JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

IN VITRO EFFICACY OF BRACKET FUNGI FOR THEIR POTENTIAL ANTIMICROBIAL ACTIVITY

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doi: 10.15414/jmbfs.2016.6.2.818-822

ARTICLE INFO

Received 27. 4. 2015
Revised 12. 11. 2015
Accepted 12. 7. 2016
Published 3. 10. 2016

Regular article

OPEN ACCESS

ABSTRACT

This study was conceptualized to reveal the anti-microbial potential of bracket fungi, *viz.* - *Ganoderma lucidum* (Curtis) Karst. and *Polyporus officinalis* (syn. *Laricifomes officinalis*) (Vill.) Fr. extracts through *in vitro* approach. The aqueous and ethanolic extracts were assessed against four phytopathogenic fungi (*Alternaria solani*, *Curvularia lunata*, *Aspergillus terreus* and *Fusarium oxysporum*) along with bacteria (*Escherichia coli* and *Bacillus subtilis*). Ethanolic extract of *G. lucidum* had significant growth inhibition effect against *A. solani*, *C. lunata*, *A. terreus* and *F. oxysporum* at 500ppm and 1000ppm concentrations. On the other hand, aqueous extract showed complete inhibition at 1000ppm and 500ppm concentrations against *A. terreus* and *F. oxysporum*. Ethanolic extract of *P. officinalis*, complete fungal growth inhibition was observed against *A. solani* and *A. terreus* at 1000ppm concentration while for *C. lunata* and *F. oxysporum*, complete inhibition was observed at 1000ppm and 250ppm concentration. Similarly, the aqueous extract of same bracket fungus, showed maximum inhibition of *A. solani*, *A. terreus* and *F. oxysporum* at 1000ppm concentration but *C. lunata* had maximum inhibition at 250ppm concentration. The antibacterial action of ethanolic extract of *G. lucidum* was observed against *E. coli* having inhibitory zone (0.09 mm) at 1000 ppm concentration and *B. subtilis* had inhibitory zone (0.05mm) at 250ppm concentration. But no inhibitory zones were observed in *E. coli* and *B. subtilis* with aqueous extract of *G. lucidum*. Whereas, the ethanolic extract of *P. officinalis* showed a maximum inhibitory zone (10 mm) at 1000 ppm concentration for *E. coli* and a remarkable inhibitory zone (0.2 mm) at 250 ppm concentration for *B. subtilis*. While an inhibitory zone (0.3 mm) was observed in *E. coli* at 250 ppm concentration of aqueous extract, but no inhibitory zone was observed for *B. subtilis* at any concentration of the aqueous extract of *P. officinalis*. Based on the study, it can be concluded that *G. lucidum* and *P. officinalis* are having considerable potential as anti-fungal and anti-bacterial action, respectively.

Keywords: Antimycotic activity, Antibacterial action, Bracket macro-fungi, Minimum inhibitory concentration, Percent fungal growth inhibition

INTRODUCTION

Human use of fungi for food preparation or preservation and other purposes is extensive and has a long history. Mushroom farming and gathering are large industries in many countries. Many fungi are producers of antibiotics such as penicillin, cephalosporin. Widespread use of these antibiotics for the treatment of bacterial diseases such as tuberculosis, syphilis, leprosy, and many others began in the early 20th century and continues to play a major part in anti-bacterial chemotherapy.

