Bulletin of Pure and Applied Sciences.
Vol.38 B (Botany), No.1. Jan-Jun 2019: P.34-48

Print version ISSN 0970 4612
Online version ISSN 2320 3196
DOI: 10.5958/2320-3196.2019.00005.3
Available online at www.bpasjournals.com

Original Article

Taxonomic Details, Antibacterial and Antioxidant Activities of *Termitomyces eurhizus* (berk) r. Heim from District Kangra, Himachal Pradesh

Devender Kumar[1*], Anand Sagar[2]

Author's Affiliation
[1,2]Department of Biosciences, Himachal Pradesh University, Shimla-171005 India.

***Corresponding Author:**
Devender Kumar
Department of Biosciences,
Himachal Pradesh University, Shimla- 171005, India

E-mail:
thakurtanudevender@gmail.com

Received on 22.01.2019
Accepted on 05.03.2019

Keywords:
Termitomyces eurhizus, Antioxidant, DPPH, Antibacterial, Mycelia, Hagem`s-Modess media.

Abstract

The aim of the present study is to investigate the growth of mycelia in different solid media and determination of antibacterial and antioxidant activity of acetone, methanol and distilled water extracts of mycelia and fruiting bodies of *Termitomyces eurhizus.* The antioxidant and antibacterial activities were evaluated by using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and Agar-well diffusion method respectively. Result showed that out of five different solid media, Hagem`s-Modess medium was found to be best solid medium for culturing the mycelia of *T. eurhizus*. Different extracts of fruiting bodies showed good antioxidant activity than the mycelial extracts. On the other hand, mycelial extracts exhibited significant antibacterial activity than the fruiting bodies extracts of *T. eurhizus*.

INTRODUCTION

Fungi are one of the most important eukaryotic organisms in the world which play very crucial roles in various ecosystem services and also influence human and human-related activities. Fungi are ubiquitous and cosmopolitan, covering an area from tropics to poles and tops of the mountain to the deep oceans[1]. As per recent estimate, the total number of fungi on the earth ranges from 5,00,000 to 9.9 million species but generally the working figure of 1.5 million species has been accepted out of which 80,060 are named[2,3]. In India, the total number of fungal species exceeds 27,000 which is considered as the largest biotic community after insects and almost one-third of the fungal diversity of the world prevails in India. Out of 1.5 million species of fungi, only around 5-10% species can be cultured artificially.[4,1].

The term "mushroom" is generally used for the fruiting bodies of macrofungi (Ascomycota and Basidiomycota) and in their life cycle they show only a short reproductive stage[5]. The total number of mushrooms on the earth are estimated to about 140,000 species out of which, only 10% (14,000) species are known[6]. Out of 14,000 known species, only 2,000 are safe for human consumption and about 650 of these possess medicinal properties[7]. In India, the total number of mushrooms are recorded around 850 species and many of them have been used in food and medicine by the local peoples[8]. Lakhanpal and his associates from a survey of mushrooms in North West Himalayas

recorded 300 Species, 59 Genera and 15 Families of Agaricales. This survey provides the list of the species occurring in the Himalayas[9].

Mushrooms remained as a delicacy in human diet since primeval time. Many of the early civilizations like Greeks, Aryans, Egyptians, Romans, Chinese and Mexican have also explored the therapeutic potential of mushrooms[10]. Mushrooms are rich source of natural antibiotics. The cell wall of the mushrooms contains glucans which are well known for their immune-modulatory properties[11] and also contains various bioactive substances such as antibacterial, antifungal, antiviral, antiparasitic, antioxidant, anti-inflammatory, antidiabetic, hypocholesterolemic, anticoagulant and hepatoprotective compounds[12,13].

Many plants have been recognized to establish the ectomycorrhizal association with the mycelium of various types of mushrooms[14]. The insect termites of sub-family Macrotermitinae make an obligate mutualistic symbiotic relationship with the genus *Termitomyces.* Termites create a specific micro-environment for the cultivation of termitophilic fungi. The older part of the comb contains semi-degraded plant materials, fungal mycelium and nodules i.e. asexual fruiting bodies covered with conidia. On the other hand, the plant materials like wood, dry grasses and leaf litter are continuously added externally in fungus comb[15,16]. *Termitomyces* are an economically valuable natural resources which provide the food, possess medicinal properties and also have many industrial applications[17,18]. *Termitomyces* also produces variety of enzymes useful in food, in leavening of bread, in processing silage and in clarification of non-citrus fruit juices[19,20]. As far as our literature survey says that the antibacterial and antioxidant activity of acetone, methanol and distilled water fruiting bodies and mycelium extract of *Termitomyces eurhizus* from Himachal Pradesh has not been published before. We have examined the taxonomic details, growth of pure culture in different solid media along with antibacterial and antioxidant activity of acetone, methanol and distilled water fruiting bodies and mycelium extract of *Termitomyces eurhizus* by employing the Agar-well diffusion method and DPPH radical scavenging activity assay respectively for determining the usefulness of this mushroom.

MATERIALS AND METHODS

To survey, collect and work out taxonomic details of *Termitomyces eurhizus*:

Survey and collection:
Specimen of *T. eurhizus* was collected from Pine forest of Bagidhar, Near Baijnath at an altitude of 1,125 meters in district Kangra of Himachal Pradesh during August and September 2018. Collection sites were visited regularly usually after every spell of rain. The fruiting bodies were collected carefully with the help of spade. The specimens were wrapped in paper bags individually and placed in a collection bag by giving it a collection number.

Taxonomic details of *T. eurhizus*:
Macroscopic studies, Microscopic studies (Photomicrographs of the slides were taken with the help of microscope LEICA DMLS2 with attached digital camera LIECA DFC320) and surface electon microscopic studies, the images of the samples were obtained on screen of Scanning Electron Microscope (JEOL, JSM-IT300).

