Comparison between antioxidant activity and bioactive compounds of *Ganoderma applanatum* (Pers.) Pat. and *Ganoderma lucidum* (Curt.) P. Karst from Iran

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Abstract

Members of *Ganoderma* genus belonging to Basidiomycota, such as *Ganoderma applanatum* and *Ganoderma lucidum* have been noticed in traditional and modern medicine and pharmacology for their medicinal properties and bioactive compounds. The present study was undertaken to determine whether there are any differences between chemical properties of *G. applanatum* and *G. lucidum*. The fresh mature fruiting bodies of fungi growing on common hornbeam (*Carpinus betulus*) were collected in Neka, a county in Mazandaran Province, Iran, and their antioxidant activity and bioactive compounds content were examined by spectrophotometer and HPLC method. According to the chemical analysis, the total phenols and flavonoids content, betulinic acid and also antioxidant activity measured by DPPH radical scavenging and FRAP methods in *G. applanatum* were higher than the other, but *G. lucidum* had higher content of total polysaccharides and proteins. Also, the two other terpenoids (oleanolic acid and ursolic acid) were very low in these two fungi.

Keywords: *Ganoderma*; medicinal properties; secondary metabolites; antioxidant activity; bioactive compounds

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Introduction

Some fungi such as Basidiomycota have been noticed in traditional and modern medicine and pharmacology, because of their medicinal properties and bioactive compounds. Members of *Ganoderma* genus such as *G. applanatum* and *G. lucidum* belong to the family Ganodermataceae, class Basidiomycota, and have biochemical and medicinal properties.

Ganoderma applanatum (the artist's bracket) is a bracket fungus with a cosmopolitan distribution. This fungus grows as a mycelium within the wood of living and dead trees. It forms fruiting bodies that are up to 100 centimeters across, hard as leather, woody-textured, and inedible. The history of medicinal uses of *G. applanatum* goes back to thousands of years ago among East Asian civilization. Also, there are

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evidences for antioxidant activity and existence of bioactive compounds such as phenolics in *G. applanatum* extracts in modern investigations (Nagaraj et al., 2014, Vazirian et al., 2014), but in contrast to *G. lucidum*, it is less investigated, especially in Iran.

Ganoderma lucidum (the lingzhi mushroom) is a large, dark mushroom with a glossy exterior and a woody texture. Its kidney-shaped cap and peripherally inserted stem gives it a distinct fan-like appearance. When fresh, the lingzhi is soft, cork-like, and flat. It lacks gills on its underside, and instead releases its spores via fine pores. Depending on the age, the pores on its underside may be white or brown. It is also an ancient traditional medicine that is highly valued in Asian countries for treatment and prevention of many diseases. The beneficial health properties of *G. lucidum* are attributed to its antioxidant capacity and bioactive compounds such as polysaccharides, terpenoids, and proteins. The results of the antioxidant assays showed that *G. lucidum* occurring in south of India were found to possess in vitro antioxidant activities and scavenged free radicals (Lakshmi et al., 2008).

Over 150 triterpenoids have been isolated from *Ganoderma* species. According to studies of Saltarelli et al. (2009) and Chien et al. (2011), the triterpenoids and polyphenols present in *G. lucidum* have been reported to show antioxidant activities. A few scientists such as Dzubak et al. (2006) have reported oleanolic acid, ursolic acid, and betulinic acid from *Ganoderma* species but their information were insufficient or inexact. So it is interesting to conduct quantitative analyses for determining these triterpenoids from *Ganoderma*. The quality control and standardization of the isolation of the terpenoids of *Ganoderma* fungi is a challenging task and HPLC is used to overcome these problems (Keypour et al., 2010).

G. lucidum is the most studied and used species of the genus *Ganoderma*, but *G. applanatum* is less investigated, especially in Iran. The purpose of this study was to estimate and compare the antioxidant activity (using DPPH and FRAP methods) and bioactive compounds (total phenolic, flavonoid, sugar, protein and triterpenoids) between *G. applanatum* and *G. lucidum* from Iran.

