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Research Article

Ganoderma colossus: Prospective Candidate of Phytochemicals and Antioxidant Sources

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ABSTRACT
The wild mushroom *Ganoderma colossus* was evaluated for its phytochemical constituents and antioxidant properties. The genus *Ganoderma* is a group of wood degrading polypore belonging to the family Ganodermataceae, it has a long history of its use in traditional medicine. The dried mushroom sample was pulverized using an electronic blender and the soxhlet apparatus was used to get a more refined extract. The phytochemical study revealed the presence of flavonoids, tannins, alkaloids, and phenolic compounds. Mushroom extract scavenged DPPH radical in a significant dose-dependent manner. These results suggest that the specimen of interest is an important source of bioactive compounds and can also act as an antioxidant.

Keywords : Wild mushroom, *Ganoderma*, Phytochemical, Antioxidant

INTRODUCTION

Free radicals are constantly formed in the human body during energy production, in the mitochondrial electron transport chain, phagocytosis, arachidonic acid metabolism, ovulation, fertilization, and xenobiotic metabolism, and from external sources such as food, drugs, smoke, and other pollutants in the environment.[1] Overproduction of free radicals can cause oxidative damage to biomolecules, (lipids, proteins, DNA), eventually leading to chronic diseases such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, post-ischemic perfusion injury, myocardiac infarction, cardiovascular diseases, chronic inflammation, stroke, and septic shock, aging and other degenerative diseases in human.[2,3] Hence antioxidants are an essential component in the diet to prevent oxidative damage of the cells and eventually to be free of these lifestyle diseases.

Discovering a natural source of antioxidants could be significant for the replacement of the artificial toxic antioxidants in today's food industry.[4] Long term use of synthetic antioxidants has got side effects like being a carcinogenic agent and other toxic effects in the body. Mushrooms have been used as a food supplement in various cultures because they contain a rich source of proteins, vitamins, fats, carbohydrates, amino acids, and minerals[5]. Medicinally, mushrooms are recognized by their extracts that give health benefits and functionally used in treatments such as antitumor, anti-cancer, antiviral, anti-hypercholesterolemia, and antidiabetic effects.[6-10] Adding antioxidant-rich fruits and vegetables to our daily diet will strengthen our ability to fight infection and disease.

The genus *Ganoderma* is a group of wood degrading polypore belonging to the family Ganodermataceae. Their fruiting bodies are hard and tough, hence not listed among the edible mushrooms. It is traditionally a popular medicinal mushroom that has been used particularly in China, Japan, and Korea for millennia to improve longevity and health.[11] There are a vast number of publications that report the abundance and variety of biological actions triggered by the primary metabolites of *Ganoderma* species such as polysaccharides, proteins, and triterpenes.[12] Extensive research over the last decade has provided evidence of the anticancer activities of both the triterpenoids isolated from *Ganoderma* species[13] and the carbohydrate-enriched crude extracts from these fungi.[14] Some species of *Ganoderma* exhibit a broad spectrum of antibacterial, immunostimulatory, cytotoxic, and antifungal activities.[14-16] The present study focused on the quantification of phytochemical constituents and the evaluation of the antioxidant activity of the wild mushroom *Ganoderma colossus* collected from the lowland forest of Thattekad, Ernakulam district of Kerala.

MATERIALS AND METHODS

Sample Collection

Ganoderma colossus (Fr.) CF Baker was collected from the lowland forest of Thattekad (10°08′N 76°41′E and altitude of 35 m to 528 m above sea level) located near Kothamangalam, Ernakulam district of Kerala state, India. The identification of the collected mushroom was validated by Dr. K Madhusudhan, Department of Botany, St. Albert's College, Ernakulam.

Sample Preparation and Extraction

The fruiting bodies of the collected mushroom were cleaned under running tap water and all the debris and soil particles adhering to it were removed. It was then air-dried at room temperature with proper ventilation. The dried material was pulverized using an electronic blender,

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sieved through a 0.5-mm mesh, and stored in air-tight plastic bottles. 100 g of the powdered sample was weighed into a 250 ml of 80% ethanol in a reflux flask and refluxed for 5 hours using a soxhlet extractor. The extracts were filtered using Whatman No.1 filter paper and subsequently, the ethanol was evaporated using a rotary evaporator to get the dry extract. The dry extracts were stored in a glass bottle at 4 ºC to prevent oxidative damage.

