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Original Article

Screening of Secondary Metabolites and Antioxidant Activity of Wild Edible Termite Mushroom

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ABSTRACT

Wild edible mushrooms produce a variety of bioactive compounds that are known to have antioxidant properties. Natural antioxidants can protect against oxidative induced free radicals without any side effects. Thus, they are consumed by people for food and nutraceutical values. The purpose of this study was to evaluate the phytochemicals and antioxidant activity of three wild edible termite mushrooms (*Termitomyces albuminosus, T. eurhizus* and *T. robustus*). Different phytochemicals were screened in the 50% ethanol, methanol and water extracts of three termite mushrooms. Total phenolic and flavonoid contents were determined by Folin-Ciocalteau and aluminium chloride method respectively. The antioxidant activity of three termite mushrooms was evaluated by DPPH assay. Qualitative screening of phytochemicals has revealed that alkaloid, steroid, fatty acid, flavonoid, saponin, tannin, carbohydrate and protein are found in the 50% ethanol, methanol and water extracts of three species of termite mushroom. A high amount of total phenolic and flavonoid content was found in the 50% ethanol extract of *T. albuminosus*, *T. eurhizus* and *T. robustus* (TPC: 50.28, 54.56 and 57.63 mg GAE/g extract; TFC: 16.30, 18.43 and 18.80 mg QE/g extract respectively). Due to high phenolic and flavonoid content, 50% ethanol extract of three termite mushrooms has shown high antioxidant activity (i.e., lowest IC_{50}: 710.00 - 714.05 µg/ml). These termite mushrooms have antioxidant properties due to the presence of bioactive secondary metabolites that can potentially be used as a source of natural antioxidants in the form of food and nutraceutical.

Key words: DPPH assay, Flavonoid, Phenolic, Phytochemical, Termite mushroom.

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INTRODUCTION

Termite mushrooms (*Termitomyces* spp.) are obligatory symbiosis with termites, grow on or near territory built by termites and their excreta. They have pink spores, termite association, subterranean elongated stipes and fruiting bodies.[1] They are tropical gilled mushrooms and good sources of wild food for termites, and mankind for high protein, crude fibre, minerals, vitamins and low fat.[2] They are consumed not only for a dietary supplement but also for therapeutics and nutraceuticals values. A variety of secondary metabolites such as phenols, flavonoids, terpenoids, etc. are found in the termite mushrooms which are strong antioxidants.[3-5]

Reactive oxygen species (ROS) are highly reactive molecules derived from cellular metabolism as well as from food, drugs, smoke and other pollutants. At normal physiological concentration, ROS are required for cellular activities, however, at higher concentrations, they cause extensive damage to cells and may cause degenerative disorders, such as ageing, atherosclerosis, diabetes, cancer and cirrhosis.[6-8] Antioxidants inhibit the harmful oxidizing free radicals which damage the living cells.[9] Naturally, living organisms are endowed with oxidative defence mechanisms but certainly need external supplements of antioxidants. However, synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, tert-butylhydroquinone and propyl gallate have toxic and carcinogenic effects.[10] Therefore, the development and utilization of more effective antioxidants of natural origin are desired. In recent years, the antioxidant properties of many plants, mushrooms and lichen have been widely reported.[11-14] To find a new natural source of antioxidants, our attention was focused on termite mushrooms. Termite mushrooms are great sources of natural antioxidant compounds they contribute directly to the antioxidative mechanism in living organisms to prevent oxidative damage. The major objectives of this research are to screen different phytochemicals, quantify the total phenolic and flavonoid content, and antioxidant activity of termite mushrooms. So, the present research promotes the sustainable use of mushrooms for food security and health.

MATERIALS AND METHODS

Material

The whole part of three species of termite mushroom (*Termitomyces albuminosus, T. eurhizus* and *T. robustus*) was collected from the natural habitats in the Chitwan National Park, central Nepal from June to August in 2018. These mushrooms were identified by N.S. Atri (Punjabi University, Patiala, India) and Hari Prasad Aryal (co-author) use their morphological and spore characters. The voucher specimens of these species were deposited at the Natural History Museum of the Tribhuvan University [accession number: *T. albuminosus* (2-2-1666), *T. eurhizus* (2-2-1668) and *T. robustus* (2-2-1672)]. The whole mushroom was washed, air-dried and crushed into fine powder.

