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GCMS ANALYSIS OF BIOACTIVE CONSTITUENTS AND EVALUATION OF ANTIMICROBIAL ACTIVITY FROM THE ETHYL ACETATE EXTRACT OF *NEOLENTINUS KAUFFMANII*

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ABSTRACT

Aim: The present investigation deals with Gas chromatography (GC) Mass Spectrometry (MS) analysis of ethyl acetate extract of dried fruiting bodies of *N. kauffmanii* and its evaluation of antimicrobial activity. **Methods:** The bioactive molecules of *N. kauffmanii* were identified by Gas chromatographic analysis. The ethyl acetate extract of *N. kauffmanii* was screened for its antimicrobial property against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumonia* and *Candida albicans* using agar well diffusion method. **Results:** Twenty four compounds were identified by GCMS. Major compounds are 7-methoxy-2,2-Dimethyl-2,3-Dihydro-4H-chromen-4-one (22.18%), 5-Methoxy-2,3-Diethylphenol (36.16%), Beta, Copaen-4-Alpha.OL (12.82%), 3-Dimethyl amino anisole (10.61%) etc., This is the first report on the composition of the different compounds of these fruiting bodies. The extract showed strong antibacterial activity by inhibiting the growth of both gram negative and gram positive bacteria. **Conclusion:** The GCMS analysis of these extract further confirm that this activity is due to the presence of antibacterial compounds present in the extract. The investigation therefore supports the traditional uses of the mushroom in the treatment of infectious diseases.

KEY WORDS: GC-MS analysis, mushroom, antimicrobial activity, *N. kauffmanii*.

INTRODUCTION

Mushroom has been playing an important role in several aspects of the human activity. Wild and cultivated mushrooms contain a large diversity of biomolecules with nutritional and

medicinal properties. Mushrooms are very popular in the market for their nutritional and medicinal uses. Mushroom volatiles are not only an important factor in the flavor, but also contain many antioxidant compounds. Antioxidant activity is a very important property for disease prevention. Mushrooms have been reported to be of therapeutic value, useful in preventing diseases such as hypertention, hypercholesterolemia, cancer and also having antibacterial and antiviral properties. These functional characteristics are mainly due to their chemical composition. [1][2][3][4][5]

In search of new drugs from the mushrooms GCMS techniques have been widely used. Smiderle et al.,[6] isolated and identified polysaccharide such as Xylomannans and β-glucan from the edible mushroom *Flammulina velutipes* using GCMS and NMR techniques.[6] GCMS is a powerful tool for qualitative and quantitative analysis of various compounds present in natural products and the techniques widely used in medical, biological and food research.[7] The active extract is subjected to isolation of active constituent(s) present in that with different analytical techniques. The analogues of isolated molecules are characterized and structural modification has been done to enhance the desired activity and minimize the unwanted side effects.

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 are consumed in the forms of capsules, tablets or extracts.[8][5] The chloroform and ethyl acetate extracts of the dried mushroom have antibacterial activity against *Streptococcus mutans* and *Prevotella intermedia*.[9] Both fruiting body and the mycelium contain compounds with wide-ranging antimicrobial activity.[10] In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources which are the good sources of novel antimicrobial chemotherapeutic agents. [11] Infusion of this macro fungus is used to prevent beriberi. In addition, the decoction is used for the treatment of abscesses and wounds.[12]

But, there is no much reports on the detailed analysis of GCMS and bioactive constituents of this mushroom material, hence, it was planned to take up detailed investigation on *N. kauffmanii* fruit body for isolation of active biomolecules and its pharmacological activities from the potent constituent.

MATERIALS AND METHODS

Extraction of mushroom powder (Soxhlet).

The shade dried, powdered 100gm mushroom material was soxhleted with ethyl acetate in a soxhlet extractor for 48 hours. The extract was concentrated to dryness in a flash evaporator (Buchi) 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. The yield of 15gm brown colored extract was obtained. The extract was stored in refrigerator at 4°C until used for experiment. 2µl of the ethyl acetate extract of *Neolentinus kauffmanii* fruit body was employed for GCMS analysis.

