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Research Article

ANTIBACTERIAL ACTIVITY, *IN VITRO* ANTIOXIDANT POTENTIAL AND GC-MS CHARACTERIZATION OF METHANOLIC EXTRACT OF *Tricholoma equestre*, A WILD MUSHROOM FROM SOUTHERN WESTERN GHATS, INDIA

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ABSTRACT

The present study was conducted to assess the antibacterial activity by disc diffusion technique, antioxidant potential by Nitric oxide scavenging as well as DPPH radical scavenging assays and GC-MS characterization of methanolic extract of *Tricholoma equestre* collected from southern Western Ghats, India. Methanol extract showed the best activity against *Enterococcus faecalis*, *Eggerthella lenta* and *Vibrio parahaemolyticus* than the standard antibiotic amoxicillin. The methanolic extracts of *T. equestre* with two complementary test systems, namely DPPH free radical scavenging and Nitric oxide scavenging activity showed inhibition EC_{50} µl values of 35.43% and 10% µg/ml, respectively. Our GC-MS analysis on the methanol extract resulted in the identification of 59 compounds for *T. equestre*. The prevalent compounds were 9,12-Octadecadienoic acid (Z,Z), Ergosta-5,8,22-trien-3-ol, (3.beta., 22E), Octadecanoic acid, 2,3-dihydroxypropyl ester, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z) -, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Octadecanoic acid, 1-Dodecanol, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, n-Pentadecanol and Dodecane. Our study is the first report on the chemical composition of this wild mushroom.

Keywords :-*Tricholoma equestre*, antibacterial activity, *in vitro* antioxidant potential, GC-MS.

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INTRODUCTION

Tricholoma equestre or *Tricholoma flavovirens*, (English name, yellow knight or man on horseback) belonging to the family Tricholomataceae is known as an eatable mushroom throughout the world [1]. *Tricholoma flavovirens* is the name used by most North American field guide authors for this species. However, it has been officially synonymized *Tricholoma flavovirens* with *Tricholoma equestre*, with the latter name prioritized [2-3].

Researchers showed antimicrobial activity of several mushrooms [4-6]. 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 [7]. Extracts from fruiting bodies

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and the mycelia of various mushrooms have been reported to have antimicrobial activity against wide range of infectious bacteria [8-9].

Antioxidant defenses in the organism against reactive oxygen species (pro-oxidants and free radicals) produced during normal cell aerobic respiration may be of endogenous (enzymatic and nonenzymatic) or dietary origin (vitamins, carotenoids and flavanoids, etc) [10]. When natural defenses are overwhelmed by an excessive generation of pro-oxidants, a situation of oxidative stress evolves and cellular and extracellular macromolecules can suffer oxidative damage, causing tissue injury [11-14] affecting immune function [15]. Increased intakes of dietary antioxidant may help to maintain an adequate antioxidant, defined as the balance between antioxidants and oxidants in living organisms [16].

In this present study, we report the results of antibacterial activity, *in vitro* antioxidant potential andGC-MS analyses of volatile and polar compounds obtained from the fruiting bodies of *Tricholoma equestre*. The results can help to characterize this wild mushroom species and elucidate the presence of some biologically active compounds and shed light upon their reported toxicity.

MATERIALS AND METHODS

Mushroom samples

The general methodology and techniques for collection, preparation and preservation of wild mushroom were followed as per standard procedures [17-18]. This wild mushroom was collected in the Fingerpost Reserve Forest of North Zone of Nilgiri, Tamilnadu in two consecutive years after the rainy days. *Tricholoma equestre*, a wild mushroom, formerly known as *Tricholoma flavovirens*, belonging to the family Tricholomataceae, were collected in the vulnerable ecosystem and brought to the lab then preserved at 4^{o}C for further studies.

Antibacterial assay

Antibacterial activity of the mushroom extracts was determined by Kirby-Bauer well diffusion assay technique [19].

Antioxidant assay

Nitric oxide scavenging activity can be estimated by the use of GriessIlIosvoy reaction [20]. DPPH radical scavenging activity of extract was determined following the method [21].

