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# NUTRACEUTICAL POTENTIAL AND ANTIMICROBIAL ACTIVITY OF *GANODERMA LUCIDUM* (FR.) P. KARST

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The crude methanol (MeOH), *n*-hexane (*n*-Hex), petroleum ether (PE), chloroform ($CHCl_3$) and Aqueous (Aq) extracts of *Ganoderma lucidum* (Fr.) P. Karst were screened to determine their bioactive constituents, antioxidant potential and antimicrobial activity. Among all the tested extracts, highest phenolics and tannins contents were observed in $CHCl_3$ (127.5 mg $100g^{-1}$) and PE fractions (78.52 mg $100g^{-1}$). The highest flavonoids moiety (84.9 mg $100g^{-1}$) and antioxidant activity (82.67%) was recorded in MeOH fractions. In vitro antibacterial and antifungal activities were evaluated by disc and well diffusion method. The maximum (72.14%) inhibitory zone was observed in $CHCl_3$ fraction with MIC value of 5.2mg $ml^{-1}$ against the bacterial strain *Staphylococcus sp.* However *E. coli* was not susceptible to PE extract while *Xanthomonas sp* was resistible to *n*-Hex and PE extracts. Likewise $CHCl_3$ fraction showed significant activity against the fungal strain *Penicillium sp* having growth inhibitory zone of 68.42% with MIC value of 41.66 mg $ml^{-1}$. However no activity was observed for *n*-Hex against *Penicillium sp* and *Aspergillus sp*., $CHCl_3$ against *Paecilomyces sp* and *Trichoderma sp* while PE ether against *Aspergillus sp*. The present findings suggest that notable antimicrobial potential of extracts might be due the presence of bioactive compounds.

**Keywords:** Antibacterial activity, antifungal activity, antioxidant activity, *Ganoderma lucidum*

## INTRODUCTION

Mushroom is fleshy, spore-bearing structure of fungi that have imperative value in nutritional, medicinal, recreational and industrial functions globally (Chang and Miles, 1992). The worldwide fungal diversity is approximately five million that can grow in a variety of habitats (Blackwell, 2011). There are about 69,000 known mushroom species of which 2000 are regarded as prime edible mushrooms, 80 of them are grown experimentally and around 20 are cultivated commercially (Chang, 1991). A reasonable number of mushrooms are widely consumed for dietary purposes and have been appreciated for their medicinal and tonic potential as well (Manzi *et al.,* 2001). However, their efficiency as nutraceutical value has merely acknowledged (Cheung *et al.,* 2003). They are capable for providing basis of physiologically functional food and important resources for the development of medicines and pharmaceutical (Sullivan *et al.,* 2006; Pala and Wani, 2011).

*Ganoderma lucidum*, belonging to family *Ganodermatacea*, is a large dark mushroom with a glossy exterior and a woody texture. The color of fruiting body varied from red, black, blue, green, white, yellow and purple (Woo *et al.,* 1999). The medicinal value of this specie is also established across the world in the formulation of nutraceutical and antioxidants rich tonic (Chang and Buswell, 1996; Saltarelli *et al.,* 2009). Various *Ganoderma* species are widely used in the synthesis of several drugs and also act as functional foods (Saltarelli *et al.,* 2009). The major chemical constituents of *G. lucidum*, such as polysaccharides, triterpenes, sterols, lectins and some proteins, have beneficial properties for the prevention and treatment of a variety of ailments. It is interesting that more than 150 triterpenes and 50 carcinostatic polysaccharides were identified during the last three decades (Fang and Zhong, 2002; Leung *et al.,* 2002; Klaus and Miomir, 2007). Medicinal mushroom exhibits paramount role in various pharmacological and biological activities such as bactericidal, fungicidal, antitumor, anticancer, immune-modulatory, heart diseases, respiratory, antihepatotoxic and antinociceptive (Ha *et al.,* 2000).*G. lucidum* play vital role in the treatment of various ailments therefore, the present research was conducted to evaluate the nutraceutical and antimicrobial potentials of *G. lucidum* collected from Swat, KPK-Pakistan. The outcome will be very helpful in producing the *G. lucidum* mushroom on commercial scale and prompt the pharmacist to develop a nutraceutical rich tonic locally.