Gas Chromatography Mass Spectroscopy Analysis

Instruments and Chromatographic Conditions

GCMS analysis was carried out on a GC 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 GCMS for different constituents. Using computer searches on a NIST REFPROP Version 9.1 database and comparing the spectrum obtained through GCMS compounds present in the plants sample were identified.

Identification of Bioactive Constituents

Interpretation on Mass-Spectrum GCMS 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.

Antibacterial activity

Test Bacteria

Staphylococcus aureus ATCC 25923, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Pseudomonas aeruginosa* ATCC 29212, *Escherichia coli* ATCC 29995 and *Klebsiella pneumonia* CCM 2318 were used as test bacteria. Nutrient Broth (NB) was used for culturing of test bacteria. All strains were stored at -20°C in the appropriate medium containing 10% glycerol, and regenerated twice before use. Gentamycin (Sigma Aldrich, India) was used as standard drug for these studies.

Preparation of Inoculum

The gram positive (*S. aureus, B.subtilis and B.cereus)* and gram negative bacteria *(P.aeruginosa*, *E.coli* and *K. pneumonia)* were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C. It is centrifuged at 10,000 rpm for 5 min and the pellets were suspended in double distilled water and the cell density was standardized spectrophotometrically (A610nm). The spore density of each bacterium was adjusted to obtain a final concentration approximately 105 spores/ml.

Agar well diffusion method

The determination of the inhibitory effect of the extracts of *N. kauffmanii* on test bacteria was carried out by agar well diffusion method. Bacterial cultures were grown at 37°C for 24 h in Nutrient Broth. The culture suspensions were adjusted by comparing against McFarland. Petri dishes with 10ml of Nutrient Agar were prepared, previously inoculated with 100µl of the culture suspension.[13][14] The wells (7.0mm) were made and the extract which is dissolved in DMSO was added to wells (100µl) and same volume (100µl) of DMSO was used as a control. The inoculated plates were incubated for 24h. After incubation, the diameter of the inhibition zone was measured with calipers. The measurements were done basically form the edge of the zone to the edge of the well.

RESULTS AND DISCUSSION

GCMS Analysis

GCMS chromatogram of the ethyl acetate extract of *N. kauffmanii* fruit body (Fig.1) showed 24 peaks indicating the presence of twenty four bioactive constituents. The active principles with their retention time (RT), molecular formula, molecular weight (MW), concentration (Peak area %) and the chemical structure were analyzed. The result revealed that 5-Methoxy-2,3-Dimethylphenol (35.16 %),7-Methoxy-2,2-Dimethyl-2,3-Dihydro-4H-Chromen-4-one

(22.18 %), Beta-Copaen-4.Alpha.-OL (12.82 %) and 3-Dimethylaminoanisole (10.61 %) were found as the 4 major constituents covering higher concentration of area in the ethyl acetate extract. The two constituents such as 3-Dimethylaminoanisole (6.78 %) and 4a,7,7-Trimethyl-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone (2.97 %) covered moderate concentration of area and remaining 18 minor constituents shown below 1.19 to 0.13 % of the concentration in the ethyl acetate extract of *N. kauffmanii.*

