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RESEARCH ARTICLE

Effect of Solvents on Phytochemicals Content and Antioxidant Activity of *Ganoderma lucidum*

Moyen Uddin Pk^{1,2,3}, Rabiul I. Talukder³, Mohammad K. I. Sarkar^{4,*}, Tasnia Rahman⁴, Rumana Pervin⁵, Matiar Rahman⁵, Elina A. Zenat⁶ and Lima Akther⁷

¹Institute of Biological Science, University of Rajshahi, Rajshahi, Bangladesh

²Department of Biochemistry & Molecular Biology, University of Rajshahi, Rajshahi, Bangladesh

³Department of Biochemistry, Primeasia University, Dhaka, Bangladesh

⁴School of Environmental Science and Management, Independent University Bangladesh (IUB), Dhaka, Bangladesh

⁵Department of Biochemistry & Molecular Biology, University of Rajshahi, Bangladesh

⁶Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh

⁷Department of Chemistry, Comilla University, Bangladesh

Abstract:

Introduction:

The aim of this study was to assess the induction of solvents on the total phenol and flavonoid content and also the antioxidant activity of *Ganoderma lucidum* extracts.

Materials & Methods:

In this study, two concentrations (100% and 75%) of diethyl ether, ethanol, butanol, chloroform, and acetone were used as extractants of *Ganoderma lucidum*. Total phenol and flavonoid contents were measured by spectrophotometric methods and 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Free radical scavenging assay was used for the investigation of antioxidant activity.

Results & Discussion:

Extractants significantly affected the % yield of extract, the quantity of phenol and flavonoids and antioxidant activity of *Ganoderma lucidum* mushroom. The highest extraction yield, around 38%, was achieved by 75% acetone, followed by 100% acetone (about 36%) and 75% chloroform (approximate 21%). Hydro-acetone extract exhibited the most significant antioxidative properties (EC₅₀ value; 645.55 µg/mL) comprised of a higher total of phenol content. In conclusion, the total phenol content encouraged the antioxidative potential of *Ganoderma lucidum* mushroom.

Conclusion:

These findings indicate that the selective extraction of *Ganoderma lucidum* shows significant biological activities.

Keywords: : Edible mushroom, Free radical scavenging, Chronic diseases, Functional foods, Antioxidative potential, *Ganoderma lucidum* mushroom.

Article History

Received: October 16, 2018

Revised: December 10, 2018

Accepted: December 18, 2018

1. INTRODUCTION

There are several chronic diseases namely cancer, diabetes, hypertension, and aging which are affected by producing free radicals during metabolism [1]. Anti-oxidants from natural sources will reduce the formation of free radicals *in vivo* while

most of the synthetic antioxidants will show side effects [2].

It has been reported that synthetic antioxidants such as Butylated Hydroxytoluene (BHT), Butylated Hydroxyanisole (BHA) and amass *in vivo* cause liver diseases and cancer [3]. Edible mushrooms, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Lentinula edodes* are available in Bangladesh and comprise different phytochemicals such as polyphenols, flavonoids, tannins, steroids [4]. It was reported that some edible mushrooms exhibited the antioxidant activity, which was

* Address correspondence to this author at the School of Environmental Science and Management, Independent University Bangladesh (IUB), Dhaka, Bangladesh
Tel: +8801711230892; E-mail: kisarkar1981@gmail.com

correlated with the total phenol content [5]. Suphaphit Boonsong *et al.* have shown that different extractants induced antioxidant potential of edible mushrooms [6]. In 2002, Yang *et al.* from China have demonstrated that tree oyster mushrooms were signal properties of free radical scavenging abilities and comprised the highest content of total phenol [7]. The free radical scavenging activity of *Ganoderma lucidum* was encouraged by the presence of total phenol content [8]. Polysaccharides, triterpenoids and other several components of *Ganoderma lucidum* illustrated antioxidant activity [8]. *Ganoderma lucidum* is presently popular and used in the production of functional foods. From several previous studies, it is known that *Ganoderma lucidum* comprises potential phytoconstituents which are concomitant with the advancement of good health as well as used for alternative medicine [9].

In this study, the induction of solvents on antioxidant activities of alcoholic and hydro-alcoholic (75%) extracts of *Ganoderma lucidum* were investigated and examined; the correlation of total phenol and flavonoid contents with DPPH scavenging potentials of 10 different extractants. Here, we also observed how extractants induced the production of percent yield of extracts of *Ganoderma lucidum* mushroom.