## MATERIALS AND METHODS

***Collection and Identification:*** *Ganoderma lucidum* was collected from the premises of Swat valley and were identified by nation eminent mycologist at the Department of Plant Pathology. All the impurities like dust, dirt and other debris material were removed by washing with tape water followed by distilled water. Then it was shade dried until complete dryness was achieved. The dried samples were grounded by using electrical grinder (Yigan, model WF130) and was stored in clean polythene bags, labeled properly and stored in refrigerator at 4°C until analysis.

***Extraction and Fractionation:*** Extraction of the powder sample was carried out by using cold maceration method. About 1.5 kg sample was dipped in 2 L methanol for several days and then concentrated under reduced pressure at 40 °C using rotary evaporator (Heidolph Laborota 4000). The crude methanol extract was dissolved in distilled water and then partitioned with *n*-hexane, petroleum ether and chloroform. The methanol-aqueous layer was separated by rotary evaporator to obtain the aqueous fraction respectively. The crude methanol extract and its fractions was kept in sterilized vials and stored at 4 °C for further analysis.

***Determination of Total Phenolics and Tannins Contents:*** Total phenol and tannin contents determination was carried out by recommended method of Grubessic *et al.* (2005). Dissolve 0.5g of each extract separately in 160ml of methanol (30%) in conical flask. Mix 30% methanol to the flask and made the volume up to 200ml. Now prepare solution-A by mixing 2ml of basic solution with 8ml of water followed by addition of 10ml of acetate buffer. Similarly, solution-B was prepared by mixing 10ml solution-A with 0.05mg casein and shake for 45 minutes followed by filtration. Take 1ml of solution-A and solution-B and mix with 0.5ml FCR and 10ml of sodium carbonate decahydrate (33%). Blank solution was prepared by dissolving 50mg casein with 10ml solution-A followed by shaking and filtration. Afterward measure the absorbance of solution-A and solution-B at 720 nm. Calculate total phenol content from the absorbance reading of solution-A, whereas, tannin content was measured from the absorbance difference of solution-A and solution-B respectively.

***Estimation of Flavonoids Moiety:*** The standard procedure of Sharma *et al*. (2010) was adopted for the determination of flavonoids moiety. Mix 5ml of each extract with 1ml water and 10ml ethyl acetate and vigorously shake for 45 minutes. Now take 100µl of each extract solution in separate test tubes and decant 90µl of phosphate buffer. Add 3ml of 1 N FCR and 4ml $Na_2CO_3$ (pH-7.5) to each test tube. Incubate the test tubes for 45 minutes and then measured the absorbance at 765nm.

***Determination of Antioxidant Activity:*** Antioxidant activity was determined using the 2, 2-diphenyl-1-pycrihydrazyl (DPPH) radical scavenging assay reported by the standard procedure of Akond *et al*. (2011). Take 0.394g DPPH in 1000ml distilled water to prepared 0.001 molar solution of DPPH. Afterwards, 500 ppm stock solution of each extracts was prepared. About 25mg of each fraction was dissolved in 50ml methanol. Mix 5ml of stock solution with 25ml methanol to make 100 ppm working solution. Afterward, add 3ml of 0.001 molar DPPH solutions in one minute with 100 ppm solutions of sample and 1ml of acetate buffer (pH 6.5). The solution was vigorously shake and allowed to stand for 30 minutes. For control solution, mix 3ml DPPH with 1ml acetate buffer solution. Antioxidant activity was determined by using the following formula.

***Anti-Bacterial Activity:*** Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by Tassou *et al.* (2000). The nutrient agar (2.8g $100ml^{-1}$) was dissolved in distilled water and then sterilized in autoclave at 121 °C for 45 minutes. The agar media was transferred to laminar flow hood and seeded in plates. The microbial inoculums from the broth were spread on the prepared nutrient plates. A Whatman No. 1 filter paper disc of 6 mm in diameter was kept on the four corners of the plates and the desired concentration of each extracts was applied. Similarly ciprofloxacin and dimethyl sulfoxide (DMSO) was used as positive and negative control. The plates were kept in incubator at 37 °C and zone of inhibition was recorded after 24 hours. The percent zone of inhibition was calculated by using the following formulae:

***Antifungal Activity:*** The antifungal activity of methanol, *n*-hexane, petroleum ether, chloroform and aqueous fractions was determined by well diffusion method. About 166.6 mg of crude extract and various fractions was taken in vial and added in 01ml DMSO. The PDA medium (20ml) was poured in sterilized environment in petri dishes to avoid contamination. Wells of 6mm in diameter and about 2cm apart, punctured in the culture medium, using sterile borers. A fungicide (Acrobat) was used for positive control and DMSO for negative control was run, in parallel. The plates were incubated for 7 days and zone of inhibition was determined by comparing the growth of test fungal culture with positive and negative control using the formula as described by (Rusman, 2006).

