Propanal, 2-methyl-	2(3h)-furanone, 5-methyl-
2-acetamido-2-deoxy-d-mannolactone	Docosanoic acid, methyl ester	1-(1-cyclohexen-1-yl) pyrrolidine	Hexadecane	Cis-13-octadecenoic acid, methyl ester
Methyl 5-oxo-2-pyrrolidinecarboxylate #	Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22e,24s)-	9-oxa-bicyclo[3.3.1] nonane-1,4-diol	1-butanamine, 3-methyl-n-(3-methylbutylidene)-	2-azacyclooctanone
Pentadecanoic acid, methyl ester	N-methylethanamine	Dodecanamide, n-(2-hydroxyethyl)-	3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione #	[(2-aminopropanoyl)amino] acetic acid #
2-pentadecanone, 6,10,14-trimethyl-	1-butoxy-3-methyl-2-butene #	Ethanol, 2-(dimethylamino)-	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	1,4-anhydrohexitol #
2,2,4,4,6,6,8,8,10,10, 12,12,14,14,16, 16,18,18,20,20- icosamethylcyclodecasiloxane #	Nonadecane	2h-pyran-2,6(3h)-dione	Oleyl alcohol, trifluoroacetate	Aziridine, 1-ethenyl-

Continue Table 10

Heptafluorobutyric acid, hexadecyl ester	(22e,24r)-24-methyl-27-norcholesta-5,7,9(11),22-tetraen-3b-ol	13-docosenoic acid, methyl ester, (z)-	(6z,9z)-6,9-pentadecadien-1-ol #	Dianhydromannitol
Hexadecanoic acid, ethyl ester	13-octadecenal, (z)-	Cyclopentadecanone, 2-hydroxy-	Pidolic acid	Hexahydro-3h-pyrrolizin-3-one
Cyclododecasiloxane, tetracosamethyl-	9-octadecenamide	1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-, [s-(z)]-	Undecanal, 2-methyl-	N-heptadecanol-1
1-naphthalenepropanol, alpha.-ethenyldecahydro-3-hydroxy-.alpha.,5,5,8a-tetramethyl-2-methylene-		9,12-octadecadienoic acid (z,z)-	Anthraergostatetraenol benzoate	4-(4-hydroxyanilino)-4-oxobutanoic acid #
Silicone oil		8-hexadecenal, 14-methyl-, (z)-	Stearic acid, 2-hydroxy-1-methylpropyl ester	1-hexanol
2-(dimethylamino) ethyl 1-adamantanecarboxylate		Docosanoic acid, docosyl ester		1,2,3,4-butanetetrol, [s-(r*,r*)]-
S-[2-[n,n-dimethylamino]ethyl] n,n-dimethylcarbamoyl thiocarbohydroximate		Octadecanamide		Pentanedioic acid, ethyl methyl ester
Cyclononasiloxane, octadecamethyl-		Tetracosanoic acid, methyl ester		2-butanethiol
9,12-octadecadienoic acid (z,z)-, 2,3-dihydroxypropyl ester				4-methoxy-2-methoxymethyl-pyrrolidine-1-carboxaldehyde
1h-purin-6-amine, [(2-fluorophenyl)methyl]-				E,e,z-1,3,12-nonadecatriene-5,14-diol
				Methanamine, n-methyl-
				L-proline, 5-oxo-, methyl ester

and 9-octadecenamide etc.

Miscellaneous category

A total of twenty-six components detected were classified under the miscellaneous category, which comprises some alkanes (ethane, 1-chloro-1-fluoro), alkenes (1-butoxy-3-methyl-2-butenre), oils (silicone oil) and siloxanes (cyclononasiloxane, octadecamethyl-) (Table-9).

Certain species-specific compounds were also identified, which were not present in any other sample. It was observed that each species revealed certain unique methanol-soluble components (Table-10).

4. Conclusion

The present work is a preliminary study on the methanol-soluble components of five wild toxic and edible mushrooms obtained by GC-MS. The peaks obtained were analyzed by the inbuilt library, provided by NIST. The components obtained were classified into alcohols, acids, aldehyde, ketones, nitrogen-containing compounds and the miscellaneous category. Furthermore, certain species-specific compounds were identified that provided the fingerprint of each sample for discrimination purposes. However, this study is limited to the identification of a few wild mushroom species. The methanol extracted profile may vary depending upon environmental factors and geographical location. Future studies could include a greater number of wild mushroom samples from different locations for more accurate species discrimination. To study species-specific compounds, selected ion monitoring techniques could prove to be more helpful.

Conflict of interest

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