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EVALUATION OF ANTIMICROBIAL ACTIVITY OF SOME OF THE SELECTED BASIDIOMYCETOUS FUNGI

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.ABSTRACT

The aim of the present study was to evaluate antimicrobial activity of *Ganoderma applanatum, Tricholoma giganteum* and *Bolbitius vitellinus*. The methanolic extract of basidiocarps and mycelial mat of all the above mentioned mushrooms was analyzed. The methanolic extracts were tested against three Gram positive, six Gram negative bacteria and two fungal cultures. Out of three mushrooms *Ganoderma applanatum* was found to be more active against bacterial cultures, followed by *Tricholoma giganteum* and *Bolbitius vitellinus*. *G.applanatum* and *T.giganteum* was found to be more sensitive to Gram Positive bacteria than Gram negative bacteria, however *B.vitellinus* was found to very active against Gram negative bacteria especially *E.coli. B.vitellinus* showed highest antifungal activity against *Candida albicans,* other two mushrooms. Hence these mushrooms can be exploited for their useful metabolites in Food and Pharmaceutical industries.

KEY WORDS: ***Ganoderma applanatum, Tricholoma giganteum*, *Bolbitius vitellinus*, Antimicrobial activity.**



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INTRODUCTION

Medicinal mushrooms have an established history of use in traditional oriental therapies. Modern clinical practice in Japan, China, Korea, and other Asian countries continues to relay on mushroom derived preparations. Edible mushrooms are among the best sources of many essential nutrients. Their nutritional value can be compared favorably with that of meat, eggs and milk[1, 2]. They are used as food stuffs containing both nutritive and medicinal values [3, 4, 5]. They play an important role in preventing diseases such as hypertension, hypercholesterolemia and cancer [6, 7]. The occurrence of antibiotics in mushrooms is less documented [8] and there are only few reviews that summaries the antibacterial activity from mushrooms [9, 10, 11, 12]. Literature survey revealed that very little work has been carried out in this regard from India. Several mushroom species belonging to the Polyporaceae family are now being regarded as the next candidate producers of valuable medicines[13]. Early studies proposed that most antibacterial components from basidiomycetous fungi were potent against Gram-positive bacteria only [14] but more recent work showed that extracts were also active against Gram negative organisms, *Proteus vulgaris* and *Escherichia coli*, in vitro[15]. Some *Agaricus* species (*A. campestris*, *A. bisporus* and *A. arvensis*) have been shown to produce compounds active against both Gram-positive and Gram-negative bacteria[11] and, extracts from *Lentinus edodes* have also been shown to be active against both types of bacteria[16]. *Ganoderma* species more often in combination with chemotherapeutic agents can be used to treat various bacterial diseases. [17] reported that methanol extract of *G.lucidum* showed remarkable antibacterial activity against *E.coli, Salmonella* species and *B.subtilis*. [18] observed that 75% of polypore fungi that have been tested show strong antimicrobial activity. [19] isolated an antifungal protein Ganodermin from *Ganoderma lucidum* and reported that it was sensitive to *Botrytis cinerea, Fusarium oxysporum, Phsalospora piricola*[20]. have studied the influence of extracts isolated from *Ganoderma lucidum* on *E.coli, Bacillus, S.aureus* and *Salmonella* species. The aqueous fruiting body extract showed maximum zone of inhibition against *Bacillus* species while least zone of inhibition was reported for *E.coli* and *Salmonella* species[21]. observed maximum antibacterial activity of methyl australate, a derivative from *G.lucidum* against *E.coli* and *Psuedomonas aeruginosa* followed by *Staphylococcus.* [22] investigated the antibacterial activity of a chloroform extract of *G.lucidum* from Iran. The results of disc diffusion tests showed that the chloroform extract had growth inhibitory effects on *B.subtilis and S.aureus*. [23] investigated the antibacterial activitly of *Lentinus tuberregium* (Fr) the exracts were more active to inhibit the growth of *Salmonella flexiner, Micrococcus luteus and Salmonella typhi.* [24] reported that the acetone extract of *Ganoderma lucidum* possessed strong antibacterial activity which was most inhibitory against *Klebsiella pneumoniae, E.coli, B.Subtilis* and *S.typhi.* The water and ethanolic extract were found to be not much effective against all the strains. [25] reported that the tooth paste made of *Ganoderma lucidum* was found to be active against *Candida albicans*. [26] reported that the polysaccharides or triterpenoids from *Ganoderma* showed activities against Herpes simple virus, Hepatitis B virus, HIV, and Epstein-Barr virus in vitro or in animal models. [27] reported the aqueous extract of *Ganoderma lucidum* showed maximum activity against *Salmonella typhi* and also reported that *Mucor indicus* and *Aspergillus flavus* was also sensitive to the extract of *Ganoderma lucidum*. .[28] reported that methanolic extract of *T. giganteum* showed activity against *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Candida albicans.* [29, 30] reported the presence of Trichogin a peptide molecule in *Tricholoma gigantium* is responsible for antifungal activity against *Fusarium oxysporum*, *Mycosphaerella arachidicola* and *Physalospora piricola*, as well as inhibitory effect on HIV-1 reverse transcriptase. The aim of present work was to carry out invitro experiments to screen antimicrobial activity of *Ganoderma applanatum, Tricholoma giganteum* and *Bolbitius vitellinus*. Since no much literature is available with regard to their activites of

