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Antioxidant Activities of *Ganoderma tropicum* in Submerged Culture

By: Aisha Umar

Department of Botany, University of the Punjab, Lahore, Pakistan
Email: Ash_dr88@yahoo.com

Abstract: *Ganoderma tropicum* is highly nutritional and popular medicinal mushroom of Pakistan. This mushroom was studied for its antioxidant activity. Total triterpenes (TT) successfully extracted by submerge technique. Potato Dextrose Agar (PDA), Glucose Peptone Agar (GPA) and Mushroom Complete Medium (MCM) were used to optim ize the cultural conditions, which enhance the maximum TT extraction. TT scavenged $DPPH^+$ radicals and showed s ignificant reducing activity. Different concentrations were used to evaluate the maximum TT scavenging ability agai nst $DPPH^+$. The antioxidant activity of MCM > PDA > GPA. Aliquot of 100 µg/ml from PDA showed 80.05 % max imum antioxidant activity while PDA 78.05% and GPA was 72.11%. TT capacity to scavenge the free radicals impr oves the body's antioxidant defense systems. The results showed that *Ganoderma tropicum* triterpenes did not posse ss significant toxicity and can be used as natural antioxidant.
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Key Words: *Ganoderma tropicum*, Antioxidant, Scavenge, DPPH, Submerge

INTRODUCTION

Ganoderma tropicum wild specie belongs to family *Ganodermatacea.* This is worldwide oriented mushroom mainly found in tropical areas of Pakistan (Pilotti et al. 2004). In health orientation and supplementation, it can replace *G.sinensis* and G. *lucidum.* Its therapeutic candidates deal coronary hepatitis and heart diseases (Wu et al., 2013). Potentially active lanostanoid triterpenes which is also called Ganotropic acid possess antioxidant activities. Triterpenes scavenege free radicals (Day, 2004). Oxidative stress stimulates the free radicals including ROS, hydrogen peroxide (H_2O_2), superoxide anion and hydroxyl radical (·OH), which causes cell signalling and maintenance of homeostasis (Devasagayam et al., 2004). Human immune system kills these radicals by their enzymatic system (Peterson, 2001) and endogenous antioxidants (Nitha et al., 2010). Now days, uncontrollable production of these radicals causes various degenerative diseases at an alarming rate (Yeh et al., 2011). This system is not sufficient to prevent the cellular damage which leads to health hazards (Pinto et al., 2005).

Presently, synthetic antioxidants are consumed to inhibit oxidation of food and extending shelf life (Wen et al., 2012). Recent report declare that synthetic antioxidants are carcinogenic and have numerous disadvantages and dangerous for human beings (Gupta & Sharma, 2006). The supreme treatment to prevent the diseases (Nagochi & Nikki, 2000; Devasagayam et al., 2007) without side effects is the production of natural products and nutraceuticals comprising antioxidant ability (Emanuel and Sultana 2013; Kamath & Rajini, 2007).

Many medicinal mushrooms are recently reported and possess significant antioxidant activity (Mathew et al., 2008: Nitha et al., 2010). *Ganoderma tropicum* is one of them. This *Ganoderma* possess triterpene with strongest antioxidant activity. They can replace the synthetic antioxidant with natural products. In this study triterpene was extracted by submerge technique. The mycelium extract of *G. tropicum* was evaluated by scavenge the DPPH free radical form.

MATERIALS AND METHODS

Isolation, Culturing and Purification of *Ganoderma tropicum*

Fruiting bodies of *G. tropicum* collected from Lahore Pakistan. It was sterilized by 0.1 % Hg_2Cl_2 for 1 min and then washed with 75 % ethanol and water (Mohita et al. 2013). Three different culture media were prepared to regulate the maximum mycelial growth of *Ganoderma* species (Table 1). These media were sterilized at 121°C for 15 min at 15 lbs in autoclave. The liquid medium was transferred separately to sterile petri plate and allowed for solidification then place the sterilized fruiting body to petri plates. Plates were incubated at room temperature (30°C) for 48 to 72 h (7 days). Kanamycin or streptomycin (0.5 mg/l) (Adebayo-Tayo et al. 2011) was used as an antimicrobial agent.