***Minimum Inhibitory Concentration (MIC):*** The crude methanol extract and its fractions showing zone of inhibition in disc and well diffusion method were used for the determination of MIC according to the standard protocol of Nostro *et al.* (2000). A stock solution of each extracts was two-fold diluted in pure DMSO in order to obtained final concentration of 83.33, 41.66, 20.83, 10.41, 5.2, 2.6, 1.3 and 0.65 mg $ml^{-1}$ respectively. Bacterial and fungal cultures were inoculated on the plates and different concentration of crude extract and various fractions were applied to analyze the visible growth of tested microbial strains.

***Statistical analysis:*** The collected data were subjected to analysis of variance (ANOVA) using Completely Randomized Design (CRD) by Statistix 8.1. The data was recorded in triplicate and means were separated by Least Significant Difference (LSD) test.

## RESULTS AND DISCUSSION

***Bioactive Constituents and Antioxidant Activity:*** The results depicted in Table 1 divulged that highest total phenolic and tannin was found in $CHCL_3$ (127.5 mg $100g^{-1}$) and PE fractions (78.52 mg $100g^{-1}$), whereas lowest was observed in *n*-Hex fraction having values of 92.9 mg $100g^{-1}$

and 60.09 mg $100g^{-1}$. Promising total flavonoids (84.9 mg $100g^{-1}$) and antioxidant activity (82.67%) was recorded in MeOH, however, lowest was examined in PE fraction having values of 51.9 mg $100g^{-1}$ and 65.23% respectively. The present results find supportive evidence from the study of Paul *et al*. (2014) who observed 37µg $ml^{-1}$ of phenol, 42µg $ml^{-1}$ of tannin and 13.75µg $ml^{-1}$ of flavonoid content in *G. lucidum*. Similarly Rajasekaran and Kalaimagal (2011) reported 42.41mg $g^{-1}$ of phenol and 13.57mg $g^{-1}$ of flavones in ethanol extract of the said mushroom. Agarwal *et al.* (2012) observed that hot water and hydro alcoholic extract of *G. lucidum* exhibited significant antioxidant property as compared to chloroform and ether extract.

***Antibacterial Activity:*** The results of the antibacterial activity of crude methanol, *n*-hexane, petroleum ether, chloroform and aqueous fractions are enlisted in Table 2. The bactericidal activity of extracts against *Escherichia coli* were in the order of Aq (67.85%) >*n*-Hex (58.92%) >MeOH (50%) > $CHCl_3$ (46.42%), while, PE failed to show inhibitory effect against the test strain. Against *Staphylococcus* species: $CHCl_3$ (72.41%) >MeOH (63.79%) >Aq (60.34%) > PE (51.72%) >*n*-Hex (41.37%). Against *Bacillus sp.* the sequence is: MeOH (67.74%) > $CHCl_3$ (64.51%) > PE (58.06%) >Aq (54.83%) >*n*-Hex (43.54%). Likewise, the antibacterial activity against *Xanthomonas* species was in the order as: Aq (59.61%) >MeOH (48.07%) > $CHCl_3$ (44.23%), however, *n*-hexane and petroleum ether exhibited no activity. Similarly, pure Dimethyl Sulfoxide (DMSO) and standard antibiotic Ciprofloxacin were used, as negative and positive control. The present results revealed that chloroform extract showed, significant activity against *Staphylococcus sp*, while, methanol and aqueous showed good activity against *Escherichia coli*, *Staphylococcus sp.* and *Bacillus sp.* Whereas, *n*-hexane and Petroleum ether exhibited moderate to low and no activity against the tested bacterial isolates.