2. METHODS AND MATERIALS

2.1. Mushroom Collection and Authentications

The fruiting bodies of *Ganoderma lucidum* were collected from Mushroom Development Institute, which belongs to the Department of Agriculture Extension, Ministry of Agriculture, Bangladesh, Savar, Dhaka-1340. The mushroom was acknowledged and authenticated by a research officer from Mushroom Development Institute, Bangladesh.

2.2. Preparation of Mushroom Extracts

Dried fruiting bodies of *Ganoderma lucidum* were blended to form fine powder and extracted using different extractants-diethyl ether (DE), 75% of DE, Ethanol (E), 75% of E, Butanol (B), 75% of B, Chloroform (C), 75% of C, Acetone (A), and 75% of acetone. Approximately 100g of mushroom powder was submerged in selected solvents and stirred gently with a 2-hour interval. After seven days, all individual fractions were collected and concentrated. Dried fractions were used to prepare the required concentrations for this study.

2.3. Determination of Total Phenolic Content

According to Turkoglu *et al.*, the total phenol content of extracts of *Ganoderma lucidum* obtained from 10 different extractants were determined [10]. In this experiment, gallic acid was used as a standard compound and the total phenol contents were calculated from a calibration curve constructed by plotting the absorbance against concentrations of the gallic acid standard. Results were expressed as the % w/w of each test. All samples were analyzed in triplicate (n=3).

2.4. Determination of Total Flavonoid Content

Total flavonoid contents were determined according to a procedure provided by Dewanto *et al.* [11]. According to this method, aliquot of diluted sample and catechin (used as a

standard) were added to 5% of sodium nitrate solution (75 mL) and gently mixed for 5 minutes after which 10% of aluminum chloride solution (0.15 mL) was added. After 10 minutes of incubation, 0.5 mL of 1M sodium hydroxide was added and final reaction volume was adjusted to 2.5 mL with double distilled water and mixed comprehensively. The absorbance of each dose of sample and standard was recorded at 510 nm and total flavonoid content was determined from a standard curve constructed from catechin absorbance from 0 to 500 mg/mL. Samples were examined in three replicates [11].

2.5. 1-Diphenyl-2-Picrylhydrazyl Scavenging Activity Assay

According to Devi *et al.* the total antioxidant activity of mushroom extracts was determined. Here, triplicate reaction mixture comprised 50 μ L of each mushroom sample and 5.0 mL of DPPH solution (0.04% w/v in ethanol) followed by 80% ethanol as a blank preparation. All test tubes were covered with Aluminum foil and after 30 minutes of incubation, discoloration of the reaction was measured at 517 nm [12]. DPPH scavenging effect was determined using the following equation;

$$\text{DPPH scavenging effect (\%)} = (\text{Ao}-\text{As})/\text{Ao} \times 100$$

Where Ao was the absorbance of control, As was the absorbance of the selected sample.

BHT was used as a positive control and for comparison with each sample's activity against free radical scavenging. The effective concentration for 50% of free radical scavenging activity of positive control and mushroom samples were determined using standard curve ($r^2=0.9994$).

2.6. Statistical Analysis

GraphPad Prism 6 (Pad Software, Inc., USA) was used to analyze data, and results were expressed as mean \pm SD. Error bars were expressed as 95% CI. One way Analysis of Variance (ANOVA) and post-hoc Tukey's Range tests were utilized to decide the distinctions among the methods. An estimation of $P < 0.05$ was thought to be statistically significant.

3. RESULTS

Fig. (1) illustrates the percentage of extract yield of *Ganoderma lucidum* directed by different solvents effect. Here we found that the maximum percentage of extract yield was produced in 75% acetone extraction method. 38.11% and 36.17% extracts of mushroom were produced for 75% acetone and acetone extraction methods accordingly.

On the other hand, second maximum yield production was found in chloroform extraction method and nearly equal amounts of the yield of extract from *Ganoderma lucidum* were found by the extraction methods of butanol solvents (5.44% for 75% butanol and 5.12% for butanol).

The ethanol extraction produces 6.75% yield of extract whereas 8.34% yield of the extract is found in 75% ethanol extraction. About 5% yield of extract was found in 75% diethyl ether extraction and the lowest percentage of yield of extract was produced by diethyl ether extraction method.

In conclusion, the percentage of yielding extract was induced by sample extraction methods as well.

Table 1 shows the total phenol and flavonoid contents of *Ganoderma lucidum* mushroom measured in selected extraction methods. Overall, the maximum total phenolic content was found in acetone extraction and ethanol extract showed the highest content of total flavonoid.

