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Research article

In vitro antioxidant activity of selected *Ganoderma* species found in Odisha, India

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Abstract: Four species of *Ganoderma* were collected from different forests of Odisha and analysed for the presence of antioxidant components. Data revealed the presence of DPPH free radical scavenging properties ranged between 91.64–95.51 % in these species. *Ganoderma applanatum* exhibited a studied for the same. Both the *Ganoderma* species exhibited higher amount of tannin content too. Alkaloid content was ranged in 3.66–5.51 $mg.gm^{-1}$ in these four species. Present study exhibited the presence of good higher amount of total phenol, ascorbic acid, carotenoid whereas lycopene and ergosterol content was found to be maximum in *Ganoderma lucidium* as compared to other mushroom species amount of flavonoid content in *Ganoderma tsugae* followed by *G. lucidium* and *G. applanatum*. This preliminary exploratory data of the present study indicated that *Ganoderma* species are good source of antioxidant components. Further extraction and purification of this component may throw light on its role as an 'antioxidant' in *Ganoderma* sp.

Keywords: Mushrooms - *Ganoderma* - Antioxidants - Flavonoids - Ergosterol.

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INTRODUCTION

Mushrooms are well known for their therapeutic properties like antibacterial, antifungal, antiviral, antitumour, immunomodulating, antiallergic, antiatherogenic, hypoglycemic, anti-inflammatory and hepatoprotective activities (Ferreira *et al.* 2010, Lindequist *et al.* 2005). These bioactivities are mainly due to β-glucans, phenolics, vitamins, organic acids and trace elements (Cheung *et al.* 2003, Khatuna *et al.* 2013, Iwalokun *et al.* 2007).

Mushrooms are also considered as home remedy to protect from various diseases elicited by oxidative stress or free radical stress (Chen *et al.* 2012). It is mainly due to the antioxidants which are widely distributed in mushrooms (Jose & Janardhanan 2000, Liu *et al.* 1997, Barros *et al.* 2007). The family Ganodermataceae presents polypore kind of Basidiomycetous fungi having a double walled basidiospore (Donk 1964). Basidiocarps of this genus possess a shiny surface which is associated with the pilocystidia embedded in an extracellular melanin matrix (Moncalvo 2000). *Ganoderma* species are ubiquitous in the world with varied characteristics, such as different shapes, size and color (red, black, blue/green, white, yellow, and purple) of the fruit body, specific host and geographical origin (Zhao & Zhang 1994, Woo *et al.* 1999, Upton 2000).

Ganoderma lucidum (Curtis) P.Karst. (Common names: Reishei, Lingzhi) is a species of Basidiomycetes that belongs to Ganodermataceae of Polyporales (Yang *et al.* 2000). Smina *et al.* (2011) reported various types of antioxidants from the *Ganoderma* which can reduce oxidative damage by directly scavenging free radicals generated in the cell. *Ganoderma lucidium* shows bioactivity against hypertension, bronchitis, arthritis, neurasthenia, hepatopathy, chronic hepatitis, nephritis, gastric ulcer, tumorogenic diseases, hypercholesterolemia, immunological disorders, scleroderma, cardiovascular disease, AIDS and cancer (Sliva 2003), beside *G. lucidium*, *G. tsugae* and *G. applanatum* is also used for the bioactivity which in turn can be well utilized for the health benefits, many reports are available regarding their anticancer, antioxidant,

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antibacterial, antiviral behavior (Mau *et al.* 2005a, Mau *et al.* 2005b, Jeong *et al.* 2008, Kim *et al.* 1998, Rym *et al.* 1999).

The main objective of the present work is to explore the antioxidant properties of some *Ganoderma* species found in Odisha.

MATERIALS AND METHODS

Collection and identification

Healthy, fresh and succulent macrofungi were collected from tropical moist deciduous and semi evergreen forests from different forest divisions of Odisha Macroscopic and microscopic examination of different parts such as pileus, stipe, veil, ring, volva, lamellae and gills etc. were focused to identify species. All of the assays were carried out using the entire mushroom. Mushrooms were cleaned and subsequently dried in the oven at 50°C for about 4 hrs. All of the dried mushrooms were ground to fine powder (100 mesh) and stored in air tight plastic container at room temperature till all the analysis.

