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Phyto-Chemical Study of Termite's Mushroom in Nepal: An Ethnomycological Approach

Research Article

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Abstract

This paper reports on the qualitative phytochemical analysis of *Termitomyces mammiformis* R. Heim belongs to family Tricholomataceae found in Tarai and Midhill of Nepal. It was collected from the termite nest of the forests in West, Centre and East of tropical, subtropical and temperate region. The sample was harvested fresh, sundried, pulverized and analyzed according to standard procedures. Screening revealed the presence of alkaloid, carotenoid, steroid, triterpenoids, fatty acid, emodins, flavonoid and coumarin in ethereal, anthracene glycoside, anthocyanadine glycoside in methanolic and tannins, saponins, glycosides, polyurenoid and polyoses in the aqueous extracts respectively. There were significant differences in the phytochemical composition of the samples collected from three ecological belts and three phytogeographic regions among the west Siwalik was the best. Its ethno mycological knowledge of different ethnic groups was also incorporated. There was definite co-relation between the traditional application of Termite's mushrooms and possession of secondary metabolites, which supports the scientific basis for the traditional medicinal system. Results showed that the consumption of wild edible mushroom that act as a good dietary supplement and it may be valuable in drug development.

Keywords: Macro-fungi; Local-medicines; Socio-economy

Introduction

All over the world more and more attention is focused on phytochemical research. Phytochemicals are the natural substance of vegetable origin, which provide arrange of drugs for a number of diseases. Many phytochemicals are reasonably heat stable and do not appreciably lost during conventional cooking. An analysis of various plant species is necessary in linking biodiversity and dietary diversity and health. Its composition data can provide useful information to promote sustainable use of biodiversity for food security and health and wellness [1].

Phytochemicals are the major bioactive compounds which provide health benefits and also found to be associated with the inhibition of atherosclerosis and cancer [2]. Edible mushrooms has become an integral part of the normal human diet and considered as nutraceutical product [3,4]. Mushrooms not only provide nutrition, but also prevent diseases and ensure good health and longevity [1]. Phytochemicals are not nutritive but make food functional. Mushroom produce a wide range of secondary metabolites having high therapeutic value, such as antioxidant, antitumor, antibacterial, antiviral, cholesterol lowering, hematological agents and immunomodulating properties [5,6]. These chemicals also used for protection against chronic diseases such as diabetes, antioxidative, anticarcinogenic, hepatoprotective properties [7,8].

Chemical screening of different species of mushrooms contain different chemicals such as Acids and alkaloids, Amatoxin, Psilocybine, Coprin, Helvellic acid, Muscarine, Ibutenic acid, which are toxic to human health either mycetism or mycotoxicoses [9]. The increased interests in consumption of mushrooms as food, in the cure of diseases, and for bioremediation have generated a lot of interest in recent time [10,11].

A high diversity of wild edible mushrooms is must important due to high climatic and floral diversity of Asia. They are consuming high by rural population as well as elsewhere. Herein, we report the chemical analysis of wild edible *T. mammiformis* mushroom collected from tropical to temperate eco-zones of Nepal.

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There are seventy five medicinal mushroom species have been reported from Nepal [12-14]. Medicinal plants are an integral part of the diverse traditional medical practices in Nepal and are codified in traditional medical systems such as Chinese, Ayurveda, Unani, Siddha, Homeopathy, Amchi, etc. [15]. Crude-drugs are commonly given in the form of powder, decoctions, and infusions or in ointment forms. *T. mammiformis* are not only an important source of food for local people but this also uses them for medicinal purpose for treatment for different types of disease and ailments (Table 1).

Hence, the preparation of monographs of wild edible mushroom *T. mammiformis* that would provide a systematic account on their phytochemical profiles is in urgent need for standardization of the traditional medicinal herbs, therapeutic benefits and their possible toxic effects. This study aimed to provide information on secondary metabolites of the *T. mammiformis* of Nepal.

Method

Ethnomycological Survey

Information were collected using an Anthropological field technique, Participatory Rural Appraisal (PRA), involving direct interviews and discussion with local people by [16] direct observation on the way different macrofungi have been collected and used. The documented information was verified by questionnaire, by consulting relevant literatures and by gathering key information from village elders. The gathered ethnomycological data was authenticated by cross questionings with other community of the same or other localities by referring the same questionnaire and samples as well as by relevant reference and specimen consultation. The survey was done at twenty-seven sites and its adjoining area of Tarai and Midhill of Nepal (Table 2).

