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Research Article

HPLC DETERMINATION OF PHENOLICS AND FREE RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACTS OF TWO POLYPORE MUSHROOMS

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ABSTRACT

Objective: Present study aimed to demonstrate free radical scavenging (antioxidant) activity of ethanolic extracts of *Ganoderma lucidum* and *Trametes hirsuta* in *in vitro* conditions.

Methods: DPPH, Nitric oxide and Superoxide oxide free radical assays were employed. Total Phenolic Content (TPC) was evaluated by Folin-Ciocalteau Method and phenolic compounds were detected by high performance liquid chromatography (HPLC).

Results: Ethanolic extracts from two mushrooms possessed significant antioxidant effects. IC_{50} values of *Trametes* ethanolic extract (TEE) and *Ganoderma* ethanolic extract (GEE) in DPPH assay were found 43.60 and 13.16 µg/ml; 70.81 and 53.71µg/ml in Nitric oxide assay and, 74.04 and 50.89 µg/ml in Superoxide assay respectively. Total Phenolic Contents determined in GEE (71.43±0.94 µg GAEs/mg extract) were higher as compared to TEE (46.01±0.98). As indicated by HPLC, the bioactive components in GEE were chlorogenic acid, gallic acid, rutin, vanillin and cinnamic acid. Comparatively, ascorbic acid, catechol and cinnamic acid were detected in TEE.

Conclusion: TPC, HPLC studies and the free radical scavenging assays suggest that the two extracts have significant antioxidant potential and proved rich sources of antioxidants.

Keywords: Antioxidants, Mushrooms, *Ganoderma*, *Trametes*, Phenolic compounds.

INTRODUCTION

Free radicals are produced in the normal natural metabolism of aerobic cells, mostly in the form of reactive oxygen species (ROS) and reactive nitrogen species (RNS). They are generally unstable and very reactive. Once formed, most of the free radicals are neutralized by cellular antioxidant defenses (enzymes and non-enzymatic molecules). Maintenance of equilibrium between free radicals and antioxidant defense is an essential condition for normal organism functioning (1)**.** Nevertheless, the equilibrium between ROS and RNS production and antioxidant defenses might be displaced either by overproduction of the free radicals or by the loss of cell antioxidant defense (2). This disequilibrium, known as oxidative stress, damage; cellular lipids, proteins and DNA thereby inhibiting their normal function (3,4**)**. The uncontrolled production of free radicals has been related to more than one hundred diseases including several kinds of cancer, diabetes, cirrhoses, cardiovascular strokes, neurological disorders and aging also (5,6,7)**.** Studies have considered the control and prevention of 70% of the chronic diseases by the maintenance of good levels of antioxidants, increasing diet quality and avoiding excessive exposure to environmental pollutants and xenobiotics, besides endogenous antioxidant defences (8). Epidemiological studies have consistently shown that a high dietary intake of fruits and vegetables is strongly associated with reduced risk of developing chronic diseases, such as cancer and cardiovascular diseases (9, 10, 11). These studies have revealed that the antioxidant activity in herbals including plant and mushroom parts was conferred by presence of significant amounts of bioactive phytochemicals. Natural antioxidants are being extensively studied for their capacity to protect organisms and cells from damage brought on by oxidative stress.