Pure culture isolation:
The cultures were raised from the fruiting bodies of healthy, shade dried and fresh specimens. The specimens were first washed with distilled water and tissues from stipe and stroma portions were cut with the help of sterilized blade. The bits of tissues (2-3 mm) were taken by sterilized forceps and dipped in 0.1% mercuric chloride solution for 5-10 second. Now the tissues were placed on filter paper to remove the excess moisture. The small bits of specimen tissues were transferred aseptically into the Petri-plates containing potato-dextrose agar (PDA) medium with the help of sterilized forceps. These were incubated at 26□ for at least 8-10 days and observed regularly for appearance of culture. The actively growing mycelium colonies were sub-culture to obtain pure culture for further studies.

Preparation of extracts of fruiting bodies and mycelium of *T. eurhizus*:
Fruiting bodies and mycelial mats of *T. eurhizus* were dried under aseptic conditions. Dried materials were pulverized by mortar and pestle. 2 g dried materials from fruiting bodies and mycelium powder were taken separately in separate Erlenmeyer flasks to which 20 ml of required solvents i.e., methanol, acetone and water were added. The flasks were covered with aluminium foil and allowed to stand for 7 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator. The extracts were collected and weighed. Finally, stock solution of conc. 50 mg/mL was prepared.

Procurement of bacteria:
Different bacteria (*Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus*) were procured from IGMC, Shimla for screening antibacterial properties of the mushroom under study.

Revival of pathogen:
The pathogens were revived in nutrient broth and stored in nutrient agar slants at 4°C.

Screening the antibacterial activity of different extracts of fruiting bodies and mycelium of *T. eurhizus*:
Different extracts (methanol, acetone and water) of *T. eurhizus* were screened using agar-well diffusion method. Nutrient agar medium (Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptone 5 g, Agar 20 g, Distilled Water 1000 mL) was used throughout the investigation. The medium was autoclaved at 121.6°C for 30 minutes and poured into Petri-plates. Bacteria were grown in nutrient broth for 24 hours. A 100 µL of bacterial suspension was spread on each nutrient agar plate. Agar-wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri-plate. The wells in each plate were loaded with 25, 50, 75 and 100% concentration of prepared extracts. The wells in the Petri-plate kept as a control contained pure solvent only. The plates were incubated at 37±2°C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were tabulated. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition zone diameter using control as standard with slight modifications[21].

$$\text{Percentage of growth inhibition (\%)} = \left(\frac{\text{Test}-\text{Control}}{\text{Standard}}\right) \text{x}100$$

Control = average diameter of bacterial colony in control.
Test = average diameter of bacterial colony in treatment sets

Screening the antioxidant activity of different extract of fruiting bodies and mycelium of *T. eurhizus*:
The free radical scavenging activity of fruiting bodies and mycelial extracts of *T eurhizus* was measured using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) as described byBlois[22] with slight modifications. Briefly, to 1 mL of different concentrations (40, 800, 120, 160 and 200 µg/mL) of mushroom or test extract, 1 mL of DPPH (0.1 mM in methanol) was added. Corresponding blank sample was prepared and ascorbic acid was used as reference standard. Mixture of 1 mL methanol and 1 mL DPPH solution (without mushroom extract) was used as control. All the tests were carried out in triplicate and the decrease in absorbance was measured at ƛ=517 nm after 30 minutes in dark using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \left(\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}\right) \times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample
Graphs were plotted against percent inhibition v/s conc. of mushroom extracts and standard ascorbic acid in order to find out the values of slope and y-intercepts. IC_{50} value (the amount of antioxidant required to decrease the initial DPPH concentration by 50%) for each extract and ascorbic acid was evaluated using the following equation given below:

$$IC_{50} = \frac{50 - Y - Intercept}{\text{Slope}}$$

RESULTS

To survey, collect and work out taxonomic details of *Termitomyces eurhizus*:

Habit and habitat:
It is a wild edible mushroom which grows on the termitarium of termites present under the soil in pine forest (*Pinus roxburghii*) (Plate I-A).

Macroscopic characters:
The fruiting body is large, at initial stages it is greyish brown then at maturity it fades to creamish brown and pale to white towards the margin. Pileus ranges from 6-10 cm in diameter. Lamellae light creamish, free to adnexed, average crowded and regular. Stipe ranges from 25-30 cm long in length, cylindrical which is swollen near the surface of soil with long tapering pseudorhiza without annulus (Plate I-B).

Microscopic characters:
Hyphae are 3-18 µm in diameter, pure white, thin walled, hyaline, branched without clamp connection. Basidia 18-26 × 6-8 µm, clavate bearing four sterigmata. Spores are 6.5-8.5 × 4-5 µm, broadly ellipsoid, hyaline and thin to slightly thickened wall (Plate IV-A to C).

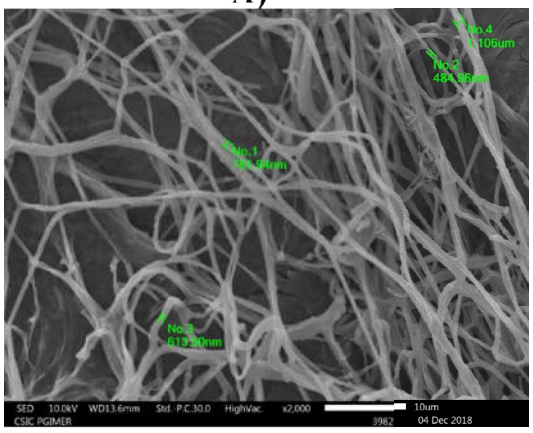
SEM studies:
Mycelium and spores were observed under Scanning Electron Microscope at magnifications 2000 X and 500 X respectively. The mycelium ranges in size from 417.11 nm-3.709 µm (Plate I-C). Spores were ellipsoidal, showed ridges and their size generally ranged from 12.43-16.16 × 15.44-29.35µm (Plate I-D).

Pure culture isolation:
Pure cultures of *T. eurhizus* were raised from the pileus and stipe portion of healthy, sundried and fresh specimens. Pure cultures were obtained on PDA medium with 100 mg/L streptomycin to inhibit the bacterial growth after incubation at 26^0C for 8-10 days. White cottony growth with thin strands of hyphae was observed and white buff mycelium became densely matted. With maturity it gained intensity and showed pinkish pigmentation (Plate II-B). Some small bits of mycelium were transferred to flasks containing liquid medium and incubated at 26^0C for 15 days. After 15 days mycelium mats were formed on the surface of the liquid medium in flasks and after that these mycelial mats were collected and dried at room temperature for further use (Plate II-F).

