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PHYTOCHEMICAL SCREENING OF TERMITE'S MUSHROOM IN NEPAL

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ABSTRACT

This paper reports the qualitative phytochemical analysis of *Termitomyces microcarpus* (Berk & Broome) R. Heim of family Tricholomataceae collected from the termite nest of the forests in west, center and east of tropical, subtropical and temperate regions of Nepal. The sample was harvested fresh, sundried, pulverized and analyzed according to standard procedures. Screening revealed the presence of volatile oil, alkaloid, carotenoid, steroid, triterpenoids, fatty acid, emodins, flavonoid, coumarin, anthracene glycoside, anthocyanadine glycoside, tannins, saponins, glycosides, polyurenoid and polyoses in the ethereal, methanolic and aqueous extracts. There were significant differences in the phytochemical composition of the samples collected from east, center and west eco-zones and tropical, subtropical and temperate climatic regions. 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.

Key words: macrofungi, different regions, *Termitomyces microcarpus*, phytochemicals

INTRODUCTION

All over the world more and more attention is focused on phytochemical research. Phytochemicals are the natural substance of vegetable origin, which provide a range 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 (Evelyn *et al*. 2006).

Phytochemicals are the major bioactive compounds which provide health benefits and also found to be associated with the inhibition of atherosclerosis and cancer (Klimczak *et al.* 2007). Edible mushrooms has become an integral part of the normal human diet and considered as nutraceutical product (Mau *et. al*. 2002, Chang and Buswell 2003). Mushrooms not only provide nutrition, but also prevent diseases and ensure good health and longevity (Evelyn *et al.* 2006). Phytochemicals make food functional,because, many mushroom reported to produce a wide range of secondary metabolites having high therapeutic value, such as antioxidant, antitumor, antibacterial, antiviral, cholesterol lowering, hematological agents and immunomodulating properties (Wasser and Weis 1999, Yang *et al*. 2002). These chemicals also used for protection against chronic diseases such as diabetes, antioxidative, anticarcinogenic and hepatoprotective properties (Johnson *et al*. 1993, Rupasinghe *et al.* 2003). Chemical screening of different species of mushrooms contain different chemicals such as Acids and alkaloids, Amatoxin,

Psilocybine, Coprin, Helvellic acid, Muscarine and Ibutenic acid, which are toxic to human health either mycetism or mycotoxicoses (Duffy 2008). 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 (Chang 1990, Bushwell and Chang 1993).

A high diversity of wild edible mushrooms is most 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 mushroom *Termitomyces microcarpus* collected from tropical subtropical and temperate eco-zones of the east, center and west Nepal.

The major chemical substances of interest in this study have been the volatile oil, alkaloid, steroid, triterpenoids, carotenoid, fatty acid, emodins, flavonoid, coumarin, anthracene glycoside, anthocyanadine glycoside, saponins, tannins, glycosides, polyurenoids and polyoses.

The Himalayan region is rich in endemic species and medicinal herbs. Seventy five medicinal mushroom species have been reported from Nepal (Adhikari 2009). 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. (Manandhar 2002). Crude-drugs are commonly given in the form of powder, decoctions, and infusions or in ointment forms. *Termitomyces microcarpus* are not only an important source of food for local people but this also use them for medicinal purpose for treatment for different types of disease and ailments such as measles, yellow fever, jaundice, inappetence, diarrhoea, constipation, stomach pain, muscular pain, delivery pain, skin diseases, mump, ear pain, cut wound etc. (table 1).

Hence the preparation of monographs of wild edible mushroom *Termitomyces microcarpus* 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 *Termitomyces microcarpus* of Nepal.

MATERIALS AND METHODS

Collection

Survey was done from 15th to 31st of May and specimens were collected from 1st June to 31st of October in 2010 and 2011, from the termite nest of the forests in west, center and east, of tropical, subtropical and temperate regions (table 1) between 26º44'08'' and 29º06'32''N latitude and 80º18'02'' an 88º08'27''E longitude of Nepal (fig. 1). The local names of specimen along with its traditional uses by native people were noted on the spot (table 2 and 3). The collected specimens were brought to the laboratory, Central Department of Botany, Tribhuvan University, Kathmandu, Nepal, for identification.

