

Screening of antioxidant properties from fruiting bodies, culture broth and mycelia of *Ganoderma lucidum*

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ABSTRACT

Ganoderma lucidum is a medicinal mushroom originated from China. It is also called lingzhi in China and reishi in Japan. The biologically active compounds of *G. lucidum* possess anti-cancer, anti-microbial and anti-oxidant activities. In the current study, growth of *G. lucidum* in Potato Dextrose Broth and free radical scavenging activity of mycelia, culture filtrate and fruiting bodies were examined. The maximum mycelia growth (0.98 ± 0.2 g/50 mL) was recorded on day 12 at static condition. The maximum antioxidant activity ($95.3 \pm 1.7\%$ and $77.9 \pm 1.37\%$) was recorded against DPPH and ABTS radicals for *Ganoderma* fruiting body extract, extracted with acetone (750 µg/mL) and methanol (1000 µg/mL concentration). Static condition recorded the maximum activity ($35.79 \pm 8.5\%$) on day 9 for culture filtrate against DPPH whereas 3rd day recorded the maximum ($48.7 \pm 1.6\%$) activity against ABTS assay. The maximum activity ($51.24 \pm 7.8\%$) recorded for the mycelia harvested on day 12 against DPPH and ($62.28 \pm 8.2\%$) against ABTS radical.

KEYWORDS: *Ganoderma lucidum*, antioxidant, radical scavenging activity, DPPH, ABTS

INTRODUCTION

Ganoderma lucidum, a woody mushroom species belonging to the family *Ganodermataceae* of class *Agaricomycetes* (Wu *et al.*, 2016, Wu *et al.*, 2019), is used as dietary supplement in the Eastern part of the world for many years with no toxic effect reported (Eo *et al.*, 1999; Liu *et al.*, 2002). It is considered as a health tonic for promoting health and longevity (Wang *et al.*, 2017).

G. lucidum is known for its bioactive compounds, therapeutic and anti-ageing properties and promotion of health (Wasser, 2005). There are many reports on the therapeutic properties of *G. lucidum* like antioxidant, anti-inflammatory, anticancer, immunomodulatory, anti-microbial and anti-diabetic activities (Gao *et al.*, 2002, 2003; Halpern, 2007; Dudhgaonkar *et al.*, 2009; Li *et al.*, 2015; Liu *et al.*, 2015; Ma *et al.*, 2015; Pan *et al.*, 2015; Chiu *et al.*, 2017; Wang *et al.*, 2017; Zheng and Chen, 2017; Yang *et al.*, 2018).

The major chemical constituents of *G. lucidum* like polysaccharides, triterpenes, sterols, lectins and some proteins are reported to have beneficial properties in the prevention and treatment of a variety of ailments. It is interesting that more than 150 triterpenes and 50 carcinostatic polysaccharides were identified from this mushroom during the last three decades (Fang and Zhong, 2002; Leung *et al.*, 2002; Klaus and Niksic, 2007).

The process of oxidation in living organisms is essential for production of energy. But during this process, oxidants and free radicals are produced which is a physiologic phenomenon in cells. The imbalance between the oxidants and antioxidants is reported to cause the oxidative stress (Sies, 2000; Ahmed, 2005). This oxidative stress leads to pathophysiological states such as neurodegeneration, cancer, cardiovascular diseases, mutagenesis and aging (Pham-Huy *et al.*, 2008; Kabel, 2014). Antioxidants are reported to scavenge the reactive oxidizing species (Chapple, 1997; Packer and Cedenas, 2007).

The antioxidant potential of *G. lucidum* was studied by many

researchers using different methods like DPPH radical scavenging activity, hydrogen peroxide radical scavenging activity, superoxide radical scavenging activity, ABTS radical scavenging activity, metal chelating ability, reducing power, lipid peroxidation assay, total antioxidant capacity, FRAP and ORAC (Baskar *et al.*, 2008; Joseph *et al.*, 2009; Kamra and Bhatt, 2012; Samarakoon *et al.*, 2013; Hasnat *et al.*, 2013; Lin *et al.*, 2015; Cör *et al.*, 2017; Dong *et al.*, 2019).