Extraction

The powder of each species of mushroom was extracted with 50% ethanol, methanol and water. A powdered sample of 20 g of each species was placed

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in an extraction thimble separately and the sample was extracted by 250 ml above-mentioned solvent using the Soxhlet extraction method.

Screening of secondary metabolites

Qualitative screening of the following secondary metabolites has been carried out using standard protocols.[15]

Volatile oil: Spot of the extract was put on filter paper by capillary and no yellow colour persists indicates the presence of volatile oil.

Alkaloid: Extract of 0.5.ml was dissolved in 1.5 ml dil. HCl and filtered. The filtrate was mixed with 2-3 drops of Mayer's reagent and Wagner's reagent was added separately and observed for five minutes. Mayer's reagent gives whitish-yellow precipitate and Wagner's reagent gives reddish-brown precipitate indicates the presence of alkaloids.

Steroid: Extract of 0.5 ml was dissolved in 5 ml chloroform and an equal volume of conc. H_2SO_4 was added from the side of the test tube. The upper yellow layer with green fluorescence indicates the presence of steroids.

Terpenoids: Extract of 1 ml was dissolved in 2 ml of chloroform and 3 ml of conc. H_2SO_4 was added, and the mixture was heated for two minutes. A greyish reddish-brown of the interface indicates the presence of terpenoids.

Fatty acids: Extract 0.5 ml was dropped on filter paper. After dropping the extract, a spot that persists on filter paper indicates the presence of fatty acids.

Emodin: Extract of 2 ml was mixed with 2 ml of 25% ammonium hydroxide and 3 ml benzene. The appearance of red colour indicates the presence of emodin.

Flavonoid: Extract of 1 ml was dissolved in 1 ml of 10% lead acetate solution gives white precipitate that indicates the presence of flavonoid.

Saponins: Extract of 1 ml was dissolved in 20 ml distilled water and mixture was shaken vigorously. The appearance of 1 cm froth for 15 minutes indicates the presence of saponins.

Tannin/Phenolic: About 2-3 drops of 5% ferric chloride solution was added to 0.5 ml of extract gives intense blue-greenish indicating the presence of tannins/phenolic.

Glycosides: Extract of 2 ml was mixed Fehling's solution A and Fehling's solution B and mixture was heated on a water bath for about two minutes. After heating, it gives a brick-red colour that indicates the presence of glycosides.

Polyurenoids: In a test tube, 2 ml extract and 10 ml acetone were added. If a thick volume was formed, 4-5 drops of hematoxylin were added. The precipitate was separated by filter and if it gives violet precipitate after centrifugation indicates the presence of polyurenoids.

Polyoses: Extract of 2 ml was placed in a porcelain basin and concentrated till to yield a residue. Then, 2-3 drops of conc. H_2SO_4 was added to the residue and allow to stand for 3-5 minutes. The appearance of red colour in the resulting solution indicates the presence of polyoses after the addition of 3-4 drops of thymolor Molisch's reagent.

Anthocyanin: Extract of 1 ml was heated with an equal volume of 10% HCl on the water bath for about 15 minutes and cool. Then, 2 ml ether was added. If the aqueous part is red and does not turn to violet at neutral pH or blue in an alkaline medium, it shows the presence of anthocyanins.

Carbohydrates: The extract of 2 ml was mixed with a 10 ml Molisch reagent. Then, 2 ml conc. H_2SO_4 was added from the side of the test tube. The formation of a violet ring at the junction of two liquids indicates the presence of carbohydrates.

Protein: Extract 0.5 ml when boiled with 2 ml of 0.25 % ninhydrin solution gives violet colour indicating the presence of protein.

Reducing sugar: Extract 0.5 ml was added with 1 ml water and boiled gently with 0.5 ml Fehling's solution A and Fehling's solution B. If it gives reddish-brick precipitate that indicates the presence of reducing sugars.

Starch: Iodine of 0.015 gm and 0.075 gm of potassium iodide were dissolved in 5 ml of distilled water and it was mixed with 2 ml of extract. A blue colour appearance indicates the presence of starch.