GC-MS analysis

Methanol extract of the fungus was analyzed by Gas chromatography and mass spectrometry (GC-MS). Gas chromatograph linked to mass spectrometer system equipped with a capillary column DB-5ms (30.0m x 0.25mm, 0.25µm film thickness) was used. The GC column oven temperature was programmed from 700^{o}C to 3000^{o}C. The initial temperature was 700^{o}C (hold time 2min) and it rose to 3000^{o}C (hold time 7min) at the rate of 100 C min^{-1}. The total run time was 32.0 min. Helium with 99.9995% purity was used as carrier gas with a constant flow of 1.51ml/min. The GC-MS interface temperature was at 2800^{o}C. Injector and detector temperatures were set at 2000^{o}C. 1µl of sample was injected in split ratio of 1:10. The MS scan range was set from 40-1000 Da. Identification of compounds was obtained by comparing the retention times with those of authentic compounds and with the spectral data obtained from data library of the corresponding compounds. Quantities of the compounds are represented as relative area percentage derived from the integrator.

RESULTS AND DISCUSSION

Antibacterial activity

The results of antibacterial activity of methanol extracts of *Tricholoma equestre* are presented in Table 1 and Fig. 1. These results indicate that methanol extract showed the best activity against *Enterococcus faecalis*, *Eggerthella lenta* and *Vibrio parahaemolyticus*. The wild mushroom species exhibited more antibacterial activity against all the test pathogens at 100 µg/ml of crude methanol extract.

There are numerous publications describing the antibacterial properties of secondary metabolites isolated from various higher Basidiomycetes [22-30]. The antibacterial activity of some Basidiomycetes mushrooms provides efficient and low-cost methods for human and plant disease control. The highest antibacterial activity occurred among members of the Ganodermatales, Poriales, Agaricales, and Stereales, and these may constitute a good source for developing new antibiotics. But the effect of Basidiomycetes secondary metabolites has been investigated mainly on human and animal disease pathogens [31].

Although the fungi are well known for the production of important antibiotic compounds, *e.g.*, penicillin, the occurrence of antibiotics in mushrooms is less well documented [32]. It was already reviewed the situation as far as edible mushrooms and other higher fungi are concerned in the production of antibacterial antibiotics [33]. But it was emphasized that polyacetylenes are common in a number of genera of higher Basidiomycetes, including such edible genera as *Pleurotus* and *Tricholoma*. Also, other types of antibacterial antibiotics, including phenolic compounds, purines and pyrimidines, quinones and terpenoids, have been listed as occurring in higher Basidiomycetes [34]. Antibacterial activity has been demonstrated to occur in phenolic and quinoid derivatives from *Agaricus bisporus* [35].

The antimicrobial activity of aqueous, methanol, hexane, and ethyl acetate extracts from edible wild and cultivated mushrooms such as *Agaricus silvicola*, *Clitocybe nebularis* and *Tricholoma equestre* against *Vibrio parahaemolyticus* and *Staphylococcus aureus*, foodborne pathogenic bacterial strains was screened and antimicrobial activity of gram-positive bacteria were more sensitive than gram-negative bacteria to fungal extracts [36].

Eggerthella lenta is an emerging pathogen which is susceptible to amoxicillin-clavulanate, cefoxitin, metronidazole, ertapenem, piperacillin-tazobactam, and meropenem; resistant to penicillin and piperacillin-tazobactam [37]. The present study is, the first of its kind, finding the antibacterial activity by crude extract of wild mushroom *Tricholoma equestre* against *Eggerthella lenta* [38-39].

Antioxidant activity

The antioxidant activity of the methanolic extracts of *Tricholoma equestre* with two complementary test systems, namely DPPH free radical scavenging and Nitric oxide scavenging activity with a characteristic absorption at 517nm and 546nm are presented in Table 2 and Fig. 2. It was found that inhibition EC_{50} µl values of methanol extracts of *T. equestre* using the two test systems were found to be 35.43% and 14.71% µg/ml, respectively (Table 2).

Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are the harmful by products generated during normal cell aerobic respiration [40]. Phenolic compounds are known to be powerful chain-breaking antioxidants and they possess scavenging ability due to their hydroxyl groups. The phenolic compounds contribute directly to the anti-oxidative action. The other antioxidant compounds include flavonoids, carotenoids, tocopherols, and others that can inhibit Fe 3+ /AA-induced oxidation, scavenge free radicals, and act as reductants. Reducing the power of a compound serves as a significant indication of its potential antioxidant activity. The presence of reducers (i.e. antioxidants) causes the reduction of Fe3+/ferrocyanide complex to ferrous form [41-42] .