Tribal information of T. mammiformis

During present study the ethnomycological information was collected from tribals viz.Baraei, Bhujel (Khawas), Bote, Chamar, Chhanthal, Darai, Damaei, Dhimal, Dusad, Gaaeene, Gurung, Jhangar, Kami, Koeeri, Kumal, Kurmi, Limbu, Magar, Majhi, Musahar, Newar, Praja (Chepang), Rai, Rajbansi, Santhali, Sarki, Sherpa, Tharu, Thakali, Thakuri and Tamang (Table 3).

Collection

Surveys were under taken and specimens collected from 15th to 31st May and from 1st June to 31st October in 2010 and 2011 respectively, from the termite nest of the forests between 26°44'08" and 29°06'32"N latitude and 80°18'02" and 88°08'27"E longitude of Nepal (Figure 1). The local names of specimen along with its traditional uses by native people were noted on the spot (Tables 1 - 3). The collected specimen was brought to the laboratory, Central

Table 1: Traditional use of *T. mammiformis* for the treatment of different types of disease and ailments in the studies area.

SN	Pathological conditions	Mode of administration
1	Measles	Pouring water in dry mushroom in pot overnight and its filtrate consumed orally, one cup thrice a day for 2/3 days. This is repeated till the recovery is evident in Magar tribe (Gulmi District).
2	Yellow fever	Pouring water in dry mushroom in pot overnight and its filtrate consumed orally, one cup thrice a day for 2/3 days. This is repeated till the recovery is evident in Tharu tribe (Kapilwastu District).
3	Jaundice	Pouring water in dry mushroom in pot overnight and its filtrate consumed orally, one cup thrice a day for 2/3 days. This is repeated till the recovery is evident in Magar tribe (Palpa District).
4	Inappetence / Abdominal disorder	Used as powdered 5-10 gm. with hot water, by mixing a pinch of salt, twice a day up to recovery in Tharu tribe (Kailali District).
5	Diarrhoea	Pouring water in dry mushroom in pot overnight and its filtrate consumed orally, one cup thrice a day for 2/3 days. This is repeated till the recovery is evident in Tharu (Rupandehi District).
6	Bleeding constipation, Wounds, Itching, Eczema etc.	For curing, its powder is used with mustard oil (in Santhan tribe in Morang District)/water (in Tharu tribe in Nawalparasi District). Parts of fruiting bodies are mashed fresh with water for several times to form a uniform paste. The paste is consumed orally after dinner for 2/3 days.
7	Muscular pain	Boiled fruits bodies are used as soup to relieve muscle pains and to maintain protect or restore immune health. It is administered in two ways- Rajbangsi (Morang) tribe uses it as curry, soup while its roasted forms are used in Chepang (Makwanpur-District) tribe.
8	Skin diseases	Mushroom is crushed in mortar/pestle and applied on skin infection twice a day for 3 to 5 as Paste form. (Magar tribe in Dang District)
9	Cut wound	Fresh extract powder/paste of fruiting bodies is used for wound healing. [(in Kumhal-Khuna tribe in Kusum (Banke District)].
10	Delivery pain	Decoction of fruiting bodies as soup or curry is given to as tonic for stimulating power. Khuna (Banke District), Kumhal (Palpa District) and Santhal (Jhapa District), Magar (Salyan District).
11	Indigestion/Stomachache	Dried powder and Black salt (Birenun) are used with hot water, twice a day up to recovery (in Khuna tribe Banke district).
12	Laziness/indolence/inactiveness	It is used to strengthen body, for increase in body healing ability and is associated with longevity. Its powder is taken one table spoon with hot water after dinner till the recovery is evident in Bote tribe (Chitwan District).
13	Stiffness of Joints	To relief from the arthritics pain, frequently used over the painful area as a paste. It is administered in two ways Santhan (Jhapa) tribe uses it as pest while Dhimal (Morang) tribe mixed with mustard oil and used as ointment for the treatment of infected parts.
14	Buccal cavity infection	Its powder is used as tooth powder with mixing mustard oil and common salt in Sherpa (Sindhuli District).
15	Cough/Cold	Decoction of fruiting bodies in hot water is given one cup in two times a day Rai, Limbu tribe, (in Ilam District).