Table 1. Determination of total phenol and flavonoid contents (% w/w).

-	TPC	TFC
DE	0.8±0.23	1.0±0.41
75% DE	1.2±0.11	1.7±0.31
EE	1.1±0.31	1.3±0.26
75% EE	1.7±0.20	1.9±0.81
BE	1.3±0.22	0.6±0.16
75% BE	0.9±0.51	0.3±0.61
CE	1.4±0.87	1.2±0.28
75% CE	1.2±0.12	0.8±0.71
AE	1.6±0.66	0.9±0.45
75% AE	2.1±0.42	1.3±0.16

Values are mean±SEM, n=3. GL- *Ganoderma lucidum*; DEGL-diethyl ether extract of GL, BEGL-Butanol extract of GL; EEGL-ethanol extract of GL; CEGL-chloroform extract of GL, and AEGL-acetone extract of GL; TPC-total phenol content; TFC-total flavonoid content.

About 2% (w/w) total phenol was determined from acetone extract and around 1.5% TPC was also calculated from butanol and chloroform extracts. On the other hand, ethanol and diethyl ether extract exhibited around 1% TPC content of *Ganoderma lucidum*.

Ethanol extract shows the highest content of total flavonoid, which is 1.3% and chloroform extract exhibits around an equal amount of TFC. On the other hand, nearly the same amount of flavonoid content was calculated in the extracts of acetone and diethyl ether and the lowest (0.6%) TFC was found in butanol extract of *Ganoderma lucidum*. From Table 1, it is clear that solvent extraction methods boost the release of total phenol and flavonoid contents from the extracts of

Ganoderma lucidum mushroom.

Fig. (2) explicates the percent inhibition of free radical scavenging activity of *Ganoderma lucidum* extracts, which are produced by different extraction methods and also compares their activity. The signal DPPH radical scavenging activity was found in acetone extract and the results were almost similar to BHT ($p>0.05$). The diethyl ether and 75% diethyl ether shared nearly equal inhibition. In Fig. (2A), the significant difference in the antioxidant activity was observed at a lower dose of DE extract, compared to BHT and approximately similar activities were found with higher concentrations of that extract. The same patterns of free radicals scavenging activities of mushroom were monitored in different solvents' extract (Figs. 2B, 2C, 2D, and 2E).

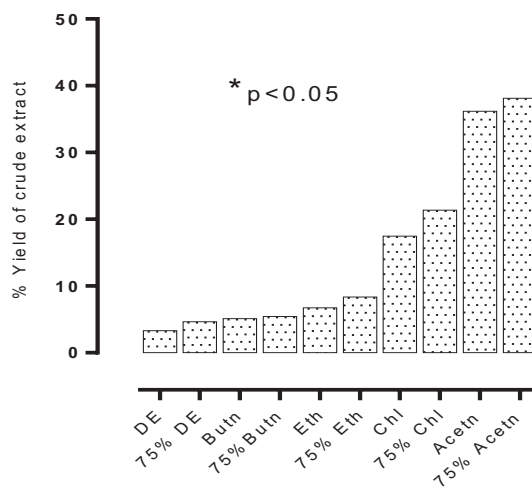


Fig. (1). Percentage of the yield of the extract by different extraction methods from *Ganoderma lucidum*. BHT-Butylated Hydroxytoluene; DE-Diethyl ether; Ethn-Ethanol; Butn-Butanol; Chln-Chloroform; Acetn-Acetone.

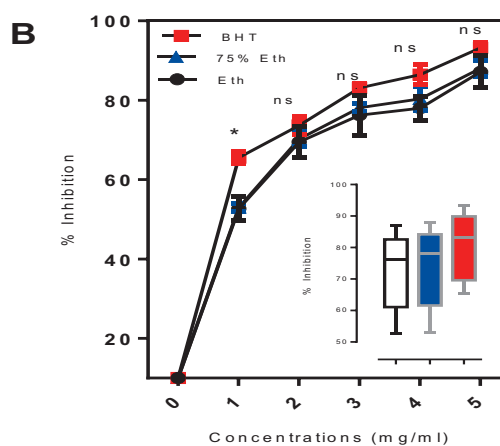
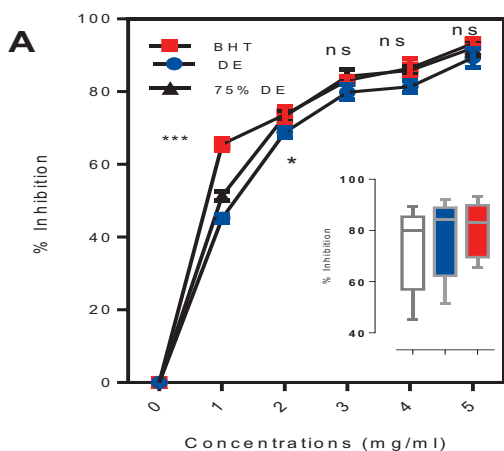


Fig. 2 cont.....

