TITLE: Mineral composition, antioxidant and cytotoxic biopotentials of wild-growing *Ganoderma* species (Serbia): *G. lucidum* (Curtis) P. Karst vs. *G. applanatum* (Pers.) Pat.

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Antioxidant and cytotoxic biopotentials of wild-growing *Ganoderma* species: *G. lucidum* (Curtis) P. Karst, *G. applanatum* (Pers.) Pat. from Serbia

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ABSTRACT

Since biochemical composition of fungal species may be significantly affected by geographical origin of the specific fungal strain that produce fruit body (basidiocarp), the aim of this work was to analyze mineral composition and chemical profile of two wild-growing medicinal fungal species: *G. lucidum* and *G. applanatum* originated from the Fruška Gora low Mountain chain (Serbia) versus their antioxidant (ABTS and A.E.A.C. assay) and cytotoxic biopotentials (MTT assay on MCF-7). Both species were analyzed for their content of macro and micro-elements determined by atomic absorption spectroscopy (AAS), while phenolic profile of ethanolic (EtOH) and aqueous (H_2O) extracts was examined by liquid chromatography coupled with mass spectrometry (LC-MS/MS).

Both species mostly contained the following ions: $K^+ > Ca^{2+} > Mg^{2+} > Mn^{2+} > Zn^{2+} > Cu^{2+} > Cr^{3+} > Ni^{2+} > Pb^{2+} > Cd^{2+} > Fe^{2+}$. Among nine phenolic compounds, vanillic acid was the most present in both extracts of *G. applanatum* while in *G. lucidum* protocatechuic acid and quinic acid were

mostly contained in EtOH extract and H_2O extract, respectively. *G. applanatum* EtOH extract showed the best reducing power of Fe^{3+} ions and $ABTS^{\cdot}$ radical scavenging activity and was also the richest in total phenolic and flavonoid content. Moreover, *G. applanatum* EtOH extract showed the best cytotoxic effect after 72 h.

Correlations between phenolic profile and biopotentials pointed to the significant impact of detected compounds on demonstrated activities. *G. applanatum* EtOH extract possess the highest biopotentials hence might be considered as a candidate for preparing new food and pharmaceutical supplements.

Key words: antioxidant activity, cytotoxicity, *Ganoderma*, LC-MS/MS, micronutrients, vanillic acid

Abbreviations

AAS (atomic absorption spectrophotometry), A.E.A.C. (Ascorbate Equivalent Antioxidant Capacity assay), AA (ascorbic acid), AAE (Ascorbic acid equivalent), ABTS (scavenging effect on $ABTS^{\cdot}$ radical), BRM (biological response modifiers), DMSO (dimethyl sulfoxide), d.e. (dry extract), d.w. (dry weight), EC_{50} (50% effective concentration), eq (equivalents), EtOH (ethanolic extract), FC (Folin-Ciocalteu reagent), GA (galic acid), GAE (gallic acid equivalent), GLPS (*Ganoderma lucidum* polysaccharide), H_2O (aqua/water extracts), LC-MS/MS (liquid chromatography coupled with mass spectrometry), LoD (limit of detection), LoQ (limit of quantitation), MeOH (methanolic extract), Q (quercetin), QE (quercetin equivalents), RSC (radical scavenging capacity), SD (standard deviation), TF (total flavonoid content), TE (Trolox equivalent), TP (total phenol content)

INTRODUCTION

Ganoderma ludicum (Curtis) P. Karst. (1881) and *Ganoderma applanatum* (Pers.) Pat. are white-rot lignicolous fungal species, mainly growing on deciduous trees (oak, beech, chestnut, maple)

and degrade lignin and cellulose in the wood mass, by using it as a main source of organic matter for their heterotrophic nutrition. In the Far East, the mostly investigated and best-known medicinal mushroom *G. lucidum* has been used for thousands of years in the alternative treatment of various diseases states (Paterson, 2006). Both *Ganoderma* medicinal fungal species have been already known to be wealth in polysaccharides (β-D-glucanes) (Batbayar et al., 2011), terpenes (ganoderic acid, ganoderiol F, ganodermanthriol) (Karaman et al., 2012), proteins (Ling Zhi-8 protein), phenols (flavonoids, phenolic acids) (Ferreira et al., 2009) and other secondary metabolites responsible for their biological effects such as antioxidant (Ferreira et al., 2009), antiviral, antibacterial, cytotoxic, anti-inflammatory and immunostimulatory (Batbayar et al., 2011).