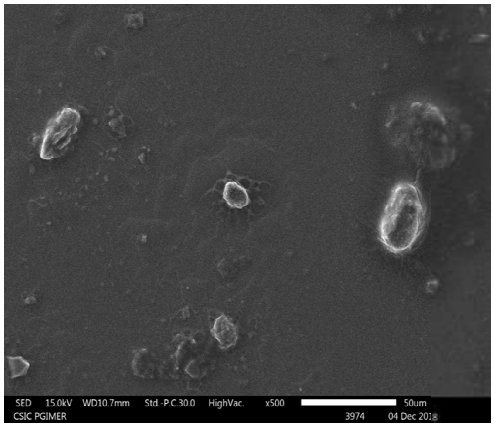
PLATE-I

A)

B)



C)



D)

A) *Termitomyces eurhizus* (Berk) R. Heim in its natural habitat.

B) Lab photograph showing macroscopic features of *Termitomyces eurhizus* (Berk) R. Heim.

C) SEM images of mycelium, **D)** SEM images of spores of *T. eurhizus*

Plate 1 A-D:

PLATE-II

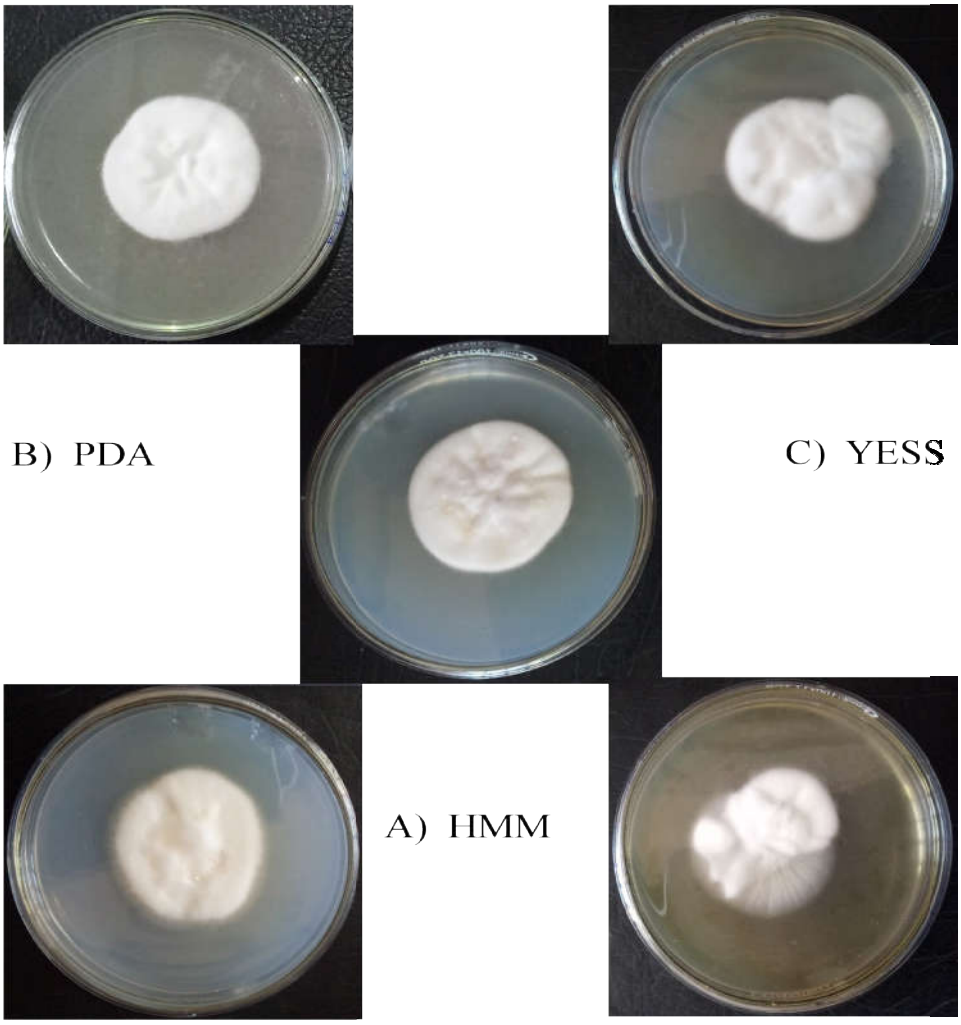
E) Pure culture of *T. eurhizus*, **F**) Mycelial mats of *T. eurhizus* collected from liquid medium.

Plate II E & F:

Recording the growth of mycelial mats of *T. eurhizus* in different solid media:

Five solid media were tested for the linear growth of *T. eurhizus*. The growth was recorded after ten days of incubation at 26□ (Plate III). The mean colony diameter of the mycelium (±SE) in different solid media is numerically and graphically represented in Table 1 and Figure 1 respectively.

PLATE-III



Growth characteristics of mycelium of *T. eurhizus* in different solid media

Plate III A-E:

Table 1: Average colony diameter of *T. eurhizus* on different solid media

Sr. No.	Name of Medium	Average diameter (cm) ± SE
1	Modified Malt Extract Agar (MMEA)	4.4 ± 0.04
2	YESS Media	4.66 ± 0.13
3	Hagam`s-Modess Medium (HMM)	4.9 ± 0.04
4	Potato Dextrose Agar (PDA)	4.83 ± 0.11
5	Dimmick Agar Extract (DAE)	4.53 ± 0.02