The antioxidant activity of the extracts from mycelia of *G. lucidum* as well as culture filtrate was determined against DPPH and ABTS by growing *G. lucidum* in Potato Dextrose Broth (PDB) incubated under static and rotary shaking conditions for 15 days. The mycelium obtained from the PDB and the organically cultivated *G. lucidum* fruiting body at MCRC was extracted with different solvents in two different conditions such as hot and 30°C and used for the study.

MATERIALS AND METHODS

Culturing in PDB: Pure culture of *G. lucidum* available at the culture collection of Shri A M M Murugappa Chettiar Research Centre, Taramani, Chennai was used in this study. Seven days old 8 mm sized mycelia disc of *G. lucidum* was used for inoculating PDB and the inoculated broth was incubated under static ($28 \pm 2^\circ\text{C}$) and shaking ($28 \pm 2^\circ\text{C}$ and 120 rpm) conditions. The mycelia was harvested from the broth at regular intervals of 3, 6, 9, 12 and 15 days. The harvested mycelium and culture filtrate samples were analyzed for their antioxidant activity.

Preparation of crude extract from fruit body and mycelium: Organically cultivated *Ganoderma* fruit body was available at Shri A M M Murugappa Chettiar Research Centre, Taramani, Chennai. The extraction of compounds from the fruiting bodies was carried out using different solvents (Ethanol, Methanol, Ethyl acetate and Acetone) under rotary shaking (120 rpm, 37°C) condition following the modified procedure of Veljovic *et al.* (2017) and hot extraction condition by refluxing method (5 hrs), the modified procedure of Cör *et al.* (2017). Extracts were transferred to crucible, evaporated at $32 \pm 2^\circ\text{C}$ and yield was recorded. Extracts were re-dissolved and

used for quantifying the antioxidant activity.

Acetone was used for the extraction of bioactive compounds from the mycelium. For this purpose Acetone was added to harvested *G. lucidum* mycelia (200 mg) in different days (3-15) and extraction done by keeping the mixture in water bath at 50°C for 6 hrs. The filtered extracts were kept for drying under room temperature (32 ± 2°C). Dried sample was taken for antioxidant activity studies.

Antioxidant activity assays: The *G. lucidum* fruit body extracts (obtained through Soxhlet and rotary process), mycelial extract and the culture filtrate (broth after removing the mycelium) were tested for their antioxidant potential against DPPH and ABTS radical scavenging activity. Investigations for DPPH radical scavenging activity of mycelial samples were carried out for the samples obtained after 6th day onwards since the mycelial sample obtained on the 3rd day was less.

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity: DPPH (3.98 mg) was dissolved in 50 mL of methanol and this served as stock of DPPH solution (0.1mM concentration). The scavenging activity for DPPH free radicals was measured according to Zhao *et al.* (2006). For this purpose to 2 mL of 0.1 mM DPPH, 1 mL of sample (extracts from different extraction methods) at different concentrations (250, 500, 750 and 1000 µg/mL concentrations) was added individually. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolourization of DPPH was determined by measuring the decrease in absorbance at 517 nm, and the DPPH radical scavenging effect was calculated according to the following equation:

$$\% \text{ scavenging activity} = [(A_0 - A_1) / A_0] \times 100$$

Where

A0 represents the absorbance of DPPH without sample solution.