Mushrooms have become attractive as functional foods and as a source of physiologically beneficial medicine having some advantages over plants (12,13). Recently, *Morchella esculenta* has been reported to show significant antimicrobial, antioxidant and antidiabetic activities *in vivo* and *in vitro* (14). *Ganoderma lucidum* (Curtis) P. Karst and *Trametes hirsuta* (Fr.) Quel. are among the polyporous, medicinal mushrooms abundantly reported from Central Indian deciduous forest areas (15,16). Central Indian Sub-tropical climatic conditions favor luxuriant growth of mushroom species but there are least reports of their pharmacological effects and bioactive principles. *Ganoderma* belongs to family Ganodermataceae and *Trametes* belong to Polyporaceae although both are polyporous mushrooms. They are predominantly found on broad leaved tree trunks and dead stumps. Whereas *Ganoderma lucidum* (Lingzhi) is a popular medicinal mushroom and have many biologically active components like triterpenoids, polysaccharides, ganoderic acids and so on, giving its antimicrobial, antioxidant, antiviral and anticancer properties (17). It has also been reported to have a number of pharmacological effects including immunomodulating, antiatherosclerotic, anti-inflammatory, analgesic, chemopreventive, antitumor, radioprotective, sleep promoting, antibacterial, antiviral (including anti-HIV), hypolipidemic, antifibrotic, hepatoprotective, diabetic, antioxidative and radical-scavenging, anti-aging, hypoglycemic, and anti-ulcer properties (18,19,20,21,22,23). There are some reports regarding antioxidant properties of *G. lucidum* methanolic (24) and aqueous (25) extracts, but also of its polysaccharides (26,27,28,29) and phenolic compounds (30). On the other hand, *Trametes* belongs to polyporaceae family with *T. versicolor, T. hirsuta* and *T. gibbosa* as its medicinally important species. *Trametes* species have been studied consistently for its anticancer and antioxidant properties; in particular, those of its isolated polysachharide fraction Krestin. Present study was aimed to evaluate the phenolic content and antioxidant potential of ethanolic extract of *Ganoderma lucidum* and *Trametes hirsuta* using different radical scavenging methods *in vitro*.

MATERIALS AND METHODS

Sampling and extraction

Naturally grown *Ganoderma lucidum* and *Trametes hirsuta* fruiting bodies were collected in June-August (monsoon), 2012 from sub-tropical deciduous forests of Madhya Pradesh (Central India). *Ganoderma* was isolated from living *Mimusops elangii;* whereas, *Trametes* from dead log of *Delonix regia.* The specimens were taken to Laboratory of Microbial Technology and Plant Pathology, Department of Botany, Dr. H.S. Gour University Sagar M.P. for identification and authentication. A specimen voucher MTPP11/45 and MTPP11/46 of the samples was deposited in herbarium of the department. The fruiting bodies were dried in an oven at 40 ^{0}C for 8 hours. The dried fruiting bodies were crushed to powder using REMI electronic blender. About 50g powder of each mushroom was taken

in 500ml of 90% ethanol in soxhlet extraction unit for extraction at 40 ^{0}C for 16-18 hours. The *Ganoderma* ethanolic extract (**GEE**) and *Trametes* ethanolic extract (**TEE**) were then rotary evaporated at 40 ^{0}C, kept in a dessicator to dry and stored at 4 ^{0}C for further use.

Chemicals

1, 1- diphenylpicrylhydrazyl (DPPH), L-ascorbic acid, Gallic acid, Folin ciocalteu's phenol reagent (FCR), Sodium carbonate, Sodium nitroprusside, methanol, chloroform and the other chemicals and reagents were purchased from Biochem Pharmaceutical Industries (Mumbai). Rutin, Catechol, α-tocopherol, Chlorogenic acid, Cinnamic acid, Vanillin, Salicylic acid and *p*-coumaric acid were purchased from Sigma-Aldrich Co. All other unlabeled chemicals and reagents were of analytical grade.

Antioxidant activity

1, 1-Diphenly-2-picrylhydrazyl (DPPH) Scavenging Assay

1, 1-diphenyl-2-picrylhydrazyl (DPPH) was used as an assay reagent and bleaching of its purple-coloured solution to yellow along with a decrease in absorbance confirm the antioxidant activity of a sample. 1000 µl of various concentration (20-100µg/ml) of the extracts (GEE & TEE) were added to 4 ml of 0.004% methanol solution of DPPH. After 20-30 min incubation period at room temperature, the absorbance was read against a blank at λ = 517 nm. Inhibition of free radical by DPPH in percent (%I) was calculated by the following formula.

% inhibition = $(A_0 – A_1 / A_0) \times 100$

Where, A_0 is the absorbance of the blank, and A_1 is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration. Tests were carried out in triplicate.