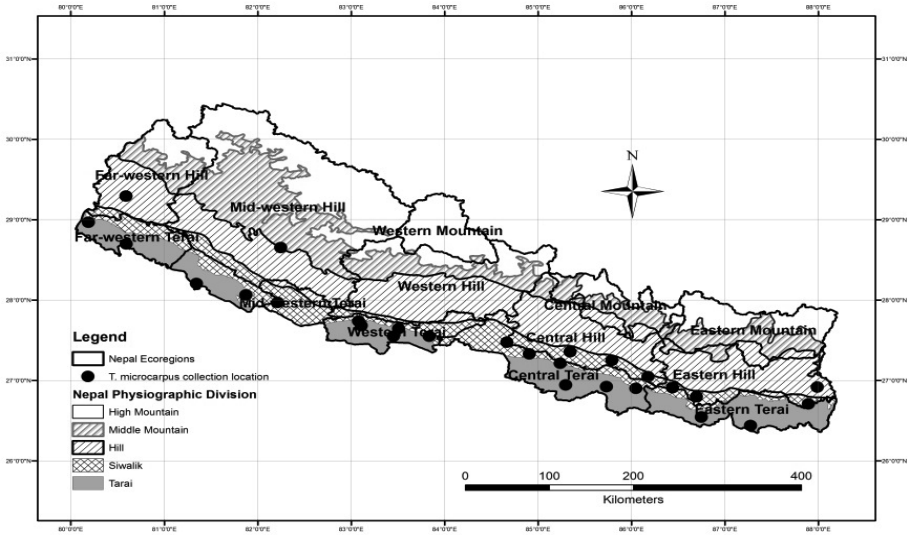

Figure 1. Map of sampling sites.

Identification

The samples were identified with the help of relevant literatures (Heim 1977, Rawla *et al.* 1983, Leelavathy and Suja 1985, Piearce 1987, Van der Weasthuisen and Eicker 1990, Pegler and Vanhaecke 1994).The voucher specimen is deposited in Tribhuvan University, Central Herbarium (TUCH).

Processing of the specimen

The mushrooms were uprooted, washed and they were oven dried for 48 hours at 40^{0}C. They were turned repeatedly to avoid the microbial growth. The samples were 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, Thapathali, Kathmandu. It was conducted according to standard methods described by Ciulei (1982). 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 40ºC.

1. The etheric extractive solution

The Petroleum extract was dried completely to water bath and dried extract dissolved in diethyl ether and then the solution was ready for test.

Test for volatile oil

The solution (2 ml) was placed in a petridish and allowed to evaporate to dryness. The dried residue gave the pleasant smell or aromatic smell indicated the presence of volatile oil (Ciulei 1982).

Test for alkaloids

The concentrated extractive solution (6 ml) was dissolved in 1.5 ml of 2N HCl and it was divided in to three test tube of 0.5 ml each. The 1st tubes were containing the standard solution. 2-3 drops of Mayer's reagent are added to the 2nd tube, and 2-3 drops of Bertrand's reagent are added to 3rd tube, if a white-yellowish (in 2nd tube) or white (3rd tube) precipitate, it indicated the presence of alkaloids (Ciulei 1982).

Test for Steroid and Triterpenes

The ethereal extractive solution (10-15 ml) was extracted 3 times each with 1.5 ml of 10% KOH in a separation funnel by gently shaking. Two solutions (a) aqueous alkaline and (b) an etheric one were obtained.

The 2nd half from the extractive solution was concentrated till it gave a residue which was dissolved in 0.5 ml of acetic anhydride and 0.5 ml of chloroform. The solutions were transformed in the dry test tube and added 1-2 ml of conc. H_2SO_4 (Liebermann-Brofad's reagent). At the contact zone two layers were formed the superior layer becomes green of steroid and lower layer of violet ring were formed denoted the presence triterpenes respectively (Ciulei 1982).