A1 represents the absorbance of DPPH with sample

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity: Scavenging activity of ABTS radical was carried out following the procedure given by Mahendran *et al.* (2012). For this purpose 7 mM ABTS solution was prepared by adding ABTS (36 mg of ABTS in distilled water) to a solution of potassium persulfate (189 mg in distilled water). To this 5 mL of ABTS and 144 µL potassium persulfate was added and kept overnight to activate the radicals. Absorbance of ABTS was adjusted to 0.7 OD at 734 nm wavelengths and it served as ABTS solution. The antioxidant activity of the samples (250, 500, 750 and 1000 µg/mL concentrations) was studied by the following standard method. For this purpose 3 mL of ABTS solution was taken in test tube to which 60 µL of sample was added and mixed vigorously for 20 minutes. The absorption was read at 734 nm. The scavenging activity of the samples was calculated by using following formula.

$$\% \text{ scavenging rate} = [(A_0 - A_1) / A_0] \times 100$$

Where

A0 represents the absorbance of control.

A1 represents the absorbance of ABTS with sample

RESULTS

Growth studies of *G. lucidum*: The maximum mycelia yield of 0.98±0.2g and 0.62±0.3g/50 mL (dry weight) was obtained in PDB on 12th day under static and shaking conditions, respectively. The study shows that the mycelia growth declined after the 12th day under both the incubation conditions (**Fig. 1**). Static condition took little longer time initially to show the notable growth compared to the shaking condition.

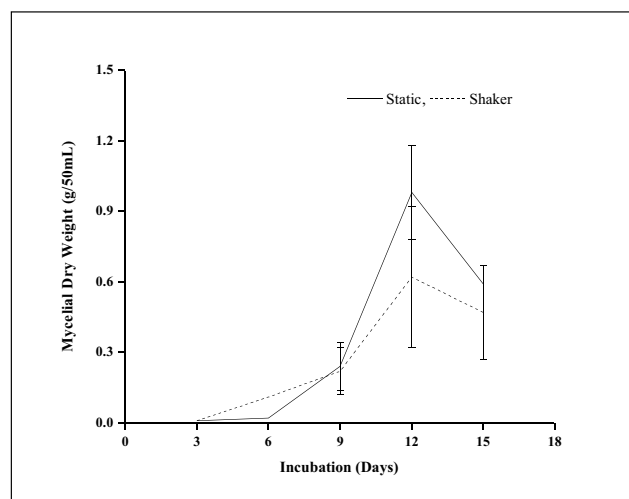


Fig. 1 Growth of *G. lucidum* in PDB

Crude extract from *G. lucidum*: The data given in **table 1** provides the results of the yield of crude extract obtained from different solvent extractions under two different extraction conditions. The maximum yield (160 mg/2g) of the extracted compounds was observed in Ethyl acetate extract and 80 mg/2g was obtained in ethanol extract using soxhlet process.

The maximum crude extract obtained through shaking process was 70 mg/2g in ethanol extract.

Table 1: Yield of *G. lucidum* extracts, extracted with different solvents

| Yield (mg / 2g) | | |
|-----------------|---------------|---------|
| Solvent | Rotary shaker | Soxhlet |
| Methanol | 40 | 60 |
| Ethanol | 70 | 80 |
| Acetone | 50 | 30 |
| Ethyl acetate | 40 | 160 |

DPPH radical scavenging activity for different solvent extracts of *G. lucidum* fruiting body: In DPPH radical scavenging activity, *G. lucidum* fruiting body extracts recorded maximum activity (95.3±1.7 %) for acetone extract extracted under shaking condition, followed by methanol extract (94.27 ± 0.3 %) extracted through soxhlet method (**Fig. 2a**). An increasing trend in antioxidant activity was observed for acetone extracts under both the conditions. Ethyl acetate extract, extracted by shaking condition have shown DPPH radical scavenging activity but there was no activity for the extracts obtained through soxhlet process in any of the

concentrations tested. Likewise, the extracts obtained using ethanol under both the conditions didn't show any activity for DPPH. The maximum activity for ethyl acetate extract recorded was $70.31 \pm 5.4\%$ at $500 \mu\text{g/mL}$ concentration.