The minimum inhibitory concentration of MeOH, *n*-Hex, PE, $CHCl_3$ and Aq fractions against different bacterial isolates are presented in table 3. The MIC value of 5.2mg $ml^{-1}$ of methanol extract was noted for *Staphylococcus sp.* and *Bacillus sp.* whereas, 10.41 mg $ml^{-1}$ was observed for *Escherichia coli* and *Xanthomonas sp*. The lowest MIC value of *n*-hexane fraction was examined against *Bacillus sp.* (5.2mg $ml^{-1}$) followed by *Staphylococcus sp.* (10.41mg $ml^{-1}$) and *Escherichia coli* (5.2mg $ml^{-1}$). However, *Xanthomonas sp.* showed high degree of resistibility at any applied concentration. In case of petroleum ether, the MIC value was found against *Bacillus sp.* (10.41mg $ml^{-1}$) followed by *Staphylococcus sp.* (21.83mg $ml^{-1}$) whereas, no activity was observed against *Escherichia coli* and *Xanthomonas sp*. Similarly, the MIC values of 5.2mg $ml^{-1}$ were noted against *Staphylococcus sp*. MIC value of 10.41mg $ml^{-1}$ against *Escherichia coli* and *Bacillus sp.*, while MIC value of 21.83mg $ml^{-1}$ was observed against *Xanthomonas sp*. The aqueous fraction has MIC value of 5.2mg $ml^{-1}$ against *E. coli*, *Staphylococcus sp.* and *Bacillus sp.* and 21.83mg $ml^{-1}$ against *Xanthomonas sp.* respectively. The MIC values of all bacterial strains ranged between 5.2mg $ml^{-1}$ to 21.83mg $ml^{-1}$. It was also investigated that the MIC values are concentration dependent against certain bacterial species. From the results it was concluded that methanol, chloroform and aqueous fraction showed prominent activity against all the test bacterial strains. Shikongo *et al.* (2013) investigated that chloroform and ethanol have maximum inhibitory zone against *Escherichia coli* (11mm) whereas, water extract exhibited 10mm zone of inhibition against *Staphylococcus aureus.* Kamra and Bhatt (2012) justified the results of our finding by testing the antimicrobial activity of *G. lucidum* extract against human pathogenic bacteria and found 13.1 to 14.76mm and 13.33 to 14.56mm diameter zone of inhibition of aqueous extract against *Bacillus subtilis* and *Streptococcus mutans* with MIC values of 31.25µg $ml^{-1}$ and 62.50µg $ml^{-1}$. Similar, results were also reported by Jonathan and Awotona (2010), who observed remarkable inhibitory zone of ethanol, methanol and water extract against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cerus* with MIC value of 1.7 and 5.0mg $ml^{-1}$. Our results also find supportive evidence from the study of Shamaki *et al.* (2012), who observed that *Corynebacterium pyogene, Bacillus subtilis* and *Klebsiela pneumoneae* strains were the most sensitive with MIC value of 12.5mg $ml^{-1}$ followed by *Pseudomonas aeruginosa* and *P. mirabilis.*

***Antifungal Activity:*** The antifungal potential of crude methanol, *n*-hexane, petroleum ether, chloroform and aqueous fraction of *G. lucidum* are depicted in Table 4. Order of the antifungal activities of the extract against *Penicillium sp.* is: $CHCl_3$ (68.42%) > PE (62.5%) >Aq (57.44%) > MeOH (55.5%). However, *n*-hexane fraction doesn't exhibit any activity against the test fungal strains. The percent inhibition against *Aspergilius sp.* was in the sequence as: $CHCl_3$ (63.63%) >Aq (46.51%) >MeOH (45.0%). *Aspergilius sp.* progressively proliferate their growth and does not show any inhibitory effect by the appliance of *n*-hexane and petroleum ether extracts. The activity of extracts against *Paecilomyces sp.* is: MeOH (58.69%) >*n*-Hex (56.15%) >Aq (53.84%) >PE (47.72%), whereas $CHCl_3$ has no fungicidal activity against the test isolates. In case of *Trichoderma sp.* the extracts activity were in the order as: PE (64.28%) >Aq (60%) >*n*-Hex (50%) >MeOH (45.45%) respectively. However, $CHCl_3$ extract was inactive against the pathogenic fungal species and failed to exhibit any inhibitory effect. Acrobat a standard antifungal drug used as positive control showed broad spectrum activity by inhibiting all the fungal strains. From the present results it was concluded that chloroform, petroleum ether and aqueous fraction exhibit good activity against *Penicillium sp., Aspergillus sp.* and *Trichoderma sp.* While, methanol and *n*-hexane fractions showed moderate to low and no activities against the pathogenic fungal strains.