Test for Carotenoid

Half volume of the etheric solution was concentrated to give a residue; 2-3 drops of a saturated solution of antimony trichloride in chloroform (Carr Price's reaction) was added. The pigment was firstly blue colour and gradually became red later. A conc. H_2SO_4 was given in the carotenoid and resulted a deep blue or green-blue coloration (Ciulei 1982).

Test for Fatty acids

The etheric solutions (2 ml) were concentrated up to 0.5 ml and were drops on a filter paper. The spot persists, after evaporation indicates the presence of fatty acids (Ciulei 1982).

Test for Emodins

The ethereal solution (2 ml) was added with 1 ml of 25 % NH_4OH (Frontage's reaction). After shaking, the solution decolorized and becomes red (Ciulei 1982).

Test for Flavonoid

The ethereal solutions (2 ml) were concentrated till a residue was obtained. The residue was dissolved in 1-2 ml of 50 % methanol by warming up metal magnesium and 2-5 drops of conc. HCl (Shibata's reaction) was added. A red or orange colour was persistent (Ciulei 1982).

Test for Coumarin

The ethereal solutions (2 ml) were concentrated till a residue was obtained, then dissolved it in hot water. After cooling, the solutions were divided in two tubes: one tube containing with

the standard and the aqueous solution of the another tube which was made alkaline with 0.5 ml of 10 % NH_4OH. The occurrence of intense fluorescence under UV light indicates the presence of coumarin (Ciulei 1982).

2. The alcoholic extractive solution

The rest of the dry samples after having been extracted with petroleum ether were extracted, by refluxing, in a flask, two or three times with alcohol. The filtrated solutions was mixed and concentrated up to 15-20 ml. The extracted constituents were identified by means of specific tests.

The ethanolic extract (15 ml) in flask and equal volume 10% HCl was added by refluxing and heated. After cooling, the solution was extracted 3 times each in a separating funnel, with 6-8 ml of ether. The etheric extractive solutions was placed together (16-20 ml) and dehydrated with dry Na_2SO_4 thus resulting an etheric and an aqueous solutions.

The etheric solutions were served to identify the anthracene glycoside whereas acidic aqueous solutions were used to identify the anthocyanadine glycoside.

Test for Anthracene glycoside

The ethereal solution (4 ml) was semi dried in a tube then 2 ml of 25 % NH_4OH was added (Borntrager reaction) by shaking. The solution (oxidized form) becomes cherry red in colour (Ciulei 1982).

Test for Anthocyanadine glycoside

The acidic solution (15 ml) added with equal volume of dist. water, the solution was extracted 3 times each in a separating funnel, with 5 ml of 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 (Ciulei 1982).

3. The aqueous extractive solution

The water soluble constituents of samples extracted with water were identified by means of specific tests.

Test for Tannins

The sample (0.5 gm.) was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1 % $FeCl_3$ solution were added. The blue black precipitate were observed, indicate the presence of tannins (Ciulei 1982).

Test for saponins

The sample (2.5 gm.) was extracted with 10 ml boiling water, then extract was shaken vigorously to froth (Frothing test) (Ciulei 1982).

Test for Glycosides

Semi dried aqueous extract (2 ml) was added with equal volume of Fehling's solution (I & II) in the ratio (1:1) and heated in water bath, it gave brick red in colour (Ciulei 1982).

Test for polyurenoids

The aqueous extract solution (2 ml) was poured in 10 ml acetone. A thick volume was formed, and then 4-5 drops of Hematoxylin was added. The precipitation was separated out by centrifugation and that was washed away with alcohol. The occurrence of a violet precipitate in these conditions denotes the presence of polyurenoids (Ciulei 1982).

Test for polyoses

The aqueous extract solution (1 ml) was concentrated with an addition of 2-3 drops of conc. H_2SO_4, then added 3-4 drops of an alcoholic solution saturated with thymol (Molisch's reagent). A red colour appears, indicates the presence of polyoses (Ciulei 1982).

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 terai, siwaliks and mahabharat range, using same test, by Pearson (1990), chi square test. Difference were considered to be significant at $p<0.05$.