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Table 2: Presence-absence of phytochemicals in different eco-zone of *T. mammiformis* in Nepal.

SN	Regions	V	A	S	T	C	F	E	Fl	Co	An	Ant	Sa	Ta	G	P	Po
1	WT	-	-	-	+	-	+++	+	-	+	+	++	+	+++	-	++	++
2	WT	-	-	-	+	-	+++	+	-	+	+	++	+	+++	-	++	++
3	WT	-	-	-	+	-	+++	+	-	+	+	++	+	+++	-	++	++
4	CT	-	+++	++	+++	++	-	++	++	+	-	-	++	+++	++	-	-
5	CT	-	+++	++	+++	++	-	++	++	+	-	-	++	+++	++	-	-
6	CT	-	+++	++	+++	++	-	++	++	+	-	-	++	+++	++	-	-
7	ET	-	+++	-	+++	+++	+++	+	+	-	-	+	++	++	+++	+++	+++
8	ET	++	+++	-	+++	+++	++	-	+	-	+	++	+++	+	+++	-	-
9	ET	-	+++	-	+++	+++	+++	+	+	-	-	+	++	++	+++	+++	+++
10	WS	-	+++	++	++	++	+	+	++	++	+	+	+++	+++	+++	++	++
11	WS	-	+++	++	++	++	+	+	++	++	+	+	+++	+++	+++	++	++
12	WS	-	+++	++	++	++	+	+	++	++	+	+	+++	+++	+++	++	++
13	CS	-	++	+	+++	+++	+	+	+	+	-	+	+++	++	+++	+	+
14	CS	-	++	+	+++	+++	+	+	+	+	-	+	+++	++	+++	+	+
15	CS	-	++	+	+++	+++	+	+	+	+	-	+	+++	++	+++	+	+
16	ES	-	++	-	+++	+++	+	+	+	+	+	+	++	++	++	+	+
17	ES	-	+++	+++	+++	+++	++	-	+++	+	+	+	+++	++	+++	+++	++
18	ES	-	++	++	+++	+++	++	+	++	+	+	+	++	++	++	++	+
19	WM	-	+++	-	+++	+++	+++	+	+	-	-	+	++	++	+++	+++	+++
20	WM	-	+++	+	+++	+++	-	+	+	-	++	+++	++	+	+++	++	++
21	WM	-	+++	+++	+++	+++	+	+	+	+	-	-	+++	+++	+++	+	+
22	CM	-	+++	++	+++	+++	-	-	+	++	-	-	+++	+++	+++	-	+
23	CM	-	+++	++	+++	+++	-	-	+	++	-	-	+++	+++	+++	-	+
24	CM	-	+++	++	+++	+++	-	-	+	++	-	-	+++	+++	+++	-	+
25	EM	-	+++	+++	+++	++	+	++	-	++	-	-	+++	++	++	+	+
26	EM	-	+++	+++	+++	++	+	++	-	++	-	-	+++	++	++	+	+
27	EM	-	+++	+++	+++	++	+	++	-	++	-	-	+++	++	++	+	+

Note. + indicate, presence of chemicals in trace amount; ++ for moderate amount; +++ for high amount and - for absence.
WT: Western Tarai; CT: Central Tarai; ET: Eastern Tarai; WS: Western Siwaliks; CS: Central Siwaliks; ES: Eastern Siwaliks; WM: Western Midhilla; CM: Central Midhilla.
Here, V: Volatile oil, A: Alkaloid, S: Steroid, T: Triterpenoid, C: Carotenoid, F: Fatty acid, E: Emodine, Fl: Flavonoid, Co: Coumarin, An: Anthracene, glycoside, Ant: Anthocyanadine glycoside Sa: Saponins, Ta: Tannins, G: Glycoside, P: Polyurenoids, Po: Polyoses..

Department of Botany, Tribhuvan University, Kathmandu, Nepal, for identification.

Identification

The samples were identified using Heim [17], Rawla et al. [18], Leelavathy et al. [19], Piearce [20], Vander & Eicker [21], Pegler and Vanhaecke [22] and on line data base such as: www.biodiversitylibrary.org [23], www.indexfungorum.org [24], www.jstor.org [25], www.mycobank.org [26], www.tropicos.org [27]. Voucher specimens are deposited in Natural History Museum Tribhuvan University. Its Accession number is NHM TU 2-2-1678.

