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Mycochemical Analysis and Antioxidant Efficacy of a Wild Edible Mushroom from the Eastern Himalayas.

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ABSTRACT

Polyphenol-rich fraction from edible mushroom, *Gomphus floccosus*, was tested for *in vitro* antioxidant activity in terms of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and chelating effect of ferrous ions, reducing power and total antioxidant capacity. Findings showed that EC_{50} values were below 1 mg /ml. Bioactive components namely, total phenols, flavonoids, β-carotene and lycopene were quantified. Estimated putative antioxidant components are in order of phenols > flavonoids > β-carotene > lycopene. High Performance Liquid Chromatography (HPLC) was performed to identify the phenols in the fraction, which detected phenolic acids, myricetin and pyrogallol. Result implies that *G. floccosus* can be a potential source of natural antioxidants which may be used as food supplement to treat various oxidative stress related diseases.
Keywords: Free radical; HPLC; mushroom; phenol; reducing power

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INTRODUCTION

The importance of oxyradicals are widely accepted not only in the aging process, but also in numerous human diseases and disorders, such as cancer, diabetes, heart ailments, inflammation, gastric ulcer, hepatic damage etc., where they have either a primary or a secondary role [1]. All living organisms are equipped with stress-response systems which help in regulating the processes of somatic maintenance and repair. But they often fall short, necessitating administration of dietary supplements to aid in combating oxidative stress and related ailments, which in turn leads to the search for antioxidants [2].

In the past few decades, several antioxidants have been explored, both natural and synthetic, which are commonly used as food additives, but the reported carcinogenic effects of synthetic antioxidants [3] have accelerated the extensive research for naturally occurring antioxidants. In the recent years, many researchers in this field of study are getting fascinated by the nutritive and medicinal properties of various edible mushrooms.

Mushrooms accumulate a variety of secondary metabolites including phenolic compounds, polyketides, terpenes and steroids. Some of these compounds bear tremendous importance for humankind, displaying a broad range of useful antibacterial, antiviral and pharmaceutical activities, at the same time bear less toxic effects [4]. A number of wild edible mushrooms have been extensively studied and found to have strong anticancer [5, 6], antimicrobial [7], antiparasitic [8], cardioprotective, [9], antiulcer [10], antidiabetic [11] and hepatoprotective [12, 13] potentiality.

In our present study, we demonstrate the antioxidative properties of polyphenol-rich extract of a wild edible mushroom, *Gomphus floccosus* (Schwein.) Singer collected from the Eastern Himalayas, a biological hotspot, and estimate the putative compounds that render the antioxidant properties to this fraction.

MATERIALS AND METHODS

Preparation of polyphenol-rich extract

Polyphenol rich fraction of *Gomphus floccosus* was extracted according to the following method [14] with a slight modification. The Basidiocarps of *Gomphus floccosus* were dried and powdered and extracted with ethanol at 25˚C for 2 days to eliminate triterpenoids, steroids and other alcohol soluble compounds. It was then filtered and the residue was similarly re-extracted. After filtration, the residue was air-dried and boiled in distilled water for eight hours to extract the water soluble biomolecules and filtered. Polysaccharides were removed from the filtrate by ethanol precipitation and centrifugation. The supernatant was then dried in a rotary evaporator and stored at 4˚C. The dried extract was re-dissolved in deionised water to obtain different concentrations for analysis.

Chemicals

BHT (butylated hydroxytoluene), L-ascorbic acid, quercetin, gallic acid, EDTA (ethylenediaminetetraacetic acid) , potassium ferricyanide, ferrous chloride, Folin-Ciocalteu reagent, DPPH (1, 1-diphenyl 1-2-picrylhydrazyl), TCA (trichlroacetic acid), ammonium molybdate and methionine were purchased from Sigma chemicals Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Determination of putative antioxidants

Total phenol, flavonoid, ß-carotene, and lycopene content in the extract were measured following the methods described by Dasgupta et al. [15]. Total phenol and flavonoid content of the sample were expressed as mg of gallic acid and quercetin equivalents per gram of extract respectively. ß-carotene and lycopene contents were calculated according to the following equations:

$$\text{Lycopene (mg/100mg)} = -0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}$$

$$\text{ß-carotene (mg/100mg)} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$$

