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Research article

Phytochemical and Antimicrobial properties of *Ganoderma lucidum* collected from Tanjore, Tamil Nadu.

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Abstract

The present investigation was carried out to evaluate the phytochemical constituents and antimicrobial potential of *Ganoderma lucidum* collected from Tanjore, Tamil Nadu. Phytochemicals such as Alkaloids, flavonoids, Steroids, terpenoids, glycocides, cardiac glycosides, tannins and phenol were present in the *G.lucidum* fruiting bodies, which exhibited moderate antimicrobial activity against most of the tested pathogens. Maximum zone of inhibition was observed against bacterial pathogens *Escherichia coli* and *Bacillus cereus* and no zone of inhibition was observed against fungal pathogen *Candida albicans.* Antioxidant activity was observed at 23.30 µg/ml in perchloric acid extract and 19.54 µg/ml in acetic acid extract.

Keywords: Antibacterial, antioxidant, *Ganoderma lucidum*

Introduction

Mushroom were widely used for medicinal purpose in China and other oriental countries for hundreds of years. Mushrooms have been widely cultivated for food and medicinal puposes (Chang 1995). At least 651 species and 7 specific taxa representing 182 genera of hetero-and homobasidiomycetes mushrooms contain antitumour or immune stimulating polysaccharides (Chang 1999). *Ganoderma* species belongs to the Basidiomycetes class, which were known to possess a variety of biochemical compounds with a wide range of biological properties like antibacterial, antitumor and antiviral agents. Genus *Ganoderma* is widely distributed in tropical areas around the world (Kleinwachter et al., 2001), reported to be a famous traditional medicinal fungi and its being used as functional food (Gao et al., 2005). *G. lucidum* is considered to be a popular folk medicine in the prevention or treatment of various diseases including hepatitis, hypertension, hypercholesterolemia, gastric cancer, arthritis and bronchitis. They were also reported to treating animal diseases (Khadila-Muandingi, 2010). Several studies have also revealed the positive biological activities including antitumour, hypoglycemic activity or anti-inflammatory effects and cytotoxicity towards hepatoma cells. Medicinal mushrooms contain diverse classes of bioactive compounds such as fatty acids, flavonoids, terpenoids, phenols, steroids and vitamins etc (Batra et al., 2013; Joseph et al., 2011; Pillai et al., 2008; Rathor et al., 2014). The dried powder of *G. lucidum* is currently used worldwide as dietary supplement (Russell *et al.* 2006), biologically active compounds such as polysaccharides, adenosine, alkaloids, mannitol, organic germanium, triterpenoids, and rare minerals (Shiao, 2003).The present study is focused on the evaluation of biological properties of *Ganoderma lucidum* collected from Tanjore, Tamil Nadu, India.

MATERIALS AND METHODS

Fruit bodies of *G.lucidum* were collected from Tanjore, Tamil Nadu, India. Salient features such as color, size, shape, texture were observed for each strain. The study of the basidiomyetes was made on macro (Kingdom: Fungi, Division: Basidiomycota, Class: Agaricomycetes, Order: Polyporales, Family: Ganodermataceae, Genus: Ganoderma) (size, color, number pores/mm, length of tubes) and microscopic characters (somatic and reproductive structures). Colors are according to Munsell (1975) and Herbaria

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abbreviations were following Holmgren et al. (1990). The collected fruit bodies were carefully stored in polythene bags and transported to the laboratory for mycological examination. Morphological observations were carried out based on the methods described (Wang et al., 2006).

Antimicrobial assay

G.lucidum were extracted with solvents of Ethanol, Diethyl ether and Choloroform. Antimicrobial activity was carried out by using agar well diffusion method (Baeur et al., 2006).

Microbial pathogens

Escherichia coli, Bacillus cereus, Staphylococcus abony, Pseudomonas aeruginosa, Micrococcus sp. and fungal strains of *Aspergillus niger, A. terrus, Candida albicans, Penicillium citrinum* and *Fusarium oxysporum* were collected from Doctor diagnostic Center, Trichy, Tamil Nadu.

Screening of Phytochemical Analysis

The phytochemical analysis was carried out for different solvents perchloric acid and acetic acid extract of *G.lucidum* with standard methods of Harborne (1984). The crude extracts were screened for the presence of alkaloids, flavonoids, triterpenoids, glycosides, anthroquinones, saponins, tannins and phenols by the method adopted by Hanaa, et al. (2008) along with steroids (Harborne, 1973) Terpenoids (Sofowara, 1993) and cardiac glycosides (Kokate et al., 2007).

