KAVAKA 58 (1) : 11-14 (2022) doi: 10.36460/Kavaka/58/1/2022/11-14

Exploring therapeutic efficacy of infusion and decoction of two wild edible mushrooms from West Bengal, India

Sandipta Ghosh, Debatri Dewanjee, Krishnendu Acharya*
Molecular and Applied Mycology and Plant Pathology Laboratory, Centre of Advanced Study, Department of Botany, University of Calcutta, Kolkata- 700 019, West Bengal, India
Corresponding author Email: krish_paper@yahoo.com
(Submitted on January 29, 2022; Accepted on March 12, 2022)

ABSTRACT

Mushrooms occupy a very unique position in the field of herbal medicine having an unlimited source of diverse therapeutically active ingredients along with nutritional values. Studies in the last few decades have illustrated that mushroom extracts and their active components have advantageous effects on a variety of biological systems. This study aimed to investigate the therapeutic potential of two different orally suitable preparations-infusion and decoction prepared from two wild mushrooms; *Tremella fuciformis* Berk and *Termitomyces heimii* Natarajan. *Tremella fuciformis* is a popular name in traditional Chinese herbal medicine while on the contrary *Termitomyces heimii* has an age-old delicacy in tribal communities of different parts of the world. Both of these preparations contained a noticeable amount of bioactive metabolites which could be ranked in the following order phenolics> flavonoids> carotenoids. Besides, the extracts showed potent free radical scavenging activity against DPPH and ABTS radicals while decoction of both species exhibited better results in terms of their chemical composition and bioactivities as well. Furthermore, both infusion and decoction displayed strong anti-inflammatory activity via inhibiting protein denaturation. Thus, the above findings suggest the use of these two formulations of *T. fuciformis* and *T. heimii* as a source of antioxidant-rich healthy beverages.

Keywords: Antioxidant, Anti-inflammatory, Flavonoids, Phenolics, *Termitomyces heimii, Tremella fuciformis*

INTRODUCTION

Mushrooms, the fruiting bodies of macrofungi, are one of the globally appraised culinary food items and known to possess diverse medicinal properties since time immemorial. Being a part of the traditional medicine of Asia, they have been extensively studied which has led to the identification of their unique biologically active ingredients (Zeb and Lee, 2021). They are considered a rich source of phenolics, terpenoids, carbohydrates (β glucan), and others thus making them a natural reservoir of bioactive pharmaceuticals (Reis *et al.,* 2017; Abdelshafy *et al.,* 2021). A diverse array of bioactivities is attributable to those metabolites present in their fruiting bodies, whose biological effect varies according to the chemical nature and process of extraction of the metabolites. It has been reported that Ayurveda and Traditional Chinese Medicine encouraged orally suitable preparations-infusion and decoction to extract active principles from botanicals (Jaiswal *et al.,* 2016). These two traditional methods are less laborious and readily prepared for providing the benefits of herbs. However, infusion and decoction of mushrooms have often been much overlooked although recently, some medicinal mushrooms including Inonotus obliquus (Chaga), Ganoderma lucidum (Reishi), some species of Cordyceps, Hericium erinaceus have been commercially sold as mushroom teas and coffee by many beverage companies in Western countries (Ghosh *et al.,* 2020).

Mushroom tea is now becoming the newest wellness trend which allows expanding their consumption beyond the culinary dish to daily health care routine. Usually, three different methods are known to brew the mushrooms; infusion, decoction and mixture. The former two processes are followed to get the maximum amount of nutrients from the fruiting bodies while the latter one mainly denotes the mixing of mushroom powder with other food products. There are primarily four popular mushroom teas available in commercial markets and each has unique health benefits. Among them, Reishi and Chaga mushrooms are difficult to consume as a whole thus brewing them as beverages and extracts are the only possible consumable form. Keeping that in mind, in this article we have made an attempt to prepare teas from two wild edible mushrooms; Tremella fuciformis and *Termitomyces heimii* following the infusion and decoction process and investigate their chemical composition and biological activities as well. Tremella fuciformis is a wild edible mushroom, extensively used in Traditional Chinese Medicine whereas *T. heimii* has a well-established medicinal value and is commonly consumed by Asians and Africans (Singha *et al.,* 2019).

MATERIALS AND METHODS

Mushroom material : Fresh wild fruiting bodies of *T. fuciformis* and *T. heimii* were collected from North 24 Parganas and Bankura district of West Bengal, India, respectively. The voucher specimens were deposited in Calcutta University herbarium with proper scientific measures bearing the accession numbers CUH386 (*T. heimii*) and CUH536 (*T. fuciformis*). The fruiting bodies were dried after a thorough cleaning and ground using a mixer grinder.