RESULTS AND DISCUSSION

In the present investigation, three samples were analyzed from the tropical, sub tropical and temperate regions of Nepal (table 3), and sixteen major chemical constituents found in the extracts of *Termitomyces microcarpus* during analysis. Frequency of high concentration on north-south gradients of alkaloids, steroid, triterpenoids, carotenoid, fatty acid, emodine, saponins, and glycoside were highest in Terai and gradually decreased in Siwaliks to Mahabharat range. Whereas, volatile oil, flavonoid, coumarin, anthracene, and anthocyanadine were almost absent (except moderate concentration of volatile oil in Terai, highest concentration of flavonoid and anthocyanadine in Siwaliks and low concentration of coumarin and anthracene in mahabharat). Herein we found, tannins and polyurenoid, their frequency concentration was highest in mahabharat and again, higher concentration in Terai and low in Siwaliks. Whereas, polyoses high concentration in mahabharat and gradually decreased in Siwaliks to Terai.

Similarly frequency of high concentration on east-west gradients of steroid, triterpenoids, fatty acids, tannins and glycoside were highest in west and gradually decreased in center to east. Whereas, in carotenoid the results showed that concentration were found highest in east and gradually decrease center to west. The alkaloid was found ample concentration in the entire region. The emodine and polyoses were found in moderate concentration in the entire region (except zero concentration of emodine in western Nepal). The volatile oil, flavonoid, anthracene and saponins were completely absent (except moderate concentration of alkaloid, flavonoid, anthracene and saponins in eastern Nepal). The anthocyanadine (except highest concentration in center) and coumarin and polyurenoid were also completely absent (except low concentration of coumarin and highest concentration of polyurenoid in western Nepal).

Likewise, there was a significant difference in volatile oil, triterpenoids, carotenoid, fatty acid, emodine, flavonoid, coumarin, anthocyanadine, anthracene, saponins, glycoside, polyuren, polyoses, and marginal significance in tannins, but no significant difference in alkaloid and steroid contains among the tested sample of three different eco-zones of east-west gradients

of tropical to temperate region of Nepal was found (table 4). Similarly there was also significant difference in all the chemicals contain among the tested sample of three different eco-zones of north-south gradients of tropical to temperate region of Nepal (table 5).

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 (Gurib-Fakim 2006, Muetzel and Becker 2006). *Termitomyces* species has ability to suppress postprandial hyperglycemia caused by prolonged high blood glucose level associated with diabetes (Moordian and Thurman 1999, Matsuura *et al.* 2002). Phytochemicals such as alkaloid, steroid, triterpenoids, flavonoid, anthracene, saponins, tannins and glycosides are also found in *Termitomyces reticulatus* (Loganathan *et al.* 2010). Similarly, fatty acids in *Termitomyces clypeatus* (Baraza *et al.* 2007) and *Termitomyces 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 (Arasmus 1995).

Mushrooms generally contain low fat and oil (Oso 1977, Okwulehie and Odunze 2004). 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 (Wasser 2002, Lindequist *et al.* 2005). Hence, they are rich sources of natural antibiotics, these wild macrofungi, possess inhibitory potential against bacteria associated with wastewater and leftover foods and also a potential source of useful drugs A 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 *Termitomyces microcarpus* popular to consume as food sources but may also be valuable in drug development.

Hence, it is necessary to identify the biological and pharmacological potential of mushrooms especially of wild edible mushrooms, which are collected indigenously. It is also necessary to do research in identifying and isolating different species of mushrooms having nutraceutical and medicinal properties to commercialize. The production at large scale would create a lot of employment opportunities especially in economically deprived rural communities.

Based on the results obtained, it can be concluded that *Termitomyces microcarpus* have high concentration of diverse phytochemicals and are of potential medicinal value. The was co-relation was found between the traditional application of mushrooms and possession of secondary metabolites. The results of this study 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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Table 1. Analysis of phytochemicals of *Termitomyces microcarpus* collected from different eco-zones of Nepal.