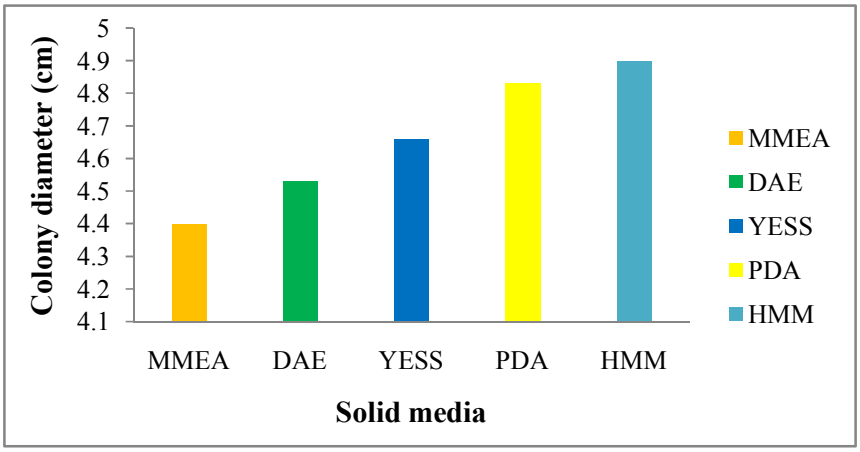


Figure 1: Average colony diameter of *T. eurhizus* on different solid media

Screening the antibacterial activity of different extracts of fruiting bodies and mycelium of *T. eurhizus*:
Antibacterial activity of *T. eurhizus* was evaluated by using Agar-well diffusion method against four pathogenic bacteria (*Escherichia coli, Listeria monocytogenes, Pseudomonas aerugenosa* and *Staphylococcus aureus*). Streptomycin (5 µg/mL) was taken as a standard and positive control. Table 2-2.2 and Figure 2.1–2.2 display results for antibacterial activity.

Screening the antibacterial activity of acetone extract of fruiting bodies and mycelium of *T. eurhizus*:
Acetone fruiting bodies extract showed maximum zone of inhibition against *L. monoctogenes* (17.51±0.10 mm at 100% concentration) and minimum zone of inhibition against *S. aureus* (8.07±0.11 mm at 25%). On the other hand, acetone mycelium extract was found to be most effective against *L. monocytogenes* (13.76±1.88 mm at 25%, 14.86±1.80 mm at 50%, 16.7±1.49 mm at 75% and 18.26±1.65 mm at 100% concentration) and less effective against *S. aureus* (9.53±0.43 mm at 25%, 10.1±0.54 mm at 50%, 11.43±0.23 mm at 75% and 11.86±0.22 mm at 100% concentration) as given in Table 2.1 and Fig. 2.1.

Screening the antibacterial activity of methanol extract of fruiting bodies and mycelium of *T. eurhizus*:
The antibacterial screening of different extracts of *T. eurhizus* observed that the methanol fruiting bodies extract was found to be most effective against *L. monocytogenes* (18.23±1.1 mm at 100% concentration) and less effective against *S. aureus* (12.32±0.32 mm at 25%). Similarly, maximum zone of inhibition was observed against *L. monocytogenes* (16.8±0.09 mm at 25%, 19.38±0.80 mm at 50%, 19.85±0.90 mm at 75% and 21.39±1.36 mm at 100% concentration) and less inhibition against *S. aureus* (11.46±0.07 mm at 25%, 14.33±0.47 mm at 50%, 14.43±0.48 mm at 75% and 15±0.09 mm at 100% concentration) for methanol mycelium extract as given in Table 3.2 and Figure 2.2. The water extract of fruiting bodies and mycelium of *T. eurhizus* had shown no inhibition against all the tested pathogenic bacteria.

Table 2: Inhibition zone shown by streptomycin (5 µg/ml) against test bacteria

Sr. No.	Test bacteria	Zone of inhibition diameter (mm) including well
1	*Escherichia coli*	21
2	*Listeria monocytogenes*	26.57
3	*Pseudomonas aeruginosa*	28.78
4	*Staphylococcus aureus*	17

Table 2.1: Antibacterial activity of acetone extracts of fruiting bodies and mycelium of *T. eurhizus*

Extract	Conntration of extract (%)	Inhibition zone diameter (mm)			
		L. monocytogenes	*S. aureus*	*P. aeruginosa*	*E. coli*
	Control	00±00	00±00	00±00	00±00
Fruiting bodies extract	25	11.23±0.56	8.07±0.11	10.85±0.32	11.84±0.53
	50	12.96±0.39	9.70±0.34	12.96±0.41	12.41±0.57
	75	16.24±0.21	9.97±0.15	13.25±0.51	14.79±0.30
	100	17.51±0.10	11.16±0.13	13.95±0.12	16.44±0.50
Mycelial extract	Control	00±00	00±00	00±00	00±00
	25	13.76±1.88	9.53±0.43	10.26±0.61	9.6±0.24
	50	14.86±1.80	10.1±0.54	11±0.47	10.43±0.07
	75	16.7±1.49	11.43±0.23	12.33±0.27	11.53±0.72
	100	18.26±1.65	11.86±0.22	13.56±0.07	12.36±0.66

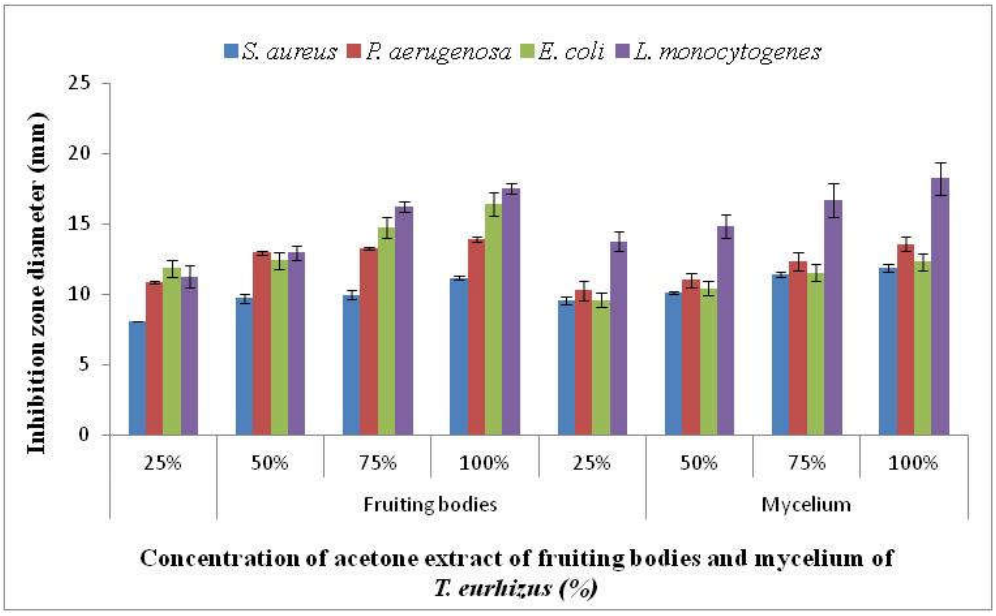


Figure 2.1: Inhibition zone diameter (mm) of acetone extract of fruiting bodies and mycelium of *T. eurhizus* against tested bacteria