Table 1: Nutritional Composition of Reagents in Culture Media

Media and Composition (g/l)			
Nutritional Reagents	**PDA**	**GPA** (Maziero et al., 1999)	**MCM** (Kim et al. 2002)
Glucose		10	20
Peptone		10	2
Yeast Extract		10	2
Malt Extract		15	
Potato	4		
Dextrose	20		
MgSO4·7H2O			0.5
K2HPO4			1
KH2PO4			0.5
Agar	15	20	20

***PDA:** Potato Dextrose Agar, **GPA:** Glucose Peptone Agar, **MCM:** Mushroom Complete Medium

Optimization of Cultural Condition

Inoculum from three different medium (Park et al. 2001) was transferred separately into 250 ml Erlenmeyer flasks containing 100 ml basal medium (Hyun et al. 2006; Kim et al. 2002). The fermenter was equipped with instrumentation for measurement and control of agitation, temperature, pH, dissolved oxygen concentration and foam. The culture medium in the fermentor, containing basal media sterilized at 112ºC for 50 min and inoculated with second preculture. Initially the pH of the medium, air flow rate, and agitation speed was 6, 0.6-8.00 vvm and 4000 rpm respectively (Tang and Zhong 2002). The dissolved oxygen (DO) level was 20% of air saturation and temperature 30°C. pH can be adjusted by 4% NaOH or 2% HCl for 7 days. After fermentor, all broth was filtered with a 60-mesh stainless steel sieve.

Production and Purification of Ganotropic Acid (GA)

Harvested mycelia (100 mg) were extracted with 95% (v/v) ethanol or 70% (v/v) methanol for 7 days. The methanol extract was evaporated to near dryness under rotatory vacuum evaporator and dissolved in 500 ml water. The water solution was extracted with chloroform. The GA in chloroform phase extracted with saturated 5% w/v $NaHCO_3$. Then the lower phase ($NaHCO_3$ phase) was acidified to pH 3.0 by adding 2 M HCl under ice-cooling condition and re-extracted with chloroform. After removal of chloroform by evaporation at 40 ◦C, GA dissolved in absolute ethanol. After evaporation a pale yellow solid material was obtained. The solid was dried in an oven to yield crude triterpenoids.

Antioxidant Activities of Ganotropic Acid

Add 3 ml of freshly prepared DPPH (2, 2-diphenyl-1-picryl hydrazyl) into different concentrations of the total triterpenes (20-100 ug/ml). Place in dark at room temperature for exactly 30 min and absorbance was measured at 515 nm. The DPPH scavenging activity was calculated by following equation:

% DPPH scavenging = Abs(t=0) - Abs(t=30) x 100 / Abs(t=0) (Wang et al. 2012)

Where: Abs(t=0) is absorbance of DPPH radical at t = 0 and *Abs(t=30)* is absorbance of DPPH radical and extracts at t = 30 (Table 2).

Statsistical Analysis.

The values were verified by means and standard deviation (SD). *P* values < 0.05 were considered statistically significant. All data were means of three measurements

Table 2: Free Radical scavenging activity of *Ganoderma tropicum* by DPPH reduction

Media Comp.	Concentration (ug/ml)	Antioxidant Activity (%)
PDA	20	19.11 ± 1.00
	40	37.05 ± 1.11
	60	41.15 ± 2.79
	80	62.12 ± 1.15
	100	78.05 ± 1.30
GPA	20	19.05 ± 1.24
	40	31.11 ± 1.15
	60	58.55 ± 3.79
	80	67.05 ± 1.40
	100	72.11 ± 1.82
MCM	20	15.02 ± 1.10
	40	38.11 ± 1.14
	60	55.05 ± 3.79
	80	72.11 ± .30
	100	80.05 ± 1.82

RESULTS AND DISCUSSION

Ganoderma tropicum is a popular medicinal and nutritional mushroom. This mushroom has recently acknowledged worldwide and captures the attention in health care (Chen et al., 2008). *G. tropicum* possessed various biological candidates that have been used as a functional food and longevity (Paterson, 2006). Dietary antioxidants is a secondary defense system (Peterson, 2001), which prevent and control the extreme production of free radicals. Appropriate quantity of antioxidants required in consumption (Alvarez et al., 2006). Natural compounds from mushroom have potent antioxidant capacity and safely assimilated in human diets than synthetic antioxidant.