Quantitative determination of phytochemical constituents in the selected sample

Determination of total phenolic content

Total phenolic compounds in the sample extracts were estimated by using Folin-Ciocalteu assay.[17] 0.1 ml of the prepared extracts was added to the test tubes containing 0.1 ml of gallic acid which was used as the reference standard compound. It was made up to a volume of 3.5 ml using distilled water. To the prepared solution, 0.125 ml of Folin's reagent was added and incubated at room temperature for 6 minutes. In each test tube, a volume of 1.25 ml of 7% sodium carbonate was added and again it was incubated at room temperature for 90 minutes. The absorbance was recorded at 725 nm against the blank.

Determination of total flavonoid content

The total flavonoid contents were estimated using the colorimetric method involving reactions with aluminum chloride.[18] In 2 ml of distilled water, 0.5 ml extract was added and it was mixed with 75 µl of 5% sodium nitrate solution and incubated at room temperature for five minutes. 150 µl of a 10% aluminum chloride solution was added and allowed to stand for 6 min. A pink colored solution was formed after adding 0.5 ml of 1 M NaOH was spectrophotometrically measured at 415 nm. Catechol was used as a standard compound for the quantification of total flavonoid content.

Determination of total tannin content

The total tannin content of the sample was estimated using a modified Prussian blue method described by author[19]. In a test tube, a 0.1ml sample was taken and 3ml of distilled (deionized) water was added. To each sample, 1ml of potassium ferricyanide was added, followed by the addition of 1ml ferric chloride and was mixed properly. After 15 minutes, the reagents and 5ml stabilizer (30 ml distilled water, 10 ml 85% H_3PO_4, 10 ml 1% gum arabic) was added to the sample and it was mixed till a stable color was obtained. The absorbance rate was measured at 700 nm spectrophotometrically.

Determination of total alkaloid content

The total alkaloid content was measured using 1,10-phenanthroline method.[20] To a test tube 1 ml of a mushroom extract, 1 ml of 0.025 M ferric chloride in 0.5 M HCl, and 1 ml of 0.05 M of 1,10- phenanthroline in ethanol were added. With a maintained temperature of 70 ± 2 ºC, the mixture was incubated for 30 minutes in a hot water bath. A red color complex was obtained and its absorbance was measured spectrophotometrically at 510 nm against the reagent blank.

Evaluation of antioxidant activity *in vitro*

The antioxidant activity of the mushroom extract against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by UV spectrophotometry at 518 nm. The activity was measured according to the method previously described by author[21]. Various concentrations of the mushroom extract were prepared using analytical methanol and vitamin C was used as an antioxidant standard. 1 ml from each extract and 3 ml of methanol were mixed with 0.5 ml of 1.0 M DPPH in methanol and allowed to react at room temperature for 30 minutes. The same amount of methanol and DPPH were mixed to prepare the blank solution. The radical scavenging activity was calculated using the following formula:

$$\text{Radical Scavenging activity (\%)} = \frac{Ab - Aa}{Ab} \times 100$$

Where A*b* is the absorption of the blank and A*a* is the absorption of the sample. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration.

Statistical analysis

For getting a concordant value, all experiments were repeated trice and the data were expressed as the mean ± standard error.

RESULTS AND DISCUSSIONS

Quantification of bioactive compounds

Quantification of phytochemicals was carried out in the wild mushroom *Ganoderma colossus* (Figure 1) collected from Thattekkad forest, Ernakulam district, Kerala. The result revealed the presence of phenolics, flavonoids, tannins, and alkaloids in appreciable amounts (Table 1). The total tannin content was recorded highest among the phytochemicals quantified and phenolic contents are the lowest. These phytochemical constituents play a significant role in elucidating the medicinal properties of the mushroom sample.