Nitric oxide Scavenging Assay

Nitric oxide (NO) ions were produced using the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extracts TEE & GEE at various concentrations and the mixture was incubated at 25 ^{o}C for 2½ hours. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthyl ethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 546 nm was measured by uv-vis spectrophotometer (Halo DB-20, Dynamica, Australia). The nitric oxide radicals scavenging activity was calculated according to the following equation:

% Inhibition = $(A_0\text{-}A_1) / A_0 \times 100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of test sample.

Superoxide Radical Scavenging Assay

Superoxide radical (O^{-2}) was generated by auto-oxidation of hydroxylamine hydrochloride in presence of NBT (nitroblue tetrazolium salt), which gets reduced to nitrite. Nitrite in presence of EDTA gives a color that was measured at 560 nm. Test solutions of the extracts TEE & GEE (20–100 µg/ml) were taken in a test tube. To this, reaction mixture consisting of 1 ml of (50mM) sodium carbonate, 0.4 ml of (24 mM) NBT and 0.2 ml of 0.1 mM EDTA solutions were added and immediate reading was taken at 560 nm. About 0.4 ml of (1 mM) of hydroxylamine hydrochloride was added to initiate the reaction then reaction mixture was incubated at 25 ^{0}C for 15 min and reduction of NBT was measured at 560 nm. Ascorbic acid was used as the reference compound. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity (31). The extracts of both mushrooms were treated in the similar manner, absorbance was recorded and the percentage of inhibition was calculated according to the following equation:

% Inhibition = $(A_0\text{-}A_1) / A_0 \times 100$

Where A_0 is the absorbance of the control (blank) and A_1 is the absorbance of test samples. All the tests were performed in triplicate and the graph was plotted with the mean values.

Total phenolic content (TPC)

Total soluble phenolics in the GEE and TEE were determined according to the method of (32) using gallic acid as a standard. 1.0 ml mushroom extract solution was taken in a volumetric flask and was diluted with 45 ml of distilled water. 1ml Folin-Ciocalteu reagent was added and mixed thoroughly. After 5 min, 2ml of Na_2CO_3 (2%) was added and the mixture was allowed to stand for 2½ hours with intermittent shaking. The absorbance of developed blue colour was measured at 760 nm. The concentration of total phenolic compounds in the mushroom ethanolic extracts was determined as µg gallic acid equivalent by using an equation that was obtained from standard gallic acid graph.

Absorbance = 0.0009 × gallic acid (µg)

Chromatographic estimation

HPLC was performed on a liquid chromatography (Alliance Waters, Millford USA) separation module 2965 coupled to Waters 2998 Photodiode array detector (DAD Milford, MA USA). Waters Spherisorb reversed phase-C18 analytical column (250 mm × 4.6 mm i.d., 5 µm; ODS2) was used at the flow rate of 0.8 ml/min. The mobile phase consisted of acetonitrile and water (70:30) adjusted with 0.05% *o*-phosphoric acid. Separation was carried out isocratically for a time period of 10 minutes injecting a volume of 20 ul of 1% mobile phase solutions of extracts and standards. The spectra were acquired in the range of 210-400 nm and chromatograms were plotted at 280 nm. HPLC data processing was performed by Empower software (Build 2154, Waters). Quantification of phytoconstituents in the sample was calculated by putting the respective values of sample and standard in the following formula:

% Content = $sample_a/standard_a \times sample_w/standard_w \times sample_v / standard_v \times potency/100\times100$

Where, *a* is peak area, *w* is weight in mg and *v* is volume in ml and potency denotes purity of the standard.

Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA) using the Origin pro 6.1 software. Means of triplicate analyses were calculated. $P<0.05$ was considered as statistically significant for all comparisons. Generalized Correlation was derived in MS Excel 2007 version.