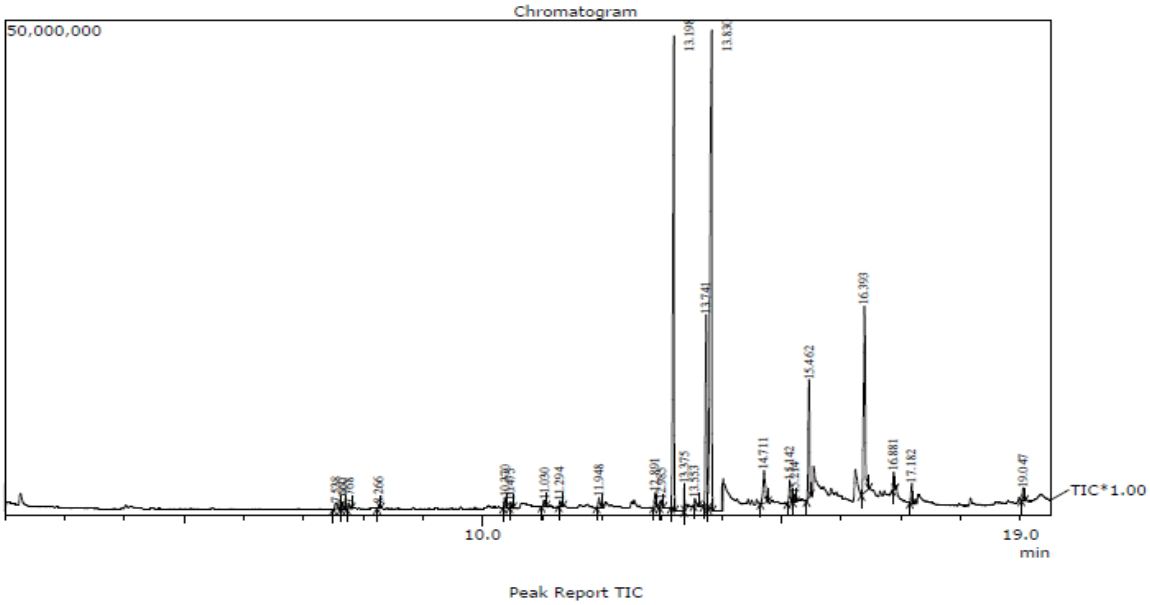

Fig 1. Chromatogram of Ethyl acetate extract of *N. kauffmanii*

Table 1: Bioactive constituents of Ethyl acetate extract of *N. kauffmanii* by GCMS analysis

Peak No	Name of the constituents	Retention Time	Peak Area (%)
1	2-Isoprophyl-2,5-Dimethylcyclohexanone-6,6-D2	7.538	0.53
2	Dodecane	7.660	0.13
3	(2-Cyclohexyl-1-Methylpropyl)Cyclohexane	7.768	0.21
4	1-Cyclohexylhexane	8.266	0.17
5	1-Hexadecene	10.370	0.52
6	Tetradecane	10.475	0.27
7	2-Norpinene, 2,6-dimethyl-6-(4-methyl-3-Pentenyl)	11.030	0.39
8	2,2-Dimethyl-2H-Chromen-7-yl Methyl Ether	11.294	0.33
9	2,4-Ditert-Butylphenol	11.948	0.71
10	1-Pentadecene	12.891	0.77
11	Hexadecane	12.985	0.42
12	7-Methoxy-2,2-Dimethyl-2,3-Dihydro-4H-Chromen-4-one	13.198	22.18
13	1-(1-Cyclopenten-1-YL)Pyrrolidine	13.375	0.48

14	2,3-Dimethyl-8-oxo-2-Nonenal	13.553	0.44
15	3-Dimethylaminoanisole	13.741	10.61
16	5-Methoxy-2,3-Dimethylphenol	13.830	35.16
17	4a,7,7-Trimethyl-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone	14.711	2.97
18	1-Nonadecene	15.142	1.19
19	Hexadecane	15.214	0.35
20	3-Dimethylaminoanisole	15.462	6.78
21	Beta-Copaen-4.Alpha.-OL	16.393	12.82
22	Hexadecanoic Acid	16.881	0.85
23	1-Nonadecene	17.182	1.04
24	1-Nonadecene	19.047	0.68

This investigation was carried out to determine the possible chemical components from *N. kauffmanii* by GCMS. The analysis revealed that the ethyl acetate extract of *N. kauffmanii* contained mainly 5-Methoxy-2,3-Dimethylphenol (35.16%) and Beta-Copaen-4.Alpha.-OL (12.82%). All identified compounds were, generally reported as having antimicrobial activity. In addition Beta-Copaen-4.Alpha.-OL compounds also having anticancer, antioxidant, antitumor, and chemo preventives. Some main constituents identified in study are reported to have antibacterial property. Therefore, antibacterial constituents from *N. kauffmanii* ethyl acetate extract could hold promise for future application in therapy. Further experiments, are planned to establish the influence of the components of these mixtures on the pharmacological activity.