Determination of total phenolic content

The total phenolic content was estimated by the Folin-Ciocalteau reagent as the method.[16] About 1 ml of extract at concentrations of 100, 80, 50 and 25µg/ml was mixed with 5 ml of 10% Folin-Ciocalteau solution and 4 ml of 7% Na_2CO_3. The reaction mixture was incubated for 30 minutes a room temperature and absorbance was measured at 760 nm. Gallic acid was used as a standard for obtaining a calibration curve. The above-mentioned procedure was applied for the gallic acid.

Determination of total flavonoid content

The total flavonoid content was determined by aluminium chloride colourimetric assay.[16] An aliquot of 1 ml of extract at the concentration of 100, 80, 50, and 25µg/ml was mixed with 4 ml of double-distilled water in a flask. Immediately, 0.3 ml of 5% sodium nitrite was added to the flask. After five minutes, 0.3 ml of 10% $AlCl_3$ and at six minutes, 2 ml of 1 M sodium hydroxide was added to the mixture. Immediately, the total volume of the mixture was made up to 10 ml by the addition of 2.4 ml double distilled water and mixed thoroughly. The absorbance of the pink-coloured mixture was measured at 510 nm. Quercetin was used as a standard for obtaining a calibration curve. The above-mentioned procedure was applied for quercetin.

Determination of antioxidant activity

Antioxidant activity was determined by using 2, 2-diphenyl-1 picrylhydrazyl free radical (DPPH).[11] About 0.5 ml of extract (prepared in methanol) at concentrations of 10, 20 and 30 µg/ml was mixed with 2.5 ml of 0.1 mM DPPH (prepared in methanol). The reaction mixture was shaken vigorously and incubated in dark for 30 minutes a room temperature. The reduction of purple DPPH into yellow in the mixture was measured at 517 nm. A control was prepared by mixing 0.5 ml of distilled water and 2.5 ml of 0.1 mM DPPH.

RESULTS

Phytochemical screening

Qualitative screening of phytoconstituents present in the ethanol, methanol and water extracts have revealed that alkaloid, steroid, fatty acid, flavonoid, saponin, tannin, carbohydrate and protein are found in all extracts of three species of termite mushroom (Table 1). The volatile oil is absent in all the extracts of the three mushrooms. Emodin, glycoside, anthocyanin, reducing sugar and starch are absent in the water extract of three termite mushrooms. Terpenoid and polyurenoid are absent in the methanol extract of *T. albuminosus* and *T. robustus*. Polyose is absent in the three extracts of *T. albuminosus* and *T. robustus*.

Total phenolic and flavonoid content

The phenolic content in the extract was calculated using the regression equation $Y= 0.0165x+0.0184$, $R^2 = 0.99$. The total phenolic content was expressed as mg gallic acid equivalent (GAE) per gram of extract. A high amount of total phenolic content (TPC) was found in the ethanol extract of three termite mushrooms (Table 2). Ethanol extract of *T. albuminosus*, *T. eurhizus* and *T. robustus* has high TPC as 50.28, 54.56

Table 1: Screening of phytoconstituents in the extract of three species of termite mushroom.