Figure 1: *Ganoderma colossus*

The presence of extracts likes triterpenoids, Colossolactone, Ganomycin, etc. from *Ganoderma colossus* has been reported by many researchers.[22] Alkaloids are basic nitrogen-containing compounds of plant origin, one of the by-products of plant metabolism. They may act as reservoirs for protein synthesis. The valuable pharmacological properties of many mushrooms have also been attributed to the presence of alkaloids on the autonomic nervous system, blood vessels, respiratory system, gastrointestinal tract, uterus, and be effective against malignant diseases, infections, and malaria.[23] Alkaloids have a wide range of pharmacological activities including antimalarial, antiasthmatic, anticancer, cholinomimetic, vasodilatory, antiarrhythmic, analgesic, anti-bacterial, psychotropic, and stimulant activities.[24-28] Alkaloids act as secondary antioxidants. In the present study, the selected mushroom has a considerable amount of alkaloid content which explains its anti-bacterial activity. Since this phytochemical is reported to have antibacterial activity.[29]

Phenolic acids are a type of aromatic compound with a phenolic ring and an organic carboxylic acid function. Phenolic acids are found in many plant and mushroom species. Phenolic compounds are antioxidants and exhibit a wide range spectrum of medicinal properties such as anti-cancer, anti-inflammatory, and diabetic effects.[30,31] They are capable of removing free radicals, formed in the human body, activating antioxidant enzymes, and inhibiting oxidation reactions. It is generally believed that plant extracts rich in bioactive compounds, particularly, phenolics, exhibit an ample level of antioxidant characteristics.[32] Phenolic acids are anticarcinogenic and antimutagenic as they protect the DNA against free radicals by inhibiting enzyme formation and inactivating mutagens. The total phenolic acid content present in the mushroom sample can be considered to contribute to the prevention of several degenerative human diseases such as Alzheimer's disease[33,34] and also finds use in other medical fields.

Flavonoids are one of the most diverse groups of natural compounds that have been shown to possess a broad spectrum of chemical and biological activities including radical scavenging properties, antiallergenic, antiviral, anti-inflammatory, and vasodilating actions.[35,36] Among the biological activities of flavonoids are actions against free radicals, free radical-mediated cellular signaling, allergies, platelet aggregation, microbes, ulcers, viruses and tumors, and hepatotoxins.[37] In the present study, a relatively high amount of flavonoid content was reported (Table 1). This significant amount of flavonoid content makes it good for natural candidates for reducing the risk of coronary heart disease.[38] Numerous studies have reported the benefits of flavonoid intake in the diet. The ingestion of a single dose (370 mg/day) showed a significant reduction in blood pressure.[39] The extract of the studied mushroom sample can be an alternative source for the treatment of diseases associated with the generation of excessive free radical and cell damage.

Tannins are polyphenols which are highly polymerized compounds present in plants, produced as secondary metabolites. They are considered as a low nutritional value in the food items, but industrially find a variety of uses. They are highly hydroxylated molecules and can form insoluble complexes with carbohydrates and protein. This function of plant tannins is responsible for the astringency of tannin-rich foods, because of the precipitation of salivary proteins.[40] Many tannin molecules have shown to reduce the mutagenic activity; carcinogen produces oxygen-free radicals for interaction with cellular macromolecules. The anticarcinogenic and antimutagenic potentials of tannins can be related to their antioxidative property which is important in protecting cellular oxidative damage including lipid peroxidation.[41] A high amount of tannins was present in the mushroom sample (Table 1) and thus it could be a potent natural source of therapeutics. Many types of research indicated that the major effect of tannins was not due to their inhibition on food consumption or digestion, but rather the decreased efficiency in converting the absorbed nutrients to new body substances.[42] In a positive aspect, we can say that for people who are looking for a weight loss diet can have these mushrooms included in their lifestyle. By consuming this tannin-rich mushroom can give a sense of fullness and no weight gained is assured, a potential supplement for the people who are overweight. Hence it can be used in weight reduction management.