**Table 1. Bioactive constituents and antioxidant activity of crude methanol extract and various fractions of *Ganoderma lucidum***

| Extracts | Total phenolic (mg $100g^{-1}$) | Total tannins (mg $100g^{-1}$) | Total flavonoids (mg $100g^{-1}$) | Antioxidant activity (%) |
|---|---|---|---|---|
| MeOH | 119.3 | 75.74 | 84.9 | 82.67 |
| *n*-Hex | 92.9 | 60.09 | 57.6 | 67.92 |
| PE | 105.2 | 78.52 | 51.9 | 65.23 |
| $CHCl_3$ | 127.5 | 72.02 | 72.2 | 73.08 |
| Aq | 115.3 | 64.01 | 81.6 | 72.69 |

MeOH = Methanol, *n*-Hex = *n*-hexane, PE = petroleum ether, $CHCL_3$ = Chloroform, Aq = Aqueous

**Table 2. Antibacterial activities of crude methanol extract and various fractions of *Ganoderma lucidum***

| Bacterial strains | Ciprofloxacin | MeOH | *n*-Hex | PE | $CHCl_3$ | Aq |
|---|---|---|---|---|---|---|
| *Escherichia coli* | 28 | 50.0 | 58.92 | R | 46.42 | 67.85 |
| *Staphylococcus sp.* | 29 | 63.79 | 41.37 | 51.72 | 72.41 | 60.34 |
| *Bacillus sp.* | 31 | 67.74 | 43.54 | 58.06 | 64.51 | 54.83 |
| *Xanthomonas sp.* | 26 | 48.07 | R | R | 44.23 | 59.61 |

MeOH = Methanol, *n*-Hex = *n*-hexane, PE = petroleum ether, $CHCL_3$ = Chloroform, Aq = Aqueous, R = Resistible

**Table 3. Minimum Inhibitory Concentration (MIC) of crude methanol extract and various fractions against bacterial strains**

| Bacterial strains | MeOH | *n*-Hex | PE | $CHCl_3$ | Aq |
|---|---|---|---|---|---|
| *Escherichia coli* | 10.41 | 20.83 | R | 10.41 | 5.2 |
| *Staphylococcus sp* | 5.2 | 10.41 | 20.83 | 5.2 | 5.2 |
| *Bacillus sp* | 5.2 | 5.2 | 10.41 | 10.41 | 5.2 |
| *Xanthomonas sp* | 10.41 | R | R | 20.83 | 20.83 |

MeOH = Methanol, *n*-Hex = *n*-hexane, PE = petroleum ether, $CHCL_3$ = Chloroform, Aq = Aqueous, R = Resistible

**Table 4. Antifungal activities of crude methanol extract and various fractions of *Ganoderma lucidum***

| Fungal | Acrobat | MeOH | *n*-Hex | PE | $CHCl_3$ | Aq |
|---|---|---|---|---|---|---|
| *Penicillium sp* | 100 | 55.55 | R | 62.5 | 68.42 | 57.44 |
| *Aspergillus sp* | 100 | 45.0 | R | R | 63.63 | 46.51 |
| *Paecilomyces sp* | 100 | 58.69 | 56.15 | 47.72 | R | 53.84 |
| *Trichoderma sp* | 100 | 45.45 | 50 | 64.28 | R | 60.0 |

MeOH = Methanol, *n*-Hex = *n*-hexane, PE = petroleum ether, $CHCL_3$ = Chloroform, Aq = Aqueous, R = Resistible

**Table 5. Minimum Inhibitory Concentration (MIC) of crude methanol extract and various fractions against fungal strains**

| Fungal | Acrobat | MeOH | *n*-Hex | PE | $CHCl_3$ | Aq |
|---|---|---|---|---|---|---|
| *Penicilliumsp* | 100 | 20.83 | R | 83.33 | 41.66 | 41.66 |
| *Aspergillus sp* | 100 | 41.66 | R | R | 83.33 | 20.83 |
| *Paecilomycessp* | 100 | 20.83 | 83.33 | 83.33 | R | 20.83 |
| *Trichoderma sp* | 100 | 83.33 | 41.66 | 41.66 | R | 83.33 |

MeOH = Methanol, *n*-Hex = *n*-hexane, PE = petroleum ether, $CHCL_3$ = Chloroform, Aq = Aqueous, R = Resistible