Phytoconstituent	Species	50% Ethanol extract	Methanol extract	Water extract
	T. albuminosus	-	-	-
Volatile oil	*T. eurhizus*	-	-	-
	T. robustus	-	-	-
	T. albuminosus	++	++	++
Alkaloid	*T. eurhizus*	+++	+++	+++
	T. robustus	+++	+++	+++
	T. albuminosus	+++	+	+
Steroid	*T. eurhizus*	+++	++	++
	T. robustus	+++	+	+
	T. albuminosus	++	-	+
Terpenoid	*T. eurhizus*	+	+	++
	T. robustus	++	-	+
	T. albuminosus	++	++	+
Fatty acid	*T. eurhizus*	++	++	+
	T. robustus	++	++	+
	T. albuminosus	+	+	-
Emodine	*T. eurhizus*	+	+	-
	T. robustus	+	+	-
	T. albuminosus	+	++	+
Flavonoid	*T. eurhizus*	+	+++	++
	T. robustus	++	+++	++
	T. albuminosus	+	+	+
Saponins	*T. eurhizus*	+	+	+
	T. robustus	++	++	+++
	T. albuminosus	+	+	+
Tannins	*T. eurhizus*	++	++	++
	T. robustus	++	+++	+++
	T. albuminosus	++	+	-
Glycosides	*T. eurhizus*	++	+	-
	T. robustus	++	+	-
	T. albuminosus	+	-	-
Polyurenoids	*T. eurhizus*	+	+	+
	T. robustus	+	-	+
	T. albuminosus	-	-	-
Polyoses	*T. eurhizus*	+	+	+
	T. robustus	-	-	-
	T. albuminosus	+	-	-
Anthocyanins	*T. eurhizus*	+	+	-
	T. robustus	+	-	-
	T. albuminosus	++	++	+
Carbohydrates	*T. eurhizus*	++	++	++
	T. robustus	++	++	+
	T. albuminosus	+	++	+++
Proteins	*T. eurhizus*	+	+++	+++
	T. robustus	+	++	+++
	T. albuminosus	++	+	-
Reducing sugars	*T. eurhizus*	++	+	-
	T. robustus	++	+	-
	T. albuminosus	+	+	-
Starch	*T. eurhizus*	+	+	-
	T. robustus	+	+	-

Presence in high amount (+++), presence in moderate amount (++), presence in little amount (+), absence (-).

Table 2: Total phenolic (TPC) and flavonoid (TFC) content in the extract of three species of termite mushroom.

Species	Solvent extract	TPC±SD (mg GAE/g extract)	TFC±SD (mg QE/g extract)
	50% Ethanol	50.28±2.59	16.30±0.72
T. albuminosus	Methanol	40.38±1.39	11.94±1.68
	Water	43.02±2.82	13.18±0.72
	50% Ethanol	54.56±1.88	18.43±0.75
T. eurhizus	Methanol	45.09±1.35	13.38±0.92
	Water	47.51±3.32	14.80±0.74
	50% Ethanol	57.63±2.43	18.80±0.76
T. robustus	Methanol	46.93±2.31	13.98±0.72
	Water	50.03±2.72	15.91±0.69

and 57.63 mg GAE/g extract respectively as compared to the methanol and water extracts.

The flavonoid content in the extract was calculated using the regression equation Y =0.0042x, R^2 = 0.9987. Total flavonoid content (TFC) was expressed as mg quercetin equivalent (QE) per gram of extract. A high amount of TFC was found in the ethanol extract of three termite mushrooms (see Table 2). Ethanol extract of *T. albuminosus*, *T. eurhizus* and *T. robustus* has high TFC as 16.30, 18.43 and 18.80 mg QE/g extract respectively as compared to the methanol and water extracts.

Antioxidant activity

The antioxidant activity of the extracts of three termite mushrooms was carried out using DPPH free radicals. Inhibition of DPPH radicals by the extracts was expressed in the percentage (Table 3). Fifty percent inhibition of DPPH radicals by the extract (IC_{50}) was calculated from the percentage inhibition of DPPH radicals at various concentrations of the extract. The lowest IC_{50} value of the extract indicates the highest antioxidant activity. Ethanol extract of *T. albuminosus*, *T. eurhizus* and *T. robustus* has the lowest IC_{50} as 714.05, 712.76 and 710.00 µg/ml respectively as compared to the methanol and water extracts (see Table 3).

Correlation between total phenolic/flavonoid content and antioxidant activity

The correlation between antioxidant activity and total phenolic/flavonoid content had been determined by plotting IC_{50} values for antioxidant activity against the total phenolic/flavonoid content of extracts of three termite mushrooms. The relationship between total phenolic/flavonoid content and antioxidant activity is shown in Figure 1. Correlation between antioxidant activity and total phenolic content revealed the coefficient of determination (R^2) of 0.9724 (Figure 1a), suggesting that the phenolic content of the extracts contributed to scavenging of 97.24% DPPH radicals. Similarly, the correlation between antioxidant activity and total flavonoid content revealed the coefficient of determination (R^2) of 0.928 (Figure 1b), suggesting that the flavonoid content of the extracts contributed to scavenging of 92.8% DPPH radicals.