3.2. Antimicrobial Activity

In recent years, multiple drug resistance in human pathogenic microorganisms has emerged as a threat to human health due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of the infectious diseases. [11] In the present study, various concentrations of ethyl acetate extracts from *N. kauffmanii* were screened for their antimicrobial activity. Antimicrobial activity against the tested bacteria was qualitatively assessed by measuring the zone of inhibition generated for each sample shown in table-2. Among them, 150µg/mL extract effectively inhibited *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus* and *C. albicans*. The results indicated that the superior antimicrobial potential of the ethanolic extract as an extractant and the solubility of the bioactive compound are the same. *L. edodes* contains several compounds that exert inhibitory effects on a wide range of microbes.[9] The chloroform and ethyl acetate extracts of the dried mushroom *L. edodes* showed antibacterial activity against gram-positive and gram-negative human pathogenic bacteria and also effectively inhibited the growth of a yeast, *Candida albicans.*[15]

Table 2: Antimicrobial Activity of Ethyl acetate extract of *N. kauffmanii*

Pathogens	Zone of inhibition (diameter in mm)			
	Concentration of methanol extract (Fruit body) (µg/mL)			
	Control	50	100	150
Bacillus subtilis	-	-	3.35 ± 1.19	7.65 ± 1.11
Bacillus cereus	-	4.5 ± 0.34	5.65 ± 0.92	8.23 ± 0.84
Staphylococcus aureus	-	-	6.26 ± 0.64	8.43 ± 0.86
E. coli	-	-	5.3 ± 1.25	7.2 ± 1.04
Pseudomonas aeruginosa	-	8.23 ± 1.13	10.9 ± 1.38	11.7 ± 1.42
Klebsiella pneumonia	-	6 .2 ± 0.58	8 ± 1.42	9.4 ± 0.32
Candida albicans	-	0.96 ± 0.83	1.9 ± 0.92	4.3 ± 1.19

Turkoglu *et al*[16] reported that the antimicrobial activity of *Laetiporus sulphureus* on some bacteria showed that gram-negative bacteria were less than gram-positive strains. The result obtained in this study with the test fungus *N. kauffmanii* fell in line with that. Dulger *et al* [12] reported that *Candida albicans* is resistant to the action of the methanolic extract of *Lepista nuda*. The culture fluid of *L. edodes* showed poor activity against *C. albicans*.[17] The ethanolic extracts of *Ramaria flava* inhibited growth of the gram-positive bacteria more than gram-negative bacteria and the yeast. [18][19] In this study we found that various concentrations of ethyl acetate extracts of *N. kauffmanii* severely inhibited the growth of some evocative human infection pathogens confirming the medicinal efficacy of the extracts and might have important applications in the pharmaceutical industries. Therefore mushrooms are now increasingly gaining worldwide recognition as a functional food and its various therapeutic, psychoactive, hallucinogenic properties.

CONCLUSION

In the present study 24 bioactive constituents have been identified from ethyl acetate extract of *N. kauffmanii* by GCMS analysis. In recent years many of the synthetic drug consumptions resulted in some side effects in the due course of administration. Thus mushroom based compounds are preferred over the synthetic one which causes minimal side effects. The presence of these medically important phytocompounds justifies the medicinal values of these mushrooms. The ethyl acetate extract of *N. kauffmanii* showed strong antibacterial activity against both gram negative and gram positive bacteria. This study revealed that the mushroom *N. kauffmanii* exhibited various levels of antimicrobial activity in different concentration of ethyl acetate extracts. In this result also clearly indicates that the presents of number of bioactive contents of the *N. kauffmanii* are promising natural antibacterial agents. So there is a need for further studies to isolate and characterize the bioactive compounds

present in *N. kauffmanii* and these metabolites can be used to develop effective drugs against these human pathogenic bacterial strains.