methanolic extract of basidiocarps of the above mentioned mushrooms. The present study reveals it biopharmaceutical properties.

MATERIALS AND METHODS

Collection of Mushrooms and identification

Basidiocarps were collected from various localities in Bangalore, [31] classification concepts were used in identification of Agaricales. *Ganoderma* species was identified using keys provided by[32].

Microorganisms tested

The pure culture of the microorganisms such as *Bacillus subtilis(2655), Proteus vulgaris(2813), Staphylococcus aureus(2127), Escherichia coli(2685), Micrococcus flavus(2376), Pseudomonas aeruginosa(5029), Salmonella abony(2257), Klebsiella pneumoniae (2706), Pseudomonas fluroscence (clinical isolate), Cryptococcus sps(3349), Candida albicans (3100)* were used which obtained from NCL, Pune.

Preparation of alcoholic extract form basdiocarps

In the present study, the dried basidiocarps mushrooms were used, where the mushroom material was made into fine powder. 10 grams of mushroom powder was subjected for extraction using 100 ml of methanol at room temperature for 24 hrs and filtered through Whatman No. 4 filter paper. The extracts were recovered by filtration and kept at 40^0c in a rotary vacuum evaporator [33]. The residue was collected and store at 4^0c for further use.

Screening of antibacterial and antifungal activity of mushroom extract

The antimicrobial activities of the extracts were determined by the Kirby-Bauer agar diffusion method according to NCCLS standards [34, 35]. All the bacterial cultures were grown in Nutrient broth and incubated at 37^0c for 24 hours. The fungal cultures were incubated at room temperature for 48 hours in Potato Dextrose Agar. Nutrient agar and Potato Dextrose Agar of about 20 ml were poured into each sterilized Petri plates aseptically and allowed to solidify. With the help of sterilized cork borer a well of 6mm diameter was made at the centre of the agar plates. The bacterial and fungal suspension was swabbed on the solidified media using sterilized swabs in the respective plates. The mushroom extract was dissolved in Dimethylsulfoxide and the suspension was sterilized by filtration through a membrane filter[36]. The mushroom extract of about 100 microliters was filled into the wells of the agar plates. The bacterial plates were incubated at 37^0 c for 24 to 48 hours. The fungal plates were incubated at 28^0c for 3-4 days. Inhibitory activity of DMSO was also tested. Nystatin was used as the reference disc for Fungi. And Penicillin and Tetracycline was used as the reference disc for bacteria. After the period of incubation the zone of inhibition was measured, tabulated and compared with reference disc. Statistical Analysis was performed using ANOVA- Analysis of variation [37] where ever applicable.

