International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (1), pp. 001-009, January, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

In vitro evaluation of anti-staphylococcal activity of *Ganoderma lucidum*, *Ganoderma praelongum* and *Ganoderma resinaceum* from Pune, India

Abdulghani Ameri[1]*, Jitendra G. Vaidya[2] and Subhash S. Deokule[3]

[1]Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahwaz, Iran.
[2]Department of Botany, University of Pune, Pune-411007, India.

Accepted 25 January, 2019

Antimicrobial activity of *Ganoderma praelongum, Ganoderma resinaceum* and *Ganoderma lucidum* were evaluated against thirty strains of clinical isolates of methicillin resistant and methicillin sensitive *Staphylococcus aureus* (MSSA) by the well agar diffusion and microtiter plate dilution. Chloroform, ethyl acetate, methanol, and water were used as extractive solvents. Standard antibiotic disks of methicillin, vancomycin, linezolid, and mupirocin were used for susceptibility testing of the test microorganisms. Maximum activity of crude extracts was exhibited by ethyl acetate. Sesquiterpeoids extract of *G. praelongum* (35.67 ± 0.62 mm) and minimum inhibitory concentration (MIC) of 0.390 – 6.25 mg/ml. Diterpenoids and triterpenoids displayed moderate activity while polysaccharides IIIa and IIIb showed weak activity. All bacterial strains were resistant to polysaccharides I and II.

Key words: *Ganoderma praelongum, Ganoderma resinaceum, Ganoderma lucidum, Staphylococcus aureus,* antimicrobial activity.

INTRODUCTION

Several species of *Ganoderma* have been used in traditional Asian medicines for thousands of years. Collectively, the *Ganoderma* are being investigated for a variety of biological activities including antitumor, immunomodulatory, cardiovascular, respiratory, antihepatotoxic and antinociceptive effect (Ha et al., 2000; Chang and Mshigeni, 2001). The responsible bioactive components belong to several chemical groups, very often they are polysaccharides or triterpenes. The best example is *Ganoderma lucidum,* which not only contains more than 120 different triterpenes but also polysaccharides, proteins and other bioactive components (Zhou and Gao, 2002; Kim and Kim, 1999). Therefore, *G. lucidum* products with different triterpenes, polysaccharides or combinations of these two groups are most likely to result in different pharmacological activities (Leung et al., 2002). Moreover, *G. lucidum* are rich in nutraceuticals, a new class of compounds with potential therapeutic values which are extracted from mycelium or fruiting bodies of mushrooms (Chang and Buswell, 1996).

One interesting aspect of *G. lucidum* performance is its antimicrobial effect due to the extracts derived from this mushroom which contain bacteriolytic enzyme, lysozyme and acid protease (Klaus and Miomir, 2007). Numerous studies on antimicrobial potential of *G. lucidum* against different microorganisms are well documented.

However, research in *Ganoderma* worldwide has been focused on only few species; with special emphasis to *G. lucidum* (80 to 90% of publications are on *G. lucidum*). The reason could be that the historical description and pictorial descriptions of Reishi / Ling zhi (the specimen described in Traditional Chinese Medicinal system) matches to *G. lucidum* and also that the cultivation protocols (liquid, solid state fermentation and artificial cultivation) have been standardized which provides the required amount of study material, while for other species, researchers have to rely on the wild collections only.

*Corresponding author. E-mail: kazem1920@hotmail.com. Tel: +98-916-611-3274.

MATERIALS AND METHODS

Samples of medicinal mushrooms were collected from various localities in and around Pune, Maharashtra, India. Mushroom samples were identified as *Ganoderma praelongum* Murrill, *Ganoderma resinaceum* Boud and *G. lucidum* gr. at Agarkar Research Institute (ARI), Pune, India and were deposited at ARI under accession numbers AMH No. 9295, AMH No. 9296 and AMH No. 9297, respectively.

Culture of *G. praelongum* Murrill mycelia (solid state)

White marginal tissue separated from the basidiocarp with a sterile surgical blade from a clean area was collected for culture. The inner tissue was then excised with a sterile surgical blade under sterile conditions. The surfaces were cut, separated and the unexposed tissue was inserted in potato dextrose agar supplemented with 50 ppm Benomyl (Fungicide Benalate, Sigma) and 20 ppm Novobiocin antibiotic (Sigma). Sufficient growth of mycelia was cultivated on sterile 300 g of *Pennisetum glaucum* grains softened by boiling in water and placed in polyethylene bags for three weeks. Following incubation, mycelia were extracted with different solvents and evaluated for antimicrobial activity.