Table 2.2: Antibacterial activity of methanol extracts of fruiting bodies and mycelium of *T. eurhizus*

Extracts	Concentration of extract (%)	Inhibition zone diameter (mm)			
		L. monocytogenes	*S. aureus*	*P. aeruginosa*	*E. coli*
	Control	00±00	00±00	00±00	00±00
Methanol Fruiting bodies extract	25	13.36±0.22	12.32±0.32	14.00±0.54	12.68±0.48
	50	15.9±0.57	13.78±0.18	16.09±0.88	14.23±0.59
	75	16.43±0.67	14.63±0.19	17.29±0.61	15.26±0.59
	100	17.03±0.83	15.33±0.27	18.23±1.1	17.41±0.26
Methanol Mycelial extract	Control	00±00	00±00	00±00	00±00
	25	16.8±0.09	11.46±0.07	13.7±0.57	13.2±0.16
	50	19.38±0.80	14.33±0.47	14.99±0.25	14.46±0.40
	75	19.85±0.90	14.43±0.48	17.38±0.15	15.23±0.40
	100	21.39±1.36	15.76±0.09	17.71±0.16	16±0.47

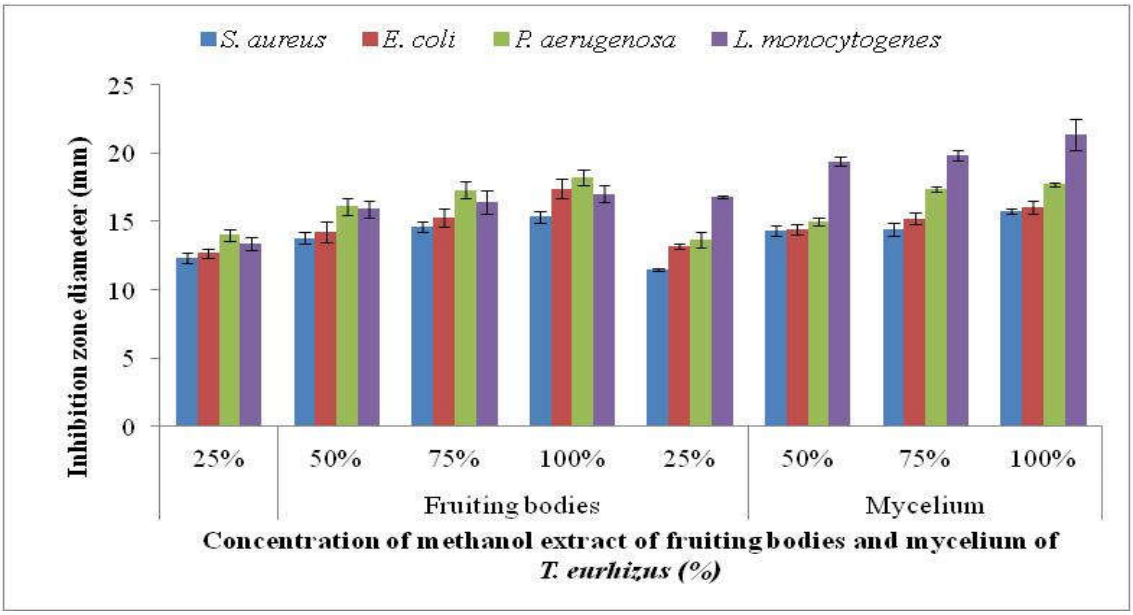


Figure 2.2: Inhibition zone diameter (mm) of methanol extract of fruiting bodies and mycelium of *T. eurhizus* against tested bacteria

Screening the antioxidant activity of different extracts of fruiting bodies and mycelium of *T. eurhizus*:

Antioxidant activity of acetone, methanol and distilled water extracts was investigated by using DPPH free radical scavenging assay and ascorbic acid in the same concentration to the extracts was taken as standard exhibiting IC_{50} value of 23.21 µg/mL (Table 3 and Figure 3).

The experimental study of different extracts of fruiting bodies of *T. eurhizus* revealed that the distilled water fruiting bodies extract was found to have higher antioxidant activity with 211.21 µg/mL IC_{50} value than acetone fruiting bodies extract with 626.38 µg/mL IC_{50} value and methanol fruiting bodies extract with 690.31 µg/mL IC_{50} value displaying least antioxidant potewntial (Table 3.1 and Figure 3.1).

The different extracts of mycelium of *T. eurhizus* were investigated and it was found that the acetone mycelium extract of *T. eurhizus* showed higher antioxidant activity (with lower IC_{50} value 711.67 µg/mL) followed by water mycelium extract (with IC_{50} value 715.57 µg/mL) and methanol mycelium extract (with IC_{50} value of 881.76 µg/mL.) (Table 3.2 and Figure 3.2).