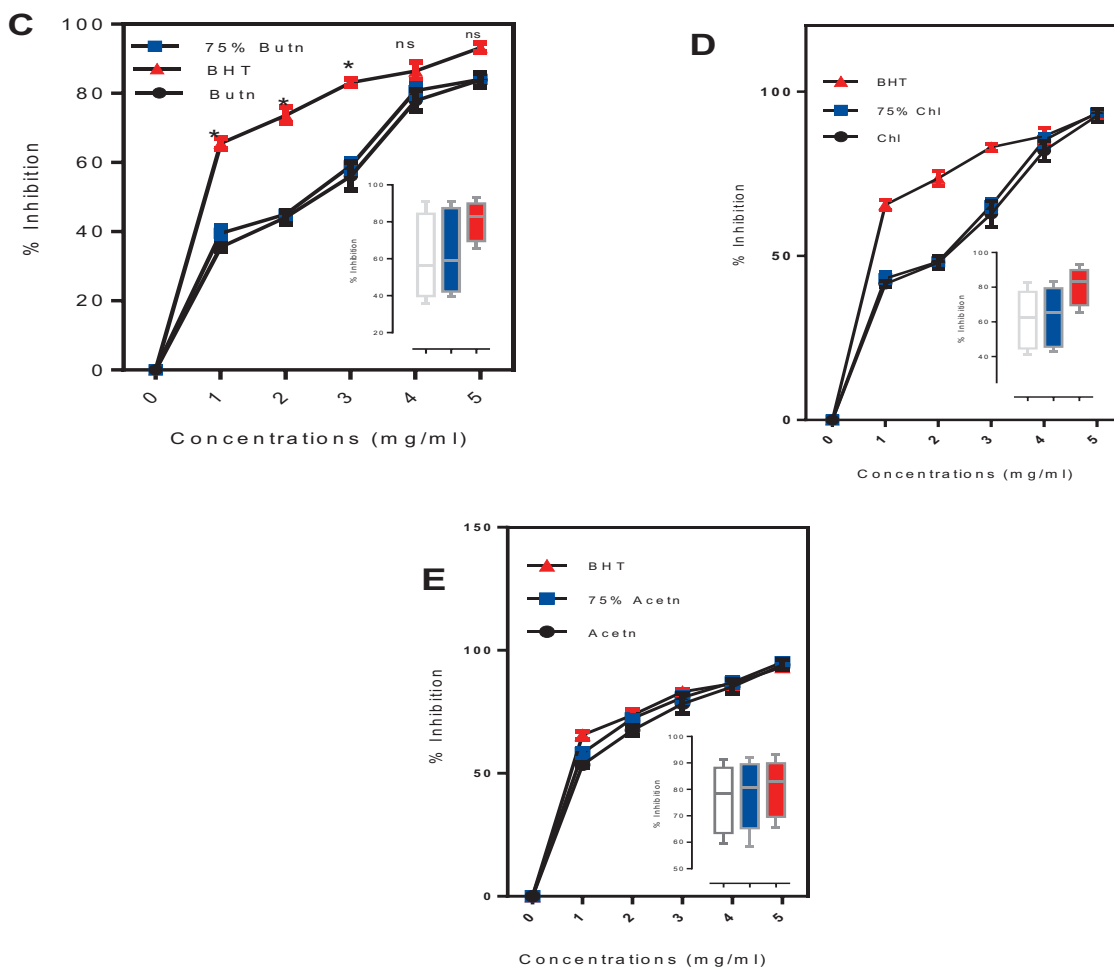


Fig. (2). *In vitro* free radical scavenging activities of different extracts of *Ganoderma lucidum*. GL- *Ganoderma lucidum*; DEGL-diethyl ether extract of GL; BEGL-Butanol extract of GL; EEGL-ethanol extract of GL; CEGL-chloroform extract of GL; and AEGL-acetone extract of GL; BHT-Butylated Hydroxytoluene; DE-Diethyl ether; Ethn-Ethanol; Butn-Butanol; Chln-Chloroform; Acetn-Acetone. The given graphs also illustrate the mean percentage of DPPH radical formation during reaction time. Acetone and ethanol extract exhibit almost equal free radical scavenging potentials as well.