Extraction

All mushroom samples were milled to a fine powder (Hammer Mill, Basant, India.). Twenty gram-portions of each of the medicinal mushrooms were extracted with 100 ml of different solvents (Harborne, 1988). Following filtration, the filtrate was concentrated under a reduced pressure (Medica Instrument MFG Co., India) until a semisolid substance was obtained. The condensate was dissolved in a required quantity of Dimethyl Sulfoxide to achieve desired concentration.

Test microorganisms

A total of 28 clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) were collected from different hospitals in Mumbai and Pune . In addition, one standard strain of methicillin sensitive *S. aureus* (MSSA) and one standard strain of MRSA were included in the study for comparison. All strains of bacteria were confirmed on KB004 HiStaph^{TM} Kit (HiMedia, Mumbai) and were subjected to antimicrobial susceptibility testing employing the Kirby-Bauer method (Bauer et al., 1966). Four antibiotics namely methicillin (5 mg), vancomycin (5 mg), linezolid (30 mg) and mupirocin (5 mg) (HiMedia, Mumbai).

Antimicrobial activity

Antimicrobial activity of crude extracts was evaluated by the well agar diffusion method (Stoke and Ridgway, 1980). Wells of 7 mm in diameter were made on each plate using sterile cork borer. Using a micropipette, 50 µl of the crude extract was introduced into each well, all plates were carried out in triplicates and control experiments were also set up by adding respective solvent as well as DMSO. In addition, vancomycin was included in the study as a positive control. Plates were incubated at 37°C for 24 h.

Extraction of bioactive components

The strain of mushroom that displayed maximum activity was further examined for antimicrobial activity of its bioactive components like diterpenoids, triterpenoids, sesquiterpenoids and various fractions of polysaccharides viz., I, II, IIIA and IIIB. For extraction of these fractions, the methods described by Harborne (1988) were adopted.

Minimal inhibitory concentration (MIC)

MIC of sesquterpenoids of *G. praelongum* was determined by microdilution method (Eloff, 1998). Using (12 X 8 wells) microtitre plates. A solution containing 50 mg/ml sesquiterpenoid was prepared. Aliquots (50 µl) of the solution and 200 µl of the inoculum were pipetted to the well labelled as A. Content were thoroughly mixed and serial dilutions were made through tube H. Standard vancomycin was used as the reference drug (2 to 8 µg/well) and methanol served as negative control. Plates were incubated for 24 h at 37°C. Bacterial growth was determined after addition of 50 µl *p*-iodonitrotetrazolium violet (0.2 mg/ml, Himedia, Mumbai).

Effect of temperature, pH and time

The effect of temperature on antimicrobial activity of sesquiterpenoids was evaluated by incubating aliquots of 1 mg/ml at 0.0, 4.0, 20., 30., 40, 50, 60, 70, 80, 90 and 100 for 30 min and 121°C for 20 min (Sharifi et al., 2006). Antimicrobial activity was determined by the disk diffusion method against two strains of MRSA. Each paper disk (Oxoid, England) was impregnated with 10 µl of sesquiterpenids extract using nutrient medium seeded with 10^6 cfu/ml of overnight growth of the respective strain of bacteria. The results were compared with that of the control (extract at zero time).

In addition, antimicrobial activity of sesquiterpenoids extract was evaluated at different pH values by the disk diffusion method against two strains of MRSA. The pH values of the extract was changed by using the following buffers: acetate (pH 3.0, 4.0 and 5.0), phosphate (6.0, 7.0 and 8.0), Tris Hcl (pH 9.0) and carbonate – bicarbonate (pH 10.0 and 11.0) (Sharifi et al., 2006).

In addition, the effect of time on antimicrobial activity of sesquiterpenoids extract was evaluated by storing the extracts at room temperature and 4°C for a period of 12 months. Results were compared with the activity of fresh extracts.

RESULTS

Antimicrobial activity of 50 mg/ml of different extracts of G. *resinaceum, G. lucidum* and *G. praelongum* fruiting bodies and *G. praelongum* biomass were tested against 30 strains of MRSA and MSSA. Maximum activity was displayed by ethyl acetate extracts of *G. praelongum* (mean 19.50 ± 0.91 mm), followed by *G. resinaceum* (mean 14.29 ± 0.14 mm), *G. lucidum* (mean 14.14 ± 0.21 mm) fruiting bodies and *G. praelongum* biomass (mean 13.69 ± 1.23 mm), respectively. Weak to moderate activities were displayed by chloroform and methanol extracts of all mushrooms against MRSA and MSSA. Aqueous extracts of all mushroom strains did not possess antibacterial activity against any of the bacterial strains (data not presented).