Antioxidant Activities

Scavenging activity of H_2O_2 radical activity

The H_2O_2 scavenging of different solvents of *G.lucidum* was determined according to the method of Ruch et al. (1989).

Scavenging activity of superoxide Dismutase

The scavenging activity of superoxide dismutase was determined by the method of Yen and Chen (1995).

Thiobarbituric Acid (TBA) Method

TBA method adopted from Sawarkar et al. (2009) was used for evaluating the extent of lipid peroxidation.

Reducing Power Assay

The mushroom extracts (50-250 µg/ml) mixed with 1 ml of distilled water were mixed thoroughly and incubated at 50ºC for 20 min. The absorbance of the reaction mixture indicated increased reducing power. The absorbance was measured at 700 nm (Singh et al., 2008)

Estimation of phenolic content

1 ml of extract solution in a volumetric flask was diluted with distilled water (10 ml). Folin-Ciocalteu reagent (1 ml) was added and the contents of the flask were mixed thoroughly. After 3 minutes, 3 ml of Na2CO3 (2%) was added, then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer. The amount of total phenolic content in the both extracts was determined in micrograms of gallic acid equivalent, using the equation obtained from the standard Gallic acid graph (Slinkard, 1977).

Result

In this study antibacterial activity of Perchloric acid, acetic acid and aqueous extracts of *G.lucidum* was examined by well diffusion method. Antimicrobial activity of Ethanol, Diethyl ether and Chloroform extract of fruiting bodies of *G.lucidum* showed moderate activity. Ethanol extract showed activity against *A.niger* (11.5 mm), *A.terreus* (12.5 mm), *F. oxysporum* (11.5 mm), *P.citrinum* (11.5 mm), *E.coli* (13.5 mm), *B.cereus* (14.5 mm), *S.abony* (13.0 mm), *P.aeruginosa* (13.1 mm) and *Micrococcus* sp (13.5 mm) but no inhibition zone was observed against *Candida albicans.*

Diethyl ether extract of fruiting bodies of *G.lucidum* also showed activity against some fungal and bacterial pathogens. Highest zone of inhibition was determined against *F.oxysporoum* (11.5 mm), *A.niger* (11.5 mm), *A.terreus* (11.5 mm), *P. citrinum* (11.0 mm), *E.coli* (15.0 mm), *B.cereus* (12.0 mm), *S.abony* (12.5 mm), *P.aeruginosa* (12.5 mm) and Micrococcus sp (13.5 mm) respectively. Diethyl ether extract also did not show any inhibition against *Candida albicans*.

Chloroform extract of *G.lucidum* did not show any inhibition against all the fungal pathogens and it showed activity against *Salmonella abony* (14.0 mm) (Table 1).

Table 1: Antimicrobial activity (Well Diffusion method) of *Ganoderma ludicum*

Name of the organism	Zone of inhibition (mm)		
	Ethanol	Diethyl ether	Chloroform
Aspergillus niger	11.5	11.5	-
Aspergillus terrus	12.5	11.5	-
Fusarium oxysporum	11.5	11.5	-
Candida albicans	-	-	-
Penicillium citrinum	11.5	11.0	-
Escherichia coli	13.5	15.0	-
Bacillus cereus	14.5	12.0	-
Staphylococcus abony	13.0	12.5	14.0
Pseudomonas aeruginosa	13.0	12.5	-
Micrococcus sp	13.5	13.5	-

Phytochemical analysis of perchloric acid extract of *G.lucidum* showed the presence of Alkaloids,

flavonoids, terpenoids, glycocides, cardiac glycosides, tannins and phenol. Steroids, Anthroquinones were found to be absent from the extract.

Phytochemical analysis of acetic acid extracts of *G.lucidum* showed the presence of Alkaloids, flavonoids, steroids, terpenoids, glycocides, cardiac glycosides and phenol. However, Anthroquinones, saponin and tannins were absent from the acetic acid extract (Table 2).

Antioxidant activity

The different extracts of *G.lucidum* were tested for their antioxidant properties in different *in vitro* assays. The percentage of inhibition showed that free radicals were scavenged by the test compounds in a concentration dependent manner.