RESULTS AND DISCUSSION

Antimicrobial activity

The methanolic extracts of the Basdiocarps of 3 different wild mushrooms were tested against three Gram positive Bacteria (*B.subtilis, S.aureus, M.flavus*), six Gram negative bacteria (*P.vulgaris, E.coli, P. aeruginosa, P.fluroscence, S.abony, K. pneumoniae)* and two different fungi *(Cryptococcus sps and C.albicans*). All the extracts showed different degree of antimicrobial activity at 1000 µg/ml as mentioned in the table 01, graph 01 and four representative photos of antimicrobial activity by Agar diffusion assay showing zone of inhibition are shown in figure 01, 02, 03 and 04. Out of three mushrooms, the methanolic extract of *G.applanatum* was found to be more active followed by *T.giganteum* and *B.vitellinus*. *G.applanatum* showed activity against most of the Bacteria, however activity was found to be highest for Gram Positive Bacteria, *B.subtilis*, followed by *S.aureus* and *M.flavus*. It even showed the activity against Gram negative bacteria, amoung Gram negative bacteria the extract showed maximum activity against, *E.coli* and *K. pneumoniae* and very less activity against *P.fluroscence* and no activity against *P.aeruginosa*. The extract of *G.applanatum* even was found to be active against *Cryptococcus sps* and *C.albicans* but the activity was very less when compared to that of standard antibiotics. [38, 39]

had reported the similar activity against Gram negative bacteria by *G.applanatum*. [40] also reported the similar activity by *G. pfeifferi.* [21] however they had reported that the activity against Gram positive bacteria were more than Gram negative bacteria. [41, 42] had documented greater resistance to Gram negative bacteria by *Ganoderma* species. This may be due to different extraction procedures followed. 5,8-epidioxy-5a,8a-ergosta-6,22-dien-3b-ol reported by[43,44] isolated from *Ganoderma applanatum* (Pers.) proved to be active against a number of gram positive and gram-negative microorganisms[38]. *Tricholoma giganteum* also showed antimicrobial activity against both Gram positive and Gram negative bacteria, however showed maximum activity against Gram positive bacteria such as *B. subtilis*, followed by *M.flavus* and *S.aureus*. Among Gram negative bacteria highest activity was against *P.vulgaris* followed by *K. pneumoniae, P.aureginosa, S.abony* and very less activity against *P.fluroscence.* Among fungi there was no activity against *Cryptococcus* sps and had little activity against *Candida albicans.* Similar report has been reported by[28]. *B.vitellinus* showed least activity when compared with *G.applanatum* and *T.giganteum*. However the extract showed more activity against Gram negative bacteria than Gram positive bacteria. E.coli was found to be more sensitive to the methanolic extract of *B.vitellinus* followed by *P.vulgaris, K. pneumoniae* and *S.abony, P.fluroscence* was found to be resistant to the extract. *Among Gram positive bacteria B.subtilis* was found to be more sensitive followed by *M. flavus and S. auereus.* The extract of *B.vitellinus* was found to be more active against *C.abicans* when compared to that of *G.applanatum and T.giganteum.* However when compared with that of standard antibiotics the antimicrobial activity of mushroom extract was found to be less.

Table 1
Antimicrobial activity of mushrooms

Test Organisms	Zone of inhibition in mm				
	G.applanatum **(1000µg/ml)**	***T.giganteum*** **1000µg/ml)**	***B.vitellinus*** **(1000µg/ml)**	**Tetracycline (30µg/ml)**	**Nystatin (30µg/ml)**
B.subtilis	42[c]	51[a]	14[e]	17[b]	0
P.vulgaris	50[a]	41[b]	25[b]	16[b]	0
S.aureus	38[e]	32[d]	9[g]	20[a]	0
E.coli	40[d]	25[f]	32[a]	8[d]	0
M.flavus	32[f]	35[c]	14[e]	20[a]	0
P. aeruginosa	0[j]	35[c]	16[d]	8[d]	0
S.abony	35[e]	30[e]	14[e]	11[c]	0
K. pneumoniae	45[b]	36[c]	19[c]	5[e]	0
P.fluroscence	11[g]	12[g]	0[h]	10[c]	0
Cryptococcus	6[h]	0[i]	0[h]	0[f]	21[a]
C.albicans	3[i]	7[h]	12[f]	0[f]	18[b]
CD(0.5)	0.3595	0.3687	0.3494	0.6381	0.4887