Polypores and bracket fungi are members of the Aphyllophorales, a group of morphologically complex, terrestrial basidiomycetes. Many of these fungi are saprobic wood decayers and as such, these fungi are most often found on logs, stumps, or other dead wood. These fungi also exhibit medicinal properties and used in remedies of various human ailments. There are some reports that revealed their medicinal usages and biological activities. **Johnston (2005)** reported that *Ganoderma lucidum* is used in TCM (Traditional Chinese Medicine) for the treatment of cancers. **Sliva (2006)** and **Stanley *et al.* (2005)** also mentioned that *G. lucidum* is popular medicinal mushroom and used in TCM in Asian countries over the past two millennia and preserve human vitality and promote longevity. *G. lucidum* is one of the most used "*herbs*" in Asia and preclinical studies have established that the polysaccharide fractions have potent effects **(Chen *et al.* 2006)**. **Lin *et al.* (2006)** referred *G. tsugae* Murrill as the Chinese mushroom '*Songshan lingzhi'*, which is cultivated in Taiwan and used extensively to treat diseases. **Stanley *et al.* (2005)** have demonstrated that *G. lucidum* induces apoptosis, inhibits cell proliferation and suppress cell migration of highly invasive human prostrate cancer cells PC-3. **Pero *et al.* (2005)** reported that combination of extracts of *Cordyceps sinensis* (Berk.) Sacc., *Grifola blazei* Gray, *G. frondosa* (Dicks.) Gray, *Trametes versicolor* (L.) Lloyd and *G. lucidum* into a formulation designed to optimise different modes of immuno-stimulatory actions and yet that would avoid metabolic antioxidant competition. The activities of hypertension, hyperglymecia, hepatitis, chronic bronchitis, bronchical asthma, liver protection and others have been demonstrated from the fruiting bodies and cultured mycelia of *G. lucidum* **(Yuen and Gohel, 2005)**. **Shieh *et al.* (2001)** concluded that the hepatic and renal protective mechanism of *G. lucidum* might be because of its superoxide scavenging effect. Later, **Lakshmi *et al.* (2006)** studied the antimutagenic activity of the methanolic extract of the fruiting bodies of *Ganoderma lucidum* occurring in South India. The activity was assayed by Ames *Salmonella* mutageniticity test using histidine mutants of *Salmonella typhimurium* tester strains. The result revealed that *G. lucidum* extract restored antioxidant defence and prevented hepatic damage. Beside this, several compounds with Biomedicinal properties like triterpenoids **(Kim and Kim, 1999)** and polysaccharides **(Bao *et al.*, 2002)** have been isolated from *Ganoderma* species.

The extract of *Ganoderma lucidum* also showed inhibitory actions against pathogenic fungi and bacteria. **Wang and Ng (2006)** isolated 'Ganodermin', an antifungal protein from fruiting bodies of *G. lucidum* (Curtis) Karst. Ganodermin inhibited the mycelial growth of *Botrytis cinerea* Pers., *Fusarium oxysporum* Schlecht. and *Physalaspora piricola* Nose. **Wang *et al.* (2006)** isolated an antifungal polypeptide from fresh fruiting bodies of *Polyporus alveolaris* (DC.) Bond. & Sing.. The antifungal polypeptide, designated as alveolarin, had demonstrated an inhibitory action on mycelial growth in *B. cinerea* (De Bary) Whetzel, *Fusarium oxysporum* Schlecht., *Mycosphaerella arachidicola* Jenkins and *Physalospora piricola* Nose.

According to **Hleba *et al.* (2014),** the methanolic fungi extracts of both fungi *Ganoderma lucidum* and *Trametes versicolor* showed the strongest antimicrobial activity against *Saccharomyces cerevisiae*. Equally, lower antimicrobial activity of fungi extracts against Gram-positive microorganisms was detected by them. But they didn't find antimicrobial activity of fungi extracts against Gram-negative bacteria and *Candida albicans*. On the other hand, antibacterial activity has been observed against Gram positive bacteria from the basidocarp extract of *G. lucidum* **(Kim *et al*., 1993)** and *G. orogonense* Murr. **(Brian, 1951)**. **Sudirman and Muziyati (1997)** observed that seven Indonesian *Ganoderma* species inhibit the growth of *Bacillus subtilis*. **Coletto and Mondino (1991)** noted that methanolic extract of the mycelial and culture extract of *G. recinaceum* Boud and *G. lucidum* inhibited *Bacillus subtilis* Cohn. *G. recinaceum* also inhibited *Staphylococcus aureus* Rosen. Ethanolic extract from *G. lucidum* mycelium demonstrated significant anti-inflammatory effects **(Kendrick, 1985)**.
There are also some studies on inhibitory and metabolic activities of polypores and bracket fungi in India. **Quereshi *et al*. (2010)** studied the antimicrobial activity of various solvent (aqueous, ethanol, methanol and acetone) extracts (40µg/ml) of *Ganoderma lucidum* against six species of bacteria, *viz*. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis Salmonella typhi* and *Pseudomonas aeruginosa*. **Sheikh *et al*. (2015)** have reported the potential antioxidative role of two mushrooms *G. lucidum* and *Trametes hirsuta* in free radical systems. **Bains *et al.* (2015)** evaluated the potential of methanolic and ethyl acetate extracts of *Agaricus* sp. *Morchella* sp. and *Canthrellus* sp. against four bacterial strains *K. pneumonia*, *P. aeruginosa*, *S. aureus* and *E. coli*. Similarly, **Sivaprakasam *et al.* (2011)** tested aqueous and methanolic extracts of *T. hirsuta* fruit body against five pathogenic fungi (*Penicillium* sp., *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus* and *Mucor indicus* and five bacterial stains (*E. coli*, *P. aeruginosa*, *Salmonella typhi*, *S. aureus* and *Streptococcus mutans*).
Polypores and bracket fungi are the major source of biologically active natural products extracted from the species of the diverse fungal phylum Basidomycota, furnishing a rich variety of active secondary metabolites and polysaccharides **(Zjawiony, 2004)**. In the search for active compounds from *Ganoderma* species, the majority of research has been performed on extracts from the fruiting body and there have been fewer studies on extracts from the liquid cultivated mycelium **(Russell and Paterson, 2006)**. It appears that there are a number of biologically active compounds to be found in the mycelium and the benefits of liquid cultivation over solid cultivation include: the ability to manipulate the cultivation medium to optimise mycelia growth; a shorter cultivation time; and less contamination. In fact, the reason that some of the Ganoderma preparations are not yet available as medicines may be from difficulties relating to mass production **(Smith *et al*., 2002)**.