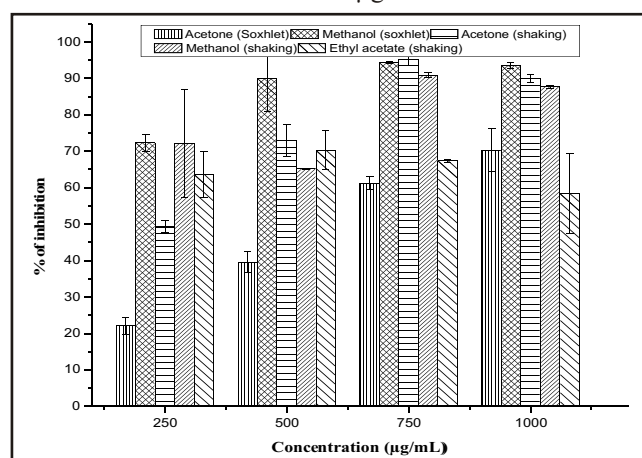


Fig. 2a: DPPH radical scavenging activity of *G. lucidum* extracted with different solvents

ABTS radical scavenging activity for different solvent extracts of *G. lucidum* fruiting body: The results of ABTS radical scavenging activity is depicted in Fig. 2b. Maximum activity ($77.9 \pm 1.37\%$) was recorded at $1000 \mu\text{g/mL}$ concentration for the methanol extract, extracted under the shaking condition. Minimum scavenging activity ($14.12 \pm 8.07\%$) against the ABTS radicals was recorded for the Methanol extract at $250 \mu\text{g/mL}$ concentration. Extraction done under shaking condition gave better results in comparison to the extraction done using soxhlet extraction method. Ethanol extract gave least activity under both the conditions of extractions.

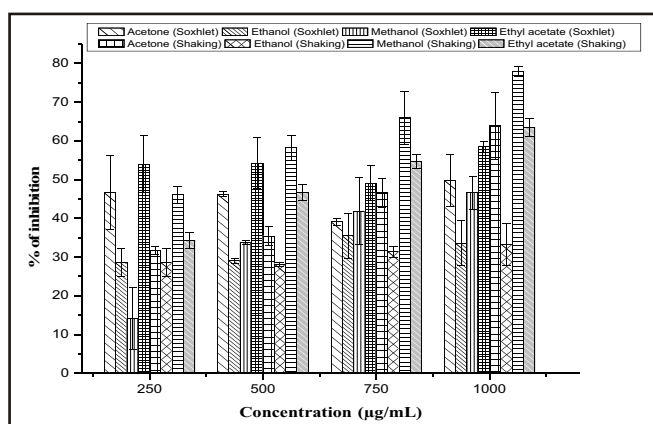


Fig. 2b ABTS radical scavenging activity of *G. lucidum* extracted with different solvents

DPPH radical scavenging activity for broth samples of *G. lucidum*: The results of DPPH radical scavenging activity of culture filtrate samples is depicted in Fig. 3a. Maximum activity ($35.79 \pm 8.5\%$) was recorded for the culture filtrate obtained from 9 day old culture incubated under static condition, followed by $34.4 \pm 16.3\%$ incubated under shaking conditions on day 9.

