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FATTY ACIDS AND VITAMIN COMPOSITION OF INDIGENOUS ISOLATES OF *TERMITOMYCETES MICROCARPUS* AND *T. HEIMII*.

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Abstract:

Nutritional value of foods plays an important role in human health. Many species of indigenous edible mushrooms in Kanyakumari district which form part of the traditional food in the tribal peoples. The indigenous Termitomycetes species was collected from Keeriparai forest area in Western Ghats of Kanyakumari district, to evaluate the vitamin and fatty acid compositions. The people have to provide a balance diet containing essential food compounds. The analysis of the extracted lipids revealed that the major unsaturated fatty acids was linoleic acid, while the predominant saturated fatty acids was palmitic acid in both T. microcarpus and T. heimii. B group vitamins were the most abundant vitamins in both samples analyzed. While vitamin D, vitamin E were the least with value of 0.3 and 0.14 mg/g and 0.08 and 0.13mg/g, respectively. Therefore, this valuable product that is often considered as an agricultural waste might be employed as a rich source of nutrients in food industries.

***Keywords**: Indigenous, Termitomycetes, vitamin, fatty acids, Western Ghats.*

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INTRODUCTION

Mushrooms are known for their characteristic biting texture and pleasant aromas. Edible mushrooms are valued for their unique taste, aroma, texture and nutritive value. Of particular significance among constituents of mushroom tissue are nitrogen compounds, especially protein, free amino acids, amines, nucleic acids, urea and chitin. Both wild and cultivated mushrooms have been consumed by humans for their nutritional and medicinal values and they have unique aroma, taste and texture [1,2]. They have been used as food and flavoring material in soups for centuries due to their unique and subtle flavour [3]. Mushrooms are now marketed along major highways and urban centers. They are also relatively much cheaper than beef, pork and chicken that contain similar nutrients. Due to their high content of vitamin, protein, mineral and low fat they are consider as 'poor man's protein'.

In recent years, it has been prove and documented in the world literature that mushrooms provide nutrition, medicine and health benefits for humans [4,5]. Wild mushrooms of Kanyakumari district usually utilize several species of mushrooms such as *Termitomycetes*, *Pleurotus*, *Auricularia*, *Lentinus*, *Volvariella* and *Agaricus* are meat substitute for the rural population [6,5,7]. Mushrooms are fungi fruit-bodies which spontaneously appear in forests and farm lands in great quantities after rain. There are edible and poisonous mushrooms and both categories possess nutritional and medicinal values.

Mushrooms includes 0.5-3.5% of fats mostly glycerides and glycolipids, less frequently phospholipids, often in the form of oil droplets within spare or in the tissue [8]. Lipids play an important role in human metabolism acting like hormones or their precursors, aiding their digestion process and providing a source of metabolic energy. Yilmaz et al., [9] reported that the fat fraction in the mushrooms is mainly composed of unsaturated fatty acids. Mushrooms are one of the best source of vitamins especially vitamin B. Mushrooms also contain vitamin C in small amounts and which are poor in vitamins A, D and E [10,11]. They are also rich in vitamin B, vitamin D and vitamin K. Mushrooms have become attractive as functional food and as a source of physiologically beneficial medicines, having therapeutic potentiality for the treatment of cancer, heart ailments, diabetes, inflammation, hepatic damage and high blood pressure [12, 13, 14]. Based on the edibility of mushrooms, two wild edible Termitomycetes species collected from the Western Ghats of Kanyakumari district to study their fatty acids and vitamin profiles.

MATERIALS AND METHODS

Mushroom Collection

The wild edible mushrooms *T. microcarpus* and *T. heimii* were collected from the Keeriparai forest area of Western Ghats of Kanyakumari district, Tamil Nadu, India. It was collected during the rainy seasons, air dried and transported to the lab for analysis. The culture was maintained on a Potato dextrose agar medium and subculture every one month and the slants were incubated at 27ºC for seven days and then stored at 4ºC.