There are several natural antioxidants available but wild mushrooms are yet to be explored in detail. The Radical scavenging activity of methanolic extracts of mushrooms was tested using a free radical, DPPH. DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition [43-44]. It was reported that *Lactarius deliciosus* revealed better antioxidant properties than *Tricholoma portentosum* (lower EC_{50} values), which is in agreement with the higher content of phenols found in the *Lactarius deliciosus* [45].

Variations in the antioxidant status in parts of the human colon are due exclusively to the fermentation processes of microorganisms in microbiota in the digestive tract [46]. The total antioxidant activity of the formulation based on lyophilized mycelia of *Pleurotus ostreatus* and *Lentinula edodes* was studied using FRAP method and ABTS method. They showed significant results on the human colon [47]. The antioxidant potential of 10 mushroom species were evaluated using nitric oxide radical scavenging assay and DPPH radical scavenging activity [48].

GC-MS Characterization

Gas chromatography and mass spectrometry (GC-MS) analyses of the *Tricholoma equestre* methanol extract led to the identification of 59 components. The prevalent compounds were 9,12-Octadecadienoic acid (Z,Z), Ergosta-5,8,22-trien-3-ol, (3.beta., 22E), Octadecanoic acid, 2,3-dihydroxypropyl ester, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z) -, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Octadecanoic acid, 1-Dodecanol, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, n-Pentadecanol and Dodecane.The methanol extract of *Tricholoma equestre* exhibited 59 major peaks in GC analysis (Fig. 3) confirming that 100% of the extract was identified and listed along with respective retention time and percentage of compound as given in Table 3.

It was studied that the primary metabolites (Amino Acids and Fatty Acids) from seven samples of wild mushrooms by GC-MS which could be used as molecular markers and possibly helped to identify the wild species of mushrooms. In addition, the secondary metabolites would be more promising as chemical markers for identification and also for therapeutic uses [49]. *Tricholoma equestre* contains 1-octen-3-ol and 3-octanol with highest levels of aldehydes [50-51]. *Agaricus placomyces* and *A. pseudopratensis*, two inedible mushrooms, show the presence of ergosterol and two Δ^7-sterols as well as 5α,8α-Epidioxi-24(ξ)-methylcholesta-6,22-diene-3β-ol that attributed to their biologically active potential [52]. 1-octen-3-ol and 3-octanone are the two main compounds with the highest amounts in the volatile compositions of straw (*Volvariellavolvacea*) and oyster mushrooms(*Pleurotus ostreatus*) [53].

Table 1. Antibacterial activity of methanol extract of *Tricholoma equestre*

Bacterial strain	**Zone of inhibition in (mm) at different concentrations (µg/ml of crude extract)**				
Gram Positive	**20**	**40**	**60**	**100**	**Amoxicillin 30µg**
Enterococcus faecalis ATCC-29212	11	12	13	17	14
Eggerthella lenta ATCC-43055	9	11	14	17	13

Gram Negative					
Vibrio parahaemolyticus MTCC-451	11	13	19	21	12

Table 2. EC_{50} values for different antioxidant assay on *Tricholoma equestre*

DPPH radical scavenging activity	Mushroom	% INHIBITION					EC_{50} µL
		10 µL	20 µL	30 µL	40 µL	50 µL	
	Tricholoma equestre	1	13	35.67	62	79	35.43
	BHA (µg/ml)	36.11	66.67	85.56	88.23	93.33	25.78
Nitric oxide scavenging activity	*Tricholoma equestre*	50	66	76	85	92	10
	Curcumin (µg/ml)	42	59	77	89	96	14.71