Graph 01
Antimicrobial activity of mushrooms

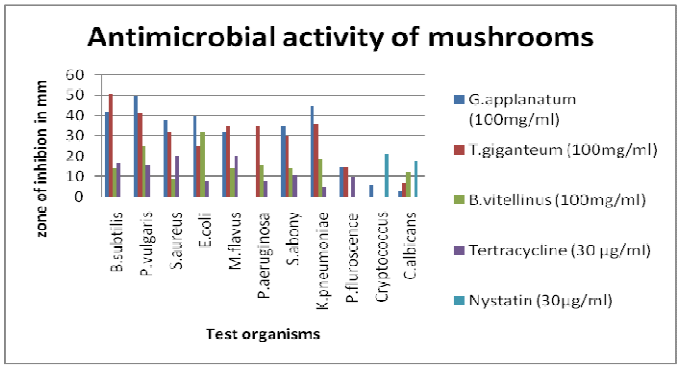


Figure 01-04
Agar diffusion assay.

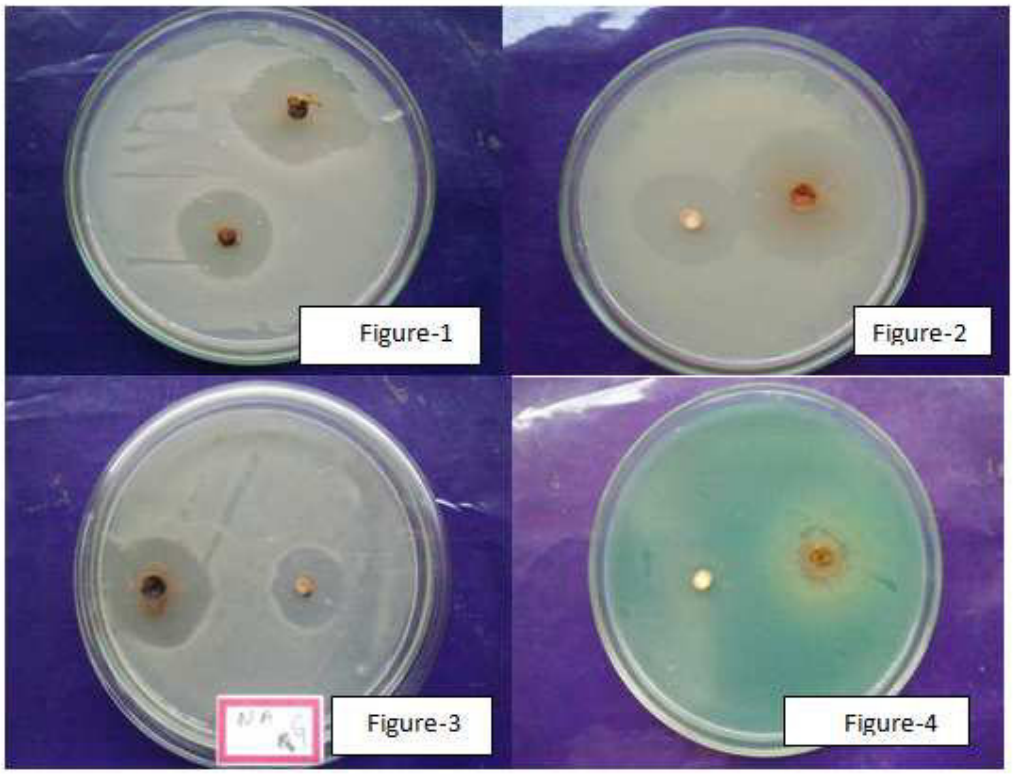


Figure 01 -04
Mushroom extract showing zone of inhibition against different bacterial cultures

CONCLUSION

In conclusion, our research suggests that *Ganoderma applanatum, Tricholoma giganteum, and Bolbitius vitellinus, c*ollected from Bangalore, has high potential; hence it would be used as natural antimicrobial agent. Hence further studies on isolation and identification of active compounds may provide a better source for developing new therapeutic agents, especially towards multiple drug resistant bacteria and fungi. Hence this property of these mushrooms can be exploited in food and pharmaceutical industries.

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