Considering the previous research reports on this antimicrobial aspects of *Ganoderma lucidum* and other species of bracket fungi and the fact that there is little or very scarce work on the antimycotic and antibacterial activities of extracts of *G. lucidum* and *Polyporous officinalis*, the present work was undertaken with an aim to find out the antimicrobial potential of these two bracket fungi against phytopathogenic fungi and bacteria through *in vitro* approach.

MATERIALS AND METHODS

Material collection

The fresh fruiting bodies of bracket fungi were collected from forest area near Solan district (situated between 76.42 and 77.20 degree East longitude and 30.05 and 31.15 degree north latitude) of Himachal Pradesh, India and sample was identified and confirmed with the help of Fungal Herbarium/Museum at National Centre for Mushroom Research and Training at Solan, Himachal Pradesh and Department of Plant Pathology, University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India.

Preparation of aqueous extracts

Aqueous extracts were prepared by adding 0.01g of dried powdered fruit bodies of *Ganoderma lucidum* and *Polyporus officinalis* in 10 ml of distilled water. The aqueous extract containing cellular debris was filtered through a fine muslin cloth to get a fine aqueous extract and then centrifuged at 1000 rpm and the supernatant was filtered through the vacuum filter unit to remove any pathogenic fungi or bacteria and then transferred to a sterilized glass container. It was considered as pure 1000 ppm solution and leveled as stock solution and from which respective concentrations (500 ppm and 250 ppm solutions) prepared by adding required amount of distilled water. These extracts were used for further investigation.

Preparations of ethanolic extracts

These were also prepared in the similar way as mentioned above but ethanol was used instead of distilled water.

Test pathogenic fungi used

Efficacy of fruiting bodies of two bract fungi as mentioned above on the four fungi *i.e. Aspergillus terreus*, *Alternaria solani*, *Fusarium oxysporum* and *Cavularia lunata* were investigated by using standard method **(Nene and Thapliyal, 1993)**.

Determination of antimycobiotic activity

For each fungi *i.e. Alternaria solani*, *Aspergillus terreus*, *Curvularia lunata*, *Fusarium oxysporum* three Petri plates were used. The technique used was poisoned food technique **(Parkash *et al*., 2005)**. All the glassware in use, are sterilized properly by autoclaving for 15 min at 121 °C. Medium was poured in the Petri plates mixed with different concentrations (*i.e.* 1000ppm, 500ppm, 250ppm and control) of sample extracts under sterile conditions. Mycelia disc were taken from pure cultures of test fungi previously grown on PDA and were placed in the centre of the plates aseptically. Suitable negative control was also kept where the mycelia disc were grown under same conditions on PDA medium without supplementation of any sample compound. For positive control, Actidion (Cycloheximide) at 1000 ppm (0.01%) was used in the medium as standard antifungal drug for comparison of antifungal action. The plates were grown at 27 °C. The efficacy in each case was determined by measuring the additional mycelial growth each time after a proximal three days. The radial growth of the colony was measured in four directions at right angle to each other and average was taken. The percentage inhibition of the fungal extract was calculated by using formula:

$$Percentage\ inhibition\ (\%) = \frac{Control - Treated}{Control} \times 100$$

where C is control and T is treatment. Data was also analyzed statistically for SEm and CV. The experiment was carried out in triplicate replications

Test bacterial strains used

The bacterial strains used were *Bacillus subtilis* (APR-4) and *Escherichia coli* (EC-1). These bacterial strains were procured from Kurukshetra University, Kurukshetra and Himachal Pradesh University, Shimla and were maintained on nutrient agar for further experiment.