Materials and Methods

Sampling and extraction

The fresh mature fruiting bodies of *G. applanatum* and *G. lucidum* were collected from the common hornbeam (*Carpinus betulus*), in Neka, a county in Mazandaran Province, Iran, in July 2018. The shade and air-dried fungi were grinded and stored in freezer at -20° C for analyses.

DPPH radicals scavenging activity assay

The antioxidant activity of fungal extract was evaluated by methanol solution of 1,2-diphenyl-2-pricrylhydrazyl (DPPH) to measure free radical scavenging activity according to Blois (1958). The absorbance of control (containing all reagents except the test compound) and test compound at 517 nm were measured with spectrophotometer (UV VIS, PG instruments, China). BHT was used as a positive control. Lower absorbance of the reaction mixture represents higher DPPH radical scavenging activity.

The antioxidant power is explained by IC_{50} as the effective concentration of extract that inhibits the formation of DPPH free radicals by 50%. IC_{50} values were obtained from linear regression analysis. Extract concentration providing 50% inhibition (IC_{50}) was measured from the graph plotted inhibition percentage against the extract concentration. Butylated hydroxytoluene (BHT) was used as the positive control.

Ferric reducing antioxidant power (FRAP) assay

FRAP method is based on reduction of Fe^{3+} to Fe^{2+} ions(Benzie and Strain, 1996). The absorbance was measured at 593 nm against a blank having all the reagents excluding the sample using spectrophotometer. Increased absorbance of the reaction mixture represents an increase in the reduction capability.

Standard curve of $FeSO_4$ concentrations (as the standard), Milli- Q and HCl (0.1% $v.v^{-1}$) was prepared using the similar procedure from which the regression formula was derived. Results were expressed in µM $FeSo_4$/g DW.

Total phenolic content (TPC) assay

Total phenolic constituent in methanol extract was assayed employing the method described in Wang et al. (2005) involving follin-Ciocalteu reagent and gallic acid as standard. The absorbance of the blue color was measured at 765 nm. The same procedure was repeated for all standard gallic acid solutions and a standard curve was obtained according to the equation. Total phenolic content was expressed as gallic acid equivalent in mg per gram of dry weight (DW) of sample using the following equation based on the calibration curve:

$y= 35.96x – 0.0096$

$R^2= 0.9986$

where y was the absorbance and x was the total phenol content in $mgGA.g^{-1}DW$ of sample.

Total flavonoid content (TFC) assay

Total flavonoid content was calculated by the method developed by Chang et al. (2002). The absorbance was determined at 765 nm. The calibration curve was prepared using quercetin as standard. Flavonoid contents were expressed as mg quercetin equivalent/g of sample dry weight (mg QE. g^{-1} DW) using the following equation based on the calibration curve:

$y = 75.931x -0.0901$

$R^2 = 0.9901$

where y was the absorbance and x was the total flavonoid content in mg QE. g^{-1} DW of sample.

Total sugar content (TSC) assay

The total sugar content of aqueous extractions was determined using the colorimetric phenol-sulfuric acid method proposed by DuBois et al. (1956). Sample absorptions at 470 nm were compared to the calibration curve of different concentrations of glucose (0.005- 0.04 $mg.ml^{-1}$).

Total protein content (TP) assay

The total protein content of extracts was measured using the method proposed by Bradford (1976). Bovine serum albumin (BSA) was used as standard. The absorbance was read at 595 nm. The total protein content was calculated according to the standard curve and its equation. The result was the protein content in 0.5 g of sample which was changed per 1 g of sample.

Quantitative analysis of triterpenoids using HPLC method

To overcome the problems related to the quality control and standardization of the isolation the terpenoids (ursolic acid, oleanolic acid and betulinic acid) of *Ganoderma* fungi, HPLC method was used. A Waters liquid chromatography apparatus consisting of a separations module (Waters 2695; USA) and a Dual Absorbance Detector (waters 996; USA) were used for the HPLC analysis. Injection was auto sampler injector equipped with a 100 µl loop. The chromatographic assay was performed on a 25 cm×4.6 mm with pre-column, Eurospher 100-5 C_{18} analytical column provided by KNAUER (Berlin, Germany) reversed phase matrix (5 µm) and elution was carried out in a isocratic system with methanol as the organic phase (solvent A) and distilled water (solvent B) with the flow rate of 1 $ml.min^{-1}$. Peaks were monitored at 210 nm wavelength. Injection volume was 20 µL and the temperature was maintained at 25° C. Calibration curves for standards were prepared for quantification. Data acquisition and integration was performed with Millennium 32 software.