Table 2. IC₅₀ values (µg/mL) of free radicals scavenging by different solvents extract of *Ganoderma lucidum*.

-	IC50 Values (µg/mL)
	DPPH Radicals Scavenging
DE	1100.8070 ± 2.43
75% DE	867.81895 ± 3.01
EE	833.79039 ± 1.87
75% EE	823.62482 ± 2.11
BE	862.91666 ± 1.34
75% BE	2029.4366 ± 3.23
ChIE	1879.0441 ± 2.00
75% ChIE	1759.8550 ± 1.11
Acetone	1266.0550 ± 1.08
75% Acetone	645.44554 ± 0.91
BHT	433.15863 ± .045

Legends: BHT-Butylated Hydroxytoluene; DE-Diethyl ether; Ethn-Ethanol; Butn-Butanol; Chln-Chloroform; Acetn-Acetone

The results obtained revealed the indicative difference of free radical scavenging potentials of solvents induced mushroom extracts. Here, it is cleared that, at the highest concentration of mushroom extract, the almost equal tendency to prevent DPPH radical's production during reaction time was demonstrated. The effective concentrations (IC₅₀) of mushroom extracts were calculated to assess their potency for reducing free radicals. The most effective crude mushroom extract was 75% acetone extract (IC₅₀ value; 645.445 µg/ml) and followed by 75% ethanol, ethanol, butanol, 75% diethyl ether, and diethyl ether extracts. On the other hand, extract of 75% butanol shows the lowest potency against the scavenging of free radicals (IC₅₀ value; 2029.4366 µg/ml). Results are shown in Table 2 as a whole.

4. DISCUSSION

In this study, we have determined total phenol and flavonoid content and antioxidant activity of *Ganoderma lucidum* mushroom extracts obtained using ten different extraction methods. The total phenol and flavonoid contents of mushroom depended on the extractants. Acetone (75%) extract showed the highest % yield of extract comprised the highest content of total phenol content while 75% ethanol extract exhibited the highest content of total flavonoids. It has been demonstrated that the phenol content of plant extract depended on the solvent used and its polarity and also shown that the acetone extract gave the highest polyphenol content [13]. Solvent polarity directly induced the phenolic solubility [14], the polyphenol extraction from plant materials was induced by extractants [15]. *Ganoderma lucidum* contains various antioxidants such as, triterpene, that was tested for its antioxidant activity [16]. There are various methods developed to assess antioxidant activity of plant materials through scavenging free radicals, inhibition of lipid peroxidation and so on [17, 18]. Here we measured the antioxidant activity of different extracts through scavenging DPPH radicals and hydro-acetone extract which showed that the signal antioxidant activity comprised high phenol content.

CONCLUSION

Extracts of *Ganoderma lucidum* obtained from hydro-acetone (EC₅₀ value; 645.44 µg/mL) and hydro-ethanol (EC₅₀ value; 823.62 µg/mL) exhibited strong antioxidative properties comprised higher phenolic content than that of diethyl ether (EC₅₀ value; 1100.80 µg/mL), 75% diethyl ether (EC₅₀ value; 867.81 µg/mL), ethanol (EC₅₀ value; 833.79 µg/mL), butanol (EC₅₀ value; 862.91 µg/mL), 75% butanol (EC₅₀ value; 2029.43 µg/mL), chloroform (EC₅₀ value; 1879.04 µg/mL), 75% chloroform (EC₅₀ value; 1759.85 µg/mL), and acetone (EC₅₀ value; 1266.05 µg/mL). Hydro-acetone extraction method produced the maximum percentage (%) yield of extract ($p < 0.05$).

From this study, it is clear that the total phenol content induced the antioxidative potential of *Ganoderma lucidum* mushroom. These findings designate that the selective extraction of phytochemicals from mushroom shows signal biological activities which can be used as a rationale for the development of alternative medicines.

DATA AVAILABILITY

The authors approve that all data essential for the findings are completely available without constraint. All pertinent data are within the paper and its backup information files.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGMENTS

The authors received no specific funding for this work. Funder provided support in the form of salaries for authors, but did not have any additional role in the study design, data collection & analysis, decision to publish, and preparation of the manuscript.

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