Estimation of Fatty Acids by Gas Chromatography

About 0.45 g of the substance to be examined was introduced into a 10 mL volumetric flask, dissolved in hexane R containing 50 mg of butyl hydroxyl toluene R per liter and diluted to 10 mL with the same solvent. Two mL of the solution was transferred into a quartz tube and evaporated the solvent with a gentle current of nitrogen R. 1.5 mL of a 20 g/L solution of sodium hydroxide in methanol, was added and covered with nitrogen capped tightly with a poly tetra fluro-ethylene lined cap, mixed and heated in a water bath for 30 min. This was 30 seconds. Five mL of saturated sodium chloride solution was added immediately, which was covered with nitrogen and capped, vortexed for at least 15 seconds. The upper layer was allowed to settle and this was transfer the same to separate tube. The methanol layer was once again shaken with 1 mL of trimethylpentane and mixed with the trimethylpentane extracts. The combined extracts were washed with two quantities each of one mL of water and dried over anhydrous sodium sulphate. Prepared two solutions for each sample for the gas chromatography.

The chromatography involved an Ashmaco (GC) flame ionization detector, carrier gas helium or oxygen for ignition purpose. Column BPX – 70 (50% cyanopropyl 50% methylsiloxane). Injection port 250ºC detector port increased by 7.0ºC per minute and the final oven temperature was 240ºC.

Vitamin Analysis

The vitamins profiles was determined following the method described by British standards and AOAC officials methods of analysis as outlined below. Vitamin C (Method derived from BSEN 14130), vitamin A (Method derived from BSEN 12823), vitamin B2 (Method derived from BSEN 14152), vitamin Niacin (Method derived from BSEN 15607), vitamin B6 (Method derived from BSEN 14164), vitamin B (Method derived from BSEN 14131), while vitamin B5 and B12 were determined using AOAC official methods of Analysis [15].

Statistical Analysis
Experimental values are given as means ± standard deviation (SD). Statistical significance was determined by one-way variance analysis (ANOVA). Differences at $P < 0.05$ were considered to be significant.

RESULT AND DISCUSSION

Mushroom is the source of extraordinary power and virility and is used in the preparation of many essential dishes [16]. It is a high value crop for domestic and export market. Thousands of years ago, fructification of higher fungi has been used as a source of food due to their chemical composition which is attractive from the nutritional point of view [10]. Edible mushrooms also provide a nutritionally significant content of vitamins (B1, B2, B12, C, D and E) [17]. It could be a source of many different nutraceutical such as unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid and carotenoids [18,19]. It is a treasure for nutrition and can substantiate the sufferings from malnutrition to some extent. Edible mushrooms are regularly regarded as a curative food having anticarcinogenic, anticholesteromic, antimicrobial, antiviral properties and prophylactic properties with regard to hypertension and heart diseases [20].

Fatty Acid Profile in *T. Microcarpus* and *T. Heimii*
Mushrooms are low in total fat content and have a high proportion of polyunsaturated fatty acids (72-85%) relative to total fat content, mainly due to linoleic acid. The high content of linoleic acids is one of the reasons why mushrooms are considered a health food [21]. Yilmaz *et al* [9] reported that the fat fraction in the mushrooms is mainly composed of unsaturated fatty acids. Fatty acids are the basic building blocks of most lipids.