Extracts preparation : Two types of teas (infusion and decoction) were prepared according to standard protocol (Martins *et al.,* 2015). For infusion, 0.1 gm dried powder was added to 20 ml of boiled distilled water and kept aside for 10 minutes in room temperature and in decoction, 0.1 gm dried powder was extracted in 20 ml distilled water which was then boiled for 10 minutes on a heating plate. Thereafter, the extracts were filtered and filtrates were store at 4^{o}C for further experimental analysis.

Determination of bioactive metabolites content: Total phenolics, flavonoid and carotenoids (β-carotene and lycopene) content of infusion and decoction were estimated following standard methods. Briefly, phenol content was estimated using Folin-Ciocalteu reagent using gallic acid as

standard and result was expressed as µg of gallic acid equivalent per mg of extract. Flavonoid content was determined by aluminum nitrate method and quercetin was used as standard. The result was presented as µg of quercetin equivalent (QE) per mg of extract. Furthermore, carotenoid content was measured spectrophotometrically in three different wavelengths as per the standard protocol (Acharya *et al.,* 2018).

Determination of antioxidant potential : The antioxidant potential of infusion and decoction of the two mushrooms was evaluated in terms of their DPPH and ABTS radical scavenging potential, chelating ability and total antioxidant capacity. The former three assays were executed in microtiter plate following the standard protocol in which ascorbic acid was used as standard in DPPH and ABTS radical scavenging activity and EDTA as standard in chelating assay (Khatua *et al.,* 2017). The total antioxidant capacity assay was determined spectroscopically using ascorbic acid as standard and result was expressed as equivalents of ascorbic acid per mg of extract (Acharya *et al.,* 2018).

In vitro* anti-inflammatory activity *: *In vitro* anti-inflammatory activity of the extracts was measured in terms of ability of the extracts to inhibit protein denaturation according to standard protocol with little modifications (Dharmadeva *et al.,* 2018). 0.5 ml reaction mixture was consisted of 0.45 ml 5% bovine serum albumin solution (BSA) and 0.05 ml of different concentrations of extracts (0.01-1 mg/ml). The test samples along with blank solution containing water instead of BSA were incubated for 20 min in room temperature followed by heating at 60^{o}C for 3 min. After cooling down, 2.5 ml phosphate buffer (pH 6.3) was added into each tube and turbidity was measured at 660 mm in spectrophotometer. Percentages inhibition of protein denaturation was calculated from the following formula and EC50 values were estimated.

Statistical analysis: All experimental results were expressed as mean±standard deviation (SD) and statistical calculation were done using Microsoft® Office Excel (Microsoft®, USA).

RESULTS AND DISCUSSION

The quantitative analysis of bioactive metabolites of infusion and decoction from *T. fuciformis* and *T. heimii* revealed the presence of noticeable amounts of phenolics, flavonoids and carotenoids. As presented in table 1, total phenol content ranged from 4.92 to 46.9 µg of gallic acid equivalent per mg of extract, decoction of both of the species showed the highest phenol content while infusion demonstrated a moderate quantity of phenolics. However, the phenolics content was comparatively higher in infusion and decoction of *T. fuciformis* than methanolic extract of cultivated *T. Fuciformis* (Lin *et al.,* 2013). It is reported that the Folin-Cicolteau reagent which is generally used to quantify phenolics in samples does not react specifically with the phenolics but with other reducing agents too thus this assay can be considered as a preliminary screening method to determine the reducing capacity of the test sample (Lizcano *et al.,* 2019). Herein, the results also reflect the reducing capacity of the teas prepared from *T. fuciformis* and *T. heimii* which was further confirmed by flavonoid quantification assay. The tea contained significant amount of flavonoids in the range between 1.38 to 11.8 µg QE/mg of extract with decoction of *T. heimii* having the highest flavonoid content (11.8 µg QE/mg of extract). A correlation between polarity of extraction solvent and quantity of phenolic compounds has been reported by many researchers, for instance, the phenolic content of hot water extract *H. erinaceus* was higher than methanolic extracts of the same species (Wong *et al.,* 2009). Furthermore, the carotenoids content also followed a similar trend being the highest in the decoction of both species and moderate in infusion. Myco-chemical composition analysis revealed that both modes of tea preparation from *T. fuciformis* and *T. heimii* were enriched with phenolic compounds indicating their potential to demonstrate therapeutic activities too. Therefore, the extracts were further subjected to determine their antioxidant and anti-inflammatory activities in various *in-vitro* systems.