Results of reducing power assay, showed that the increase in reducing power of the perchloric acid extract of *G.lucidum* at 18.32 µg/ml, when compared to acetic acid extract of *G.lucidum* at 13.05 µg/ml. Hydroxyl radical scavenging activity revealed that antioxidant level was observed with 23.30 µg/ml in perchloric acid extract and at 19.54 µg/ml concentration in acetic acid extract. The antioxidant activity was observed for Superoxide dismutase activity in 3.01 and 6.10 µg/ml of extracts respectively. Lipid peroxidation activity revealed that, with increasing of the mushroom extract concentration, the former also increased. The antioxidant activity was recorded as 20.14 and 15.04 µg/ml respectively ~~of~~ for the two extracts of *G.lucidum*. Phenolic content aids in free radical scavenging activity. In this study in the two different extracts of perchloric acid and acetic acid, the *G.lucidum* extract was quantified as 12.1 and 10.3 µg/ml respectively (Table 3).

Table 2: Phytochemical analysis of *Ganoderma lucidum*

S.NO	Test name	Perchloric acid	Acetic acid	Water
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Steroids	-	+	-
4	Terpenoides	+	+	+
5	Glycosides	+	+	+
6	Cardiac glycosides	+	+	+
7	Anthroquinones	-	-	-
8	Saponins	+	-	-
9	Tannins	+	-	+
10	Phenols	+	+	+

Table 3: Antioxidant activity of *Ganoderma lucidum*

Test name	Perchloric acid (100µg/mL)	Acetic acid (100 µg/mL)
Thiobarbituric acid methods (Lipid peroxidation)	20.14	15.04
Reducing power	18.32	13.05
Phenolic content (free radical scavenging activity)	12.01	2.01
Elavonoids (hydroxyl radical scavenging activity)	23.30	19.54
Saponins (SOD activity)	3.01	6.01

Discussion

Ganoderma lucidum is a widely known Chinese mushroom used for centuries to treat various diseases in the East Asia and also possess numerous therapeutic properties, such as anti-tumor, anti-inflammation, immunomodulation, anti-oxidation. (Dudhgaonkar et al., 2009; Sudheesh et al., 2010). It has been reported that triterpenes and polysaccharides were the major bioactive components reported from *G. lucidum* and they have antitumour effects via anti-metastasis, anti-angiogenesis, apoptosis-inducing, cell cycle arrest (Liu and Zhong, 2011). Phytochemical constituents such as saponins, tannins, terpenoids and phlobatannins were extracted from *G.lucidum*, which showed antimicrobial activity against *Micrococcus* sp, *Pseudomonas* sp, *Enterococcus faecalis* (Rajesh and Dhanasekaran, 2014), Similar observation of terpenoids, steroids, flavonoids and saponins extracted from *G.lucidum* fruit bodies exhibited antifungal, antioxidant and anti-platelet aggregation activities (Kumar et al., 2015). In the present investigation, mushroom *G.lucidum* fruiting bodies were used for extraction of phytochemicals for biotechnological applications. Phytochemicals such as Alkaloids,

flavonoids, Steroids, terpenoids, glycocides, cardiac glycosides, tannins and phenol were present in the *G.lucidum* fruiting bodies, which exhibited the antimicrobial and anti-oxidant activity. Two solvents Ethanol and Diethyl ether extract of fruiting bodies of *G. lucidum* showed antimicrobial activity against some fungal pathogens *A.niger, A.terreus, F.oxysporum, P.citrinum* and it showed activity against all the bacterial pathogens *E.coli, B.cereus, S.abony, P.aeruginosa* and *Micrococcus* sp. Among the pathogens used only pathogen *Candida albicans* showed resistance to all the extracts obtained from this study. In aqueous extracts, less activity was observed against few pathogens.

Earlier studies of *Ganoderma* extract displayed potent antifungal activity against *Candida* spp. and *Aspergillus* spp. but it failed to show any antibacterial activity (Kumar et al., 2015). In this study *G.lucidum* showed activity against most of the tested pathogens but it did not display inhibition zone against *Candida albicans.* Recently, Mehta and Jandaik et al. (2012) revealed that methanol extract of *G.lucidum* fruit bodies showed maximum zone of inhibitory activity. Similar kind of observation were recorded by Kamra and Bhatt (2012) methanol and aqueous extract of *G.lucidum* fruit bodies showed potent inhibitory activity in the concentration of 0.5mg/100µl and 1.0mg/100µl. Phytochemical constituents such as phenols and flavonoids present in the *G.lucidum* were reported to have strong antioxidant properties (Kamra and Bhatt, 2012). In the present study our results were in agreement with the earlier findings and phenols, saponins and flavonoids present in the *G.lucidum* showed antioxidant activity. It is proved based on the earlier findings and the present investigation that the phytochemicals constituents present in the *G.lucidum* account for antimicrobial and antioxidant properties but further work is needed to evaluate the compounds present in the extract for pharmaceutical applications.

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