The minimum inhibitory concentration of MeOH, *n*-Hex, PE, $CHCl_3$ and Aq fractions against fungal isolates are presented in Table 5.The MIC value of 21.83mg $ml^{-1}$of methanol extract was observed against *Penicillium sp.* and *Paecilomyces sp.* followed by *Aspergillus sp.* (41.66mg $ml^{-1}$) and *Trichoderma sp*. The *n*-hexane fraction has MIC value of 41.66mg $ml^{-1}$ and 83.33mg $ml^{-1}$ against *Trichoderma sp* and *Paecilomyces sp*. However, no activity was reported against *Penicillium sp.* and *Aspergillus sp.* at any test concentration. Petroleum ether has MIC value of 41.66mg $ml^{-1}$ against *Trichoderma sp.* and 83.33mg $ml^{-1}$against *Penicillium sp.* and *Paecilomyces sp.*, whereas *Aspergillus sp*. was resistible. The MIC values of 41.66 and 83.33mg $ml^{-1}$ was recorded against *Penicillium sp.* and *Aspergillus sp.,* while, *Paecilomyces sp.* and *Trichoderma sp.* were resistible to the subject mushroom extracts. The aqueous fraction exhibit prominent activity with MIC values of 20.83mg $ml^{-1}$ against *Paecilomyces sp.* and *Aspergillus sp.* Likewise MIC value of 41.66mg $ml^{-1}$ and 83.33mg $ml^{-1}$ was recorded against *Penicillium sp.* and *Trichoderma sp.* respectively. The MIC values of fungal strains ranges from 20.83 to 83.33mg $ml^{-1}$. However, no activity was noted at concentration of 5.2 and 10.41mg $ml^{-1}$. Among all mushroom extracts, only methanol and aqueous fraction showed remarkable activity against all the fungal species. Nithya *et al.* (2013) reported, 16.8mm and 3mm zone of inhibition of diethyl ether and aqueous extract against *Aspergillus niger* and *Fusarium oxysporum*. The results of our findings were in conformity with the study of Jonathan and Awotona (2010), who observed promising antimicrobial activities of ethanol, methanol and water extracts of *G. lucidum.* The MIC value for ethanol extract against different fungal species ranged between 2.0 and 6.0mg $ml^{-1}$, respectively.

## CONCLUSION

*G. lucidum* is a well-known medicinal mushroom with a long and notable range of applications. With its growing popularity, many studies on *G. lucidum* composition, cultivation and reputed effects are being carried out

including, anticancer effects, blood glucose regulation, antioxidant, antibacterial, antifungal, antiviral effects and protection against liver and gastric injury. The crude methanol extract and various fractions of *G. lucidum* contain appreciable amount of phenols, tannins and flavonoids content which might be responsible for various pharmacological activities especially for antioxidant and antimicrobial activities.

## REFERENCES


Agarwal, K., G. S. Chakarborthy and S. Verma. 2012. *In vitro* antioxidant activity of different extract of *Ganoderma lucidum.* Inter. J. Pharma. Sci. 3: 2278-8328.

Akond, A. S. G. M., L. Khandaker, J. Birthhold, L. Gates, K. Peters, H. Delong and K. Hossain. 2011. Anthocyanin, total phenol and antioxidant activity of common bean. Am. J. Food. Technol. 6: 385-394.

Blackwell, M. 2011. The Fungi: 1, 2, 3 ... 5.1 million species. American. J. Bot. 98:426-438.

Chang, S. T and J. A. Buswell. 1996. Mushroom nutraceuticals. World. J. Microbio. Biotech. 12:473-476.

Chang, S. T and P. G. Miles. 1992. Mushroom biology, New discipline. J. Mycologist. 6:64-65.

Chang, S. T. 1991. Mushroom biology and mushroom production. Mushroom. J. Tropics. 11:45-52.

Cheung, P. C. K. 2003. Chemical evaluation of some lesser-known edible mushroom mycelia produced in submerged culture from soymilk waste. Food. Chem. 60:61-65.

Fang, Q. H and J. J. Zhong. 2002. Submerged fermentation of higher fungus *Ganoderma lucidum* for production of valuable bioactive metabolites-ganoderic acid and polysaccharides. J. Biochemical. Engine. 10:61-65.

Grubesic, R. J., J. Vukovic, D. Kremer and S. Vladimir-Kvezevic. 2005. Spectrophotometric method for polyphenols analysis: prevalidation and application on plantago L. species. J. Pharm. Biomed. Anal. 39: 837-842.