The ability and great potentials of perennial basidioms of lignicolous fungal species for accumulation of absorbed metals through mycelia indirectly, from the soil, through the wood, to their fruit bodies make these wood-decaying fungal species good sources of metal ions e.g. micronutritients which functional role is to act as catalisators or cofactors of various enzymes for fungal metabolism to be carried out (Manavalan et al., 2015).

Considering direct or indirect impact of oxidative stress to emerging development of many diseases (Kaur et al., 2011), natural antioxidants are the focus of contemporary scientific investigations. However, undisputed antioxidant activity of *G. applanatum* and *G. lucidum* (Ferreira et al., 2009; Zengin et al., 2015) cannot be attributed confidently to specific compound. Namely, it is considered that any biological effect is a consequence of the present synergism of all components (Yang et al., 2014). Considering recent literature data, the antioxidant effect of medicinal mushrooms is due to the presence of various phenolic compounds (Yildiz et al., 2015),

but also to some non-phenolic compounds, specifically referring to the terpenes (Ma et al., 2011) and polysaccharides (Heleno et al., 2012) depending on investigated activity.

Fungal metabolites were also documented to possess cytotoxic activities against different cancer cell lines by expressing multi-level inhibitory effect on breast, prostate and colon cancer as well as on human hepatoma cells (Cheng and Sliva, 2015). There are evidences that the mechanism of this activity is based on inhibition of cell growth and induction of apoptosis of the secretion of vascular endothelial growth factor and angiogenesis by prevention of cell migration (Cheng and Sliva, 2015). Low molecular weight compounds is assumed to be the major secondary metabolites which influence the processes of apoptosis, angiogenesis, metastasis, cell-cycle regulation and signaling cascade reaction (Hu et al., 2002; Nguyen et al., 2015).

In addition, high-molecular weight compounds (polysaccharides, polysaccharid-protein complexes) from medicinal mushrooms are very significant for exhibition of antitumor activity, due to their increased solubility in water (Zeidman et al., 2005). These molecules are also well-known to promote their antitumor activity in animals and humans acting as immune modulators - biological response modifiers (BRM), since they promote natural and acquired immunity of host organism itself. BRMs are isolated from over a 30 fungal species up to date, but only few have been found their path to clinical researches, among them, GLPS-polysaccharide from *G. lucidum* (Paterson, 2006). Three potential mechanisms for *Ganoderma* polysaccharides have been declared recently for anticancer effect by stimulation of immune cells themselves, leading to mononuclear leukocyte production of cytokines (or cytotoxic T-lymphocytes) and by production of interleukin 2 and activation (Kao et al., 2013; Paterson, 2006), stimulation of the production of macrophages, NK cells, and T-lymphocytes, antioxidative action and prevention of DNA strand breaks (Kao et al., 2013). Basic *Ganoderma* bioactive polysaccharides are β-(1-3)-D-

glucopironan with 1-15 units of β-(1-6) monoglycosil - branched chains, along with glycoproteins and heteropolysaccharides ganoderans A, B and C (Camargo and Kaneno, 2011). Triterpenes of lanostane skeleton have been shown to inhibit growth and invasive behavior of cancer cells, by induction of cell cycle arrest at the G1 phase by the down-regulation of Cyclin D1, and at the G2 phase by suppressing the activity of PKC as well as by induction of apoptosis in cancer cell lines via mitochondria-dependent pathways followed by activation of the caspase cascade and also act as an anti-oxidant by scavenging free radicals and enhancing innate anti-oxidant enzymes (Kao et al., 2013).