DISCUSSION

The qualitative phytochemical screening of the extracts of three termite mushrooms has shown the presence of alkaloid, steroid, protein, carbohydrate, tannin, flavonoid, triterpenoid, fatty acid, saponin, glycoside, reducing sugar, starch, emodine, polyurenoid, polyoses and anthocyanin. However, volatile oil has not been found in three species of termite mushroom. Many similar types of phytochemicals were screened in the *Termitomyces* species in the previous research that confirm to a certain degree with the phytochemical screening of selected *Termitomyces* species.[17-21] These phytoconstituents play a significant role in the medicinal properties, so that termite mushrooms are used as food for nutrition, low calories, rich sources of water-soluble vitamins and dietary fibres. Therefore, these wild edible termite mushrooms have been largely used by ethnic people for the treatment of rheumatism, diarrhoea, gonorrhoea, stomach upset and hypertension.[2,17]

The quantification of more amount of phenolic and flavonoid content in the 50% ethanol extract of three termite mushrooms was revealed due to an increase in its polarity by the addition of 50% water which extracts more compounds.[21] The presence of phenolic and flavonoid content exhibits a wide range of spectrum of medicinal properties by reducing the reactive oxygen species which may lead to anticancer, anti-inflammatory, antidiabetic, antiallergenic and antiviral properties.[22,23] Thus, these selected termite mushrooms may be good alternatives for the treatment of diseases associated with excessive free radical generation and damage.

Table 3: Percentage inhibition of DPPH free radicals and IC_{50} values of the extract of three species of termite mushroom.

Species	Extract	Concentration (µg/ml)	Inhibition percentage	IC_{50} (µg/ml)
T. albuminosus	50% Ethanol	10	1.64	714.05
		20	2.56	
		30	3.01	
	Methanol	10	1.28	720.60
		20	2.10	
		30	2.65	
	Water	10	1.37	719.72
		20	2.10	
		30	2.74	
T. eurhizus	50% Ethanol	10	1.83	712.76
		20	2.65	
		30	3.11	
	Methanol	10	1.46	717.65
		20	2.28	
		30	2.74	
	Water	10	1.55	715.27
		20	2.28	
		30	2.92	
T. robustus	50% Ethanol	10	1.92	710.00
		20	2.65	
		30	3.20	
	Methanol	10	1.55	716.60
		20	2.37	
		30	2.92	
	Water	10	1.64	714.93
		20	2.47	
		30	3.01	

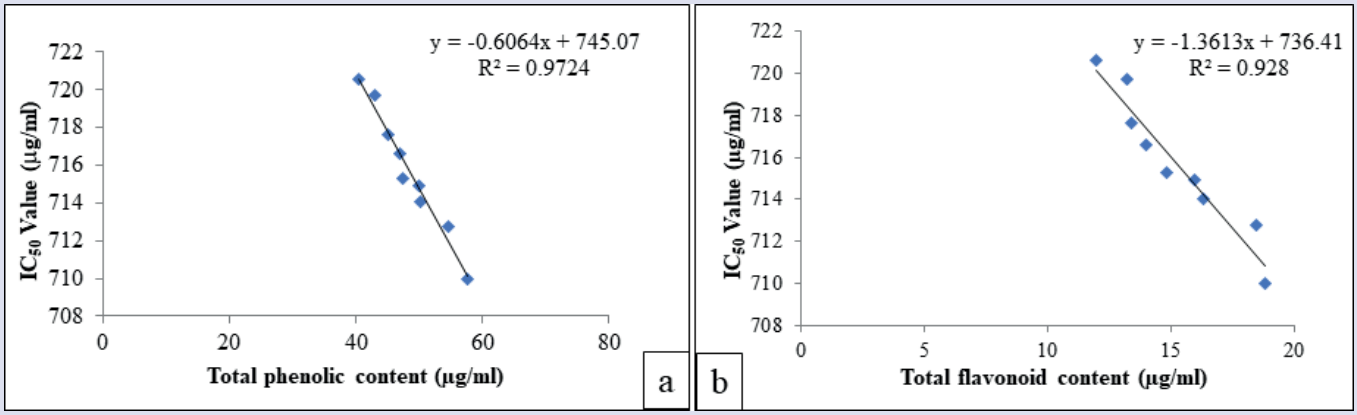

Figure 1: Correlation between IC_{50} values of antioxidant activity and total phenolic content (a) and total flavonoid content (b).