Statistical Analysis

All experiments were replicated for three times to obtain mean values using IBM SPSS Statistics 21.

Results

IC_{50} value of *G. applanatum* was found to be at about 0.4 mg/ml and lower than *G. lucidum*, but its FRAP amount was higher. Therefore, this sample had higher antioxidant activity for the

Table 1
Comparison between antioxidant activity measured by DPPH (shown as IC_{50}) and FRAP methods, total phenolic (TPC), total flavonoid (TFC), total sugar (TSC), and total protein (TP) contents of *Ganoderma applanatum* and *Ganoderma lucidum*

	IC_{50} ($mg.ml^{-1}$)	FRAP ($\mu MFeSo_4/g$)	TPC (mg GA.g^{-1})	TFC (mgQ.g^{-1})	TSC ($mg.g^{-1}$)	TP ($mg.g^{-1}$)
G. applanatum	0.415044	107.4667	6.709924608	1.372561931	23.32240015	9.192309362
G. lucidum	1.745815	38.78872	3.173155358	0.628333619	23.70827706	16.29558902

Table 2
Comparative values of antioxidant activity and bioactive compounds values for extracts of species of *Ganoderma* in India, Taiwan, and Poland according to Acharya et al. (2015), Rajoriya et al. (2015), Lin et al. (2015), Gąsecka et al. (2016)), and the present study in Iran for *G. applanatum* and *G. lucidum*

	G. applanatum	***G. lucidum***	***G. tsugae***	**The present study (Iran)**
DPPH ($mg.mL^{-1}$)	6 (India)	0.9 (Taiwan); 1.3 (India); 9 (India)	10 (India)	0.415044 1.745815
FRAP ($\mu MFeSo4.g^{-1}DW$)	-*	-	-	107.4667 38.78872
TPC (mg GA.$g^{-1}DW$)	11.6 (India)	9 (India); 22.3 (India); 28 (Poland); 41 (Taiwan)	9 (India)	6.709924608 3.173155358
TFC (mgQ.$g^{-1}DW$)	0.4 (India) 45	0.5 (Poland); 0.6 (India); 2.1 (India) 4.8 (India)	0.8 (India)	1.372561931 0.628333619
TSC ($mg.g^{-1}DW$)	-	112.5 (Taiwan)		23.32240015 23.70827706
TP ($mg.g^{-1}DW$)	-	-	-	9.1923.9362 16.29558902

*- These compounds for these species have not yet been measured by scientists

Table 3
The comparison of terpenoid acids content between *Ganoderma aplanatum* and *Ganoderma lucidum* growing on *Carpinus betulus*

	Ursolic acid ($mg.g^{-1}$)	Oleanolic acid ($mg.g^{-1)}$	Betulinic acid ($mg.g^{-1}$)
G. applanatum	trace	trace	1.546035
G. lucidum	trace	trace	1.264011

ability of scavenging free radicals. Also, higher total phenolic and total flavonoid contents were recorded for *G. applanatum*. According to this study, *G. lucidum* had higher amount of total sugar and total protein content (Table 1).

The comparative values of antioxidant activity and bioactive compounds content for the extract of *G. applanatum* and *G. lucidum* in Iran evaluated in this study and the results reported by other authors in different countries for different species of *Ganoderma* are shown in Table 2, where different values were observed for present study. In some cases, Iranian samples had higher values than those in other countries.