Antimicrobial susceptibility testing conducted against target microorganisms are depicted in Table 1. A wide range of activity (8.0 to 30.0 mm) was observed by mupirocin (30 mg) against *S. aureus*, however, four strains were resistant. Intermediate activity was shown by

Table 1. Antimicrobial sensitivity testing of *S. aureus* (diameter of zone of inhibition in millimeter).

Strains code	Standard Antibiotics			
	Methicillin (30 mg)	Mupirocin (30 mg)	Vancomycin (30 mg)	Linezolid (30 mg)
MRSA-1	R	11.0 ± 0.7	12.0 ± 0.81	25.0 ± 1.63
MRSA-2	R	11.0 ± 0.7	12.0 ± 1.63	25.0 ± 1.77
MRSA-3	R	9.0 ± 1.41	11.0 ± 1.41	26.0 ± 0.4
MRSA-4	R	8.0 ± 0.4	13.0 ± 1.22	24.0 ± 2.44
MRSA-5	R	8.0 ± 0.81	10.0 ± 1.08	26.0 ± 1.47
MRSA-6	R	10.0 ± 0.81	14.0 ± 1.22	24.0 ± 0.81
MRSA-7	R	9.0 ± 1.41	14.0 ± 1.63	25.0 ± 2.44
MRSA-8	R	8.0 ± 0.81	12.0 ± 0.81	26.5 ± 1.22
MRSA-9	R	9.0 ± 1.41	12.0 ± 0.81	25.0 ± 1.41
MRSA-10	R	9.0 ± 1.63	14.0 ± 0.4	25.0 ± 2.04
MRSA-11	R	9.0 ± 1.41	12.0 ± 0.81	27.0 ± 2.04
MRSA-12	R	8.0 ± 0.4	13.0 ± 1.47	33.0 ± 0.81
MRSA-13	R	12.0 ± 1.47	13.5 ± 1.22	30.0 ± 1.22
MRSA-14	R	R	15.0 ± 1.22	28.0 ± 0.81
MRSA-15	R	11.0 ± 1.63	14.0 ± 0.81	26.0 ± 0.81
MRSA-16	R	9.0 ± 1.08	15.0 ± 0.81	25.0 ± 2.44
MRSA-17	R	8.0 ± 0.81	16.5 ± 0.4	29.0 ± 2.44
MRSA-18	R	8.0 ± 0.84	12.0 ± 0.81	27.0 ± 1.08
MRSA-19	R	24.0 ± 0.7	14.0 ± 1.47	26.0 ± 1.22
MRSA-20	R	8.0 ± 0.81	16.0 ± 1.22	30.0 ± 1.22
MRSA-21	R	R	13.0 ± 1.41	24.0 ± 4.08
MRSA-22	R	9.0 ± 1.47	15.0 ± 0.81	27.0 ± 1.22
MRSA-23	R	25.0 ± 1.08	18.0 ± 1.22	25.0 ± 3.26
MRSA-24	R	25.0 ± 0.81	13.0 ± 1.22	28.0 ± 1.22
MRSA-25	R	R	14.0 ± 0.4	27.0 ± 1.22
MRSA-26	R	R	16.0 ± 2.04	32.0 ± 0.81
MRSA-GBT	R	30.0 ± 0.81	14.0 ± 2.44	30.0 ± 0.4
MSSA- GBT	14.0 ± 0.81	12.0 ± 0.4	14.0 ± 0.81	25.0 ± 0.81
ATCC No 29737	15.5 ± 0.95	10.0 ± 0.81	15.0 ± 0.81	28.0 ± 0.81
ATCC No. 9144	R	8.0 ± 1.63	15.0 ± 1.63	25.0 ± 3.26
Mean	-	11.84	13.73	26.78
SD	-	6.22	1.69	2.31

SD: standard deviation, n = 6, R: resistant.

vancomycin (30 mg) with a narrow range of 10.0 to 18.8 mm. Nonetheless, maximum activity was exhibited by linezolid (30 mg) with a range of 24.0 to 33.0 mm. And with the exception of two MSSA that were included for comparison, all strains of MRSA showed resistance to methicillin (30 mg).