Considering all the above facts about medicinal features of *Ganoderma* species world over, taking into account that they are also present as autochthonous fungal species in the Balkan region, including Serbia, the aim of this study was to investigate the chemical characterization and antioxidant and cytotoxic potentials of these two *Ganoderma* species strains (*G. lucidum* and *G. applanatum*) as possible novel potent sources of natural bioactive substances that could be used as nutriceuticals and pharmaceuticals in regular medicinal treatment in the futures.

MATERIALS AND METHODS

Fungal material

The whole mature air- dried basidioms of wild-growing fungi: *Ganoderma applanatum* (Pers.) Pat. 1887 and *Ganoderma lucidum* (Curtis) P. Karst. 1881 were collected at the locality Morović woods (Fruška Gora, Serbia) on September in a year 2010. Fungal species were determined at Department of Biology and Ecology, Faculty of Natural Sciences and Mathematics, University of Novi Sad by authority of Maja Karaman and both voucher species were deposited at

Herbarium of the University of Novi Sad - BUNS Herbarium, under number (12-00714, 12-00715).

Preparation of fungal extracts

All experiments were performed using EtOH and H_2O extracts of both fungal species. Powdered samples (30.00 g) of dried basidioms were macerated with 300 ml of 95% ethanol on a rotary shaker (Sekljalnik S400 W Chopper, Gorenje) for 72h at 120 rpm, for EtOH extracts. Maceration for H_2O extracts was performed with boiled distillated water, followed by incubation at 80 °C for 60 min in water bath (Elektromedicine, Ljubljana, Slovenia). Obtained organic filtrates of EtOH extracts were rotary-evaporated (unit Bűchi R-210, Flawil, Switzerland) at 35 °C to dryness, whereas H_2O extracts were lyophilized (Christ Alpha 1-2 LD Freeze Dryer, Switzerland) for 72-96h at ice condenser temperature –55 °C. All extracts were stored at +4 °C prior to analysis, after process of freeze-drying reaching final concentration at 10% (w/w).

AAS detection of macro- and micro-elements

Preparation of sample solutions and determination of selected macro- (K, Ca and Mg) and micro-elements (Cu, Ni, Cd, Pb, Cr, Mn, Fe and Zn) were determined in powdered samples of *G. applanatum* and *G. lucidum* by flame AAS. Approximately 0.3 g of oven-dried (70 °C for 24h) material were ground and homogenized in a laboratory mill and then digested in 10 ml of nitric acid and 2 ml 30% (w/v) hydrogen peroxide using a microwave-assisted digestion system (D series; Milestone, Bergamo, Italy) for 45 min at 180 °C with power of microwave of 900 W. Homogenates were then diluted to 25 ml with deionized water. Pre-treated samples were processed by Atomic Absorption Spectrophotometer (model FS AAS240/GTA120, Varian)

using the acetylene/air burner flame technique (with an atomization temperature of about 2300 °C) for Cu and Mg quantification, while a nitrous oxide (N_2O)-acetylene flame (with a temperature of about 2700 °C) was used for Ca content determination. By using single element hollow-cathode lamps concentrations of Cu, Mg and Ca were determined at 324.8, 285.2 and 422.7 nm, respectively and expressed in mg/kg dry weight (DW) of fungi material.

LC-MS/MS screening of the selected phenols

Screening of selected phenolic compounds was performed according to Orčić *et al.* (2014). The Agilent 1200 series liquid chromatograph was used for separation of all analyzes, using a Zorbax Eclipse XDB-C18 RR 4.6mm x 50mm x 1.8mm (Agilent Technologies) reversed-phase column held at 40 °C. Detection was carried out by means of Agilent series 6410B Triple Quad tandem mass spectrometer with electrospray ionization (ESI). MassHunter ver. B.03.01. software (Agilent Technologies) was used for instruments control and data analysis. The binary mobile phase consisted of 0.05% formic acid(A) and methanol (B) and was delivered at a flow rate of 1 mL/min. Gradient elution was performed using the following solvent gradient: starting with 70%A/30% B,reaching30%A/70%Bin 6.00 min, then100%Bat 9.00min, holding until 12.00 min, with re-equilibration time of 3 min. The injection volume for all samples was 5 mL. ESI parameters were: drying gas (N_2) temperature, 350 °C; flow, 9 L/min; nebulizer gas pressure, 40 psi; capillary voltage, 4 kV, negative polarity. All compounds were quantified in dynamic MRM mode (multiple reaction monitoring mode). Compound-specific, optimized LC-MS/MS parameters are given in (supplementary data). The mix of stock solutions was prepared, with concentration of each compound being 100 mg/mL, and then, subsequently serially diluted in methanol-water (3:7), giving working standard solutions with concentration ranging from 0.0015