Table 1: Quantitative estimation of phytochemicals

Sample	Phytochemical			
	Phenolic	Flavonoid	Tannin	Alkaloid
	---µg/mg---			
Ganoderma colossus	12.42 ± 0.57	219.83 ± 1.14	392.22 ± 0.79	120.63 ± 0.91

Each value is expressed as mean ± standard error done in triplicates

***In vitro* antioxidant activity**

The antioxidant activity of *Ganoderma colossus* was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. This method is widely used to test the ability of compounds to act as hydrogen donators or free radical scavengers, to evaluate the antioxidant capacity. The parameter IC_{50}, an efficient concentration value is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes a 50% loss of the DPPH activity. The results of the antioxidant activity of the wild mushroom *Ganoderma colossus* are represented in Table 2. It exhibited the strongest antioxidant activity with a value of 66.36% at a concentration of 300 µL. The IC_{50} values of *Ganoderma colossus* were found to be 210.81 µg/ml, while that of the standard ascorbic acid value was 120 µg/ml.

The results indicated the proton-donating ability of the mushroom extracts which could serve as free radical inhibitors or scavengers and can also be served as primary antioxidants. This assumption is supported by a study that says that the strong anti-radical potency possessed by *Dorstenia psilurus* and *Dorstenia ciliate* against the DPPH test might be the basis of their strong therapeutic efficacy in traditional medicine.[43] The presence of bioactive compounds (like phenolic acids, flavonoids, tannins, and alkaloids) and high antioxidant activities can be the reason for their nutritional and therapeutic uses. Antioxidants are also used as food additives to help guard against food deterioration, also frequently used in industrial products as stabilizers in fuels and lubricants to prevent oxidation. The results confirm that mushrooms can find many applications in pharmacology and drug development.

Table 2: Radical scavenging activity (%) of *Ganoderma colossus* at different extract concentrations

Concentration (µg/ml)	*Ganoderma colossus*	Ascorbic acid (standard)
	Percentage inhibition	
100	29.74 ± 0.61	46.54 ± 0.11
200	49.62 ± 0.17	65.94 ± 0.25
300	66.36 ± 0.14	81.64 ± 1.01
IC_{50}	210.81 µg/ml	120 µg/ml

Each value is expressed as mean ± standard error done in triplicates.

CONCLUSION

Genus *Ganoderma* has a long history of usage in traditional medical fields. This study was focused on elucidating the phytochemical constituents and antioxidant activity of the mushroom *Ganoderma colossus* collected from Thattekkad forest, Ernakulam district Kerala. The results of phytochemical analysis proved that *Ganoderma colossus* has a considerable amount of bioactive compounds like alkaloids, phenolic acids, flavonoids, and tannins. The antioxidant activity studied on the same mushroom species revealed that it has a high reduction potential i.e. hydroxyl radical scavenging activities. These results suggest that the specimen of interest is an important source of bioactive compounds and can also act as antioxidants. Even though it appears to be a promising source of nutritional and therapeutic values, further analysis of its efficiency as a drug, as an alternative of synthetic food supplements, its long term effects on the body and other vast disciplines is recommended. An extensive clinical trial is essential in evaluating its effects on human health and specialized investigation on the effect of all the individual extracts of the mushroom in the treatment of different types of diseases is also suggestible research in the future which can yield a positive response. Hence it can find numerous applications in pharmacology and pharmaceutical research.

REFERENCES

1. Unekwu HR, Audu JA, Makun MH, Chidi EE. Phytochemical screening and antioxidant activity of methanolic extract of selected wild edible Nigerian mushrooms. Asian Pacific Journal of Tropical Disease 2014 ; 4:153-157.
2. Bhuvaneswari S, Murugesan S, Subha TS, Dhamotharan R, Shetty N. In vitro antioxidant activity of marine red algae *Chondrococcus hornemanni* and *Spyridia fusiformis*. Journal of Chemical and Pharmaceutical Research 2013 ; 5(3):82-85.
3. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition 2002 ; 18(10):872-879.
4. Vidović S, Zeković Z, Mujić I, Lepojević Ž, Radojković M, Živković J. The antioxidant properties of polypore mushroom *Daedaleopsis confragosa*. Central European Journal of Biology 2011 ; 6(4):575-582.
5. Jiskani MM. The energy potential of mushrooms. The Dawn economic and business review 2001 ; 4:15-21.
6. Wasser SP. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Applied Microbiology and Biotechnology 2002 ; 60(3):258-274.
7. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life sciences 2004 ; 74(17):2157-2184.
8. Gao Y, Zhou S, Huang M, Xu A. Antibacterial and antiviral value of the genus *Ganoderma* P. Karst. Species (Aphyllophoromycetideae): a review. International Journal of Medicinal Mushrooms 2003 ; 5(3):235–246.
9. Vetvicka V, Vetvickova J. Effects of yeast-derived β-glucans on blood cholesterol and macrophage functionality. Journal of Immunotoxicology 2009 ; 6(1):30-35.
10. Gao Y, Lan J, Dai X, YE J, Zhou S. A phase I/II study of Ling Zhi mushroom *Ganoderma lucidum* (W. Curt.: Fr.) Lloyd (Aphyllophoromycetideae) extract in patients with type II Diabetes Mellitus. International Journal of Medicinal Mushrooms 2004 ; 6(1):33-39.
11. Cheng CR, Yang M, Yu K, Guan SH, Wu XH, Wu WY, Sun Y, Li C, Ding J, Guo DA. Metabolite identification of crude extract from *Ganoderma lucidum* in rats using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry. Journal of Chromatography B 2013; 941:90-99.