Results of bioactive components of *G. praelongum* fruiting bodies are presented in Table 2. As shown in this table, the most effective activity was exhibited by sesquiterpenoids extract of fruiting bodies (17.33 to 35.67 mm). Moderate activity was displayed by triterpenoids and diterpenoids (12.25 to 16.67 mm) and (14.33 to17.33 mm), respectively. The polysaccharide IIIa and IIIb fractions showed weak to moderate activity (9.33 to 12.67 mm) and (7.0 to 12.67 mm), respectively. All strains of bacteria were resistant to polysaccharide fractions I and II. Mycelial sesquiterpenoids extract showed moderate activity as compared to fruiting bodies.

Minimal inhibitory studies showed a low range of 0.390 to 6.25 mg/ml and 1.256 to 12.5 mg/ml for sesquiterpenoid extract of fruiting body and mycelium, respectively. This low value of MIC indicates that these components possess promising potential to combat some of the antibiotic resistant bacteria including the multi - drug resistant MRSA. However, further evaluation and phytochemical analysis to isolate and identify the bioactive component is required.

Studies on the effect of different temperatures showed

Table 2. Antimicrobial activity of sesquiterpenoid components of *G.praelongum* fruiting bodies and mycelium against *S. aureus*.

Strain code	Sesquiterpene fruiting body (30 mg/ml)	Sesquiterpene biomass (30/ml)	Vancomycin (30 mg) (+ control)	Methanol (30 mg) (- control)
MRSA-1	30.0 ± 1.63	14.33 ± 0.47	12.0 ± 0.81	NA
MRSA-2	25.33 ± 0.62	15.33 ± 1.24	12.0 ± 1.63	NA
MRSA-3	21.67 ± 3.09	14.33 ± 0.47	11.0 ± 1.41	NA
MRSA-4	26.67 ± 1.54	14.67 ± 0.47	13.0 ± 1.22	NA
MRSA-5	30.67 ± 1.24	14.67 ± 0.47	10.0 ± 1.08	NA
MRSA-6	24.33 ± 1.24	14.0 ± 0	14.0 ± 1.22	NA
MRSA-7	33.33 ± 0.47	14.0 ± 0.81	14.0 ± 1.63	NA
MRSA-8	25.0 ± 1.63	12.6 7± 0.47	12.0 ± 0.81	NA
MRSA-9	19.67 ± 1.24	12.33 ± 0.47	12.0 ± 0.81	NA
MRSA-10	20.33 ± 2.05	13.67 ± 1.24	14.0 ± 0.4	NA
MRSA-11	18.5 ± 1.63	15.0 ± 0.81	12.0 ± 0.81	NA
MRSA-12	18.33 ± 3.29	14.67 ± 0.47	13.0 ± 1.47	NA
MRSA-13	19.5 ± 1.22	14.0 ± 1.08	13.5 ± 1.22	NA
MRSA-14	17.33 ± 2.09	13.0 ± 1.41	15.0 ± 1.22	NA
MRSA-15	19.67 ± 1.54	9.67 ± 0.94	14.0 ± 0.81	NA
MRSA-16	32.33 ± 1.24	14.67 ± 1.69	15.0 ± 0.81	NA
MRSA-17	31.0 ± 1.41	**15.67** ± 0.47	16.5 ± 0.4	NA
MRSA-18	21.67 ± 1.24	15.33 ± 0.94	12.0 ± 0.81	NA
MRSA-19	20.0 ± 0.4	14.0 ± 1.63	14.0 ± 1.47	NA
MRSA-20	19.67 ± 1.24	7.0 ± 0	16.0 ± 1.22	NA
MRSA-21	33.0 ± 0.81	15.0 ± 1.63	13.0 ± 1.41	NA
MRSA-22	**35.67** ± 0.62	7.0 ± 0	15.0 ± 0.81	NA
MRSA-23	33.67 ± 0.47	7.0 ± 0	18.0 ± 1.22	NA
MRSA-24	24.33 ± 0.62	12.33 ± 0.94	13.0 ± 1.22	NA
MRSA-25	25.0 ± 0.81	7.0 ± 0	14.0 ± 0.4	NA
MRSA-26	25.67 ± 0.47	14.0 ± 1.63	16.0 ± 2.04	NA
MRSA-GBT	26.5 ± 0.4	11.0 ± 1.47	14.0 ± 2.44	NA
MSSA- GBT	34.5 ± 2.27	12.67 ± 0.94	14.0 ± 0.81	NA
ATCC No.29737	26.5 ± 0.4	15.33 ± 1.24	15.0 ± 0.81	NA
ATCC No. 9144	22.67 ± 2.46	12.33 ± 0.94	15.0 ± 1.63	NA
Mean	25.41	12.88	13.73	-
SE	5.47	2.65	1.69	-

SD: standard deviation, n = 6, NA: no activity.

that sesquiterpenoids retains its activity at temperatures up to 80°C, however, activity gradually decreased above 90°C. Strong antibacterial activity of sesquiterpenoids was observed at pH 6.0 to 7.0. Sesquiterpenoids antibacterial activity was not drastically effected at 4°C storage, nonetheless, storage at room temperature resulted in drastic loss of activity.