RESULTS

Scavenging of DPPH radicals by GEE and TEE

DPPH is extensively used reagent in determination of free radical scavenging activity *in vitro*. Its purplish colour turns yellow and fades away to pale when it receives hydrogen from increased concentrations of antioxidants. TEE and GEE in present study showed variable DPPH radical-scavenging activities in a concentration dependent manner (Fig. 1). In particular, GEE showed strong antioxidant activity at concentrations higher than 20 µg/ml, though weaker than that of ascorbic acid. At 100 µg /ml concentration of GEE, 82.76±1.06% of the DPPH was inhibited with IC 50 value of 13.615 µg /ml (Table 1). TEE scavenged 71.88±0.84% DPPH radicals at the level of 100 µg /ml. Both the extracts exhibit significant DPPH scavenging potential when compared with ascorbic acid taken as standard.

Nitric oxide scavenging effect of GEE and TEE

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide.GEE scavenged 63.46±1.11 % nitric oxide radical at 100µg/ml, with IC_{50} value of 53.71 µg /ml. On the other hand, TEE showed inhibition per cent of 58.87±0.78 to nitric oxide

and at 100 µg/ml concentration with IC_{50} value of 70.81 µg/ml respectively (Table 1). Both the extracts showed inhibiton of nitric oxide radicals in a concentration dependent manner (Fig. 2). These results were found to be statistically significant (P < 0.05).

Mushroom extract	Total Phenolic Compounds (µg GAEs/mg extract)	Antioxidant assay	IC_{50} Values (µg/ml)	Correlation coefficients (r)*
TEE	46.01 ± 0.89 *a*	DPPH	43.60	0.988
		Nitric – oxide	70.81	0.996
		Super - oxide	74.04	0.998
GEE	71.43 ± 0.94 *b*	DPPH	13.615	0.995
		Nitric-oxide	53.71	0.996
		Super – oxide	50.89	0.985
Ascorbic acid	_____	DPPH	4.306	___
		Nitric – oxide	18.82	
		Super - oxide	26.46	

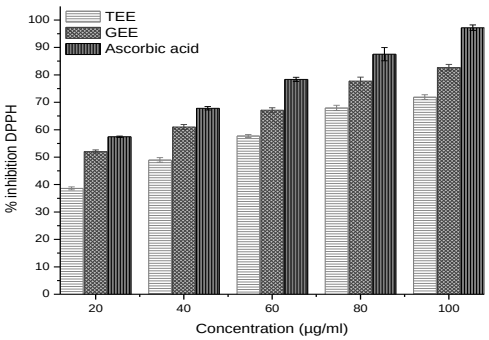

Fig. 1: DPPH scavenging activity of TEE, GEE and Ascorbic acid. Values are mean±SD (n=3); p<0.05

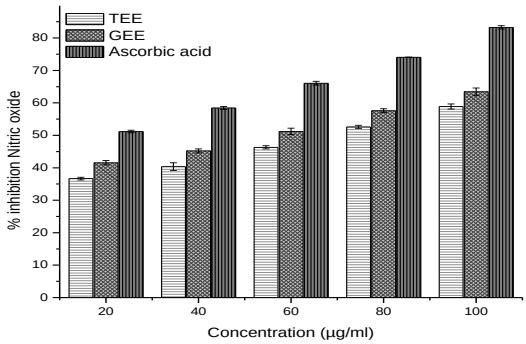

Fig. 2: Nitric oxide scavenging activity of TEE, GEE and Ascorbic acid. Values are given as mean±SD (n=3); p<0.05

Superoxide scavenging effect of GEE and TEE

The scavenging abilities of mushroom extracts on superoxide radicals are shown in Figure 3. GEE showed significantly higher superoxide radical scavenging abilities (66.00±0.89 %) than TEE (57.14±0.54) at the level 100 µg/ml in the reaction mixture. Nevertheless, they revealed significantly lower scavenging potential when compared with ascorbic acid. IC_{50} values of the test samples are given in table 1. Although TEE also displayed good antioxidant activity in all the antioxidant assays, GEE exhibited far better results when compared with standard antioxidant. Regression analysis showed the linear relationship and hence concentration dependent curves in all the radical scavenging models.