12. Hapuarachchi KK, Wen TC, Jeewon R, Wu XL, Kang JC, Hyde KD. Mycosphere Essays 7. *Ganoderma lucidum*-are the beneficial anti-cancer properties substantiated? Mycosphere A 2016; 7:305–332.
13. Wu GS, Guo JJ, Bao JL, Li XW, Chen XP, Lu JJ, Wang YT. Anti-cancer properties of triterpenoids isolated from *Ganoderma lucidum*–a review. Expert opinion on investigational drugs 2013; 22(8):981-992.
14. Osińska-Jaroszuk M, Jaszek M, Mizerska-Dudka M, Błachowicz A, Rejczak TP, Janusz G, Wydrych J, Polak J, Jarosz-Wilkołazka A, Kandefer-Szerszeń M. Exopolysaccharide from *Ganoderma applanatum* as a promising bioactive compound with cytostatic and antibacterial properties. Biomed Research International 2014; 2014:1-10.
15. De S Pereira-Jr JA, Rodrigues DP, Peixoto-Filho RC, Bastos IVGA, De Oliveira GG, Araújo JM, Melo SJ. Contribution to Pharmacognostic and Morphoanatomical Studies, Antibacterial and Cytotoxic Activities of *Ganoderma parvulum* Murrill (Basidiomycota, Polyporales, Ganodermataceae). Latin American Journal of Pharmacy 2013; 32(7):996-1003.
16. Sivaprakasam E, Kavitha D, Balakumar R, Sridhar S, Kumar JS. Antimicrobial activity of whole fruiting bodies of *Trametes hirsuta* (Wulf. Fr.) Pil. against some common pathogenic bacteria and fungus. International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(3):219-221.
17. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology. Academic press 1999; 299:152-178.
18. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis 2002; 10(3):178-182.
19. Graham HD. Stabilization of the Prussian blue color in the determination of polyphenols. Journal of agricultural and food chemistry 1992; 40(5):801-805.
20. Singh DK, Srivastava B, Sahu A. Spectrophotometric determination of Rauwolfia alkaloids: estimation of reserpine in pharmaceuticals. Analytical sciences 2004; 20(3):571-573.
21. Pal J, Ganguly S, Tahsin KS, Acharya K. In vitro free radical scavenging activity of wild edible mushroom, *Pleurotus squarrosulus* (Mont.) Singer. Indian Journal of Experimental Biology 2010; 48(12):1210-1218.
22. Hapuarachchi KK, Cheng CR, Wen TC, Jeewon R, Kakumyan P. Mycosphere Essays 20: Therapeutic potential of *Ganoderma* species: Insights into its use as traditional medicine. Mycosphere 2017; 8(10):1653-1694.
23. Trease GE, Evans WC. Pharmacognosy: a physicians guide to herbal medicine. Ed 13, UK: Bailliere Tindall, London, 1989, 176-180.
24. Kittakoop P, Mahidol C, Ruchirawat S. Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis and smoking cessation. Current topics in medicinal chemistry 2014; 14(2):239-252.
25. Russo P, Frustaci A, Del Bufalo A, Fini M, Cesario A. Multitarget drugs of plants origin acting on Alzheimer's disease. Current medicinal chemistry 2013; 20(13):1686-1693.
26. Sinatra RS, Jahr JS, Watkins-Pitchford JM. The essence of analgesia and analgesics. Cambridge University Press, Cambridge, 2010, 550.
27. Cushnie TT, Cushnie B, Lamb AJ. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. International Journal of Antimicrobial Agents 2014; 44(5):377-386.
28. Robbers JE, Speedie MK, Tyler VE. Pharmacognosy and pharmacobiotechnology. Williams & Wilkins, Maryland, 1996, 337.