Table 3. Chemical composition of methanolic extract of *Tricholoma equestre*

Constituent	RT	Area %	Peak
1,3,5-Triazine-2,4,6-triamine	6.174	0.51	1
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	7.296	0.40	2
1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	8.604	1.86	3
1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	8.685	0.86	4
Benzeneacetic acid	8.923	1.60	5
Silane, tirmethl (2-pentenyloxy)-, (Z)-	11.865	0.38	6
1-Dodecanol	11.976	3.37	7
.beta.-D-Glucopyranose, 1,6-anhydro-	12.11	1.24	8
Phenol, 2,4-bis (1,1-dimethylethyl)	12.466	0.56	9
Dodecanoic acid	13.016	0.19	10
Butanedial, dioxime	13.402	1.30	11
Diethyl Phthalate	13.484	0.99	12
d-Talonic acid lactone	13.841	0.71	13
1-Trimethylsiloxy-2-(1-cyclohexenyl) ethane	14.294	0.28	14
n-Pentadecanol	14.368	2.70	15
Dodecane	14.821	2.29	16
Adenine	15.252	0.45	17
4,8-Dimethylbicyclo[3.3.1] nonane-2,6-dione	15.884	0.15	18
Pentadecanoic acid	16.3	0.36	19
Phthalic acid, 5-methylhex-2-yl butyl ester	16.448	0.11	20
Hexadecanoic acid, methyl ester	16.976	0.88	21
10-Methyl-9-nonadecene	17.035	0.17	22
n-Hexadecanoic acid	17.332	6.57	23
Dibutyl phthalate	17.377	1.49	24
Estra-1,3,5 (10) - trien-17 .beta. -ol	17.614	0.19	25
3,5-di-tert-Butyl-4-hydroxyphenylpropionic acid	17.659	0.49	26
9,12-Octadecadienoic acid, methyl ester	18.595	1.87	27
9-Octadecenoic acid, methyl ester	18.647	0.23	28
Methyl stearate	18.877	0.50	29
9,12-Octadecadienoic acid (Z,Z) -	18.981	22.86	30
Octadecanoic acid	19.197	3.52	31
9,12-Octadecadienoic acid (Z,Z) -	19.368	0.40	32
9,12-Octadecadienoic acid (Z,Z) -	20.155	0.16	33
Cyclopropaneoctanal, 2-octyl-	20.905	0.18	34
9-Octadecenamide, (Z)-	20.905	0.34	35
2-Propenoic acid, 2-(dimethylamino) ethyl ester	21.663	0.34	36
Butyl 9,12-octadecadienoate	21.789	0.20	37
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	22.079	2.91	38
Oxycyclohexadecan-2-one, 16-methyl	22.161	0.53	39
4a, 8a-(Methanothiomethano) naphthalene, 1,4,5,8-tetrahydro-	23.171	0.34	40

Isoquinoline, 3,4-dihydro-	23.372	0.29	41
9,12-Octadecadienoic acid (Z,Z) -, 2-hydroxy-1-(hydroxymethyl) ethyl ester	23.439	6.03	42
(R) - (-) - 14-Methyl-8-hexadecyn-1-ol	23.505	1.22	43
Octadecanoic acid, 2,3-dihydroxypropyl ester	23.639	8.13	44
Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	23.706	1.74	45
(-)-cis-3,4-Dimethyl-2-phenyltetra hydro-1,4-thiazine	24.1	0.37	46
Piperazine, 1,4-bis[(1,1-dimethylethyl) thio] -	24.159	0.72	47
Hexanoic acid, nonyl ester	24.345	0.41	48
Oxazole, 2-(3-methoxyphenyl) -5-phenyl-	25.474	0.80	49
2-(Acetoxymethyl) -3- (methoxycarbonyl) biphenylene	25.667	0.12	50
5-Methyl-2-phenylindolizine	26.477	0.10	51
Anthracene, 9,10-dihydro-9,9,10-trimethyl-	26.848	0.64	52
Ergosta-5,8,22-trien-3-ol, (3 .beta., 22E) -	27.145	11.19	53
5,6-Dihyroergosterol	27.249	0.98	54
Benzo [h] quinoline, 2,4-dimethyl-	27.346	0.49	55
Cholest-8 (14) en-3-ol, 4-methyl-, (3 .beta., 4 .alpha., 5.alpha) -	27.762	0.65	56
Naphthalene, 1,1' -methylenebis-	28.713	0.34	57
Benzo [h] quinoline, 2,4-dimethyl-	31.365	0.87	58
1,2-Bis (trimethylsilyl) benzene	31.699	0.43	59