Table 3: Free radical scavenging activity (%) of the control i.e. L-Ascorbic acid at different concentrations

	Concentration (µg/mL)	% Inhibition	IC_{50} value (µg/mL)
Ascorbic acid (control)	40	54.49	23.21
	80	63.2	
	120	71.91	
	160	79.77	
	200	92.41	

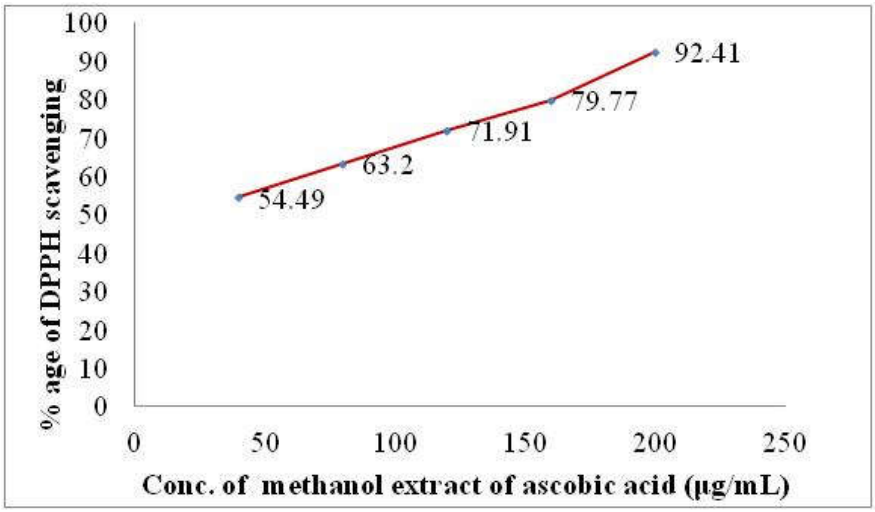


Figure 3: Free radical scavenging activity of L-Ascorbic acid

Table 3.1: Free radical scavenging activity (%) of the different extracts of fruiting bodies of *T. eurhizus* at different concentrations

Conc. (µg/mL)	Acetone extract (%)	IC_{50} value (µg/mL)	Methanol extract (%)	IC_{50} value (µg/mL)	Water extract (%)	IC_{50} value (µg/mL)
40	6.91±0.002	626.38	5.27±2	690.31	18.48±0.008	211.21
80	10.63±0.25		7.03±0.54		25.87±0.01	
120	12.94±0.41		9.57±0.005		30.80±0.14	
160	17.37±1		12.69±0.01		39.01±0.16	
200	18.08±0.00		16.4±1.2		49.69±0.026	

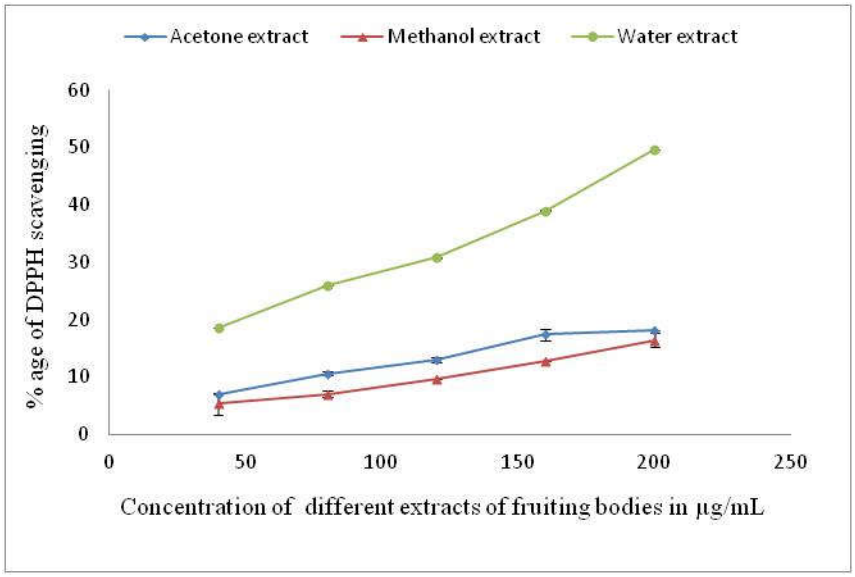


Figure 3.1: Free radical scavenging activity of the different extracts of fruiting bodies of *T. eurhizus*

Table 3.2: Free radical scavenging activity (%) of the different extracts of mycelium of *T. eurhizus* at different concentrations

Conc. (µg/mL)	Acetone extract (%)	IC_{50} value (µg/mL)	Methanol extract (%)	IC_{50} value (µg/mL)	Water extract (%)	IC_{50} value (µg/mL)
40	3.7±0.008	711.67	3.11±0.03	881.76	4.42±0.7	715.57
80	6.9±0.112		5.67±0.54		6.73±0.05	
120	9.42±0.5		6.59±1		9.23±0.6	
160	12.45±0.01		9.34±0.8		13.07±0.21	
200	14.64±0.45		12.45±0.36		14.8±1.4	

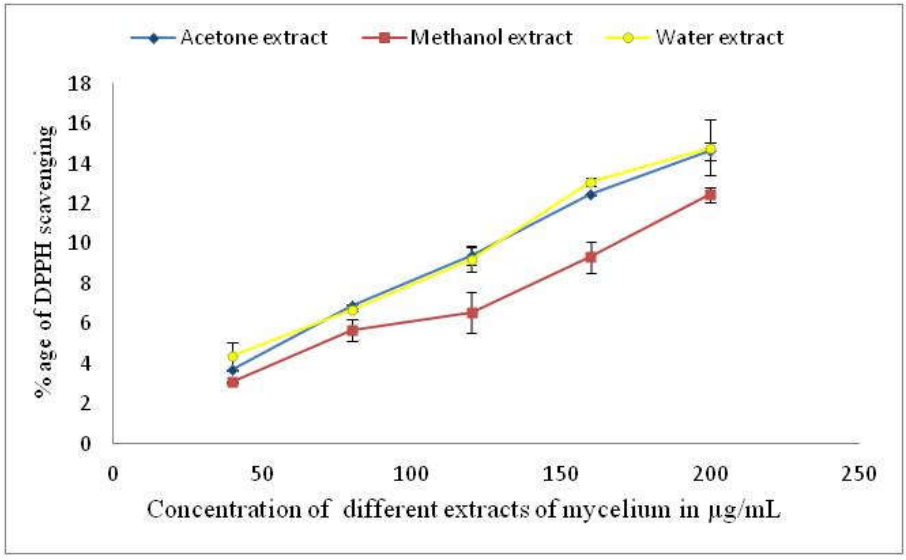


Figure 3.2 Free radical scavenging activity of the different extracts of mycelium of *T. eurhizus*

DISCUSSION

Mushrooms collected from the Bagidhar near Baijnath in District Kangra were used to carry out all the experimental work. Macroscopic characters (shape, colour and size of pileus and stipe) and microscopic characters (SEM, characteristics of spore, hyphae and basidia) observed in present study were almost similar to the taxonomic studies carried out by Purkayastha and Chandra[23]; Pegler and Vanhaecke[24]; Karun and Sridhar[25].