Antioxidant components

Total Phenolic Content: Total phenolic content in the wild mushrooms were estimated through folin phenol method as described by Singleton and Rossi (Moradali *et al.* 2006). The optical density was measured at 765 nm using spectrophotometer (Analytic Jena).

Ascorbic acid content: The ascorbic acid content in the wild mushrooms was determined by volumetric method (Singleton & Rossi 1965). The amount of ascorbic acid in mg 100 g^{-1} sample is calculated by using formula; 0.5 mg/V_1 ml × V_2 /5×100/ weight of the sample×100, when V_1 is the standard ascorbic acid consumed against dye.

Flavonoids: The flavonoid content in dried sample was estimated by using aluminum chloride colorimetric technique and expressed in terms of quercetin equivalents per gram (Harris & Ray 1935).

Beta carotene and Lycopene: The concentration of β-carotene and lycopene in mushroom extracts was estimated by spectrophotometer following Nagata and Yamashita (1992), Chang *et al.* (2002) & Barros *et al.* (2007).

Carotenoid: The carotenoid content was estimated in 500 mg of dried mushroom powder treated with 10 ml of 80% acetone and centrifuged at 3000 rpm for 10 minutes at 4°C. The quantity of carotenoid was calculated (Arnon 1949).

Tannins: Tannic acid was served as a standard and tannin content was estimated at 760nm according to Schanderl (1970) and expressed in $mg.gm^{-1}$.

Alkaloids: Total alkaloid was estimated after extraction with glacial acetic acid and ethanol and precipitated with Draggendroff's reagent. The residue treated with sodium sulfide and thiourea solution and optical density was measured at 435 nm for alkaloid estimation (Srividya & Mehrotra 2003).

Ergosterol: Total ergosterol was estimated with chloroform and methanol mixture followed by $NaCl_2$ and glacial acetic acid treatment. Finally sterol content was estimated by using ferric chloride reagent and measuring absorbance at 550 nm (Sadasivam & Manickam 1996).

Antioxidant assay

Free radical scavenging activity: The DPPH free radical scavenging activity was estimated in the methanolic extracts by colorimetric method (Chan *et al.* 2007). 1 ml of methanolic extract was added with 2 ml of DPPH solution (1:2) and incubated for 30 min. in dark after vigorous shaking. Absorbance was measured at 517 nm and scavenging activity of each extract was calculated.

Reducing power ability: Each mushroom extract (0.5–4 $mg.ml^{-1}$) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH: 6.6) and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20 minutes. After 2.5 ml of 10% trichloroacetic acid was added, the mixture was centrifuged at 2000 rpm for 10 minutes. The upper layer (5 ml) was mixed with 5 ml of deionized water and 1 ml of 0.1% ferric chloride, and the absorbance was taken at 700 nm (Analytik Jena) spectrophotometer. Ec-50 value was calculated in $mg.ml^{-1}$ at 0.5 optical density against reagent blank (Oyaizu 1986).

Ferric Antioxidant Reducing Power (FRAP): 100 µl of the methanolic extract was mixed with 3 ml of FRAP reagent and incubated in the room temperature in dark for 10 minutes and finally absorbance was read at 593 nm (Analytik Jena) spectrophotometer. FRAP value was expressed in terms of mg $AEAC.gm^{-1}$ of sample (Benzie & Strain 1996, Athavale *et al.* 2012).

RESULTS AND DISCUSSIONS

In the present study four species of *Ganoderma* (*i.e. G. lucidium*, *G. tsugae*, *G. applanatum* and *Ganoderma* sp.) were collected and analyzed for the antioxidant properties. Radical scavenging activity was seen best in the *G. tsugae* (95.51%) and *Ganoderma* sp. (94.43%) at the IC50 value of 12 mg.ml^{-1} and 10 mg.ml^{-1} respectively (Table 1). Thus the assay used to test the radical scavenging activity shows that scavenging activity is directly proportional to the concentration of the sample. Since free radicals are the causal agents for the oxidative stress and different aliments *Ganoderma* samples used in the present studies can with stand for the health benefits. Presence of ergosterol in range of 0.028–0.45 mg.gm^{-1} in these *Ganoderma* species is also revealed in the present studies (Mattila *et al.* 2002).

Table 1. Representing antioxidant activity of different wild *Ganoderma* species from Odisha, India.