Determination of antibacterial activity

The antibacterial susceptibility test was carried out using agar well diffusion test **(Perez *et al*., 1990)**. The bacteria were cultured overnight at 35 °C in nutrient agar **(Zhang *et al*., 2004)**. The final cell concentrations of bacterial rods were in the range of 10^6-10^7 CFU ml^{-1}.
The activity was checked against two pathogens *i.e. Escherchia coli* (EC-1) and *Bacillus subtilis* (APR- 4) and quantified using MIC (Minimum Inhibitory Concentration). Four nutrient agar plates (two for ethanolic and aqueous culture of each bacterium) were prepared and the media was made to settle down for 10 minutes. Wells were punched in the plates with the help of borer of size 8mm to have uniform wells but the medium was not removed. The bacterial culture of respective strains were spread on the nutrient agar medium with the help of cotton swab and left for 5 minutes. Later, the medium from the wells was removed and the aqueous and ethanolic extracts of different concentrations (*i.e.*1000ppm, 500ppm, 250ppm) were poured into the different wells in the respective Petri plates with the help of micropipette. No extract was added in to the negative control well. For positive control, Ciprofloxacin (0.05% = 500ppm) was used in a single well as standard antibacterial drug for comparison of antibacterial action in a separate Petri plate. The plates were incubated at 37^0C for overnight and inhibition zone were recorded. The effect of fungal extract was expressed in terms of average diameter of the zone of inhibition measured in millimeter. Each test was carried out in triplicate replications.

RESULTS AND DISCUSSION

Effect of *Ganoderma lucidum* (ethanolic extract) on test fungi

There was complete inhibition of mycelia growth at 250ppm, 500ppm, 1000ppm for *Alternaria solani*. *Aspergillus terreus*, *Fusarium oxysporum* were also completely inhibited at 1000ppm and 500ppm but minimum inhibition observed at 250ppm; whereas *Curvularia lunata* was also completely inhibited at 1000ppm and 500ppm and 250ppm concentration. In control sets, however no fungal or mycelia inhibition was observed.

Table 1 Effect of *Ganoderma lucidum* (ethanolic extract) on mycelial growth of different fungi

S. no.	Concentration (ppm)	*Alternaria solani* (% Inhibition)	*Aspergillus terreus* (% Inhibition)	*Curvularia lunata* (% Inhibition)	*Fusarium oxysporum* (% Inhibition)
1.	Actidion (Cy) 1000	99.8 ± 0.10	100 ± 0.0	100 ± 0.0	100 ± 0.0
2.	Control (without extract)	0 ± 0	0 ± 0	0 ±0	0±0
3.	250	100 ±0	82±0.626	100±0	93.6±0.379
4.	500	100±0	100±0	100±0	100±0
5.	1000	100±0	100±0	100±0	100±0

Effect of *Ganoderma lucidum* (aqueous extract) on test fungi

In case of *Alternaria solani*, minimum mycelial inhibition was observed at 250ppm, 500ppm and maximum at 1000ppm whereas *Aspergillus terreus* was not completely inhibited at any concentrations. A minor mycelial inhibition percent was observed in case of *A. terreus* but *Curvularia lunata* and *Fusarium oxysporum* were completely inhibited at 1000ppm, 500ppm and 250ppm.