Present study proposed that the best radial mycelial growth was supported by Hagam`s-Modess medium (4.9 cm) followed by Potato dextrose agar medium (4.83 cm), YESS medium (4.66 cm), Dimmick agar extract medium (4.53 cm) and Modified malt extract agar medium (4.4 cm) respectively.

Screening antibacterial activity

Methanol extract of fruiting bodies showed maximum zone of inhibition of 17.03, 15.33, 18.23 and 17.41 mm against the *L. monocytogenes, S. aureus, P. aeruginosa* and *E. coli*, respectively at 100% concentration than the acetone extract of fruiting bodies which showed the maximum zone of inhibition of 17.51, 11.16, 13.95 and 16.14 mm for *L. monocytogenes, S. aureus, P. aeruginosa* and *E. coli,* respectively.

Methanol extract of mycelium was found to possess high activity against all the tested bacteria. The maximum inhibition zones of 21.39, 15.76, 17.71 and 16 mm were observed for *L. monocytogenes, S. aureus, P. aerugenosa* and *E. coli,* respectively. While the acetone extract of mycelium showed maximum inhibition zone of 18.26, 11.86, 13.56 and 12.36 mm against the *L. monocytogenes, S. aureus, P. aeruginosa* and *E. coli* respectively. The water extract of mycelium and fruiting bodies of *T. eurhizus* were also investigated but no zone of inhibition was found against all the tested bacteria.

Antimicrobial activity of methanol extract of fruiting bodies of some wild edible mushroom including *T. eurhizus* from West Bengal were evaluated by Giri *et a.l*[26] against some microorganisms. Results displayed that majority of mushrooms showed antimicrobial activity. *T. eurhizus* showed positive inhibition against *E. coli* and *Proteus vulgaris*. Kumari *et al*.[27] investigated the antimicrobial activity of methanol extract of *Termitomyces heimii* against *Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumonia, E. coli* and *Pseudomonas*. Results showed highest zone of inhibition against *E. coli* i.e. 22 mm at 200 µg/mL concentration and lowest zone of inhibition (10 mm) was observed in *S. aureus* and *Pseudomonas*. Therefore, it can be cocluded that *T. eurhizus* displayed antibacterial activity against *E. coli, S. aureus* and *P. aerugenosa.*

Screening antioxidant activity

In present investigation, the antioxidant potential of *T. eurhizus* was measured by using DPPH free radical scavenging assay. The acetone, methanol and water extract of fruiting bodies showed IC_{50} value of 626.38, 690.31, 211.21 µg/mL respectively and 711.67, 881.76, 715.57 µg/mL values for acetone, methanol and water extract of mycelium of *T. eurhizus* respectively. The extracts of fruiting bodies of *T. eurhizus* were found to exhibit good antioxidant potential than mycelium extracts. Water extract of fruiting bodies of *T. eurhizus* displayed significant IC_{50} value of 211.21 µg/mL. Tibuhwa[28] analysed the antioxidant activity of methanol extract of six termiterium mushrooms along with *T. eurhizus* from Tanzania by using the DPPH free radical scavenging assay and observed EC_{50} value of 0.36 mg/mL.

Termitomyces medius was examined for antioxidant activity by using the DPPH free radical scavenging assay by Mitra *et al*.[29]. Results showed that EC_{50} value of ethanol extract of *T. medius* was 0.5±0.04 mg/mL. Mitra *et al*.[30] analyzed the antioxidant activity of the ethanol extract of *T. microcarpus* by using the DPPH free radical scavenging assay at 517 nm and EC_{50} value was found to be 0.60±0.01 mg/mL.

Kumari *et al*.[27] investigated the antioxidant potential of methanol extract of mycelia of *Termitomyces heimii* by DPPH free radical scavenging assay and showed higher radical scavenging activity which was 87.32% at 400 µl/mL due to the presence of phenolic compounds content and EC_{50} value was around 2.63 mg/mL in the extract. Present investigations on *T. eurhizus* revealed good antioxidant potential which was approximately similar and higher than the above discussed *Termitomyces* species by different workers.

CONCLUSION

The results of our study confirm the Hagam`s-Modess medium as the best solid media for culturing the mycelia of *T. eurhizus*. The different extracts of fruiting bodies showed good antioxidant activity than the mycelial extracts of *T. eurhizus*. While mycelial extracts exhibited significant antibacterial activity than the fruiting bodies extracts. *T. eurhizus* present a valuable source of antimicrobial and antioxidant agents, but require more studies to improve the growth conditions, extraction methods to retrieve larger amounts of biologically active compounds and isolation of new compounds from *T. eurhizus* which are responsible for antioxidant and antimicrobial activity. Present investigations on *T. eurhizus* have established base for the future studies which can be undertaken nutritive analysis of fruiting bodies and phytochemical analysis of the mycelium.

ABBREVIATIONS

DPPH: 2,2-diphenyl-1-picrylhydrazyl; DAE: Dimmick Agar Extract; HMM: Hagam`s-Modess Medium; MMEA: Modified Malt Extract Agar; PDA: Potato Dextrose Agar.

ACKNOWLEDGEMENT

Authors want to put on record their gratitude to the Chairperson, Department of Biosciences, Himachal Pradesh University Shimla for providing the lab facilities and also the local people for shearing their valuable information.

CONFLICT OF INTRUST: There is no conflict of interest.