DPPH free radicals have been used for the determination of the antioxidant activity of the extracts of termite mushrooms. The extract with an inhibitory concentration (IC_{50}) is less than 10 mg/ml has an antioxidant potential,[24] and it donates a hydrogen atom to scavenge the free radicals. As such, three species of termite mushrooms have IC_{50} values ranging from 710.00 to 719.72 µg/ml, therefore they have potent antioxidant activity. The phytochemicals especially phenolic and flavonoids are potential antioxidants.[11] It has been demonstrated that a strong correlation between the antioxidant activity of the extracts and their phenolic and flavonoid content. More amount of total phenolic and flavonoid content in the extracts have shown strong antioxidant activity. The termite mushrooms have strong antioxidant activity against tested free radicals. The differences in the antioxidant activity of various solvent extracts may be the result of the extraction of different bioactive substances. In the literature, there are several data for the antioxidant activity of wild mushrooms such as *Agaricus*, *Psilocybe*, *Ganoderma*, *Pleurotus*, *Clitocybe*, *Lepista*, *Amanita*, *Termitomyces*, *Auricularia* etc. are strongly supported the present research.[10,13,18,24-28] Although, some wild mushrooms are edible attests of utility by local inhabitants as they have antioxidant activity.[3,29-31] Hence, the importance of antioxidants in food as protective agents to help humans in the reduction of oxidative damage. Hence, in the growing demand for food and pharmaceutical industries, such wild termite mushrooms can be the important candidates.[2,13,32]

CONCLUSION

In conclusion, termite mushrooms have several secondary metabolites which have significant antioxidant activity. Hence, termite mushrooms can be widely used in food and their proper utilization for food security. They have also opened the way of their use in the pharmaceutical industry.

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REFERENCES

1. Aryal HA, Budhathoki U, Lakhey PB. Phytochemical study of *Termitomyces robustus* (Beeli) R. Heim in Nepal. Bull Dep Plant Resour. 2014;36:92-7.
2. Ugbogu EA, Emmanuel O, Salem AZM, Elghandour MMMY. Nutritional composition of *Termitomyces robustus* (Agaricomycetes) and *Lentinus squarrosulus* (Mont.) singer in South East Nigeria. Agrofor Syst. 2018;94:1291-300.
3. Gebreyohannes G, Nyerere A, Bii C, Sbhatu DB. Investigation of Antioxidant and Antimicrobial Activities of Different Extracts of Auricularia and *Termitomyces* Species of Mushrooms. Sci World J. 2019;7357048.
4. Maity P, Nandi AK, Pattanayak M, Manna DK, Sen IK, Chakraborty I, *et al.* Structural characterization of a heteroglycan from an edible mushroom *Termitomyces heimii*. Int J Biol Macromol. 2020;151:305-11.
5. Mitra P, Mandal NC, Acharya K. Polyphenolic extract of *Termitomyces heimii*: antioxidant activity and phytochemical constituents. J Consum Prot Food Saf. 2015;11:25-31.
6. Lushchak VI. Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact. 2014;224:164-75.
7. Nita M, Grzybowski A. The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. Oxid Med Cell Longev. 2016;1-23.
8. Salman KA, Ashraf S. Reactive Oxygen Species: A link between chronic inflammation and cancer. Asia-Pacific J Mol Biol Biotechnol. 2013;21:41-9.
9. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118-26.
10. Kosanic M, Ranković B, Dasic M. Antioxidant and Antimicrobial Properties of Mushrooms. Bulg J Agric Sci. 2013;19:1042-8.
11. Chand MB, Paudel MR, Pant B. The antioxidant activity of selected wild orchids of Nepal. J Coast Life Med. 2016;4:731-6.
12. Egwim E, Elem R. Proximate composition, phytochemical screening and antioxidant activity of ten selected wild edible Nigerian mushrooms. Am J Food Nutr. 2011;1:89-94.
13. Kalyoncu F, Oskay M, Sag H, Erdog TF, Tamer AU. Antimicrobial and Antioxidant Activities of Mycelia of 10 Wild Mushroom Species. J Med Food. 2010;13(2):415-9.
14. Sharif S, Shahid M, Mushtaq M, Akram S, Rashid A. Wild mushrooms: A potential source of nutritional and antioxidant attributes with acceptable toxicity. Prev Nutr Food Sci. 2017;22(2):124-30.
15. Harborne JB. Phytochemical Methods. Chapman and Hall Ltd, London. 1985;37.