In this experiment, submerge culture technique was selected because mycelium of *G. tropicum* liked to grow fantastically with maximum production therapeutic agent called ganotropic acid. Ganotropic acid a triterpene was bitter in taste but have many medicinal impacts in folk and pharmacy medicines. In this work three different growth media (**PDA:** Potato Dextrose Agar, **GPA:** Glucose Peptone Agar, **MCM:** Mushroom Complete Medium) were used to evaluate the maximum production of triterpene. Triterpene possessed antioxidant activity which scavenges free radicals. DPPH (2,2-diphenyl-1-picryl hydrazyl) is a most common form of free radical and a reducing assay. It is quick method to evaluate the antioxidant capacity of extracts. Its absorbance was 515 nm in UV spectroscopy (Aquino et al., 2001). When DPPH interact with assay of triterpene after 30 min, its value become reduced which indicated that extract possessed antioxidant ability. This ability was determined by % DPPH scavenging formulae. In this experiment 3 ml of DPPH was added into different concentrations of triterpene assay (20, 40, 60, 80 and 100 µg/ml) (Fig 1, 2, 3) (Table 2).

Aliquot from PDA after seven days were transferred to liquid basal media (100 ml), the colour variation indicated the secondary metabolites production, which has strong antioxidant activities. Aliquot of 100 µg/ml showed 78.05 % maximum antioxidant activity (Fig 2) while 100 µg/ml from GPA was 72.11% (Fig 3). MCM was a complete and highly nutritive for *G. tropicum.* MCM 100 µg/ml has 80.05% antioxidant capacity (Fig 1). Minimum concentration of 20 µg/ml of PDA and GPA has nearly equal ability to scavenge free DPPH radical (Fig 2, 3). While the same concentration of MCM has lower value than PDA and GPA. Antioxidant potential 40 µg/ml of GPA of *G. tropicum* was 31.11% of this work is near to *Ganoderma lucidum* (30.1%) and greater than *Schizophyllum commune* (27.6%) (Noorlidah et al., 2012; Zhu et al., 1999). Sminaa et al., (2011) used 100 µg/ml of *G. lucidum* showed significant DPPH scavenging activity i.e., 81.81%. Their results were match with similar concentration of MCM used in this study.

Huang (2000) reported that DPPH scavenging effects of *Antrodia camphorata* was 31% at concentration of 0.5 mg/mL. Antioxidant ability to scavenge DPPH of *Ganoderma tsugae* was about 42% at the concentration of 0.2 mg/mL (Yen and Wu 1999), which was comparable dry matter of filtrate (DMF) of fruiting body of *Antrodia camphorata* (45 %) (Song and Yen 2002). *Cordyceps militaris* extracts (CME) was weak in scavenging the DPPH than *Cordyceps sinensis* (CSE) (Won and Park 2005)

Concentration of 20 µg/ml from PDA and GPA (Fig 2, 3) has potential like *Hericium erinaceus* (17.7%) (Noorlidah et al., 2012), *Volvariella volvaceae* (17.4%), *Termitomyces heimii* (16.4%), *Pleurotus*

florida (16.6%), *Auricularia auricular-judae* (16.9 %) *and Pleurotus flabellatus* (18.4) (Puttaraju et al., 2006). MCM extract of 20 µg/ml possessed 15.02 % antioxidant activity (Fig 1). This result harmonized with *Agrocybe* sp. (15.0%), *Pleurotus eryngii* (15.6 %) and *Lentinula edodes* (15.9 %) (Puttaraju et al., 2006). Antioxdnt activity to scavenge DPPH of PDA, GPA and MCM of G. tropicum in any concentration was maximum than *Flammulina velutipes* (12.0 %), *Pleurotus sajor-caju* (14.6 %) and *Pleurotus cystidiosus* (14.6 %) (Puttaraju et al., 2006)