Identification and quantification of phenols and flavonoids by HPLC

A HPLC system (Agilent, USA) equipped with an Agilent DAAD detector and an Agilent Eclipse plus C18 column (100 mm 9 4.6 mm, 3.5 lm) was used to identify and quantify some phenols and flavonoids in *GfPre*. The mobile phases were (A) acetonitrile and (B) 0.1 % phosphoric acid. The linear gradient conditions were as follows: 0–5 min, 10 % A in B; 5–15 min, 10–20 % A in B; 15–25 min, 20-90 % A in B with a flow rate of 0.8 ml min^{-1}. The injection volume was 20 µl. UV–Vis absorption spectra were recorded on-line from 190 to 600 nm during the HPLC analysis [16]. Samples were injected three times into the sample loop, and the mean of the peak areas of individual compounds was taken for quantification. Solutions of each standard, at various concentration levels, were injected into the HPLC system. The peak areas and thus the calibration curves and response factors were recorded under the same conditions as for the samples. Gallic acid, caffeic acid, vanillic acid, p-coumaric acid, ferulic acid, cinnamic acid, chlorogenic acid, myricetin, salicylic acid, quercetin, pyrogallol and kaemphferol (Sigma Aldrich, MO, USA) were used as standards. The DAAD detection was conducted at 278 nm for the quantification [17]. Concentrations were calculated by comparing peak areas of reference compounds with those in the samples run under the same elution conditions.

Determination of antioxidant activity

To determine the overall antioxidative potential of *GfPre*, the total antioxidant capacity was tested. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was used to determine the free radical- scavenging activity of *GfPre*. The ability of the extract to reduce ferric iron and chelate ferrous iron were tested in accordance with previously established methods [15]. EC_{50} values were calculated for each of these tests.

RESULTS AND DISCUSSION

In the present study, total phenols, flavonoids and β-carotene were found to be the major antioxidant components, whereas, lycopene was found in lower amounts. The total phenolic content of *GfPre* was 17.5 µg/mg. Compared to some of the previously studied mushrooms, it was much higher than that were found in the hot water extracts of *P. eous* [18], *R. laurocerasi* [14], *P. citrinopileatus*, [19] and *P. flabellatus*, [15] which were reported to be 8.77 µg/mg, 7.05 µg/mg, 8.62 µg/mg and 13.12 µg/mg respectively. *P. squarrosulus* was found to contain marginally higher amount of phenols, 18.1 µg/mg [20]. Some other naturally occurring antioxidant components, like *β*-carotene, lycopene and flavonoids are known to possess strong antioxidative characteristics [21]. In this study *β*-carotene was found in considerable amounts, i.e. 1.67 µg/mg while lycopene content was lower (0.557 µg/mg), These results were found to be higher than that of the methanolic extract of *P. Squarrosulus*, which were 570 ng and 225 ng per mg respectively [20], and polyphenol-rich extract of *R. Laurocerasi*, *0*.01 and 0.007 µg/mg respectively [14]. The estimated flavonoid content of *Gfpre* was 4.62 µg/mg, which is much higher than *R. laurocerasi*, where it was reported to be 2.4 µg/mg [14]. Total phenols, flavonoids and *β*-carotene were the major naturally occurring antioxidant components estimated in this study. The higher amounts of these components in this extract might explain its more effectiveness in antioxidant activities.

Identification and quantification of phenols and flavonoids by HPLC

Table 1: Phenolic compounds quantified in *GfPre*

Peak no.	RT (min)	Compound	Concentration (µg /mg)
1	3.214	Gallic acid	0.998
2	7.712	Caffeic acid	1.931
3	8.195	Vanillic acid	0.503
4	9.767	Ferulic acid	0.189
5	10.599	Myricetin	3.899
6	11.23	Salicylic acid	1.07
7	12.242	Cinnamic acid	0.352
8	12.804	Pyrogallol	69.259

As shown in Figure 1, eleven components were analysed of which eight phenolic constituents were determined in *GfPre*. Our findings revealed that the dominant phenolic compound in *EfraGf* was pyrogallol

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(69.259 mg/gm of dry weight of mushroom). Along with it, caffeic acid, myricetin and salicylic acid were identified in comparatively higher proportions (1.931, 3.899 and 1.07 mg/gm of dry weight respectively). Most components that were detected in *EfraGf* were phenolic acids. Apart from the ones named before, the remaining components were vanillic acid, gallic acid, ferulic acid and cinnamic acid. The concentrations of these identified phenolic acids are shown in table 1.