Table 2 Effect of *Ganoderma lucidum* (aqueous extract) on mycelial growth of different fungi

S. no.	Concentration (ppm)	*Alternaria solani* (% Inhibition)	*Aspergillus terreus* (% Inhibition)	*Curvularia lunata* (% Inhibition)	*Fusarium oxysporum* (% Inhibition)
1.	Actidion (Cy) 1000	50.5 ± 0.10	70.6 ± 0.05	70.0 ± 0.06	60.5 ± 0.06
2.	Control (without extract)	0 ± 0	0 ± 0	0 ±0	0±0
3.	250	82±0.622	05±1.030	100±0	100±0
4.	500	82±0.622	04±044	100±0	86±0.533
5.	1000	87±0.536	05±1.030	100±0	100±0

Effect of *Polyporus officinalis* (ethanolic extract) on test fungi

In *Alternaria solani* and *Aspergillus terreus* there was a complete mycelia inhibition observed at 1000ppm and minimum inhibition was observed at 500ppm, 250ppm concentrations respectively. Whereas *Fusarium oxysporum* had maximum mycelia inhibition at 1000ppm and 500ppm followed by 250ppm concentration but *Curvularia lunata* was completely inhibited at 1000ppm, 500ppm and 250ppm concentrations.

Table 3 Effect of *Polyporus officinalis* (ethanolic extract) on mycelial growth of different fungi

S. no.	Concentration (ppm)	*Alternaria solani* (% Inhibition)	*Aspergillus terreus* (% Inhibition)	*Curvularia lunata* (% Inhibition)	*Fusarium oxysporum* (% Inhibition)
1.	Actidion (Cy) 1000	99.8 ± 0.10	100 ± 0.0	100 ± 0.0	100 ± 0.0
2.	Control(without extract)	0 ± 0	0 ± 0	0 ±0	0±0
3.	250	75±0.737	95±0.48	100±0	90±0.32
4.	500	89±1.025	92±0.418	100±0	94±0.362
5.	1000	100±0	100±0	100±0	94±0.36

Effect of *Polyporus officinalis* (aqueous extract) on test fungi

In case of *Fusarium oxysporum*, *Curvularia lunata* and *Alternaria solani*, there were maximum mycelia inhibition observed at 1000ppm conc. while minimum mycelia inhibition was observed at 500ppm, 250ppm concentrations respectively. Whereas a little mycelia inhibition was observed at 1000ppm conc. but there was very little mycelial inhibition observed at 250ppm, 500ppm concentrations respectively.

Table 4 Effect of *Polyporus officinalis* (aqueous extract) on mycelial growth of different fungi

S. no.	Concentration (ppm)	*Alternaria solani* (% Inhibition)	*Aspergillus terreus* (% Inhibition)	*Curvularia lunata* (% Inhibition)	*Fusarium oxysporum* (% Inhibition)
1.	Actidion (Cy) 1000	50.5 ± 0.10	70.6 ± 0.05	70.0 ± 0.06	60.5 ± 0.06
2.	Control (without extract)	0 ± 0	0 ± 0	0 ±0	0±0
3.	250 ppm	81±0.642	1.0±0.0	74±0.766	84.7±0.47
4.	500 ppm	82±0.622	1.5±1.0	78±0.702	88±0.518
5.	1000 ppm	87±0.536	5.7±1.030	82±0.622	89.7±0.58

Effect of *Ganoderma lucidum* (ethanolic and aqueous extracts) on test bacteria

The minimum inhibitory concentration (MIC) was analyzed for aqueous and ethanolic extracts against two bacterial strains *Bacillus subtilis* (strain APR-4, Gram positive) and *Escherichia coli* (EC-1, Gram negative). The antibacterial action of ethanolic extract of *G. lucidum* was observed against *E. coli* having inhibitory zone (0.09 mm) at 1000 ppm concentration and *B. subtilis* had inhibitory zone (0.05mm) at 250 ppm concentration respectively. The aqueous extract of *G. lucidum* had shown zero antibacterial activity/potential against *E. coli* and *B. subtilis* at any test concentrations.

Table 5 Activity of aqueous and ethanolic extracts of *Ganoderma lucidum* against different bacteria

S.no.	Bacterial Type	Bacterial strain	Type of extract	Minimum Inhibitory conc. (MIC)	Inhibition zone (mm)
1.	Gram negative	*Escherichia coli*	Ethanol Extract	1000	0.09± 0.06
2.	Gram positive	*Bacillus subtilis*	Ethanol Extract	250	0.05± 0.06
3.	Gram positive	*E. coli*	Aqueous Extract	-	No inhibitory zone
4.	Gram positive	*B. subtilis*	Aqueous Extract	-	No inhibitory zone

Effect of *Polyporus officinalis* (ethanolic and aqueous extracts) on test bacteria

The ethanolic extract of *P. officinalis* showed a maximum inhibitory zone (10mm) at 1000 ppm concentration for *E. coli* and a remarkable inhibitory zone (0.2 mm) at 250 ppm concentration for *B. subtilis*. While an inhibitory zone (0.3 mm) was observed in *E. coli* at 250 ppm concentration of aqueous extract; no inhibitory zone was observed for *B. subtilis* at any concentration of the aqueous extract of *P. officinalis*.