Several studies extracted the ganoderic acids and check their antioxidant activities (Keypour et al., 2010; Wang and Liu, 2008). Fruiting body of *Boletus edulis* composed of ergosterol (Mattila et al., 2002), which have high antioxidant capacity (Ribeiroa et al., 2008) than *Coprinus comatus*, *Agaricus bisporus*, *Pleurotus eryngii* but lower than *Pleurotus citrinopileatus* (Tsai et al., 2007). Literature preferred PDA for growth of *Ganoderma* species but these results indicated that MCM would be best growth media for maximum production of therapeutic candidates (Fig 4). This study evident that *G. tropicum* triterpenes was devoid of toxicity and possessed outstanding antioxidant property.

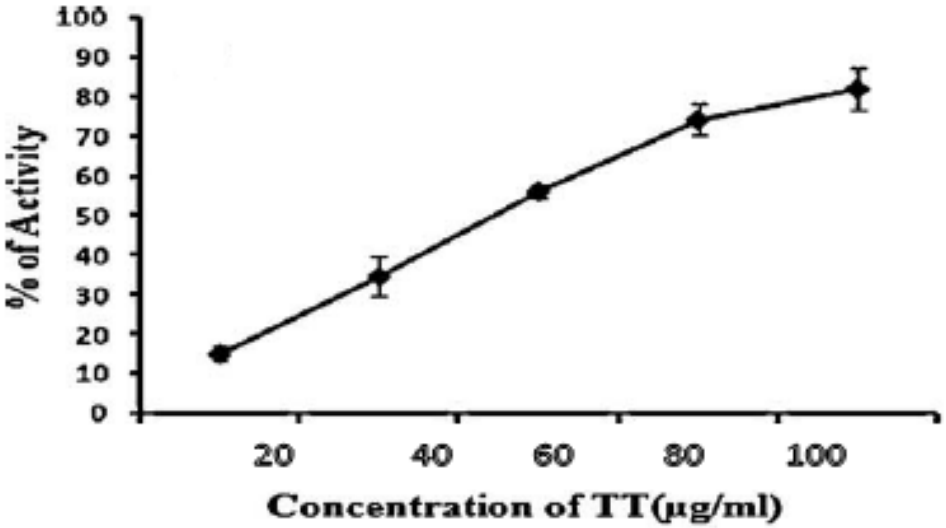

Fig 1. DPPH radical scavenging activity MCM

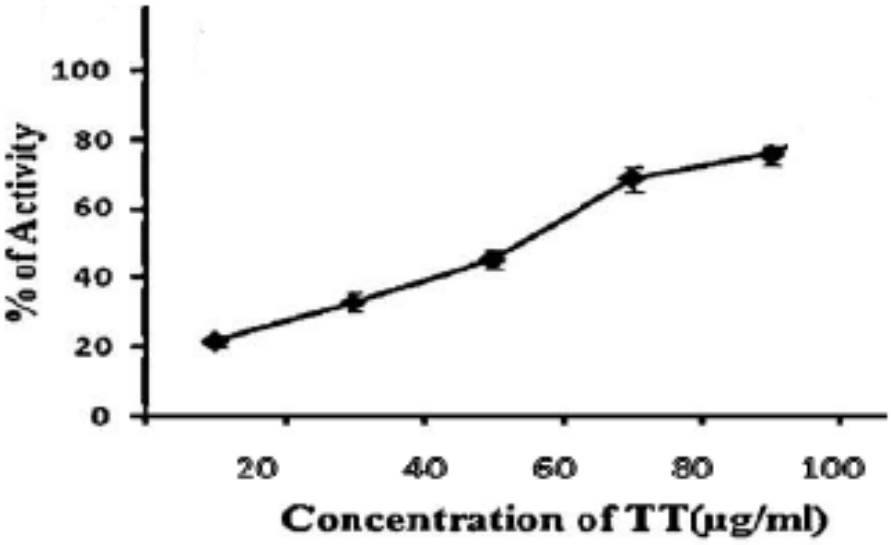

Fig 2. DPPH radical scavenging activity PDA

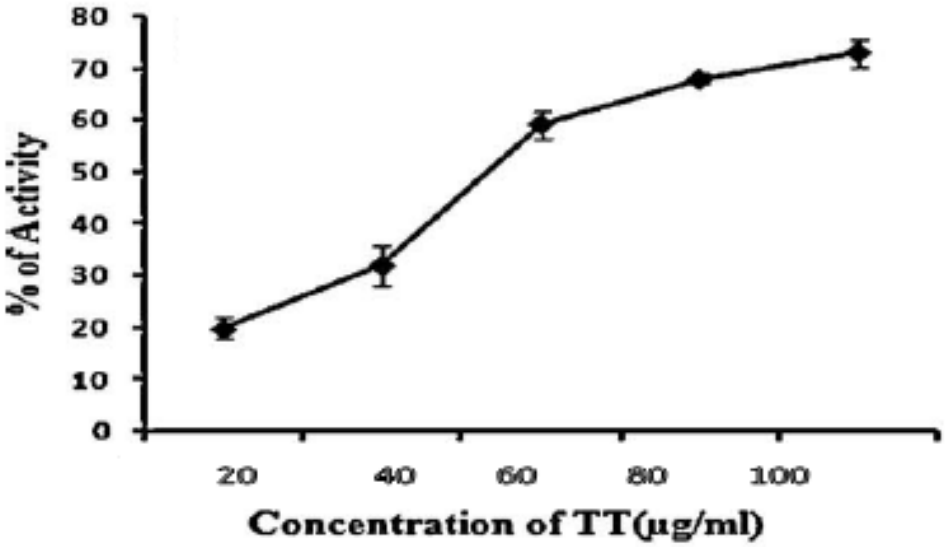

Fig 3. DPPH radical scavenging activity GPA

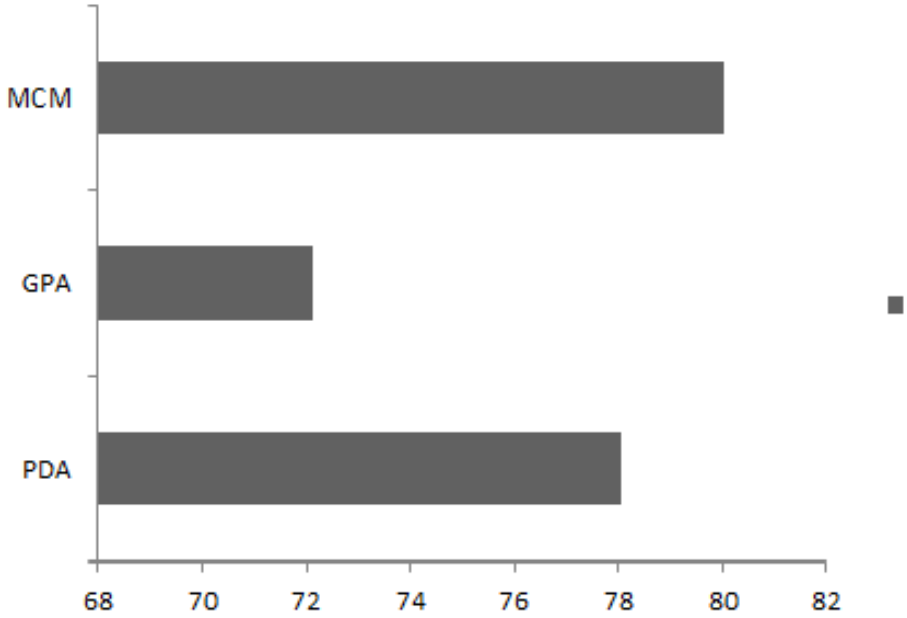

Fig 4: Percentage Antioxdant Activity of PDA, GPA and MCM

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