µg/mL do 25.0 µg/mL, which were used for construction of the calibration curves. Concentrations of standard compounds in extracts were determined from the peak areas by using the equation for linear regression obtained from the calibration curves (R^2 gt; 0.995).

Antioxidant activity analysis

Antioxidant activity was determined by standard antioxidant assays, considering examination of scavenging activity to $ABTS^{\cdot}$ radical according to Arnao *et al.* (2001) and reducing power of fungal extracts (Ascorbate Equivalent Antioxidant Capacity-A.E.A.C. assay) according to Yen and Chen (1995). Ascorbic acid for A.E.A.C. and trolox for $ABTS^{\cdot}$ were used to construct the standard curve, and results were expressed as mg ascorbic acid equivalents/g of dry weight (mg AAE/g d.w.) and mg trolox equivalents/g of dry weight (mg TE/g d.w.). Each analysis was performed three times.

TP and TF content

TP and TF were determined according to Singleton *et al.* (1999) and Chang *et al.* (2002). All assays were measured on spectrophotometer (Multiscan EX Thermo Labsystems, RS-232C, Model 355. (ThermoLabsystems, Helsinki, Finland). Absorbance was read at 690 nm. TP is expressed as mg gallic acid equivalents/g of dry weight (mg GAE/g d.w.).

Absorbance was measured at 414 nm, after incubation of 30 min. The results are expressed as mg quercetin equivalents/g of dry weight (mg QE/g d.w.).

Cytotoxic activity / MTT assay

Antiproliferative activity of EtOH and H_2O fungal extracts was evaluated on estrogen-dependent breast cancer cell line (MCF-7) according to Mosmann (1983). Ellagic acid and DMSO were used as negative control agents. Cancer cells viability was monitored during the incubation period of 24h (acute) and 72h (chronic) for extracts concentrations in the range from 50–250 μg/mL.

Cell cytotoxicity was expressed as a percentage of the corresponding control value. The 50% effect concentration (EC_{50}) values, defined as the concentration that inhibits 50% of cell growth were extrapolated from concentration-response curves.

Statistical analysis

The data were reported as mean values ± standard deviation (SD). EC_{50} values were determined by the linear regression analysis of obtained RSC and values of the concetration of TP and TF (Microsoft Excel programme forWindows, v.2007 and Origin 8).Statistical analysis was determined using one-way analysis of variance (ANOVA) using software system STATISTICA (StatSoft, Inc. (2011), version 10.0 (www.statsoft.com).The differences between control and experimental samples were determined by the Tukey's test.

RESULTS AND DISCUSSION

AAS detection of selected macro- and micro-elements

Results obtained for macro- and micro-elements of *Ganoderma* species are presented in **Table 1**. Among analyzed macro-elements, the content of Ca^{2+} and K^{+} examined in both analyzed species was for twice to three time higher than for Mg^{2+} ions, indicating them to be the most relevant for fungal organism. Both species of fungi mostly accumulated heavy metal ions of Zn^{2+}, Mn^{2+},

Cu^{2+} (≈ 20 μg/g d.w.) and Cr^{3+} (≈18 μg/g d.w.) while Cd^{2+} and Fe^{2+} were detected in very low quantity (**Table 1**). Namely, *G. applanatum* was the richest in Ca^{2+} (2.62 mg/g d.w.), followed by K^{+} (2.18 mg/g d.w.) and Mg^{2+} (0.91 mg/gd.w.) while *G. lucidum* contained mostly K^{+} (3.66 mg/g), followed by Ca^{2+} (1.66 mg/g) and Mg^{2+} (0.84 mg/g).