16. Paudel MR, Chand B, Karki N, Pant B. Antioxidant activity and total phenolic and flavonoid contents of *Dendrobium amoenum* Wall. ex Lindl. Bot Orient Plant Sci. 2015;9:20-6.

17. Aryal HP, Budhathoki U. Ethnomycology of *Termitomyces* spp. R. Heim for its medicinal importance in Nepal. Med Plants. 2014;6:128-37.

18. Mitra P, Mandal NC, Acharya K. Phytochemical characteristics and free radical scavenging activity of ethanolic extract of *Termitomyces microcarpus* R. Heim Der Pharm Lett. 2014a;6:92-8.

19. Mitra P, Mandal NC, Roy A, Acharya K. Phytochemical Study and Antioxidative Property of Ethanolic Extract from *Termitomyces clypeatus*. J Appl Pharm Sci. 2016;6:120-4.

20. Mitra P, Sarkar J, Mandal NC, Acharya K. Phytochemical analysis and evaluation of antioxidant efficacy of ethanolic extract of *Termitomyces medius*. Int J Pharm Sci Rev Res. 2014b;27:261-6.

21. Oboh G, Shodehinde SA. Distribution of nutrients, polyphenols and antioxidant activities in the pilei and stipes of some commonly consumed edible mushrooms in Nigeria. Bull Chem Soc Ethiop. 2009;23:391-8.

22. Paudel MR, Chand MB, Pant B, Pant B. Assessment of antioxidant and cytotoxic activities of extracts of *Dendrobium crepidatum*. Biomolecules. 2019;19(9):478.

23. Paudel MR, Chand MB, Pant B, Pant B. Antioxidant and cytotoxic activities of *Dendrobium moniliforme* extracts and the detection of related compounds by GC-MS. BMC Complement Altern Med. 2018;18(1):134.

24. Fadeyi OG, Assogba FM, Chabi DCB, Yorou NS, Gbenou JD. Ethnomycology, myco-chemical analyzes and antioxidant activity of eleven species of the genus *Amanita* (Basidiomycota, fungi) from Benin (West Africa). J Pharmacogn Phytochem. 2019;8:335-41.

25. Azizur MR, Abdullah AM, Yasmin NL, Salman S. Proximate analysis, phtochemical screening and antioxidant activity of different strains of *Ganoderma lucidum* (Reishi Mushroom). Open J Biol Sci. 2020;5:024-7.

26. Dhamodharan G, Mirunalini S. A detail study of phytochemical screening, antioxidant potential and acute toxicity of *Agaricus bisporus* extract and its chitosan loaded nanoparticles. J Pharm Res. 2013;6:818-22.

27. Nkadimeng SM, Nabatanzi A, Steinmann CML, Eloff JN. Phytochemical, cytotoxicity, antioxidant and anti-inflammatory effects of psilocybe natalensis magic mushroom. Plants. 2020;9(9):1127.

28. Rahimah SB, Djunaedi DD, Soeroto AY, Bisri T. The phytochemical screening, total phenolic contents and antioxidant activities *in vitro* of white oyster mushroom (*Pleurotus ostreatus*) preparations. Open Access Maced J Med Sci. 2019;7(15):2404-12.