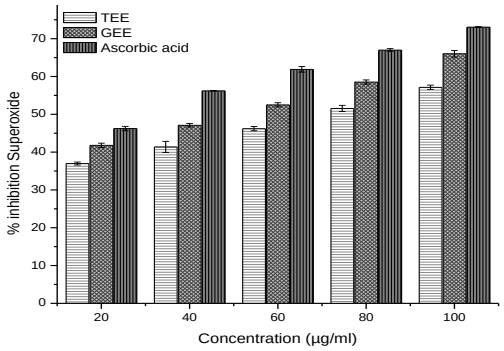

Fig. 3: Superoxide radical scavenging activity of TEE, GEE and Ascorbic acid. Values are presented as mean±SD (n=3); p<0.05.

Total Phenolic Content

Phenolic compounds are well-known, naturally occurring antioxidants and are believed to be beneficial to human health (33). A recent study has demonstrated that an ethanolic extract of white button mushroom (*Agaricus bisporus*) possessed potent antioxidant activity associated with its phenolic components (34). TPC was expressed in terms of ug GAE/mg of the extract. Whereas GEE possessed 71.43±0.94 µg GAE/mg of the extract; TEE showed the presence of 46.01±0.98 µg GAE/ mg of the extract (Table 1). In addition, positive correlation coefficients obtained between TPC and inhibition percentage clearly indicated phenolic compounds responsible for the antioxidative effects of test mushroom extracts.

Table 1: Total phenols (µg GAEs/mg extract) and IC_{50} (µg/ml) values of GEE, TEE and Ascorbic acid

Values are mean ± S.D., (n = 05). Letters ***a*** and ***b*** denote the significant difference of means at P< 0.05;

* generalized correlation between TPC and antioxidant activity

HPLC analysis

As the study dealt with the antioxidant activity, HPLC studies evaluated the % content and indicated the presence of bioactive compounds reported in earlier studies. HPLC indicated the presence of five phenolic and related compounds in GEE; only three compounds were detected in TEE by matching the retention times of the peaks with the standard antioxidants (Table 2). The chromatogram profiles are given in Fig. 4 and Fig. 5.

Table 2: HPLC studies of TEE and GEE indicating the presence of Phenolic compounds

Mushroom extract	Bioactive compound	Retention time	Peak area	% Content
TEE	Catechol	3.277	181299	0.05
	Cinnamic acid	4.179	1618204	0.21
	Ascorbic acid	4.834	933860	4.0
GEE	Chlorogenic acid	2.911	1966299	0.7
	Rutin	3.360	1305066	2.82
	Vanillin	3.523	570217	2.02
	Cinnamic acid	4.163	693998	0.09
	Gallic acid	4.717	3426102	2.82

DISCUSSION

Antioxidants donate an electron to stabilize a free radical. When a free radical is neutralized it is not capable of damaging our cells. Hence, modern health science is very much concerned with the antioxidants from natural sources to fight as defense mechanisms against various diseases and ailments. Antioxidants protect our body against free radicals and, mushrooms are rich source of these antioxidants (35, 36, 37). Different extracts from mushrooms have

expressed different behaviors towards free radical scavenging. Jones and Janardhan (38) demonstrated that *G. lucidum* can trap number of free radicals. Russell and Paterson (39) reported that triterpenoids are the main chemical compounds in *G. lucidum*. Other workers have demonstrated that polysaccharides from *Ganoderma* and *Trametes* have substantial antioxidant activity. A diverse group of bioactive components have been detected, isolated and investigated for different antioxidant assays from mushrooms and it is established that they introduce hydroxyl groups and other hydrogen and electron donating groups to scavenge the free radical species. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule, with an absorption band around 515-528 nm and thus, it is a useful reagent for evaluation of antioxidant activity of compounds. Antioxidants reduce the DPPH radical to a yellow coloured compound, diphenylpicrylhydrazine, and the extent of the reaction will depend on the hydrogen donating ability of the compounds. Results revealed that GEE expressed excellent scavenging capacities in all the models whereas TEE showed least but good scavenging capacities when compared with ascorbic acid. GEE performed much better than earlier studies on other mushrooms by various workers. Rai *et al*., reported IC_{50} value for ethanolic extract of *Armillaria mellea* against DPPH as 107.07 µg/ml, while GEE in our study demonstrated IC_{50} value as 13.615 µg/ml. *Armillaria* extracts in the same study exhibited no inhibition on Superoxide radicals (40). Our study determined significant inhibition of superoxide (59.10±1.32% and 57.28±0.76) at 100µg/ml concentration of the TEE and GEE extracts. In a recent study, *Solanum surattense* plant extract exhibited higher inhibition percentages on superoxide radicals when compared to ascorbic acid in the range of 50-250 µg/ml, but reported DPPH inhibition similar to our results (41). Hence, our results are rather significant against such studies. According to Kamiyama *et al.*, acetone extract of *Trametes versicolor* exhibit highest antioxidant activity at 54.9±0.1% at the level of 500 µg/ml followed by methanol extract at 40.0±0.2 % against DPPH radical (42). Results obtained with TEE and GEE 71.81 ± 0.89 and 84.12 ± 0.67 % inhibition, are much better than other studies (43,24) carried at the level of 100 µg/ml against DPPH radical.