Among all detected heavy metals, only Cr^{3+} was present in the largest amount in both species, resulting in 8/5 times and 20/10 higher abundance of this element in comparison to Pb^{2+} and Cd^{2+} in *G. applanatum*/*G. lucidum,* respectivelly. In general, *G. lucidum* has proved to be slightly better accumulator of microelements than *G. applanatum* (except Mn^{2+}).

Results obtained for all elements, that comprise the similar amount of detected ions in both fungal species, can be explained by a consequence of common habitat features that have significant impact on qualitative and quantitative mineral contents of fungi. This supports the fact that lignicolous (wood-decaying) fungi absorb ions both directly from the substrates they grow on (wood) and indirectly from the soil where wood grows.

These results were in accordance to previously reported data (Karaman and Matavulj, 2005) for lignicolous fungi since both *Ganodema* species expressed the affinity for the accumulation of macro elements such as K^{+} and Ca^{2+}. The species *G. applanatum* showed almost two times higher accumulation ability for Ca^{2+} than *G. lucidum*, which probably indicate its high content level in wood substrata.

According to recent review for trace element contents in European fungal species (Kalač, 2010), results obtained in this paper for *G. lucidum* mainly agree for all examined metals, except for Cr^{3+} and Fe^{2+} (**Table 1**). In the present study the content of Cr ions was almost four times higher than maximal value recently reported (5 μg/g d.w.) while the content of Fe^{2+} was about 100 times lower (≈ 30-50μg/g d.w.) for the same species (Kalač, 2010). These results are not in

accordance with results obtained before (Kalač, 2010; Karaman and Matavulj, 2005) hence indicate autochtonous *G. lucidum* species as super accumulator of Cr^{3+} ions.

Considering results obtained in relation to earlier data from the same geographical origin (Karaman and Matavulj, 2005), but different habitat, it is clear that environmental habitat properties, which can be also related to pollution, could be the main influence factors for a level of metal content in fungal basidioms. Furthermore, analyzed species could have a strong potential in biomonitoring of atmosphere and especially soil pollution (Karaman and Matavulj, 2005).

LC-MS/MS screening of selected phenols

Forty-five phenolic compounds were quantified using LC-MS/MS technique, among which nine of them were detected in *G. applanatum* and *G. lucidum* extracts. Amounts of detected phenolic compounds are presented in **Table 2**. However, examined compounds, which could not be quantified, might be present in amount lower than the limits of quantification (LOQ).

Generally, phenolic profile was dependent of fungal species and type of extracts resulting in nine phenolic acids and aesculetin, derivate of coumarin. Only, *G. applanatum* EtOH contained all detected phenolic compounds. Protocatechuic and quinic acids were detected in all examined extracts, while p-coumaric and caffeic acids were present only in EtOH extracts of both fungal species. The phenolic acid p-hydroxibenzoic acid was detected in EtOH extract of *G. applanatum* as well as in both, EtOH and H_2O extracts of *G. lucidum*. Syringic acid was present in both extracts of *G. applanatum*, while in *G. lucidum* was not detected. Vanillic acid, gallic

acid and aesculetin were detected in both *G. applanatum* extracts and in EtOH one of *G. lucidum*.

Generally, vanillic acid showed the highest content in both EtOH and H_2O extract of *G. applanatum* (11.40 μg/g and 4.50 μg/g d.w.), while protocatechuic acid (22.20 μg/g d.w.), was detected in largest amount in EtOH extract of *G. lucidum* and quinic acid (2.5 μg/g d.w.) in H_2O.

Previously detected phenolic components of *G. lucidum* were the following phenolic acids and flavonoids: protocatechuic acid, gallic acid, 5-sulfosalicylic acid, quercetin, kaempferol, myricetin, catechin, hesperetin, pyrogalol, γ-tocopherol (Kim et al., 2008; Yildiz et al., 2015; Zengin et al., 2015). In this paper the presence of only two phenolic acids was confirmed (protocatechuic and gallic acids), but also the existence of others such as p-OH-benzoic, p-coumaric, vanillic, caffeic, quinic and siringinic acids, and aesculetin, a derivative of coumarin.