Fig 1. Antibacterial activity of methanolic extract of *Tricholoma equestre*

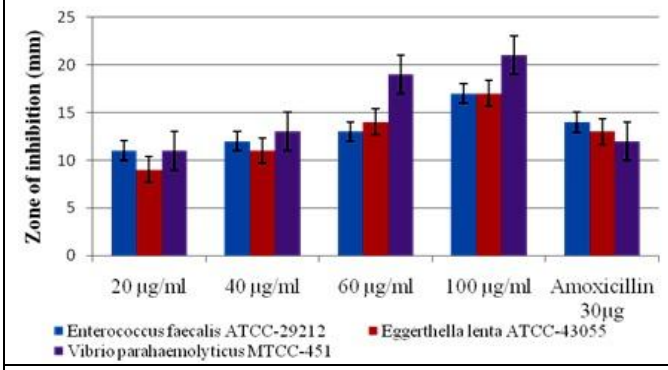


Fig 2. Antioxidant assay on *Tricholoma equestre*

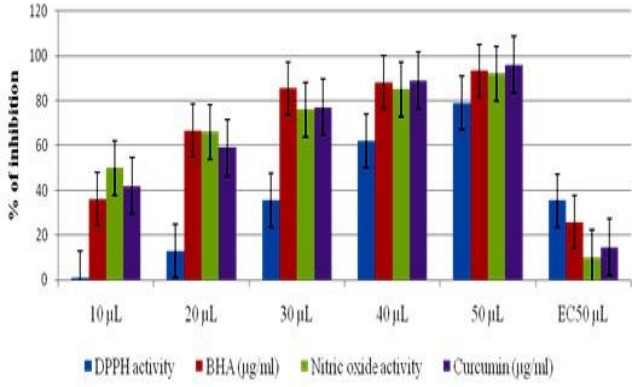
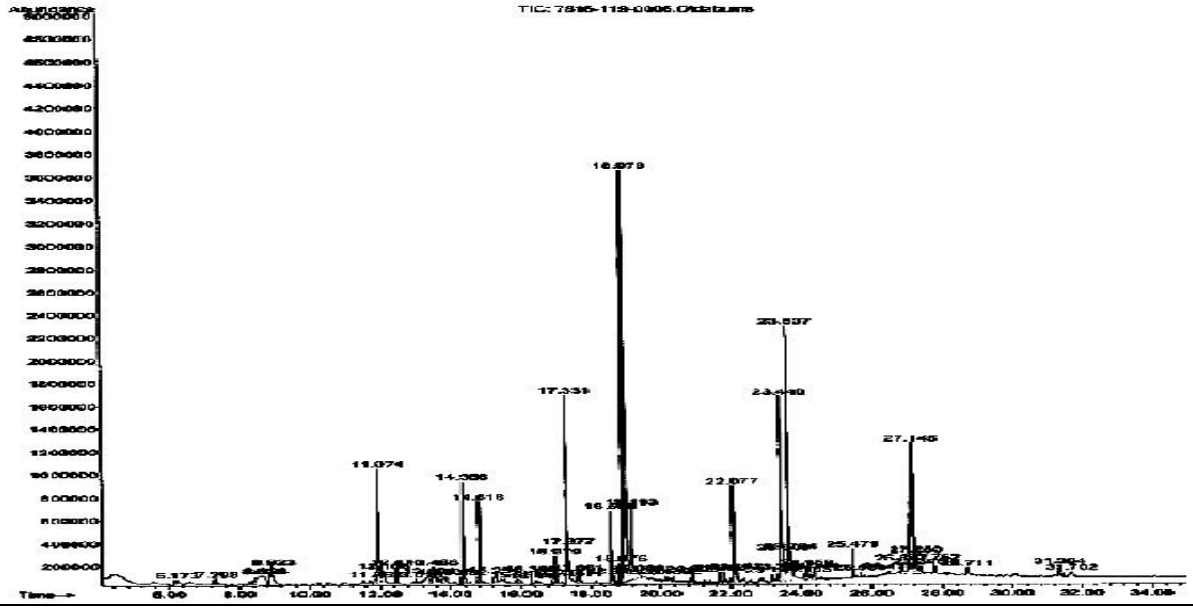


Fig 3. GC-MS spectrum of methanolic extract of *Tricholoma equestre*



CONCLUSION

The antibacterial activity, antioxidant potential and GC-MS characterization of methanolic extract of *Tricholoma equestre* collected from southern Western Ghats, India were studied. From the result it was concluded that, methanolic extract of *T. equestre* exhibited dose dependent inhibition against *Enterococcus faecalis, Eggerthella lenta* and *Vibrio parahaemolyticus*. All the concentrations of methanolic extract of *T. equestre* showed strong antioxidant activity. From the study it was found that, 59 different compounds were identified from this wild mushroom - *T. equestre*. The antibacterial activity, antioxidant potential and various chemicals present in this mushroom warrants further study and it may promising to lead to discover potent drugs to cure various diseases.

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Nil

CONFLICT OF INTEREST

No interest

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