Ha, T. B., C. Gerhauser, W. D. Zhang, N. C. Line and I. Fouraste. 2000. New lanostanoids from *Ganoderma lucidum* that induce NADPH: quinine oxidoreductase in cultured hepalcic7 murine hepatoma cells. Plant. Medic. 681-684.

Jonathan, S. G and F. E. Awotona. 2010. Studies on Antimicrobial Potentials of three *Ganoderma* species. Afr. J. Biomed. Res. 13:133-139.

Kamra, A., A. B. Bhatt. 2012. Evaluation of antimicrobial and antioxidant activity *of Ganoderma lucidum* extracts against human pathogenic bacteria. Int. J. Phar. Pharma. Sci. 4:359-362.

Klaus, A and N. Miomir. 2007. Influence of the extracts isolated from *Ganoderma lucidum* mushroom on some microorganisms. Proceed. Nat. Sci. 113:219-226.

Leung, S. W. S., K. Y. Yeung, Y. L. S. Ricky and Y. K. Man. 2002. *Ganoderma lucidum* research the past, present and future pespectives. Beijing medical University Press. pp: 1-9.

Manzi, P., L. Gambelli, S. Marconi, V. Vivanti and L. Pizzoferrato. 2001. Nutrients in edible mushrooms. Food. Chem. 65:477-482.

Nithya, M., V. Ambikapathy and A. Panneerselvam. 2013. Studies on antimicrobial potential of different strains of *Ganoderma lucidum* (Curt: Fr.) P. Karst.Int. J. Pharm. Sci. Rev. Res. 21:317-320.

Nostro, A., M. P. Germano, V. D. Angelo, A. Marino and M. A. 2000. Cannatelli Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity.Lett. Appl. Microbiol.30:379-384.

Pala, S.A. and A.H. Wani. 2011. Mushrooms: The entities with multifarious medicinal properties. J. Pharm. Res. 4: 4721-4726.

Paul, S., A. R. J. Singh and C. S. Sasikumar. 2014. An antioxidant and bioactive compound studies of *parmelia perlata, Ganoderma lucidum and phellinu signiarius*-supplimentory drug. Asia. J. Pharm. Tech. Innovation. 2:13-22.

Rajasekaran, M. and C. Kalaimagal. 2011. In Vitro antioxidant activity of ethanolic extract of a medicinal mushroom, *Ganoderma lucidum.* J. Pharm. Sci. Res. 3: 1427-1433.

Rusman, Y. 2006. Isolation of new secondary metabolites from sponges associated and plant derived endophytic fungi. PhD thesis. Heinrich Heine University Dusseldorf.

Saltarelli, R., P. Ceccaroli, M. Lotti, A. Zambonelli, M. Buffalini, L. Casadei, L. Vallorani and V. Sticchi. 2009. Biochemical characterization and antioxidant activity of mycelium of *Ganoderma lucidum* from central Italy. Food. Chem. 116:143-151.

Shamaki, B. U., Y. A. Geidam, F. Abdulrahman, A. O. Ogbe and U. K. Sandabe. 2012. Evaluation of phytochemical constituents and in vitro antibacterial activity of organic solvent fractions of *Ganoderma lucidum* methanolic extract. Int. J. Med. Plants. Res. 1: 26-31.

Sharma, R. J., S. R. Chaphalkar and A. D. Adsool. 2010. Evaluating antioxidant potential, cytotoxity and intestinal absorption of flavonoids extracted from medicinal plant. Int. J. Biotechnol. App. 2:1-5.

Shikongo, L. T., P. M. Chimwamurombe, H. R. Lotfy and M. Kandawa-Schulz. 2013. Antimicrobial screening of crude extracts from the indigenous *Ganoderma lucidum* mushrooms in Namibia. Afr. J. Microbiol. Res. 7: 4812-4816.

Sullivan, R., J.E. Smith and N.J. Rowan. 2006. Medicinal mushrooms and cancer therapy: Translating a traditional practice into Western medicine. Perspect. Biol. Med. 49: 159-170.

Tassou, C., K. Koutsoumanis and J. G. J. E. Nychas. 2000. Inhibition of *Salmonella enteritidis* and *Staphylococcus spaureus* in nutrient broth by mint essential oil. Food. Res. Int. 33:273-280.

Woo, Y.A, H.J. Kim, J.H. Cho and H. Chung. 1999. Discrimination of herbal medicines according to geographical origin with near infrared reflectance spectroscopy and pattern recognition techniques. J. Pharm. Biomed. Anal. 21: 407-413.