Table 6 Activity of aqueous and ethanolic extracts of *Polyporus officinalis* against different bacteria

S.no.	Bacterial strain	Type of extract	Minimum Inhibitory conc. (MIC%)	Inhibition zone (mm)
1.	*Escherichia coli*	Aqueous Extract	250	.03 ± 0.006
2.	*Bacillus subtilis*	Aqueous Extract	-	No zone of inhibition
3.	*E. coli*	Ethanol Extract	1000	10± 0.0
4.	*B. subtilis*	Ethanol Extract	250	.02± 0.006

Effect of Ciprofloxacin as a positive control on test bacteria

Ciprofloxacin (0.05%= 500ppm) in ethanol and distilled sterilized water were taken as positive controls and it showed maximum zone of inhibitions (18.4mm) against *E. coli* and (20.2 mm) against *B. subtilis* in ethanolic extract while maximum zone of inhibitions (13.0 mm) against *E. coli* and (12.0 mm) against *B. subtilis* in aqueous extract (see Table -7). But relatively antibacterial activity was low in sample extracts in comparison to standard positive control drug.

Table 7 Activity of aqueous and ethanolic extracts of Ciprofloxacin as a positive control against different bacteria

S.no.	Bacterial strain	Type of extract	Minimum Inhibitory conc. (MIC in ppm)	Inhibition zone (mm)
1.	*E. coli*	Ciprofloxacin (50µg/100µl) (in Ethanol)	500	18.4 ± 0.041
2.	*B. subtilis*	Ciprofloxacin (50µg/100µl) (in Ethanol)	500	20.2 ± 0.06
3.	*E. coli*	Ciprofloxacin (50µg/100µl) (aqueous)	500	13.0 ± 0.04
4.	*B. subtilis*	Ciprofloxacin (50µg/100µl) (aqueous)	500	12.0 ± 0.02

Several previous researches have proved the antimicrobial activity of *Ganoderma lucidum* (basidiocarp and methanolic/ ethanolic extracts) against a wide range of zoo- and phyto-pathogenic fungi (*Botrytis cinerea*, *Fusarium oxysporum*, *Physalaspora piricola*, etc.) and a few bacteria (*Bacillus subtilis*, etc.) **(Sudhirman and Mujiyati, 1997; Coletto and Mondino, 1991; Kim and Kim, 1999)**. On the other hand, inhibitory action on mycelial growth in *Botrytis cinerea*, *Fusarium oxysporum*, *Mycosphaerella arachidicola* and *Physalospora piricola* was demonstrated by an antifungal polypeptide from fresh fruiting bodies of *Polyporus alveolaris* designated as '*alveolarin'*. Similarly the positive antimicrobial effect was seen in case of *G. lucidum* and *P. officinalis* against the test microbes. So, the present study is exceptional on the aspect that it deals with the antimycobiotic potential of *G. lucidum* against some different phyto-pathogenic fungi as stated above; along with prospecting the potential of an unreported bracket fungus *i.e. Polyporus officinalis* (as a source of anti-fungal and anti-bacterial compounds.

To conclude this, the first report on the screening of the antimicrobial activity against the four fungal pathogens, *viz.* - *Aspergillus terreus*, *Curvularia lunata*, *Alternaria solani* and *Fusarium oxysporum* and antibacterial activity against the two bacteria, *viz.* - *Bacillus subtilis* and *Escherichia coli* can serve as prospective potential aspect for much needed novel antibiotics. Further work is needed toward the evaluation of their antimicrobial potential against a wider range of microorganisms, identification of the bioactive principles and elucidation of their mechanism of action.

Acknowledgements: Authors are thankful to Rain Forest Research Institute (ICFRE), Jorhat, Assam, India and Shoolini Institute of Life Sciences and Business Management, Solan, Himachal Pradesh, India for providing the all possible facilities to carry out the experiment. They also thank Dr. Dinesh Kumar Chatanta for providing the culture of *Bacillus subtilis* (APR-4) for this study.