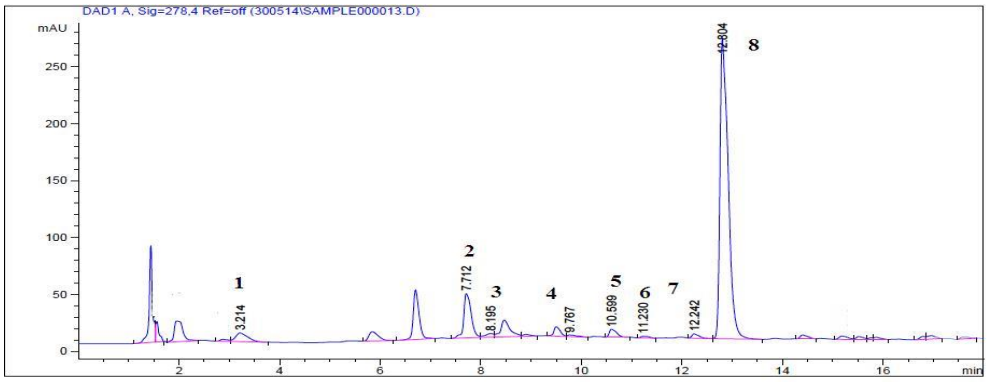

Figure 1: HPLC chromatogram of polyphenol-rich extract of *Gomphus floccosus* (*GfPre*) (MP: mobile phase, peaks: 1: gallic acid, 2: caffeic acid, 3: vanillic acid, 4: ferulic acid, 5: myricetin, 6: salicylic acid, 7: cinnamic acid, 8: pyrogallol

Total antioxidant capacity

Total antioxidant activity of the extract was estimated, using ascorbic acid as standard. On analysis, it was found that 0.5 mg of *Gfpre* is as functional as approximately 43 ± 3.7 µg of ascorbic acid, expressed as 43 µg ascorbic acid equivalents (AAE). The total antioxidant capacity of *Gfpre* may be attributed to their chemical composition and phenolic content. A recent study by Jayaprakasha et al. [22] showed that some bioactive compounds from citrus fruits had strong total antioxidant activity, which was probably due to the presence of flavonoids, carotenoids and ascorbic acid.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity

DPPH is a stable free radical and possesses a characteristic absorbance at 517 nm, which decreases significantly on exposure to radical scavengers by providing hydrogen atoms or electrons to become stable diamagnetic molecules. Upon treatment with an increasing concentration of the *Gfpre*, a marked decrease in absorption was observed, indicating a potent DPPH scavenging ability of the extract (Figure 2). EC_{50} of DPPH radical scavenging activity was 0.193 ± 0.02 mg/ml. When compared with our earlier investigations, the EC_{50} value of polyphenol-rich fraction of *Gomphus floccosus* (*Gfpre*) was lower than the polyphenol-rich extract of *Russula laurocerasi* [14], *Amanita vaginata* [23] and *Russula albonigra* [24]. *Ganoderma tsugae* [25] and *Pleurotus flabellatus* [15] were also reported to have a higher EC_{50} value than *Gfpre* while that of *Pleurotus eous* was much higher [18]. Thus it can be said that the polyphenol-rich extract of *Pleurotus flabellatus* has significant DPPH radical scavenging ability.

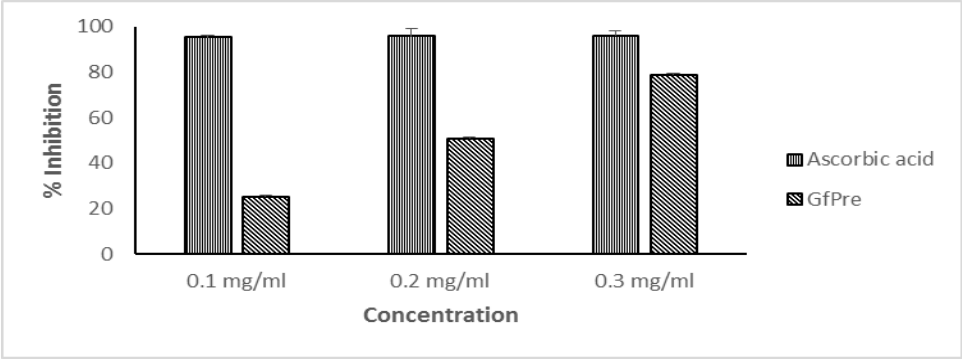

Figure 2:. DPPH radical scavenging activity of *Gfpre* compared with that of the standard, ascorbic acid. Values are the mean ± standard deviation of three separate experiments, each in triplicate.