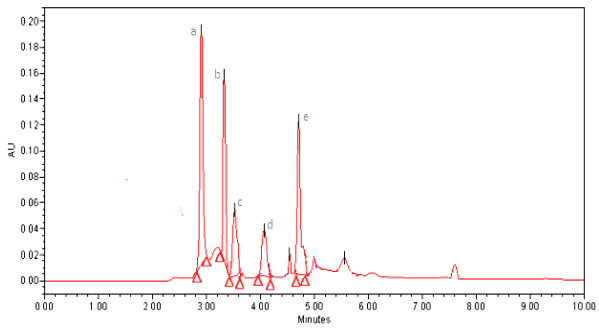

Fig. 4: HPLC profile of *Ganoderma* ethanolic extract

a) chlorogenic acid b) rutin c) vanillin d) cinnamic acid e) gallic acid.

Nitric oxide plays as a potent pleitropic inhibitor of physiological processes such as smooth muscles. It is an essential bioregulatory molecule required for neural signal transmission, immune response, control vasodilation and control of blood pressure. However, the its elevated concentrations lead to several pathological conditions, including cancer. NO has an unpaired electron, hence is a free radical. Compounds in TEE and GEE have probably donated a single electron to nitric oxide thereby neutralizing it. In a study carried out by Pal *et al*., cold water, hot water and methanolic extracts exhibited NO-scavenging dose-response curve, speculating that EC_{50} value for hot water 14 fold greater than that of methanolic extract of *Pleurotus squarrosulus* (44). Our results have proven more excellent for all the scavenging systems.

Phenolic derivatives are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The antioxidant properties of phenolic compounds are mainly because of their redox potential, which allow them to act as reducing agents, hydrogen donators, metal chelators and singlet oxygen quenchers (45). Phenolic compounds have specific health effects, even though they are non-nutritive compounds. In our diet they provide health benefits associated with reduced risk of chronic diseases that may be due to their ability to reduce agents by donating hydrogen and quenching singlet oxygen. Studies have shown that the phenolic compounds in mushrooms may contribute directly to antioxidative action. In many studies, correlation between phenolic compounds and antioxidative capacities has been described (46,47,48). The antioxidant property of many plant extracts has been attributed to their phenolic contents and/or structures. Positive correlation between TPC and % inhibition in three different assays clearly indicated that phenolic contents are responsible for the antioxidant activity of the test samples (Table 1).

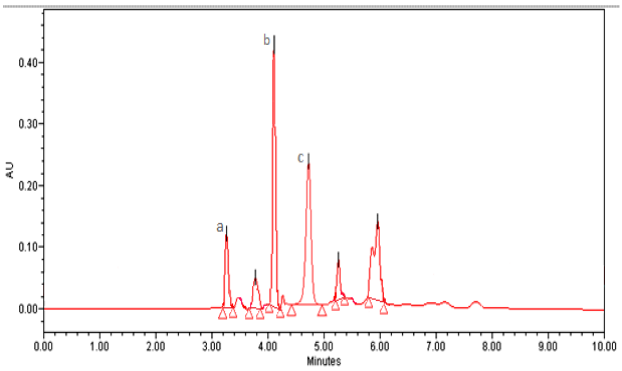

Fig. 5: HPLC profile of *Trametes* ethanolic extract.