Processing of the specimen

The mushroom was uprooted, washed and they were oven dried for 48 hours at 40°C. They were turned repeatedly to avoid the microbial growth. The sample was pulverized using a manual blender and stored in a labeled air-tight container before analysis.

Phytochemical Screening

The experiment was carried out in Laboratory of Department of Plant Resource, Kathmandu, Government of Nepal. It was conducted according to standard methods described by Ciulei, [28] (Table 4). Briefly, 10 gm of powdered sample from each site was first extracted with petroleum ether using Soxhlet extractor until 6 hr or until colour change, then with ethyl alcohol and finally with water. The obtained solutions in each extraction process were filtered through whatman filter paper No.1 and concentrated up to 20-25 ml using rotary evaporator at 40°C.

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Table 3: *T. mammiformis,* pronounce in different region of the country.

SN	Local name	Mycophagous, Ethnic groups	Regions
1	Sadaka	Awadhi	WT
2	Bagale	Praja	WS
3	Sangraeino	Doteli	WM
4	Vavanethi	Kumhal	CT
5	Dewale	Magar	CS
6	Kaltauke	Khash	CM
7	Bemtee	Tharu	ET
8	Dhamere	Dhimal,	ES
9	Kalunge	Rai, Limbu	EM

Table 4: Methodology of phytochemical screening.

SN	Chemical constituents	Test
1	Volatile oil	2 ml petroleum extract dissolved in diethyl ether evaporated to dryness. Pleasant smell or aromatic smell indicates presence of volatile oil.
2	Alkaloids	0.5 ml of extract + 1.5 ml HCl + Mayer' reagent, if it gives whitish- yellow ppt. and 0.5 ml of extract + 1.5 ml HCl + Bertrand reagent gives white ppt. indicates the presence of alkaloids.
3	Steroid &Triterpenes	15 ml of extract + 1.5 ml of 10% KOH + 0.5 ml acetic anhydride + 0.5 ml chloroform + Liebermann-Brofad's reagent. At the contact zone of test tube two layers were formed, the upper became green of steroid and lower of violet of triterpenes respectively.
4	Carotenoid	15 ml extract evaporated to dryness. Addition of 1 ml of antimony trichloride first became blue then red in colour. After addition of 1 ml conc. H_2SO_4,it became deep blue, indicates the presence of carotenoid.
5	Fatty acids	Spot persists on filter paper after dropping the 0.5 ml concentrated extract.
6	Emodins	2 ml of extract + 1 ml of 25% NH_4OH. It became red in colour.
7	Flavonoid	2 ml extract evaporated to dryness. Addition of 1 ml of methanol + piece of Mg + 0.5 ml of conc. HCl. It became orange in colour.
8	Coumarin	Addition of 2ml d/w in conc. extract + 10% NH_3. The occurrence of intense fluorescence under UV light indicates the presence of coumarin.
9	Anthracene glycoside	4 ml of ethanolic extract + 2 ml 25% NH_4OH. It became cherry red in colour.
10	Anthocyanadine glycoside	10/10 ml methanolic extract + 10% HCl + H_2O + 5 ml ether. The solution became red and turns neither to violet at a neutral pH, nor to green or blue in an alkaline medium, indicate the presence of anthocyanadine.
11	Tannins	20 ml of aqueous extract + 0.5 ml of 0.1% $FeCl_3$. The blue black precipitate were observed, indicate the presence of tannins.
12	Saponins	2.5 ml of aqueous extract + 10 ml of hot water. Persistence of froth, after shaken vigorously.
13	Glycosides	2/2 ml of semi dried aqueous extract + Fehling Solution (I & II). It gave brick red in colour, after heating.
14	Polyurenoids	2 ml of aqueous extract + 10 ml acetone + 1 ml Hematoxylin. The occurrence of a violet ppt. after centrifugation.
15	Polyoses	1 ml of aqueous extract + 0.5 ml H_2SO_4 + 1 ml methanol + Molisch's reagent gives red colour indicates the presence of polyoses.

Table 5: Results of P and X^2 on the variation of phytochemicals along with phytogeographic north-south gradients (Tarai, Siwaliks and Midhillaregion) with its frequency of each chemical at different eco zone North-South gradient (Tarai, Siwalik and Midhilla).