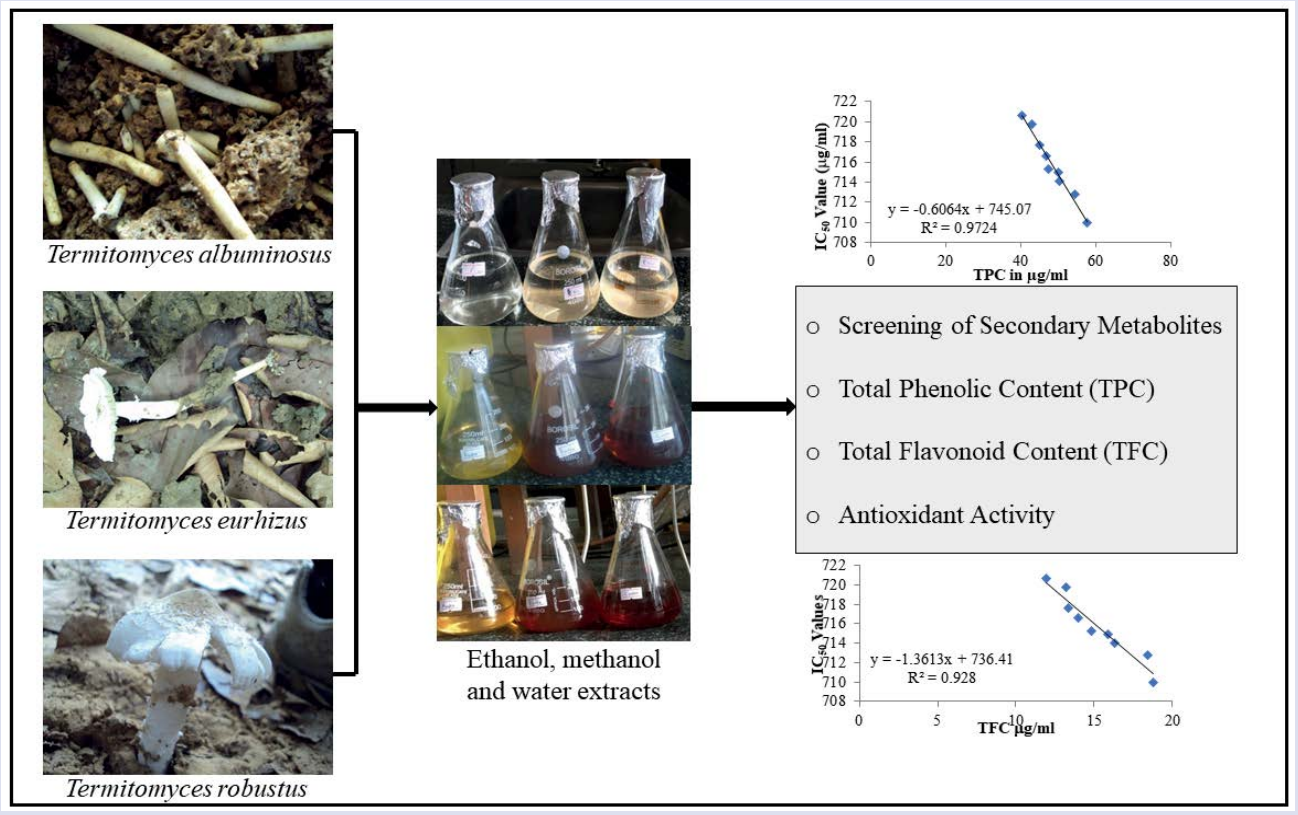
29. Acharya K, Khatua S, Ray S. Quality assessment and antioxidant study of Pleurotus djamor (Rumph. ex Fr.) Boedijn. J Appl Pharm Sci. 2017;7:105-10.

30. Asamoa AA, Essel EA, Agbenorhevi JK, Oduro IN. Effect of Processing Methods on the Proximate Composition, Total Phenols and Antioxidant Properties of Two Mushroom Varieties. Am J Food Nutr. 2018;6:55-9.

31. Thu ZM, Myo KK, Aung HT, Clericuzio M, Armijos C, Vidari G. Bioactive phytochemical constituents of wild edible mushrooms from Southeast Asia. Molecules. 2020;25(8):1972.

32. Gan CH, Amira N, Asmah R. Antioxidant analysis of different types of edible mushrooms (*Agaricus bisporous* and *Agaricus brasiliensis*). Int Food Res J. 2013;20:1095-1102.

GRAPHICAL ABSTRACT



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