DISCUSSION

Many *Ganoderma* species have been studied for different therapeutic properties as antitumor and antiviral agents but far less investigations have been carried out on their antibacterial potential. Extracts from *Ganoderma applanatum* (Smania et al., 1999) and *Ganoderma pfeiffer*i (Monthana et al., 2000) have been shown to possess significant antibacterial activity against *Escherichia coli*. Sheena et al. (2003) reported that methanol extract of *G. lucidum* showed remarkable antibacterial activity against *E. coli*, *Salmonella* species and *Bacillus subtilis*. Furthermore, Keypour et al. (2008) investigated the antibacterial activity of chloroform extract of *G. lucidum* from Iran which showed growth inhibitory effects toward *B. subtilis* and *S. aureus*. Phytochemical analysis of the extract revealed the presence of lipid derivatives including sterols and triterpenoid acids. In addition, methanol extract of *G. lucidum* from India have

been shown to possess efficient antibacterial activity against MRSA (Prasad and Wesely, 2008). However, there appears to have been no investigations of antibacterial activity on aqueous or organic extracts from fruiting body or mycelium of *G. praelongum*.

In the present study, the aqueous extract of all mushrooms studied exhibited least antibacterial activity than the organic extracts. Maximum zones of inhibition were displayed by ethyl acetate extracts of all three mushrooms. These results are consistent with the report of Cowan (1999) who showed that the most active components are generally water insoluble, hence it is expected that low polarity organic solvents would yield more active extracts.

Since the fruiting body and mycelium of *G. praelongum* exhibited the highest effect of antibacterial activity against MRSA, it was decided that only this strain of *Ganoderma* would be further studied. This does not suggest that *G. lucidum* and *G. resinaceum* did not possess antimicrobial activity. Therefore, this is one of the first account of antibacterial activity from *G. praelongum* fruiting body and mycelial extracts against MRSA.

It is reported that many antimicrobial components such as terpenes, lectins, polysaccharides etc. act on the bacterial cytoplasmic membrane (Lin and Chou, 1984; Yang et al., 2002). The polysaccharide components were found to be the bioactive principles which play an important role in antibacterial activity. However, in our study, maximum zone of inhibition was exhibited by sesquiterpenoids of fruiting body and mycelial extracts. This supports the findings of Donnelly et al. (1985) and Ishikawa et al. (2001) who showed that sesquiterpenoids to be one of the antibacterial agents in mushrooms.

Assessment of sesquiterpenoid extracts activity at different temperatures showed that they can withstand temperatures up to 80°C without drastic loss of antibacterial activity which implies that they are thermoresistant. Exhibition of maximum zones of inhibition at pH 6.0 to 7.0 indicates that they exert their highest effect at optimal pH value for bacterial growth, and they retain their antibacterial growth when stored at low temperature (4°C).

Conclusion

With an increasing number of bacteria developing resistance to commercial antibiotics, such as MRSA, extracts and derivatives from mushrooms hold great promise for novel medicines in modern times. It is apparent from the present study that mushroom extracts from *G. praelongum* could be employed alone or in combination with commercial antibiotics to combat several diseases causing pathogenic bacteria. Moderate activity of vancomycin against some of the strains and the resistance of a few strains toward mupirocin is an indication of a rise in resistance of *S. aureus* towards these two antibiotics, leaving linezolid as the only drug of choice for treatment of MRSA infections.

ACKNOWLEDGEMENTS

The authors gratefully wish to acknowledge the assistance and cooperation of Dr. Pradhan (Joshi Hospital, Pune), Dr. D. V. Gokahle (NCL, Pune), Dr. Anand Joshi (National Burns Hospital Airoli, Thane), Dr. Bikash (GenomeBioTech, Pune), for providing test microorganisms.

REFERENCES

Bauer KS, Turck (include initials) (1966). Am. J. Clin. Path. 45: 493.

Chang ST, Buswell JA (1996). Mushroom nutriceuticals. World J. Microbiol. Biotechnol., 12(5): 473-476.