REFERENCES

BAINS, A., TRIPATHI, A., SHARMA, A. 2015. Antibacterial and Antioxidant properties of Wild Mushrooms collected from Himachal Pradesh. *International Journal of Biology, Pharmacy and Allied Sciences*, *4*(10), 6161-6170.

BAO, X.F., WANG, X.S., QUN, D., FANG, J.N., LI, X.Y. 2002. Structural features of immunologically active polysaccharides from *Ganoderma lucidum*. *Phytochemistry*, *59*, 175-181. http://dx.doi.org/10.1016/s0031-9422(01)00450-2

BRIAN, P.W. 1951. Antibiotics produced by mushroom. *The Botanical Review*, *17*, 357-430. http://dx.doi.org/10.1007/bf02879038

CHEN, X., HI, Z., YANG, X.X., HUANG, M., GAO, Y., TANG, W., CHAN, SY. 2006. Monitoring of immune responses to a herbal immuno-modulator in patients with advanced colorectal cancer. *International Immunopharmacology*, *6*, 499-508. http://dx.doi.org/10.1016/j.intimp.2005.08.026

COLETTO, B.M.A., MONDINO, P. 1991. Antibiotic activity in Basidiomycetes: V. Antibiotic activity of mycelia and cultural filtrates. *Allionia* (*Turin*), *30*, 61–64.

JOHNSTON, N. 2005. Medicinal mushroom cuts off prostrate cancer cells' blood supply. *Drug Discovery Today*, *10*, 23-24. http://doi:10.1016/S1359-6446(05)03657-3

KENDRICK, B. 1985.The fifth Kingdom. Mycologue Publication: Waterloo, Ontario, Canada, 375 p. ISBN-10: 0969223706.

KIM, B.K, CHO, H.Y., KIM, J.S., KIM, H.W., CHOI, E.C. 1993. Studies on constituents of higher fungi of Korea(LXVIII).Antitumour components of the cultured mycelia of *Ganoderma lucidum*. *The Korean Journal of Physiology and Pharmacology*, *24*, 203-212. http://dx.doi.org/10.1007/bf02861310

KIM, H.W., KIM, B.K. 1999. Biomedicinal triterpenoids of *Ganoderma lucidum* (Curt.:Fr.) P.Karst. (Aphyllophoromycetideae). *International Journal of Medicinal Mushrooms*, *1*, 121-138. http://dx.doi.org/10.1615/intjmedmushrooms.v1.i2.20

LAKSHMI, B., AJITH, T.A., NAYANA, J., JANARDHANAN, K.K. 2006. Antimutagenic activity of methanolic extract of *Ganoderma lucidum* and its effect on hepatic damage caused by benzo(a)pyrene. *Journal of Ethnopharmacology*, *107*, 297-303. http://dx.doi.org/10.1016/j.jep.2006.03.027

LIN, J.Y., CHEN, M.L., CHIANG, B.L., LIN, B.F. 2006. *Ganoderma tsugae* supplementation alleviates bronchoalveolar inflammation in an airway sensitization and challenge mouse model. *International Immunopharmacology*, *6*, 241-251. http://doi:10.1016/j.intimp.2005.08.009

LUKÁŠ, H., VUKOVIĆ, N., PETROVÁ, J., KAČÁNIOVÁ, M. 2014. Antimicrobial Activity of Crude Methanolic Extracts from *Ganoderma lucidum* and *Trametes versicolor*. *Animal Science and Biotechnologies*, *47* (2), 89-93.

NENE, Y.L., THAPLIYAL, P.N. 1993. Evaluation of fungicides. In: fungicides in plant disease control (3rd ed.). Oxford and IBH publishing Co. Pvt. Ltd.: New Delhi, 531 p. ISBN: 8120402227.

PARKASH, V., AGGARWAL, A., SHARMA, S., GUPTA, S., GUPTA, A. 2005. Antifungal activity of *Withania somnifera* L Dunal on some Phytopathogenic Fungi. *Bulletin of the National Institute of Ecology*, *15*, 215-218.

PEREZ, C., PAULI, M., BAZERQUE, P. 1990. An antibiotic assay by the agar-well diffusion method. *Acta Biologiae et Medecine Experimentalis*, *15*, 113-115.