Considering the pharmaceutical role of phenolic compounds and reducing ability of the infusion and decoction as preliminary confirmed from spectrophotometric method, the antioxidant potential of those extracts was assessed in four different *in vitro* systems. Both infusion and decoction displayed a dose-dependent radical scavenging activity against DPPH and ABTS radicals (Fig. 1). Decoction of *T. fuciformis* and *T. heimii* inhibited 90% and 80% DPPH radicals respectively; whereas infusion also demonstrated quite comparable percentage of inhibition at the highest concentration (240 µg/ml). Similar trend was also observed for ABTS radical scavenging assay while 90% ABTS radicals were scavenged by decoction of *T. heimii* at the concentration of 120 µg/ml. However, the result was comparable with the higher EC50 values ranging between 0.8-20.3 mg/ml observed in DPPH radical scavenging activity of different non polar extracts of *T. fuciformis* thus indicating the efficiency of infusion and decoction method to provide better activity in comparison to non-polar solvent extraction procedure (Li *et al.,* 2014). Besides, infusion and decoction also exhibited strong chelating activity with decoction of both *T. fuciformis* and *T. heimii* having low EC50 values as compared to the infusion (Table 2). Furthermore, the total antioxidant capacity was measured which indicated higher values for decoction of *T. Heimii* (0.063 ± 0.005µg AAE/mg of extract) followed by decoction of *T. fuciformis* (0.001 ± 0.0002 µg AAE/mg of extract), *T. heimii* infusion (0.006 ± 0.001 µg AAE/mg of extract) and *T. fuciformis* infusion

Table 1: Chemical composition of teas from *Tremella fuciformis* and *Termitomyces heimii*

Mushrooms	Teas	Phenol (µg GAE/mg of extract)	Flavonoid (µg QE/mg of extract)	β carotene (µg /mg of extract)	Lycopene (µg /mg of extract)
Tremella fuciformis	Infusion	4.92 ± 0.51	1.388 ± 0.61	0.00047±0	0.0018 ± 0.0004
	Decoction	10.21 ± 2.84	10.46 ± 1.02	0.0153 ± 0.001	0.002 ± 0.0006
Termitomyces heimii	Infusion	28.27 ± 2.23	8.33 ± 0.94	0.018 ± 0.002	0.003± 0.0007
	Decoction	46.97 ± 1.52	11.8 ± 0.89	0.0303 ± 0.001	0.025 ± 0.001

Data from three repetitions with mean ± standard deviation.

Table 2: Antioxidant activity of infusion and decoction obtained from *Tremella fuciformis* and *Termitomyces heimii*

Experiments	*Tremella fuciformis*		*Termitomyces heimii*	
	Infusion	Decoction	Infusion	Decoction
DPPH radical scavenging activity (µg/ml)	108 ± 1.05	58 ± 1.12	110 ± 0.98	120 ± 0.58
ABTS radical scavenging activity (µg/ml)	115 ± 0.74	94 ± 0.46	28 ± 1.12	34 ± 0.59
Chelating activity (µg/ml)	104 ± 0.41	25 ± 0.78	33 ± 1.07	22 ± 1.25

Data from three repetitions with mean ± standard deviation.