Antioxidant activity

Based on results obtained (**Table 3**) for ABTS assay, EtOH extracts demonstrated better scavenging effect than H_2O extracts for both fungal species, among which EtOH extract of *G. applanatum* has pronounced the greatest activity (328.80mg TE/g d.w.). Moreover for A.E.A.C. assay, the same extract showed the highest reducing power of Fe^{3+} ions (143.26 mg AAE/g d.w.). Generally statistical analysis separated extracts for all assays preformed, including TP and TF content in the following order: *G. applanatum* EtOH > *G. lucidum* EtOH > *G. applanatum* H_2O > *G. lucidum* H_2O. Hence, the species *G. applanatum* proved to be better source of natural antioxidative agents than *G. lucidum*.

According to results of Karaman *et al.*, 2010 *G. lucidum* showed greater antioxidant capacity than *G. applanatum*, which is not proved by our results. However, the fact that different extracts were analyzed before (MeOH and chloroformic) in comparison to the EtOH and H_2O in this study, we assumed that various fungal components could be isolated by different solvents applied, causing different effects on antioxidative activities. Furthermore, biochemical and other biopotentials of wild-growing macrofungi are highly influenced by geographical origin, environmental and habitat factors of the specific species. Furthermore, according to Lee *et al.*, 2007 EtOH fungal extracts showed better antioxidant activities than H_2O ones, what is also confirmed by our results.

Statistically significant positive correlation coefficient (R^2, $p<0.05$) between antioxidant assays and TP and TF content obtained (**Table 3**), supports previously reported data (Liu et al., 2009; Slivova et al., 2004).

According to higher obtained TP and TF content for EtOH extracts (**Table 3**), we assumed that the polarity of the extraction solvent mostly affect the level of phenolics (Karaman et al., 2010; Rajasekaran and Kalaimagal, 2011). These results for TF could be also explained by the higher presence of metal ions in basidioms since they can have tendention to complex flavonoids (e.g. Cr^{3+}; that was measured in the highest amount) (Nagaraj et al., 2014). Moreover, these correlations could explain the impact of phenolic acids as the main compounds dedicated to the manifested antioxidative activities, especially in ABTS assay, what is in agreement of data of Zengin *et al.* (2015) who obseved strong correlation between the TP content and RSC. The best antioxidative activity obtained for *G. applanatum* EtOH extract could be also connected with the highest phenolic quantity of polyphenolic constituents in this extract (**Table 2**).

Lower correlations, but statistically significant (R^2<0.5) noticed for both extracts of *G. applanatum* for A.E.A.C. assay (**Table 3**) could be the consequence of impact of some other non-phenolic compounds to this Ferric chelating activity and its possible mechanism of obtained activities.

Cytotoxic activity

For both fungal species, EtOH extracts showed higher antiproliferative activity than H_2O ones, which can be explained by higher content of phenolics in EtOH extracts and can be realized by the highest correlations obtained between cytotoxic activity and TP and TF in examined fungal extracts (**Table 4**). A lower correlation for TF and cytotoxic activities indicates minor effects of flavonoids to demonstrated activities. The other possible compounds that may have strong cytotoxic effects of *Ganoderma* species are triterpenes, such as ganoderic acids (ganoderic acid AM_1, B, D, F and K) which have been previously detected in *G. lucidum* (Cheng et al., 2010; Yue et al., 2010) and correlated with activation of estrogen receptors (Shimizu et al., 2009). The possible mechanism of antiproliferative activity of EtOH extract of *G. lucidum* on MCF-7 can be explained by apoptosis in human breast cancer cells which might be mediated through up-regulation of pro-apoptotic BAX protein pathway (Hu et al., 2002).

However, there is the assumption that the polysaccharides are responsible for the antiproliferative activity of examined H_2O extracts, while terpenoids are even thought to lead to a proliferative effect on MCF-7 cells (Shimizu et al., 2009), although such a proliferative activity was not presented in this study.