29. Idowu TO, Onawunmi GO, Ogundaini AO, Adesanya SA. Antimicrobial constituents of *Chrysophyllum albidum* seed cotyledons. Nigerian Journal of Natural Products and Medicine 2003; 7:33-36.
30. Hamzah RU, Egwim EC, Kabiru AY, Muazu MB. Phytochemical and in vitro antioxidant properties of the methanolic extract of fruits of *Blighia sapida*, *Vitellaria paradoxa* and *Vitex doniana*. Oxidants and Antioxidants in Medical Science 2013; 2(3):217-223.
31. Nagavani V, Madhavi Y, Rao DB, Rao PK, Rao TR. Free radical scavenging activity and qualitative analysis of polyphenols by RP-HPLC in the flowers of *Couroupita guianensis* Abul. Electronic Journal of Environmental, Agricultural and Food Chemistry 2010; 9(9):1471-1484.
32. Sultana B, Fatima B, Mushtaq M. In vitro synergism of antimutagenic and antioxidant activities of *Phoenix dactylifera* fruit. Food Science and Biotechnology 2014; 23(3):881-887.
33. Amić D, Davidović-Amić D, Bešlo D, Trinajstić N. Structure-radical scavenging activity relationships of flavonoids. Croatica chemica acta 2003; 76(1):55-61.
34. Ejelonu OC, Akinmoladun AC, Elekofehinti OO, Olaleye MT. Antioxidant profile of four selected wild edible mushrooms in Nigeria. Journal of Chemical and Pharmaceutical Research 2013; 5(7):286-295.
35. Parajuli S, Pun NT, Parajuli S, Jamarkattel-Pandit N. Antioxidant Activity, Total Phenol and Flavanoid Contents in Some Selected Medicinal Plants of Nepal. Journal of Health and Allied Sciences 2012; 2(1):27-31.
36. Pereira DM, Valentão P, Pereira JA, Andrade PB. Phenolics: From Chemistry to Biology. Molecules 2009; 14:2202-22011.
37. Kinsella JE, Frankel E, German B, Kanner J. Possible mechanisms for the protective role of antioxidants in wine and plant foods: physiological mechanisms by which flavonoids, phenolics, and other phytochemicals in wine and plant foods prevent

or ameliorate some common chronic diseases are discussed. Food technology (Chicago) 1993 ; 47(4):85-89.

38. Hertog MG, Feskens EJ, Kromhout D, Hollman PC, Katan MB. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. The lancet 1993 ; 342(8878):1007-1011.
39. Balzer J, Rassaf T, Heiss C, Kleinbongard P, Lauer T, Merx M, Heussen N, Gross HB, Keen CL, Schroeter H, Kelm M. Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients : a double-masked, randomized, controlled trial. Journal of the American College of Cardiology 2008 ; 51(22):2141-2149.
40. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition reviews 1998 ; 56(11):317-333.
41. Chang ST, Miles PG. Edible mushrooms and their cultivation : The nutritional attributes and medicinal value of edible mushrooms. Ed 1, CRC press, Minnesota,1989, 27-40.
42. Ozcan T, Akpinar-Bayizit A, Yilmaz-Ersan L, Delikanli B. Phenolics in human health. International Journal of chemical engineering and applications 2014 ; 5(5):393.
43. Kansci G, Dongo E, Genot C. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) test demonstrates antiradical activity of *Dorstenia psilurus* and *Dorstenia ciliata* plant extracts. Food/Nahrung 2003 ; 47(6):434-437.