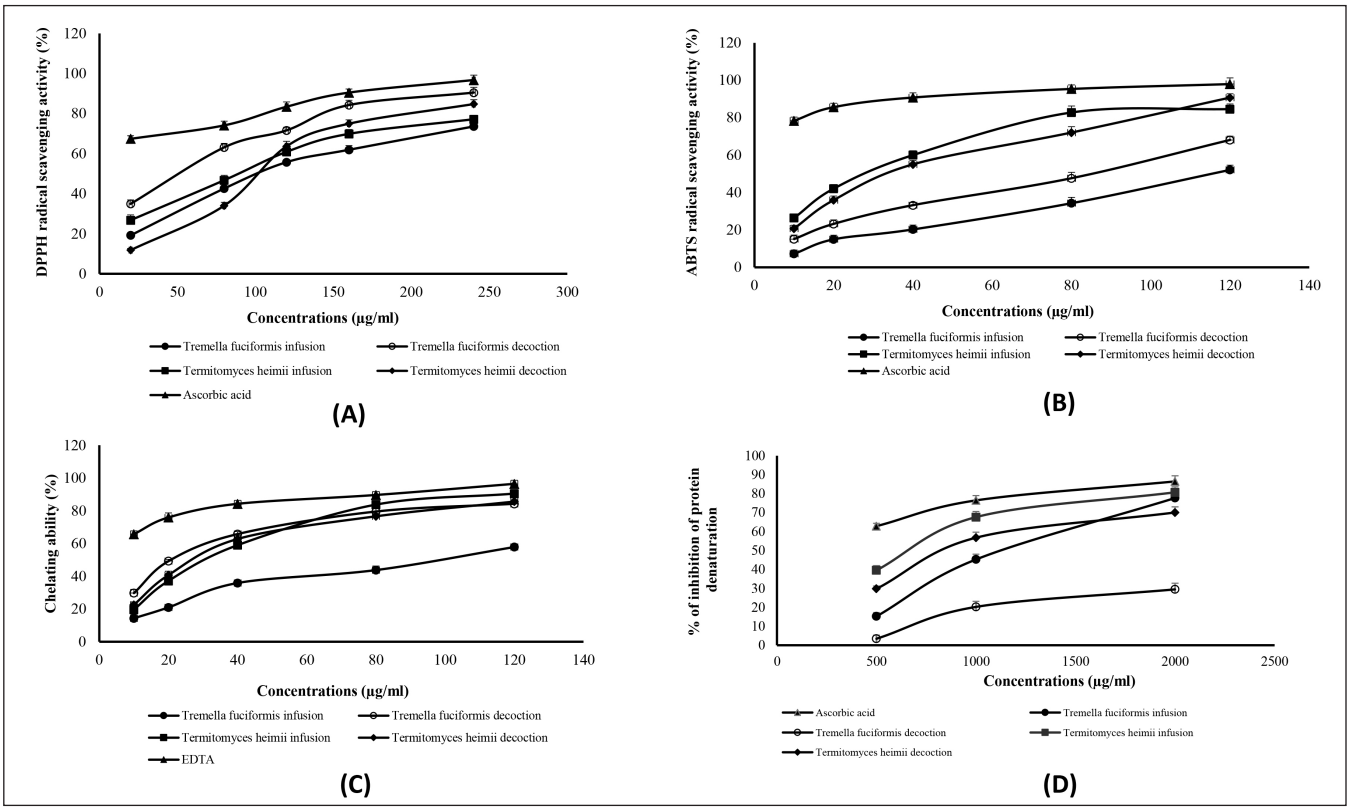

Fig. 1: Graphical representation of antioxidant and anti-inflammatory activity of *Tremella fuciformis* and *Termitomyces heimii* (A) DPPH radical scavenging activity (B) ABTS radical scavenging activity (C) Chelating activity (D) Anti-inflammatory activity

(0.0012 ± 0.0005µg AAE/mg of extract).

It is evident that inflammation has been associated with many chronic disorders including rheumatoid arthritis, cancer and others. Several anti-inflammatory drugs have the potential to inhibit heat-induced protein denaturation which is considered a major regulatory factor in certain arthritic and inflammatory diseases (Saso *et al.,* 2001). Therefore, in this study, both infusion and decoction were assessed for their anti-inflammatory activity in terms of inhibition of protein denaturation. As shown in fig. 1D, a dose-dependent inhibition of albumin protein denaturation was observed for all extracts excluding *T. fuciformis* decoction, although at the maximum concentration, *T. fuciformis* decoction displayed only 27% inhibition. The maximum inhibition (80.64%) was observed for *T. heimii* infusion at the concentration of 2000 µg/ml whereas other extracts also showed quite good inhibitory activity with 77% and 70% at the same concentration tested for *T. fuciformis* infusion and *T. heimii* decoction, respectively. However, with respect to EC50 value the efficiency of the abovementioned extracts can be ranked in the following order *T. heimii* decoction (865 ± 1.02 µg/ml) > *T. heimii* infusion (900 ± 0.89 µg/ml) > *T. fuciformis* infusion (1500 ± 0.75 µg/ml).

CONCLUSION

The present investigation showed promising therapeutic properties of infusion and decoction of *T. heimii* and *T. fuciformis* in terms of their antioxidant and anti-inflammatory activity. However, previously there is no such documentation related to their bioactive metabolites composition and therapeutic properties of tea formulations (infusion and decoction). All of these extracts were found to be enriched with phenols, flavonoids and carotenoids which belong to the category of health-promoting constituents of edible mushrooms. Extracts were also capable of showing inhibitory activity against free radicals and chelating ferrous ions. Moreover, extracts also had shown potent anti-inflammatory activity suggesting their application as a remedy of inflammatory disease as well as a readily available source of antioxidants in form of energizing beverages.