G. lucidum extracts demonstrated the best acute cytotoxicity (24h) (148.40 μg/mL) what is in accordance with data for *G. lucidum* (Hu et al., 2002; Shimizu et al., 2009; Kao et al., 2013;

Yue et al., 2006), while *G. applanatum* showed the best chronic cytotoxic activity (72h) (**Table 4**). EtOH extracts of *G. lucidum* showed three time stronger inhibition effect on proliferation (EC_{50}=148.40±1.03μg/mL) than for the same extract type for Chinese species previously described (EC_{50}=521 μg/mL) (Cheng et al., 2010).

According to lower EC_{50} values obtained, *G. applanatum* species seems to possess better cytotoxicity effects than *G. lucidum* especially EtOH extracts after 72h (EC_{50}=84.71±1.01 μg/mL) which cytotoxic activity was similar to ellagic acid (**Table 4**), which can be attributed to the wealthy phenolic profile of this species determined in this study or terpenoid profile that should be investigated in the future.

Conclusion

In conclusion, after investigation of two autochtonous *Ganoderma* species on their antioxidant and cytotoxic biopotentials, the impact of phenolic compounds such as vanillic, protocatechuic and p-hydroxibenzoic phenolic acids is of the main importance. Beside phenolic acids in cytotoxic activities also some other compounds may contribute to their activities. Fungal phenolic compounds may be easily extracted and applied for therapeutic purposes in the form of functional ingredients, preferably for chronically diseases which are associated with oxidative stress.

Determination of eleven macro- and micronutrients with domination of: Cr, Cu, Mn and Zn ions pointed to *Ganoderma* species as good sources of micro nutrients that can be applied in regular human diet.

In general, *G. applanatum* has demonstrated better biopotential as a source of natural products such as antioxidant and anti-cancer agents than *G. lucidum*, which could point to this

species in a manner of extraordinary source of fungal pharmaceuticals. Despite the fact that both *G. lucidum* and *G. applanatum* species are the favorable subject of numerous scientific studies that confirm their benefits as nutriceuticals, there are many *Ganoderma* strains that are still unexplored.

Furthermore, the investigations of chemical profile and bio-potential of autochthones species from different geographical regions is of great importance worldover. They should combined biological and chemical investigations about their mycochemical profile, and biotechnological potentials as food supplements or remedies.

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Table 1 Content of macro - and micro-elements of two *Ganoderma* species

Fungal species	macro-elements (mg/g)		
	K^{+}	Ca^{2+}	Mg^{2+}
G. applanatum	2.18±0.17[a]	2.62±0.31[a]	0.91±0.17[a]
G. lucidum	3.66±0.42[b]	1.66±0.16[b]	0.84±0.11[a]

	micro-elements (µg/g)							
	Cu^{2+}	Ni^{2+}	Cd^{2+}	Pb^{2+}	Cr^{3+}	Mn^{2+}	Fe^{2+}	Zn^{2+}
G. applanatum	22.05±4.76[a]	2.51±0.39[a]	0.82±0.09[a]	2.22±0.27[a]	17.46±3.06[a]	43.00±8.78[a]	0.37±0.08[a]	21.09±2.63[a]
G. lucidum	22.05±1.73[a]	4.18±1.16[a]	1.79±0.06[a]	3.70±0.93[a]	18.52±0.79[a]	21.34±1.88[b]	0.62±0.12[a]	35.69±3.27[b]

Values are expressed as mean ± SD

[a,b] means in the same column for the specific element not sharing the same superscript are significantly different ($p<0.01$)

Table 2 LC-MS/MS detection of phenolic compounds in examined extracts

Compound	Amount of compound detected (µg/g of dry fungal sample)			
	*G.applanatum*EtOH	*G.lucidum* EtOH	*G.applanatum* H_2O	*G.lucidum* H_2O
Phenolic compound				
p-hydroxybenzoic acid	3.82	8.30	<0.03*	1.90
Protocatechuic acid	6.40	22.20	1.40	0.90
p-Coumaric acid	0.316	0.50	<0.20*	<0.20*
Vanillic acid	11.40	6.30	4.50	<4.00*
Galic acid	2.10	0.50	0.40	<0.80*
Aesculetin	4.70	0.90	0.90	<0.20*
Caffeic acid	1.90	1.70	<0.20*	<0.20*
Quinic acid	2.90	6.20	2.50	2.50
Syringic acid	9.80	<3.60*	3.00	<1.60*