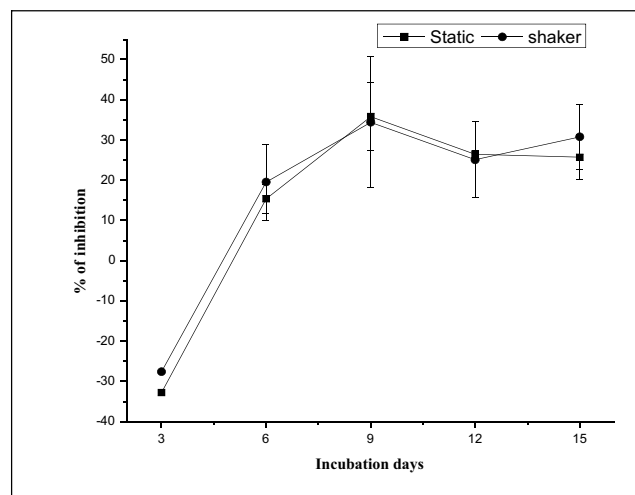


Fig. 3a. DPPH radical scavenging activity of culture filtrate sample

ABTS radical scavenging activity for broth samples of *G. lucidum*: The results of ABTS radical scavenging activity of culture filtrate is represented in Fig. 3b. Maximum activity was recorded for the culture filtrate samples obtained from 3 day old culture kept under both the conditions. No significant variation was observed in the results obtained under static and shaking conditions on day 3.

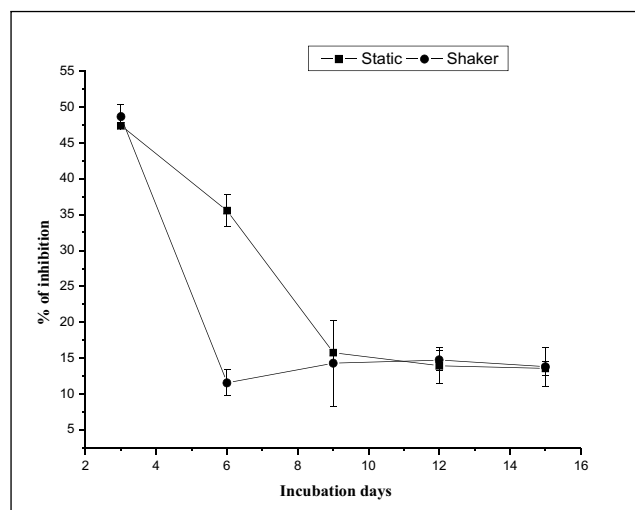


Fig. 3b. ABTS radical scavenging activity of culture filtrate

DPPH radical scavenging activity for mycelia samples of *G. lucidum*: The result of DPPH radical scavenging activity of mycelial extracts are depicted in the following graphic presentation (Fig. 4a).

The maximum activity ($51.24 \pm 7.8\%$) was recorded for mycelia grown under shaking condition on the 12th day whereas the extracts obtained through static conditions have shown maximum activity on the 15th day ($43.5 \pm 1.5\%$). Samples incubated under shaking conditions gave the better activity compared to the sample incubated under static conditions.

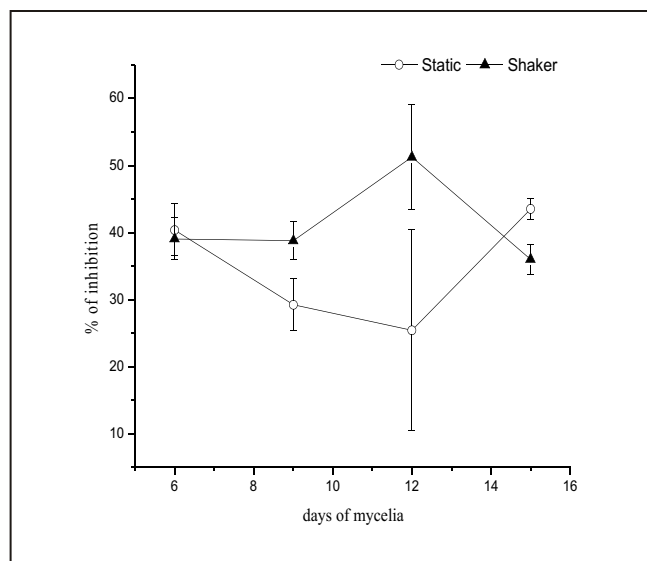


Fig. 4a. DPPH radical scavenging activity of different day's mycelium

ABTS radical scavenging activity for mycelia of *G. lucidum*: The maximum ABTS radical scavenging activity ($62.28 \pm 8.2\%$) was recorded on day 15 under static condition. Minimum activity was recorded on the 9th day mycelia ($17.04 \pm 3.9\%$) and ($12.36 \pm 0.21\%$) under both static and shaking conditions, respectively. Better scavenging activity was obtained under static condition as compared to the shaking condition (**Fig. 4b**).