Anthocyanadine	Anthracene	Tannins	Saponins	Glycoside	Polyurenoid	Polyoses	N
0.133 (9.80)	0.041 (9.975)	0.396 (4.077)	0.132 (7.067)	0.096 (7.875)	0.126 (9.964)	0.006 (18.25)	27

Table 6: Results of P and X^2 on the variation of phytochemicals along with phytogeographic east-west gradients (East, Central and West region) with its frequency of each chemical at different eco zone.
East west gradient (East, Central and West Region).

Volatile oil	Alkaloid	Carotenoid	Steroid	Triterpenoid	Fatty acid	Emodine	Flavonoid	Coumarin
0.354 (2.077)	0.064 (8.905)	0.126 (7.20)	0.011 (16.50)	0.004 (15.429)	0.004 (19.039)	0.046 (9.675)	0.301 (7.22)	0.423 (3.877)

Anthocyanadine	Anthracene	Tannins	Saponins	Glycoside	Polyurenoid	Polyoses	N
0.133 (9.8)	0.016 (12.225)	0.007 (14.115)	0.132 (7.067)	0.024 (11.25)	0.0001 (24.857)	0.003 (19.75)	27

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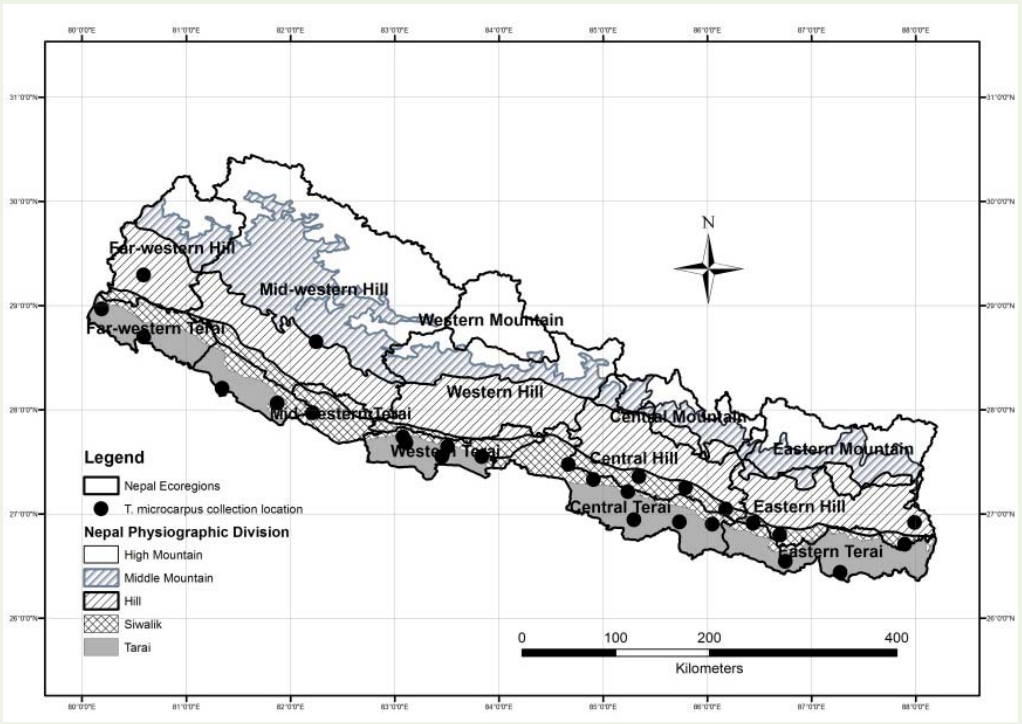

Figure 1: Map of sample collection site.

Statistical Analysis

Variation of concentration of individual chemical compounds in east, center and west Nepal was tested. Similarly those variations were tested among the sample of Tarai, Siwalik and Midhill, using same test, by Pearson's [29] chi square test. Difference were considered to be significant at $p<0.05$ (Tables 5 & 6).