REFERENCES

1. Manoharachary, C., Sridhar, K., Singh, R., Adholeya, A., Suryanarayanan, T.S., Rawat, S. and Johri, B.N. 2005. Fungal biodiversity: Distribution, conservation and prospecting of fungi from India. *Current Science*. 89: 58-71.
2. Kirk, P.M., Cannon, P.F., David, J.C. and Stalpers, J.A. 2001. Ainsworth & Bisby`s dictionary of the fungi, 9th edition CAB international, Wallingford.
3. Wasser, S.P. 2002. Medical mushrooms as a source of antitumour and immunostimulating polysaccharides. *Appl. Microbiol. Biotech.* 60: 258-274.
4. Sarbhoy, A.K., Agarwal, D.K. and Varshney, J.L. 1996. Fungi of India (1982-1992). CBS Publisher and Distributer, New Delhi.
5. Das, K. 2010. Diversity and conservation of wild mushroom in Sikkim with special reference to Barsey Rhododendron Sanctuary. *NeBio-An International Journal of Environment and Biodiversity*. 1: 1-13.
6. Hawksworth, D.L. 2001. Mushroom: the extent of unexplored potential. *International Journal of Medicinal Mushrooms*. 3: 333-337.
7. Rai, M., Tdke, G. and Wasper, S.P. 2005. Therapeutic potential of mushroom. *Natural Product Radiance.* 4: 246-257.
8. Deshmukh, S.K. 2004. Biodiversity of tropical basidiomycetes as source of novel secondary metabolites. In Jain PC (ed). Microbiology and Biotechnology for Sustainable Development. CBS Publishers and Distributeors, New Delhi, India. 121-140.
9. Lakhanpal, T.N. 2014. Mushroom Biodiversity in India: Prospects and Potential. Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8).

10. Wasson, G.R. 1969. Soma-Devine mushroom of immortality XIII. New Court Brace and World Inc. New York. 318.
11. Kupra, J., Anke, T., Oberwinler, G., Schramn, G. and Steglich, W. 1979. Antibiotics from basideomycetes VII. *Crinipellis stripitaria* (Fr.) Pat. *Journal of Antibiotics*. 32: 130-135.
12. Suzuki, H., Liyama, K., Yoshida, O., Yamazaki, S., Yamamoto, N. and Toda, S. 1990. Structural characterization of the immunoactive and antiviral water-solublized lignin in an extract of the culture medium of *Lentinus edodes* mycelia (LEM). *Agricultural and Biological Chemistry*. 54: 479-487.
13. Thatoi, H. and Singdevsachan, S.K. 2014. Diversity, nutritional composition and medicinal potential of Indian mushrooms: A review. *African Journal of Biotechnology*. 13: 523-545.
14. Semwal, K.C., Bhatt, R.P. and Upadhyay, R.C. 2005, The Genus *Amanita* from Garhwal Himalaya region of India. *Mushroom Research*. 14: 50-55.
15. Light, H.D.F., Andersen, A. and Aanen, D.K. 2005. *Termitomyces* sp. associated with the termite *Macrotermes natalensis* has a heterothallic mating system and multinucleate cells. *Mycological Research*. 109: 314-318.
16. Srivastava, B., Dwivedi, A.K. and Pandey, V.N. 2012. Sociobiology and natural adaption of termite and *Termitomyces* in different forest division of Gorkhpur region. *Environment Pharmacology and Life Science*. 2: 32-36.
17. Ogundana, S.K. and Fagade, O.E. 1982. Nutritive value of some Nigerian edible mushrooms. *Food Chemistry*. 8: 263-268.
18. Tibuhwa, D.D. 2012a. Termitomyces species from Tanzania, their cultural properties and unequalled Basidiospores. *Journal of Biology and Life Science*. 3.
19. Khowala, S., Ghosh, A.K. and Sengupta, S. 1992. Saccharificationof xylem by an amyloglucosidase of *Termitomyces clypeatus* and synergism in the presence of xylanase. *Applied Microbiology and Biotechnology*. 37: 287-292.
20. Ghorai, S., Banik, S.P., Verma, D., Chowdhury, S., Mukherjee, S. and Khowala, S. 2009. Fungal biotechnology in food and feed processing. *Food Research International*. 42: 577-587.
21. Prakash, V., Rana, S. and Sagar, A. 2016. Studies on antibacterial activity of leaf extracts of *Rhododendron arboretum* and *Rhododendron companulatum*. *Int. J. Curr. Microbiol. App. Sci*. 5: 315-322.
22. Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. *Nature*. 181:1199-1200.
23. Purkayastha, R.P. and Chandra, A. 1975. *Termitomyces eurhizus*- A new Indian edible mushroom. *Transactions of the British Mycological Society*. 64: 168-170.
24. Pegler, D.N. and Vanhaecke, M. 1994. Termitomyces of southeast Asia. *Kew Bulletin*. 49: 717-736.
25. Karun, N.C. and Sridhar, K.R. 2013. Occurrence and distribution of *Termitomyces* (Basidiomycota, Agaricales) in the Western Ghats and on the west cost of India. *Czech Mycology*. 65: 233-254.
26. Giri, S., Biswas, G., Pradhan, P., Mandal, S.C. and Acharya, K. 2012. Antimicrobial activity of basidiocarps of wild edible mushrooms of West Bengal, India. *International Journal of PharmTech Research*. 4: 1554-1560.
27. Kumari, S.G., Kumar, R.P.K. and Geeta, S. 2017. In vitro antioxidant and antibacterial activity of edible mushroom (*Termitomyces heimii*). *International Journal of Development Research*. 7: 17531-17535.
28. Tibuhwa, D.D. 2012. Antiradical and antioxidant activities of methanolic extracts of indigenous termitarian mushroom from Tanzania. *Food Science and Quality Management*. 7:13-23.
29. Mitra, P., Sarkar, J., Mandal, N.C. and Acharya, K. 2014. Phytocheemical analysis and evaluation of antioxidant efficacy of ethanolic extract of *Termitomyces medius*. *International Journal of Pharmaceutical sciences Review and Research*. 27: 261-266.
30. Mitra, P., Mandal, N.C. and Acharya, K. 2016. Mycochemicals and antioxidant activity of polyphenol-rich fraction of *Termitomyces microcarpus*. *International Food Research Journal*. 23: 2384-2389