Species	DPPH (%)	IC50 (mg.ml^{-1})	FRAP (mg AEAC.gm^{-1})
Ganoderma lucidium	93.19	9	2.29±0.14
Ganoderma tsugae	95.51	10	1.82±0.01
Ganoderma applanatum	91.64	6	2.43±0.05
Ganoderma sp.	94.43	12	0.24±0.04

The phenolic content of *Ganoderma* sp. was ranged in 9–11.60 mg.gm^{-1} whereas highest flavonoid content was observed in *G. tsugae* (0.84±0.37 mg.gm^{-1}). A very high amount of Ascorbic acid *i.e.* 1.40±0.01 and 1.1±0.16 mg.gm^{-1} was found in *G. applanatum* and *G. lucidium*, respectively (Table 2). More or less similar quantities of carotenoid content ranged in 3.48–4.65 mg.gm^{-1} were observed in all *Ganoderma* sp. studied except *G. applanatum* (7.43±0.29 mg.gm^{-1}). All the four species of *Ganoderma* showed a good amount of alkaloid and tannin content.

Table 2. Analysis of antioxidant components of wild *Ganoderma* species from Odisha, India.

S. No.	Parameters	*G. lucidium*	*G. tsugae*	*G. applanatum*	*Ganoderma* sp.
1	Total Phenolic	9.00±0.30	9.00±0.10	11.60±0.20	11.40±0.10
2	Flavonoids	0.63±0.15	0.84±0.37	0.62±0.13	0.38±0.06
3	Ascorbic acid	1.10±0.016	0.70±0.017	1.40±0.011	0.60±0.017
4	Carotenoids	4.47 ±0.05	4.65±0.73	7.43±0.29	3.48±0.49
5	β-Carotene	3.63±0.19	1.66±0.16	3.30±0.77	1.60±0.43
6	Lycopene	0.224±0.029	0.188±0.002	0.177±0.014	0.043±0.004
7	Ergosterol	0.49±0.067	0.47±0.067	0.44±0.094	0.28±0.044
8	Alkaloids	3.66±0.10	4.50±1.15	4.40±0.25	5.51±0.16
9	Tannins	2.29±0.14	1.82±0.01	2.43±0.05	0.24±0.04

The presence of phenolic compounds in *Ganoderma* confirms the observations of Rawat *et al.* (2013) and Celik *et al.* (2014). Phenolic compounds are responsible factors of antioxidant properties in many mushrooms and plants (Ferreira *et al.* 2009, Barros *et al.* 2008). Data revealed good radical scavenging properties by these mushroom species may be due to good phenolic content also (Table 2). Flavonoids are the group of phenolic compounds which were assumed to be accumulated only in the plants but not by the animal or any fungi (Ferreira *et al.* 2009) however a good amount of flavonoid content was recorded in present studies. Flavonoid content in all the four species of *Ganoderma* ranged from 0.84 to 0.38 mg.gm^{-1} which is also supported by the studies of Logananthan *et al.* (2010) and Barros *et al.* (2008) and can be compared with the edible mushroom varieties. Due to the presence of flavonoid content these species may be considered as curing agent for various cardiovascular, anti-proliferative, detoxification and anti-inflammatory type of diseases (Le 2002).

Findings of carotenoid content in the *Ganoderma* species in good amount made them important at par with fruits and vegetables (Mangels *et al.* 1993). A higher amount of Beta carotene and lycopene was exhibited in these species and corroborated with the reports of Robaszkiewicz *et al.* (2010), Pal *et al.* (2010) and Celik *et al.* (2014). Ascorbic acid is considered to exert a protective role against many oxidative stress related ailments such as cardiovascular, cancer, neurodegenerative problems and cataract (Halliwell 1996). Ascorbic acids ranged from the 1.40–0.60 mg.gm^{-1} of the sample which is comparable to the reports of Barros *et al.* (2008). Tannins are polyphenolic compounds responsible for various bioactivities *viz.* antimicrobial, antioxidative and antitumor

activities (Hatano *et al.* 2006, Yoshizawa *et al.* 1987, Okuda & Ito 2011). Data presented in table 2 regarding tannin content in the four species of *Ganoderma* ranged between 2.29–0.24 $mg.gm^{-1}$ is well compared with the results of Puttaraju *et al.* (2006) and Onuoha *et al.* (2010).

The present study suggests the *Ganoderma* species are the good source of antioxidant compounds. Further, elucidation and purification of these compounds may lead towards the findings of new class of antioxidants useful for the development of health care agent.

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