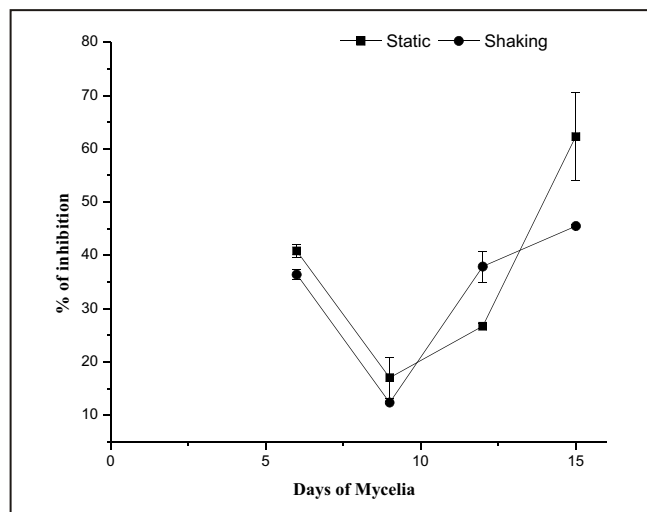


Fig. 4b. ABTS radical scavenging activity of different days of mycelium

DISCUSSION

The present investigation is exclusively focused on the screening of antioxidant properties of mycelium and culture filtrate harvested on different day's time interval and its comparison with the antioxidant activity of the fruiting body. On the basis of the results obtained it can be concluded that crude extract of fruiting body exhibited better activity against both DPPH and ABTS radicals in comparison to mycelia and culture broth. Maximum activity ($95.3 \pm 1.7\%$ and 77.9 ± 1.3

%) was recorded by fruiting body against DPPH and ABTS radicals, respectively. In culture broth samples maximum activity ($35.79 \pm 8.5\%$) was there on 9th day when incubated under static condition against DPPH and $48.7 \pm 1.6\%$ on 3rd day when incubated under shaking condition against ABTS radical. Mycelia harvested on day 12 recorded the maximum activity ($51.24 \pm 7.8\%$) against DPPH radical extracted under shaking condition and $62.28 \pm 8.2\%$ against ABTS radical extracted under static condition.

The available literatures show that the crude extracts of *G. lucidum* prepared using water as well as organic solvents exhibited antioxidant activities. Agarwal *et al.* (2012) reported better activity for crude extract of *G. lucidum*, extracted with hot water compared to hydro alcoholic, chloroform and petroleum ether extracts. It was reported that hot water extract recorded the maximum activity for both DPPH ($> 90\%$) and ABTS ($> 80\%$) radical scavenging activity at $500 \mu\text{g/mL}$ concentration. During the present investigations crude extract of *G. lucidum* fruiting body, extracted with acetone recorded the maximum activity ($95.3 \pm 1.7\%$) at $750 \mu\text{g/mL}$ concentration. The results obtained by Kalyoncu *et al.* (2010) corroborated with similar such results obtained by Agarwal *et al.* (2012) who documented better scavenging activity of crude water extract of *G. lucidum* against DPPH (21.5%) and ABTS (70.71%) at 1 mg/mL concentration. On the other hand, Stajkovic *et al.* (2014) compared the DPPH free radical scavenging activity of two fruiting bodies collected from Serbia and China and reported 0.17 and $0.13 \text{ g/mL IC}_{50}$ respectively for methanol extracts. Presently in the methanol extract more than 70% activity was recorded at $250 \mu\text{g/mL}$ concentration, extracted under both the conditions. Likewise, Samarakoon *et al.* (2013) also reported the antioxidant activity of $2.09 \text{ mg/mL IC}_{50}$ for ethyl acetate extract of *G. lucidum* fruiting body and $1.1 \text{ mg/mL IC}_{50}$ for methanol extract. In the present study Ethyl acetate extract exhibited 63% activity at $250 \mu\text{g/mL}$ concentration whereas methanol extract recorded $>90\%$ activity at $750 \mu\text{g/mL}$ concentration. Cör *et al.* (2017) reported maximum inhibition of DPPH radicals for *G. lucidum* fruiting body extract when hot acetone solvent was used, followed by hot ethanol, methanol, water and hexane extracts. The antioxidant activity of 0.48% - 23.66% was obtained using DPPH free radical scavenging method with fruiting body extract.