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Reducing power

Reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. The increasing absorbance at 700 nm by measuring the formation of Perl's Prussian Blue indicates an increase in reducing ability. A steady increase in reducing power was observed (Figure 3). Results showed that EC_{50} for the reducing power of *Gfpre* was of 0.63 ± 0.06 mg/ml. *Gfpre* was found to be a potent reducing agent, with an EC_{50} value of 0.63 mg/ml. Compared with the reducing powers of previously studied edible mushrooms from our previous reports, the polyphenol-rich extract of *Gomphus floccosus* was an excellent reducer of ferric ions. The reducing power of polyphenol-rich extracts of different edible mushrooms in descending order are *G. floccosus* > *P. flabellatus* [15] > *R.* albonigra [24] > *A. vaginata* [23] > *Russula laurocerasi* [14].

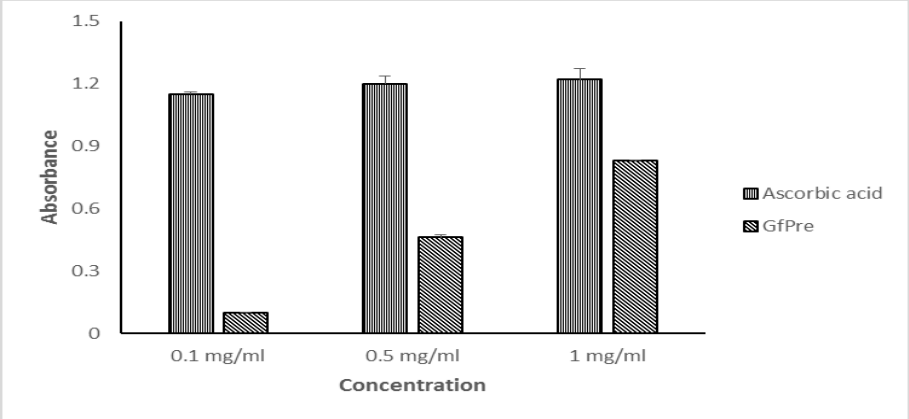

Figure 3: Reducing power of *Gfpre* with respect to that of ascorbic acid used as standard. Values are the mean ± standard deviation of three separate experiments, each in triplicate.

Chelating effect on ferrous ions.

At 100-500 µg/ml the chelating effects of the *Gfpre* was between 32% and 90% (Figure 4). It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential thereby stabilising the oxidised forms of metal ions [21]. The ferrous ion chelating ability of *Gfpre* was effective and the EC_{50} value was found to be 0.16 mg/ml. Our previous investigations have shown that the EC_{50} value of the polyphenol-rich extract for *R. laurocerasi* [14] was 0.58 mg/ml, which is higher, while the EC_{50} value for *A. vaginata* [23] was much higher than that of *Gfpre*. *Gfpre* showed a better EC_{50} to that of *R. albonigra* [24]. Hence, the studied mushroom extract shows higher interference with the formation of ferrous and ferrozine complex and can be considered as a good chelator of ferrous ions.

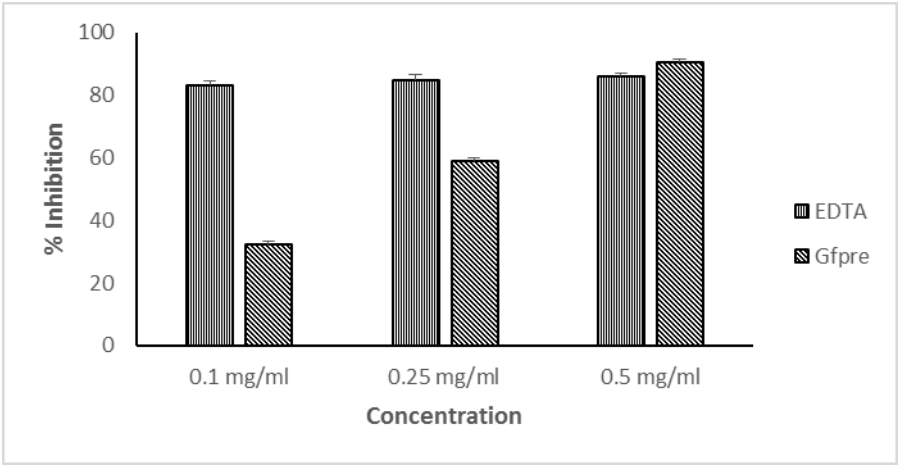

Figure 4: Chelating effects of *Gfpre* on ferrous ions compared with that of EDTA, used as standard. Values are the mean ± standard deviation of three separate experiments, each in triplicate.