SN	Place	Region	Alt (m)	FT	V	A	S	T	C	F	E	Fl	Co	An	Ant	Sa	Ta	G	P	Po
1.	Attariya	WT	197	TESF	0	1	3	3	1	3	0	0	0	0	0	1	2	3	3	1
2.	Jhalari	WT	330	Ag.Fld	0	1	3	3	1	3	0	0	0	0	0	1	2	3	3	1
3.	B.N. Park	WT	140	TESF	0	1	3	3	1	3	0	0	0	0	0	1	2	3	3	1
4.	Neoulapur	WT	125	PL	0	1	3	3	1	3	0	0	0	0	0	1	2	3	3	1
5.	Karhiya	CT	223	TDRF	0	3	1	3	3	0	2	1	0	0	0	3	2	3	2	2
6.	Chiliya	CT	110	CMF	0	3	1	3	3	0	2	1	0	0	0	3	2	3	2	2
7.	Dharapani	CT	145	Ag.Fld	0	3	1	3	3	0	2	1	0	0	0	3	2	3	2	2
8.	Damak	ET	152	TDRF	2	3	0	3	3	2	0	1	0	1	2	3	1	3	0	0
9.	Daulatpur	ET	141	Ag.Fld	2	3	0	3	3	2	0	1	0	1	2	3	1	3	0	0
10.	Pathari	ET	205	TSF	2	3	0	3	3	2	0	1	0	1	2	3	1	3	0	0
11.	Shreepur	WS	560	TESF	0	3	0	3	3	0	0	1	0	0	0	2	1	3	3	2
12.	Kachanapur	WS	495	TESF	0	3	0	3	3	0	0	1	0	0	0	2	1	3	3	2
13.	Amaliya	WS	510	TESF	0	3	0	3	3	0	0	1	0	0	0	2	1	3	3	2
14.	Bageshwary	CS	475	TDRF	0	2	3	3	3	1	1	0	0	0	3	3	0	3	1	0
15.	Dohothe	CS	500	STDHF	0	2	3	3	3	1	1	0	0	0	3	3	0	3	1	0
16.	Patharkot	CS	500	TESF	0	2	3	3	3	1	1	0	0	0	3	3	0	3	1	0
17.	Jaljale	ES	500	TDRF	0	1	1	3	3	3	2	2	0	0	0	3	2	3	1	2
18.	Jaljale-Rajdevi	ES	565	TDRF	0	1	1	3	3	3	2	2	0	0	0	3	2	3	1	2
19.	Gaighat	ES	675	TDRF	0	1	1	3	3	3	2	2	0	0	0	3	2	3	1	2

20.	Amargadhi	WM	1828	LTMBF	0	1	1	1	1	0	0	1	1	0	0	1	2	0	2	2
21.	Amargadhi	WM	1828	LTMBF	0	1	1	1	1	0	0	1	1	0	0	1	2	0	2	2
22.	Surkhet	WM	1314	LTMBF	0	1	1	1	1	0	0	1	1	0	0	1	2	0	2	2
23.	Dawwanne	CM	705	TEF	0	1	1	3	3	1	1	1	0	1	0	0	2	3	1	2
24.	Dawwanne	CM	735	TEF	0	1	1	3	3	1	1	1	0	1	0	0	2	3	1	2
25.	Dawwanne	CM	740	TEF	0	1	1	3	3	1	1	1	0	1	0	0	2	3	1	2
26.	Peepalbot	EM	1290	S-C-F	0	1	1	2	2	1	1	1	0	2	0	0	2	2	1	2
27.	Maipokhari	EM	2224	LTMBF	0	1	1	2	2	1	1	1	0	2	0	0	2	2	1	2
28.	Harkate	EM	1077	LTMBF	0	1	1	2	2	1	1	1	0	2	0	0	2	2	1	2

Note: 1 indicate, presence of chemicals in trace amount; 2 for moderate amount; 3 for high amount and 0 for absence.

WT=Western Terai; CT=Central Terai; ET=Eastern Terai; WS=Western Siwaliks; CS=Central Siwaliks; ES=Eastern Siwaliks; WM=Western Mahabharata; CM=Central Mahabharata.

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.

Reg= Region, FT= Forest Type, B. N. = Bardiya National Park. , Ag. Fld. = Agricultural Field. TESF= Tropical evergreen sal forest, PL= Pasture land, TDRF=Tropical Deciduous riverine forest, CMF= Community Managed Forest, TSF= Tropical Sal forest, STDHF= Subtropical deciduous hill forest, LTMBF = Lower Temperate mixed broadleaved forest, TEF= Tropical evergreen forest, S-C-F= Schima-Castanopsis Forest.