Results

In the present investigation, three samples were analyzed from each region (Table 3), and fifteen major chemical constituents have been found in the extracts of *T. mammiformis* during analysis, where volatile oil is almost absent, among the tested samples. Generally frequency of high concentration on north-south gradients of alkaloids, steroid, triterpenoids, carotenoid, coumarin, saponins and glycoside are highest in Midhill and gradually decrease Siwalik to Tarai range (except steroid in Tarai) (Appendix IA), whereas fatty acid, tannin polyurenoid and polyoses are gradually decreases from Tarai to Midhill through Siwalik (except fatty acid and polyoses). Herein we found emodine almost equal in the entire eco zone (except in siwaliks) and flavonoid, anthracene glycoside and anthocyanadine glycoside almost absent (except flavonoid in Siwalik & anthracene & anthocyanadine glycoside in Midhill, respectively). Similarly frequency of high concentration on east-west gradients of alkaloid, steroid, triterpenoids, carotenoid, emodine, polyurenoid and polyoses are highest in east and gradually decrease centre to west (Appendix IB), (except steroid, polyurenoid& polyoses in centre and emodine in west, respectively). Whereas in fatty acid, saponins, tannin and glycoside the result shows that concentration were found highest in west and gradually decrease to the east through center (except fatty acid in centre & tannin in the east & west). The flavonoid, anthracene and anthocyanadine glycoside are almost absent (except flavonoid in east & anthracene & anthocyanadine glycoside in west respectively). Herein we found coumarinequal in the entire eco zone.

Likewise, there was significant difference in alkaloid, steroid, triterpenoids, fatty acid, coumarin, anthracene glycoside and polyoses, contains among the tested sample of three different eco-zones of north-south gradients of tropical to temperate region of Nepal (Table 5). Similarly there was also significant difference in steroid, triterpenoids, fatty acid, emodine, anthracene glycoside, tannin, glycoside, polyurenoid and polyoses contain among the tested sample of three different eco-zones of east-west gradients of tropical to temperate region of Nepal (Table 6).

Discussion

The plants which are rich in a wide variety of secondary metabolites are generally superior in medicinal property and exhibit physiological activity at a particular dose [30,31]. *Termitomyces* species has ability to suppress postprandial hyperglycemia caused by prolonged high blood glucose level associated with diabetes [32,33]. Phytochemicals such as alkaloid, steroid, triterpenoids, flavonoid, anthracene, saponins, tannins and glycosides are also found in *T. reticulates* [34]. Similarly, fatty acids in *T. clypeatus* [35] and *T. letestui* their essential fatty acids are required for the promotion of a variety of body biochemical function. These are potential nutritional food for individuals susceptible to diabetes [36].

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Mushrooms generally contain low fat and oil content [37,38]. This statement also supports our experimental work. Because of it, they are recommended as good source of food supplement for patients with cardiac problems or at risk with lipid induced disorders. Mushrooms need antibacterial and antifungal compounds to survive in their natural environment [6,39]. Hence, they are rich sources of natural antibiotics, these wild macro-fungi; possess inhibitory potential against bacteria associated with wastewater and leftover foods and also a potential source of useful drugs. Still now large number of the unknown species of mushrooms whose health promoting properties are unknown. This is because there are little or no information about these mushrooms and their medicinal potentials. Phytochemicals are responsible for their nutritional and therapeutic uses. These results therefore not only make these wild edible mushrooms *T. mammiform* is popular to consume as food sources but may also be valuable in drug development.

T. mammiformis have food and medicinal significance, used as vegetable, has therapeutic use and its soup is also used as tonic (Table 4). Hence, it is necessary to identify the biological and pharmacological potential of mushrooms especially of wild edible mushrooms, which are collected indigenously (Table 1). It is also necessary to do research in identifying and isolating different species of mushrooms having nutraceutical and medicinal properties to commercialize. The production in large scale level would create a lot of employment opportunities especially in economically deprived rural area which plays a vital role in enrichment of the socio- economic life. Besides their consumption, the local medicine also paves the way for the upbringing new industries.

Based on the result of these finding, it can be concluded that *T. mammiformis* have high concentration of diverse phytochemicals and are potential of medicinal value. The species contain different chemical concentration of bioactive compounds even in same ecological zone. Concentration of chemicals may be affected by climatic variation. There was co-relation between the traditional application of mushrooms and possession of secondary metabolites. This result may be useful to future workers to select a group of plants having similar chemical constituents to isolate biologically active principle or prepare remedies for particular case. Bioactive compounds with antibacterial properties can also be sourced from this underutilized macrofungi present in wild state.

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