According to the results of the presence of triterpenoids using HPLC method, *G. applanatum* and *G. lucidum* contained betulinic acid (shown as B) with 1.546035 and 1.264011 $mg.g^{-1}$ values, respectively, but the other two terpenoids

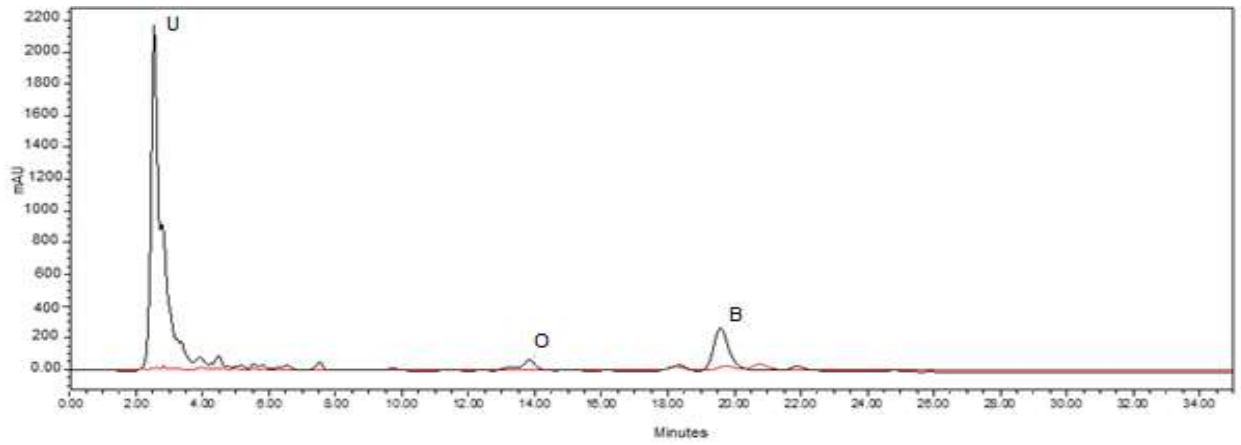

Fig. I. High performance liquid chromatography (HPLC) results of terpenoids (ursolic acid: U; oleanolic acid: O; betulinic acid: B) of methanol extract of fruiting bodies of *Ganoderma applanatum* growing on *Carpinus betulus*; the mobile phase comprised of methanol (solvent A) and distilled water (solvent B) at flow-rate of 1 mL.min^{-1}

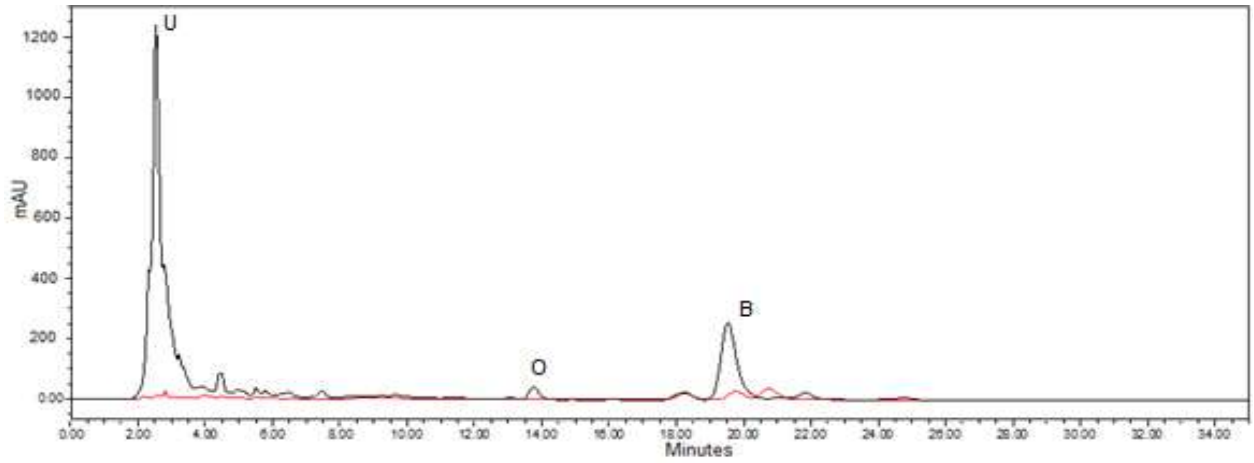

Fig. II. High performance liquid chromatography (HPLC) results of terpenoids (ursolic acid: U; oleanolic acid: O; betulinic acid: B) of methanol extract of fruiting bodies of *Ganoderma lucidum* growing on *Carpinus betulus*; the mobile phase comprised of methanol (solvent A) and distilled water (solvent B) at flow-rate of 1 mL.min-1.