Table 2. Traditional uses of *Termitomyces microcarpus* for the treatment of different types of diseases and ailments in the study areas.

SN	Pathological condition	Application for the remedy of diseases and ailments
1.	Measles	Pouring water in dry mushroom in pot overnight and its filtrate used for drinking purpose.
2.	Yellow fever	Pouring water in dry mushroom in pot overnight and its filtrate used for drinking purpose.
3.	Jaundice	Pouring water in dry mushroom in pot overnight and its filtrate used for drinking purpose.
4.	Inappetence / Abdominal disorder	Used as soup.

5.	Diarrhoea	Pouring water in dry mushroom in pot overnight and its filtrate used for drinking purpose.
6.	Constipation	Dry mushroom is used as vegetable; for this they are kept in air tight Box in unseasoned (in Kumhal-Khuna community in kusum, Banke).
7.	Muscular pain	Used as soup.
8.	Skin diseases	Used as Pest
9.	Cut wound	To heal cut and wounds, using pest.
10.	Delivery pain	Soup, curry, used as tonic for stimulating power. Khuna, Kumhal and Santhal communities.
11.	Indigestion/Stomachache	Dried powder and Black salt (Birenun) is taken with hot water.
12.	Laziness/indolence/inactiveness	To make body energetics, dry stick mushrooms are used a vegetable
13.	Stiffness of Joints	To relief from the arthritics pain, frequently used dry stick mushrooms.
14.	Buccal cavity infection	Its powder is used as tooth powder with mixing mustard oil and common salt.

Table 3. Local names of *Termitomyces microcarpus* in different regions of Nepal.

SN	Local name	Mycophagous groups	Regions
1.	Nakaphunani	Awadhi	WT
2.	Masino Bagale	Praja	WS
3.	Sanu Sangraeino	Doteli	WM
4.	Rai	Tharu	CT
5.	Jhari	Kumhal	CT
6.	Jhari	Magar	CS
7.	Chichimira	Khash	CM
8.	Kirkounle	Khash	ET
9.	Sano Dhamere	Dhimal	ES
10.	Kanike Kalunge	Rai	EM

Table 4. Results of P and X^2 on the variation of phytochemicals along with phytogeographic north-south gradients (Terai, Siwaliks and Mahabharat Region) with its frequency of each chemical at different eco-zones.

North-south gradient (Terai, Siwalik and Mahabharat)

Volatile oil	Alkaloid	Carotenoid	Steroid	Triterpenoid	Fatty Acid	Emodine	Flavonoid	Coumarin
0.049 (6.048)	0.003 (15.944)	0.012 (12.889)	0.02 (11.613)	0.003 (16.121)	0.008 (15.378)	0.024 (11.284)	0.012 (12.889)	0.029 (7.093)

Anthocyanadine	Anthracene	Tannins	Saponins	Glycoside	Polyurenoid	Polyoses	N
0.016 (12.158)	0.014 (12.515	0.016 (12.158)	0.001 (28.133)	0.003 (16.121)	0.005 (18.333)	0.005 (18.333)	28

Table 5. Results of P and X^2 on the variation of phytochemicals along with phytogeographic east-west gradients with its frequency of each chemical at different eco-zones.

East-west gradient (eastern, central and western regions)

Volatile oil	Alkaloid	Carotenoid	Steroid	Triterpenoid	Fatty Acid	Emodine	Flavonoid	Coumarin
0.029 (7.093)	0.104 (7.681)	0.001 (22.556)	0.096 (7.880)	0.014 (12.515)	0.001 (21.6)	0.001 (20.581)	0.044 (9.778)	0.049 (6.048)

Anthocyanadine	Anthracene	Tannins	Saponins	Glycoside	Polyurenoid	Polyoses	N
0.012 (12.772)	0.009 (13.576)	0.056 (9.211)	0.001 (28)	0.014 (12.515)	0.001 (28.467)	0.032 (10.578)	28

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