Chang ST, Mshigeni KE (2001). Muschroom and Human health: their growing significance as potent dietary supplements. University of Namibia, Windhoek, Namibia, p. 79.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12(4): 564-582.

Donnelley DM, Abe F, Coveney D, Fukuda N, O' Reilly J, Polonsky J, Prange T (1985). Antibacterial sesquiterpenes aryl esters from Armillaria mellea. J. Nat. Prod., 48: 10-16.

Eloff JN (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica., 64: 711-713.

Ha TB, Gerhauser C, Zhang WD, Ho-Chong-Line N, Fouraste I (2000). New lanostanoids from *Ganoderma lucidum* that induce NAD(P)H: quinine oxidoreductase in cultured hepalcic7 murine hepatoma cells. Planta Medica., 66(7): 681- 684.

Harborne JB (1988). Phytochemical Methods, a guide to modern techniques of plant analysis. 3rd. ed., Springer (India) Private Limited. pp. 120-121.

Ishikawa NK, Fukushi Y, Yamaji K, Tahara S, Takahashi K (2001). Antibacterial cuparene – type sesquiterpenes, enokipodins C and D, from a mycelial culture of Flammulina velutipes. J. Nat. Prod., 64: 932-934.

Keypour S, Riahi H, Moradali MF, Rafati H (2008). Investigation of the antibacterial activity of a chloroform extract of Ling Zhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (W.Curt.: Fr) P. Karst. (Aphyllophoromycetideae) from Iran. Inter. J. Med. Mushrooms., 10(4): 345-349.

Kim HW, Kim BK (1999). Biomedicinal triterpenoids of *Ganoderma lucidum* (Curt.:Fr.) P. Karst. (*Aphyllophoromycetideae*). Inter. J. Med. Mushrooms., 1(2): 121-138.

Klaus A, Miomir N (2007). Influence of the extracts isolated from *Ganoderma lucidum* mushroom on some microorganisms. Proceedings of National Science, Matica Srpska Novi Sad., 113: 219-226.

Leung SWS, Yeung KY, Ricky YLS, Man YK (2002). Lingzhi (*Ganoderma*) research the past, present and future perspectives. In: *Ganoderma*: Genetics, Chemistry, Pharmacology and Therapeutics. ZB Lin (Ed), Beijing medical University Press, Beijing. pp. 1-9.

Lin JY, Chou TB (1984). Isolation and Characterization of a lectin from edible mushroom, *Volvariella volvacea*. J. Biol. Chem., 96(1): 35-40.

Mothana RAA, Jansen R, Julich WD, Lindequist U (2000). Ganomycin A and B, new antimicrobial farnesyl hydroquinones from the basidiomycete *Ganoderma pfifferi*. J. Nat. Prod., 63(3): 416-418.

Prasad Y, Wesely WEG (2008). Antibacterial activity of the bio - multidrug (*G.lucidum*) on multidrug resistant *S. aureus* (MRSA). Advanced Biotech. March ed., pp. 9-16.

Sharifi A, Bhosle SR, Vaidya JG (2006). Evaluation of crude sesquiterpenoid extract of *Phellinus fastuosus* as a natural preservative Hind. Antibiotic, Bull. 47(48): 20-23.

Sheena N, Ajith TA, Mathew A, Janardhanan KK (2003). Antibacterial activity of three macrofungi, *Ganoderma lucidum*, *Navesporus*

floccosa and *Phellinus rimosus* occurring in South India. Pharmaceutical Biology., 41(8): 564-567.
Smania A (Jr), Delle MF, Smania EFA, Cuneo RS (1999). Antibacterial activity of steroidal compounds isolated from *Ganoderma applanatum* (Pers.) Pat. (Aphyllophoromycetideae) fruit body. International J. Med. Mushrooms., 1: 325-330.
Stoke JE, Ridgway GL (1980). Clinical Bacteriology. Edward Arnold Ltd. London.
Yang BK, Kim DH, Jeong SC, Das S, Choi YS, Shin JS, Song SC, Song CH (2002). Hypoglycemic effect of a *Lentinus edodes* exo-polymer produced from a submerged mycelial culture. Biosci. Biotechnol. Biochem., 66(5): 937-942.
Zhou S, Gao Y (2002). The immunomodulating effects of *Ganoderma lucidum* (Curt.:Fr.) P.Karst (LingZhi, Reishi Mushroom) (Aphylloromycetidae). Int. J. Med. Mushrooms., 4: 1-11.