(oleanolic acid: O; ursolic acid: U) were very low in these two fungi (Table 3, Figs. I and II).

Discussion

The antioxidant activity was measured using DPPH method to evaluate free radicals scavenging shown as IC_{50}. IC_{50} indicates the extract concentration providing 50% inhibition of DPPH free radicals. The lower the IC_{50}, the higher the antioxidant power.

Oxidants and antioxidants have different physicochemical properties and may react with each other. Also, there are different explanations on different methods of antioxidant assays and there might be some other chemical moieties other than phenolics. Therefore, it is worthwhile to analyze the extracts by other methods such as FRAP assay. This method is based on the ability of the sample to reduce Fe^{3+} to Fe^{2+} and chelating Fe^{2+}. Iron is an essential mineral for normal physiological activity of the human body, but excess can cause cellular damage. The ferrous ion is well known as the most effective pro-oxidant component in food systems. Also, the good chelating effect would be beneficial and removal of free ion from circulation could be a promising approach to prevent oxidative stress induced disease. Ferrous ion reacts with ferrozine and forms violet color Ferrozin-Fe^{2+} complex. Chelating compounds in the extract prevent the formation of Ferrozin-Fe^{2+} complex, which leads to decrease in the intensity of violet color (Nagaraj et al., 2014).

A lot of chemical compounds have antioxidant activity but in this study, it seems that total phenolic and flavonoid compounds had an important role in antioxidant activity of the studied fungi because we confirmed higher total phenolic and flavonoid compounds content and also antioxidant activity measured as IC_{50} content and FRAP method for *G. applanatum*. This suggests that there is a relation between total phenolic and flavonoid compounds content with antioxidant activity documented by pervious authors such as Pourmorad et al. (2006), Saltarelli et al. (2009), Modi et al. (2014), and Acharya et al. (2015) in *Ganoderma* genus.

This is the first comparative study on the bioactive compounds of *Ganoderma* species growing on similar host trees. The comparative values of antioxidant activity and bioactive compounds content for the extract of *G. applanatum* and *G. lucidum* growing in Iran were evaluated in this study and the results reported by other authors in different countries for different species of *Ganoderma* are shown in Table 2, where different values were observed in the present study and in some cases Iranian samples had higher values than other countries samples.

HPLC is one of the most important methods for the extraction and purification of terpenoids and has also been used for the extraction and purification of oxidized terpenoids for decades, especially for *G. lucidum* species (Lin and Shiao, 1987). A comparison between ganoderic acids of two Iranian and Chinese cultivars of *G. lucidum* showed that geographical and climatic conditions affect the bioactive compounds of this fungus (Keypour et al., 2010).

Poomsing et al. (2013) and Ha et al. (2015) also found that habitat, species, fungus age, harvest season, cultivar, and growth media had an influence on the content of terpenoid compounds of *G. lucidum* and other species. This is why two species that grew on the same host trees were included in the present project.

Oleanolic acid, ursolic acid, and betulinic acid are terpenoids that some researchers have noted in the fungi of the genus *Ganoderma*. Dzubak et al. (2006), for example, have referred to oleanolic acid in *G. lucidum* and discussed its medicinal properties, based on earlier literatures and research studies. Among the sources that Dzubak et al. (2006) cite to support their statement is Kozai et al. (1987) where only pharmacological properties of oleanolic acid were addressed and the presence of these substances in *G. lucidum* was not dealt with. In order to investigate the presence of these terpenoids in *Ganoderma* fungi, the relevant analyses were carried out in two species of *Ganoderma* in this study.

In this study, *G. applanatum* had more antioxidant activity and also more betulinic acid. A positive relation between the amount of terpenoids and antioxidant activity was documented by pervious authors such as Lin et al. (2015) and Smina et al. (2016) on *G. lucidum* extract.

This study suggests that *G. applanatum* is an ideal fungus for antioxidant activity and production of phenolics, flavonoids, and betulinic acid while *G. lucidum* is an ideal fungus for production of polysaccharides and proteins. Although there has been a great deal of focus in recent years on *Ganoderma lucidum* as an anticancer fungus, this research suggests that *Ganoderma applanatum* is a better option for the production of anticancer drugs because of its greater antioxidant properties.

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