Bold number: amount of qualified phenolic compounds in examined extracts
* number: detected compound – peak observed, concentration is lower than the LoQ (limit of quantification), but higher than the LoD (limit of detection)

Table 3 Antioxidant activity, TP and TF and correlations between TP and TF versus antioxidant activities of two *Ganoderma* species (mean ± SD)

Assay		*G. applanatum*		*G. lucidum*	
		EtOH	**H_2O**	**EtOH**	**H_2O**
ABTS (mg TE/g d.w.)		328.80±1.16 [a]	58.48±2.27 [c]	151.40±1.07 [b]	23.30±2.15 [d]
A.E.A.C. (mg AAE/g d.w.)		143.30±1.20 [a]	52.37±1.16 [c]	26.38±1.28 [b]	39.85±1.41 [d]
TP (mg GAE/g d.w.)		191.76±1.30[a]	21.07±0.42[c]	60.11±1.98 [b]	11.38±0.67 [d]
TF (mg QE/g d.w.)		17.47±0.79[a]	8.34±0.90[d]	10.82±0.37[b]	9.08±0.41[c]
		correlation coefficient - R^2*			
ABTS	TP	0.99*	0.89*	0.98*	0.80*
	TF	0.99*	0.87*	0.91*	0.94*
A.E.A.C.	TP	0.74*	0.73*	0.99*	0.91*
	TF	0.66*	0.76*	0.90*	0.92*

Legend:TP- total phenol content, TF- total flavonoid content
a,b,c,d- different letters in the same row indicate significant difference between extracts ($p<0.01$)
R^2* - all values are statistically significant($p<0.05$)

Table 4 Antiproliferative activities of two *Ganoderma* species and correlations between TP and TF versus cytotoxic activities - EC_{50} (mean ± SD)

Extracts		EC_{50} values (µg/mL)			
		24h		**72h**	
		EtOH	**H_2O**	**EtOH**	**H_2O**
G. applanatum		100.56±0.71[b]	278.59±1.03[d]	84.71±1.01[a]	139.22±1.13[c]
G. lucidum		148.40±1.03[a]	238.62±0.95[d]	164.22±1.08[b]	214.15±1.30[c]
Ellagic acid		63.09±1.05[a]		49.62±1.04[b]	
DMSO		51.81±1.28[a]		37.53±0.98[b]	
		correlation coefficient - R^2			
MTT 24h	TP	0.98*	0.67*	0.90*	0.99*
	TF	0.71*	0.72*	0.44	0.43
MTT 72h	TP	0.99*	0.69*	0.91*	0.75*
	TF	0.73*	0.71*	0.44	0.44

Legend: EC_{50}–extract concentration required to inhibit cell growth by 50%, TP- total phenol content, TF- total flavonoid content

a,b,c,d- different letters in the same row indicate significant difference between extracts ($p<0.01$)

* -values are statistically significant ($p<0.05$)

Supplementary data

LC-MS/MS data for standard compounds

Compound	Retention time (min)	Fragmentor voltage (V)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)
p-hydroxybenzoic acid	1.08	80	137	93	10
Protocatechuic acid	0.79	105	153	109	9
p-Coumaric acid	1.69	90	163	119	9
Vanillic acid	1.24	100	167	108	15
Gallic acid	0.58	90	169	125	10
Aesculetin	1.13	105	177	133	15
Caffeic acid	1.18	100	179	135	10
Quinic acid	0.52	150	191	85	20
Syringic acid	1.31	90	197	182	7

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Title of the article

ANTIOXIDANT AND CYTOTOXIC BIOPOTENTIALS OF WILD-GROWING GANODERMA SPECIES: G. LUCIDUM (Curtis) P. Karst., G. APPLANATUM (Pers.) Pat. FROM SERBIA

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