REFERENCES

Abdelshafy, A.M., Belwal, T., Liang, Z., Wang, L., Li, D., Luo, Z. and Li, L. 2021. A comprehensive review on phenolic compounds from edible mushrooms: Occurrence, biological activity, application and future prospective. *Crit Rev Food Sci Nutr.* **61:** 1-21.

Acharya, K., Ghosh, S. and Biswas, R. 2018. Total phenolic contents and antioxidant effects of infusion and decoction from Lepista sordida (Schumach.) Singer. *FABAD J Pharm Sci.* **43:** 1724.

Dharmadeva, S, Galgamuwa, L.S., Prasadinie, C. and Kumarasinghe, N. 2018. In vitro anti-inflammatory activity of Ficus racemosa L. bark using albumin denaturation method. *Ayu.* **39(4):** 239-242.

Ghosh, S., Chatterjee, T., Sardar, A., Chatterjee, I., Bose, A., Dasgupta, A., Malitha, A. and Acharya, K. 2020. Antioxidant properties and phytochemical screening of infusion and decoction obtained from three cultivated *Pleurotus* species: A comparative study. *Jordan J. Pharm. Sci.* **13:** 121-129.

Jaiswal, Y., Liang, Z. and Zhao, Z. 2016. Botanical drugs in Ayurveda and Traditional Chinese Medicine. *J. Ethnopharmacol.* **194:** 245-259.

Khatua, S., Ghosh, S. and Acharya, K. 2017. A simplified method for microtiter-based analysis of *in vitro* antioxidant activity. *Asian J Pharmacol.* **11:** S327S335.

Li, H., Lee, H-S., Kim, S-H., Moon, B. and Lee, C. 2014. Antioxidant and anti-inflammatory activities of methanol extracts of *Tremella fuciformis* and its major phenolic acids. *J. Food Sci.* **79:** C460-C468.

Lin, W.Y., Yang, M.J., Hung, L.T. and Lin, L.C. 2013. Antioxidant properties of methanol extract of a new commercial gelatinous mushrooms (white variety of Auricularia fuscosuccinea) of Taiwan. *Afr. J. Biotechnol.* **12:** 6210-6221.

Lizcano, S.C., Dávila, J.A. and Hernández, V. 2019. Fruit agroindustrial wastes for preparing beverages for medicinal purposes by supercritical fluid extraction technology: Andes berry (Rubus glaucus Benth.) case. *Production and Management of Beverages*. 151-177.

Martins, N., Barros, L., Santos-Buelga, C., Silva S, Henriques, M. and Ferreira, I.C.F.R. 2015. Decoction, infusion and hydroalcoholic extract of cultivated thyme: Antioxidant and antibacterial activities, and phenolic characterisation. *Food Chem.* **167:** 131-137.

Reis, F.S., Martins, A., Vasconcelos, M.H., Morales, P. and Ferreira, I.C.F.R. 2017. Functional foods based on extracts or compounds derived from mushrooms. *Trends Food Sci. Technol.* **66:** 48-62.

Saso, L., Valentini, G., Casini, M.L., Grippa, E., Gatto, M.T., Leone, MG. and Suvestrini, B. 2001. Inhibition of heat-induced denaturation of albumin by nonsteroidal anti-inflammatory drugs (NSAIDs): Pharmacological implications. *Arch. Pharm. Res.* **24:** 150-158.

Singha, K., Sahoo, D.K., Banerjee, A. and Mohapatra, P.D. 2019. *Termitomyces* Mushrooms: A Natural Resource of Biomolecules with Enormous Bioactive Potentials for Biotechnological Exploitation. In: *Biotechnology for Sustainable Utilization of Bioresources*, pp 295-308 Daya Publishing House, New Delhi.

Wong, K.H., Vikineswary, S., Abdullah, N., Kuppusamy, U.R. and Naidu, M. 2009. Effects of cultivation techniques and processing on antimicrobial and antioxidant activities of Hericium erinaceus (Bull.:Fr.) Pers. Extracts. *Food Technol. Biotechnol.* **47:** 47-55.

Zeb, M. and Lee, C.H. 2021. Medicinal properties and bioactive compounds from wild mushrooms native to North America. *Molecules* **2021. 26:** 251.