CONCLUSION

As a result of the study, the polyphenol-rich extract of *Gomphus floccosus* was found to be an effective antioxidant in different *in vitro* assays including ferrous iron chelating, ferric iron reducing, DPPH free radical scavenging and total antioxidant activity. As observed in our results, the EC_{50} values for all conducted antioxidant activities were well below 1 mg/ml. Analysis of putative antioxidants in the extract revealed the presence of phenolic acids and flavonoids. In the era, where natural; antioxidants are mostly being sought, the polyphenol-rich extract of *Gomphus floccosus* proved potent in *in vitro* systems and can thus be suggested as a natural additive in food and pharmaceutical industries.

REFERENCES

[1] Rai M, Biswas G, Mandal SC, Acharya K. New Central Book Agency (P) Ltd., India, 2011, pp. 479-496.
[2] Sultana B, Anwar F, Przybylski R. Food Chem 2007; 104(3): 1106-1114.
[3] Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniquchi K, Tsuda S. Mutat Res Genet Toxicol Environ Mutagena 2002; 519(1-2): 103-109.
[4] Asatiani MD, Elisashvili V, Songulashvili G, Reznick AZ, Wasser SP. Progress in Mycology, 2010; 311-326.
[5] Chatterjee S, Biswas G, Acharya K. Aust J Crop Sci 2011; 5: 904-911.
[6] Chatterjee S, Biswas G, Chandra S, Saha GK, Acharya K. Int J Pharm Sci Rev Res 2014; 26(2): 189-196.
[7] Rai M, Sen S, Acharya K. Int J PharmTech Res 2013, 5(3): 949-956.
[8] Mallick S, Dutta A, Dey S, Ghosh J, Mukherjee D, Sultana SS, Mandal S, Paloi S, Khatua S, Acharya K, Pal C. Exp Parasitol 2014; 138: 9-17.
[9] Biswas G, Rana S, Sarkar S, Acharya K. Pharmacologyonline 2011; 2: 808-817.
[10] Chatterjee A, Khatua S, Chatterjee S, Mukherjee S, Mukherjee A, Paloi S, Acharya K, Bandyopadhyay SK. Glycoconjugate J 2013; 30(8): 759-768.
[11] Biswas G, Acharya K. Int J Pharm Pharm Sci 2013; 5: 391-394.
[12] Acharya K, Chatterjee S, Biswas G, Chatterjee A, Saha GK. Int J Pharm Pharm Sci 2012; 4: 85-288.
[13] Chatterjee S, Datta R, Dey A, Pradhan P, Acharya K. Res J Pharm Tech 2012; 5(8): 1034-1038.
[14] Khatua S, Roy T, Acharya K. Asian J Pharm Clin Res 2013; 6(4): 156-160.
[15] Dasgupta A, Sherpa AR, Acharya K. J Chem Pharm Res 2014; 6(5): 1059-1065.
[16] Yao L, Jiang Y, Datta N, Singanusong R, Liu X, Duan J, Raymont K, Lisle A, Xu Y. Food Chem 2004; 84: 253-263.
[17] Seeram NP, Henning SM, Niu Y, Lee R, Scheuller HS, Heber D. Agric Food Chem 2006; 54: 1599-1603.
[18] Sudha G, Vadivukkarasi S, Rajan BIS, Lakshmanan P. Food Sci Biotechnol 2012; 21(3): 661-668.
[19] Lee YL, Huang GW, Liang ZC, Mau JL. LWT-Food Sci Technol 2007; 40(5): 823-833.
[20] Pal J, Ganguly S, Tahsin KS, Acharya K. Indian J Exp Biol 2010; 48(12): 1210-1218.
[21] Sowndhararajan K, Joseph JM, Manian S. Int J Food Prop 2013; 16(8): 1717-1729.
[22] Jayaprakasha GK, Girennavar B, Patil BS. Bioresour Technol 2008; 99(10): 4484-4494.
[23] Paloi S, Acharya K. Int J PharmTech Res 2013; 5(4): 1645-1654.
[24] Dasgupta A, Ray D, Chatterjee A, Roy A, Acharya K. Res J Pharm Biol Chem Sci 2014; 5(1): 510-520.
[25] Mau JL, Tsai SY, Tseng YH, Huang SJ. LWT-Food Sci Technol 2005; 38: 589-597. doi:10.1016/j.lwt.2004.08.010