PERO, R.W., AMIRI, A., SHENG, Y., WELTHER, M., RICH, M. 2005. Formulation and *in vitro/in vivo* evaluation of combining DNA repair and immune enhancing nutritional supplements. *Phytomedicine*, *12*, 255-263. http://dx.doi.org/10.1016/j.phymed.2004.01.008

QURESHI, S., PANDEY, A.K., SANDHU, S.S. 2010. Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts. *People's Journal of Scientific Research*, *3*(1), 2010 9-13.

RUSSELL, R., PATERSON, M. 2006. Ganoderma – A therapeutic fungal biofactory. *Phytochemistry*, *67*, 1985–2001. http://dx.doi.org/10.1016/j.phytochem.2006.07.004

SHEIKH, I.A., VYAS, D., LONE, R., SINGH, V. 2015. *Ganoderma lucidum* and *Trametes hirsuta* as potent antioxidants in free radical systems *in vitro*. *World Journal of Pharmacy and Pharmaceutical Sciences*, *4*(5), 1695-1710.

SHIEH, Y.H., LIU, C.F., HUANG, Y.K., YANG, J.Y., WU, I.L., LIN, C.H., LI, S.C. 2001. Evaluation of the hepatic and renal-protective effects of *Ganoderma lucidum* in Mice. *The American Journal of Chinese Medicine*, *29*, 501-507. http://dx.doi.org/10.1142/s0192415x01000526

SIVAPRAKASAM, E., KAVITHA, D., BALAKUMAR, R., SRIDHAR, S., KUMAR, J. S. 2011. Antimicrobial activity of whole fruiting bodies of *Trametes hirsuta* (Wulf. Fr.) Pil. against some common pathogenic bacteria and fungus. *International Journal of Pharmaceutical Sciences and Drug Research*, *3*(3), 219-221.

SLIVA, D. 2006. *Ganoderma lucidum* in cancer research. *Leukemia Research*, *30*, 767-768. http://dx.doi.org/10.1016/j.leukres.2005.12.015

SMITH, J.E., ROWAN, N.J., SULLIVAN, R. 2002. Medicinal mushrooms: a rapidly developing area of biotechnology for cancer therapy and other bioactivities. *Biotechnology Letters*, *24*, 1839–1845.

STANLEY, G., HARVEY, K., SLIVOVA, V., JIANG, J., SLIVA, D. 2005. *Ganoderma lucidum* supresses angiogenesis through the inhibition of secretion of VEGF and TGF-B1 from prostrate cancer cells. *Biochemical and Biophysical Research Communications*, *330*, 46-52. http://dx.doi.org/10.1016/j.bbrc.2005.02.116

SUDHIRMAN, L.I., MUJIYATI, S. 1997. Preliminary detection of antimicrobial activity of fruiting bodies' extract of tropical *Ganoderma* sp.. In: Proceedings of the 1st International Symposium on *Ganoderma lucidum* in Japan. Toyoigakusha Co. Ltd., Tokyo; p. 154-159.

WANG, H., NG, T.B. 2006. Ganodermin, an antifungal protein from fruiting bodies of the medicinal mushroom *Ganoderma lucidum*. *Peptides*, *27*, 27-30. http://doi:10.1016/j.peptides.2005.06.009

WANG, X.M., YANG, M., GUAN, S.H., LIU, R.X., XIA, J.M. 2006. Quantitative determination of six major triterpenoids in *Ganoderma lucidum* and related species by high performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, *41*, 838-844. http://dx.doi.org/10.1016/j.jpba.2006.01.053

YUEN, J.W., GOHEL, M.D. 2005. Anticancer effects of *Ganoderma lucidum*: a review of scientific evidence. *Nutrition and Cancer*, *53*, 11-17. http://doi:10.1207/s15327914nc5301_2.

ZHANG, Y.J., NAGAO, T., TANAKA, T., YANG, C.R., OKABE, H., KOUNO, I. 2004. Antiproliferative activity of the main constituents from *Phyllanthus emblica*. *Pharmaceutical Bulletin*, *27*, 251-255. http://dx.doi.org/10.1248/bpb.27.251

ZJAWIONY, J.K. 2004. Biologically Active Compounds from Aphyllophorales (Polypore) Fungi. *Journal of Natural Products*, *67*(2), 300-310. http://dx.doi.org/10.1021/np030372w