The antioxidant activity of 35% was recorded during the present study using culture filtrate (PDB) on 9th day which is in conformity with the similar results presented by Cilerdzic *et al.* (2016). In the culture broth studied by these authors for antioxidant activity it was reported that culture maintained in malt dextrose medium (MDM), incubated for 21 days, recorded maximum activity (39.6%) with 100% broth (MDM) concentration (Cilerdzic *et al.*, 2016). As compared the number of days to obtain the activity was reduced during the present investigations.

While working with the mycelia extract, it was observed that 11 days old mycelia extracted with culture broth exhibited 70% scavenging activity at 0.5 mg/mL concentration whereas Asatiani *et al.* (2007) reported that the residue extracted with

ethanol resulted in 77% scavenging activity at 1.5 mg/mL concentration. Likewise, Darsih *et al.* (2019) also reported that the scavenging of DPPH radical by methanol extract of mycelia cultivated on PDB (22.140±3.25 %) was higher than obtained in the mycelia cultured on sweet corn media (12.840±2.03 %) at 0.2 mg/mL.

It was shown by Yan *et al.* (2019), that the GLP (*G. lucidum* polysaccharide) in the *G. lucidum* fruit body extract exhibited strong antioxidant effects, including scavenging of DPPH• and HO•, with IC₅₀ values of 0.55 mg/mL and 0.76 mg/mL, respectively. In another report by Mahendran *et al.* (2012), it was reported that crude EPS exhibited higher DPPH radical scavenging activity. Heleno *et al.* (2012) extracted phenolic compounds and polysaccharides from mycelia and fruiting bodies, and spores of *G. lucidum*; and reported that polyphenols extracted from mycelia and fruiting bodies showed the better scavenging activity than the polysaccharides against the DPPH and reducing power assay but the results were vice versa in spores. The phenolic extract of mycelia grown in solid MMN media recorded IC₅₀ at 0.9 mg/mL concentration whereas phenolic extract of mycelia grown in liquid MMN (Melin Norkans media) media recorded 1.32 mg/mL IC₅₀ value. Tan *et al.* (2018) reported 65-68% radical scavenging activity against DPPH radicals for polysaccharides produced under the heat stress from the *G. lucidum*. Presently free radical scavenging activity was reported for fruiting body powder, mycelia and culture broth of *G. lucidum* grown for different days. The outcome of the experiment is encouraging and further studies in cost effective medium will provide path for preparing mushrooms as food supplements.

CONCLUSION

In the present study, the maximum antioxidant activity was observed in *G. lucidum* fruiting body. The culture filtrate as well as mycelia has also shown antioxidant activity although it is not at par with the activity obtained from the extract prepared using fruit bodies. The crude EPS of the fruiting body of *G. lucidum*, mycelium and exopolysaccharides in the culture filtrate might have induced the antioxidant activity as reported in several other reports. Further optimization studies are required to produce nutrient rich mycelium. The potent antioxidant activity of *G. lucidum* mushroom indicates the beneficial effects of mushroom. The report by Celal, (2019) stated that *G. lucidum* is a natural antioxidant source which can be consumed.

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