a) Catechol b) cinnamic acid c) ascorbic acid

These results were partly supported by HPLC, where peak signals corresponded to retention times of standard phenolic compounds (Fig. 4 & 5). HPLC studies detected antioxidant compounds mainly as benzoic acid and cinnamic acid derivatives including flavonoids. The flavonoids contain a number of phenolic hydroxyl groups attached to ring structures, which confer the antioxidant activity. Rutin was detected as one of the flavonoids in a good quantity 2.821% (table 2) in GEE which has not been reported earlier in *Ganoderma lucidum* and *Trametes hirsuta*. On the contrary, Park and Lee (49), reported 20 phenolic compounds from tryptophan induced *Ganoderma neo-japonicum* cultures. However, studies carried out in Portugal and India showed the presence of rutin in *Cantharellus cibarius* and *Pleurotus ostreatus.* Significantly higher scavenging effect of GEE may be attributed to rutin which is a known antioxidant. Antioxidant activity could also be related to other phenolic compounds like cinnamic acid, vanillin, chlorogenic acid, ascorbic acid and gallic acid present in our mushroom samples reported by other workers (50,51). Significant differences in the antioxidant potentials within the test samples and standard can be discussed on the basis of TPC and synergistic antioxidant effects of phenolic compounds. Wei and Griensven (52), while correlating ROS scavenging and ROS generating effects with TPC of a number of mushrooms, speculated phenolic compounds least relative to ROS generation than polysaccharides. However, a mixture of polysaccharides and phenolics contributed to increased ROS generation. This indicated some bioactive components along with phenolics in TEE act as pro-oxidants which lowered its % inhibition as compared to GEE and standard. As compared to the contents of total phenolics in TEE (46.01±0.9 ug/mg), the highest contents of total phenolics in GEE (71.43 ug/mg), might be the key components accounting for its better antioxidant effects. According to Mau *et al.*, (24) total phenols are responsible for the antioxidant properties of mushrooms. Since, the extracts mainly contained phenolic compounds with one or more hydroxyl groups, it could be suggested that free radicals were scavenged due to hydrogen donating or single electron transfer. Higher phenolic contents in our ethanolic extracts are in agreement with the findings of Hu *et al.*, (33) reporting highest phenolic content (91.5 ug GAE/mg extract), and no polysaccharide and protein content in ethanolic extract of *Inonotus obliquus*. Furthermore, mushrooms were isolated from two different hosts viz, *Mimusops* tree stem and dead stump of *Delonix* as described previously

(supplementary figures). Plants defend themselves from pathogen using a variety of mechanisms including rapid induction of localized necrosis at the site of infection, e.g., hypersensitive response (HR), increased expression of defense-related proteins or pathogenesis related proteins (PRs), production of antimicrobial compounds, lignin formation and the oxidative burst (53,54,55). Host difference may be responsible for the variation in the phenolic contents and other bioactive components in the test mushroom extracts. In addition, nature (live or dead) of the substrate could have accounted for the difference in the composition of phenolic compounds in TEE and GEE. Therefore, the antioxidant potentials were also found to be different. Nevertheless, the actual mechanism for which the two mushrooms varied in types and quantities of bioactive compounds is still unknown.

In conclusion, GEE and TEE exhibited significant antioxidant potentials when compared with standard. HPLC indicated that phenolic compounds from the extracts were mainly responsible for the antioxidant potential. It is interesting to note further the presence of flavonol glycosides like rutin which could have contributed better antioxidant property in *Ganoderma lucidum* in present study. Thus, given the antioxidant capacity of the fruiting bodies of *Ganoderma lucidum* and *Trametes hirsuta*, and their high phenolic content, it could be concluded that they are valuable asset for the welfare of humankind. Authors are correlating antioxidant activity with other therapeutic properties of mushrooms.

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