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REFERENCES

1. Chovot, V.L., Opletal, L., Jahodar, A.V., Patel, C.G., Dacke and Blunden, G. Ergota-4,6,8,22, tetraen-3-one from the edible fungus *Pleurotus ostreatus*, (Oyster mushroom). Phytochemistry, 1997; 45: 1669-71.
2. Gunde-Cimerman, N. Medicinal value of the Genus *Pleurotus* (Fr.) P. katst (Agaricales. I, basidiomycetes). International Journal of MedicinalMushroom, 1999; 1: 69-80.
3. Manzi, P.A., Aguzzi., Pizzzo ferrato, L. Nutritional value of mushrooms widely consumed in Italy. Food Chemistry, 2001; 73: 321-325.
4. Johnsy, G. and Kaviyarasan, V. Antimicrobial and antioxidant properties of *Trametes gibbosa* (pers) Fr. Journal of pharmaceutical Research, 2011; 4(11): 3939-3942.
5. Johnsy, G, Kaviyarasan, V. Evaluation of antioxidant activities and Determination of Bioactive compounds in two wild edible *Termitomycetes* (*T. microcarpus and T. heimii*). World Journal of Dairy and food Sciences 2014; 9(1): 10-19.
6. Fhernanda, R., Smiderle, Elaine, R., Carbonero., Caroline, G., Mellinger., Guilherme, L., Sassaki., Philip, A.J., Gorin and Marcello Lacomini. Structural characterization of a polysaccharide and a β-glucan isolated from the edible mushroom *Flammulina velutipes*. Phytochemistry, 2006; 67(19): 2189-2196.
7. Kaluzna and Czaplinska. GC-MS analysis of biologically active compounds in cosmopolitan grasses. Acta Chromatographica, 2007; 19.
8. Filipa, S.R., Lillan, B., Ricardo, C.C., Ana C., Leo, J.L.D., Marina, S., Isabel C.F.R.F. The Methanolic extract of *Cordyceps militaries* (L.) Link fruiting body show antioxidant, antibacterial, antifungal and antihuman tumor cell lines properties. Food chemical Toxicology, 2013; 62: 91-98.
9. Hirasawa, M., Shouji, N., Neta, T., Fukushima, K. and Takada, K. 1999. Three kinds of antibacterial substances from *Lentinus edodes* (Berk.) Sing. (Shiitake, an edible mushroom). International Journal of Antimicrobial Agents, 1999; 11: 151-157.

10. Jong, S.C and Birmingham, J. M. Medicinal and therapeutic value of the Shiikate Mushroom. Advances in Applied Microbiology, 1993; 39: 153-184.
11. Karaman, Y., Bin, S.A., Gu, F., llu¨ ce M., g˘u¨ tc¸u¨ O¨, S_engu¨ HlM, Adıgu¨ Z.A. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. Journal of Ethnopharmacology. 2003; 85: 213-235.
12. Dulger, B., Ergul, C. C. and Gucin, F. Antimicrobial activity of the macrofungus *Lepista nuda*. Fitoterapia, 2002; 73: 695-697.
13. Tumay Yaltirak., Belma Aslim., Sahlan Ozturk and Hakan Alli. Antimicrobial and antioxidant activities of *Russula delica* Fr. Food Chemical Toxicology, 2009; 47:2052-2056.
14. Adeloye O Adewale., Akinpelu A David., Ogundaini O Abiodun and Obafemi A Craig. Studies on antimicrobial, antioxidant and phytochemical analysis of *Urena lobata* Leave extract. Journal of Physical and Natural Sciences, 2007; 1(2).
15. Stamets, P. Mycomedicinals: An Informational Treatise on Mushrooms. Mycomedia Productions, 2002, Olympia, WA
16. Turkoglu, A., Duru, M.E. and Mercan, N. Antioxidant and antimicrobial activity of *Russula delica* Fr: an edible wild mushroom. Eurasian Journal of Analytical Chemistry, 2007; 2: 54-67.
17. Hatvani, N. Antibacterial effect of the culture fluid of *Lentinus edodes* mycelium grown in submerged liquid culture. International Journal of Antimicrobial Agents. 2001; 17(1): 71-74.
18. Gezer, K., Duru, E., Kivrak., Tukaglu, A., Mercan, N., Turkoglu, H. and Gukan, S. Free radical scavenging capacity and antimicrobial activity of wild edible mushroom from Turkey. African Journal of Biotechnology, 2006; 5(20): 1924-1928.
19. Manjamalai, A., Yasaswini Narala., Aiswarya Haridas and V.M. Berlin Grace. Antifungal, anti-inflammatory and GC-MS analysis of methanolic extract of *Plectranthus Amboinicus* leaf. International Journal of Current Pharmaceutical Research, 2011; 3: 2.