Table 1 presents the fatty acids composition of the oil extracted from the *T. heimii* and *T. microcarpus*. Gas chromatography analysis is indicates that linoleic acid is the predominant unsaturated fatty acid, while palmitic acid is the predominant saturated fatty acids present in both mushroom. Statistical results indicate significant differences in fatty acid composition of these two edible mushrooms ($P<0.05$). The amount of the linoleic acid of the *T. heimii* (2.054%) was significantly more than *T. microcarpus* (1.61%). Alpha linolenic acid was more in *T. microcarpus* (1.069%) compared to the *T. heimii* (0.98%). More amount of palmitic acid was present in the *T. microcarpus* (1.129%) when compared to the *T. heimii* (1.265%). In particular, the unsaturated fatty acids oleic and linolenic acids were also reported as main fatty acids in *Flammulina velupites* from Korea [22].
Oleic acid is a bioactive compound and strongly inhibits the activity of human telomerase in a cell free enzymatic assay with an IC_{50} value of 8.6μm [23]. Oleic acid is a monounsaturated fatty acid of the omega-9 family [24]. More amount of oleic acid is present in *T. heimii* (1.113%) when compared to the *T. microcarpus* (0.82%).The predominant fatty acids in the *T. heimii* and the *T. microcarpus* in decreasing order were linoleic, palmitic oleic acid, alpha linolenic acid respectively that are in agreement with the findings of Barros et al., [25]. More or less same amount of moroctic acids was present in both mushrooms. The fatty acid contents observed in *T. heimii* and *T. microcarpus* were similar to those in the other mushrooms such as *A. bisporus*, *Pleurotus ostreatus* and *Armillaria mella* [26].

Table 1: Fatty acid Profile in *T. Microcarpus* and *T. heimii*

Fatty acids	*T. microcarpus*	*T. heimii*
Palmitic acid	1.265±0.17	1.129±0.09
Stearic acid	0.69±0.22	0.92±0.056
Oleic acid	0.82±0.001	1.113±0.188
Linolenic acid	1.61±0.16	2.054±0.26
Alpha Linolenic acid	1.069±0.14	0.98±0.05
Moroctic acid	0.155±0.07	0.15±0.069
Margaric acid	0.54±0.09	0.209±0.006

Data are expressed as mean ±SD (n=4)

Table 2: Vitamin Profile of *T. Microcarpus* and *T. Heimii*

Vitamins	T. heimii (mg/g)	T. microcarpus (mg/g)
Vitamin A (Retinol)	0.26±1.15	0.18±0.9
Vitamin D (Calciferol)	0.3±0.17	0.14±0.07
Vitamin E (Tocopherol)	0.08±0.04	0.13±0.05
Vitamin K (Phytonadione)	0.002±0.002	0.002±0.003
Vitamin B1(Thiamine)	1.18±0.23	1.31±0.50
Vitamin B2 (Riboflavin)	0.31±0.39	0.056±0.029
Vitamin B3 (Niacinamide)	2.24±0.45	2.39±0.6
Vitamin B5 (Calcium pantothenate)	1.32±0.59	1.49±0.25
Vitamin B6 (Pyridoxin)	0.035±0.054	0.014±0.007
Vitamin B12 (Cyanocobalamide)	0.026±0.018	0.014±0.005
Vitamin C (Ascorbic acid)	0.56±0.02	0.47±0.02

Data are expressed as mean ±SD (n=4)

Vitamin Profile of *T. Microcarpus* and *T. Heimii*

Mushroom an important source of vitamins such as, thiamine, riboflavin, pyridoxine, cyanocobalamine, niacinamide, folic acid and calcium pantothenate. Vitamin content is an important factor in the overall nutritional value of food. Because of its antioxidant and therapeutic ascorbic acid (vitamin C) is a valuable food component [26].

The results of vitamins composition obtained from the studied mushroom are shown in table 2. Interestingly, the B group vitamins were recorded in significant quantities in both *T. microcarpus* and *T. heimii*. Vitamin B3 (Niacinamide) was the most abundant vitamin with the value of 2.24 and 2.39 mg/g while vitamin B1 (Thiamine) 1.18 and 1.31 mg/g and vitamin B12 (cyanocobalamide) were the least with values of 0.026 and 0.014 mg/g, respectively. Earlier report [10,27] also confirm the abundance of vitamin B in mushrooms particularly thiamine, riboflavin, pyridoxine, pantotene acid and folic acid. The concentration of vitamin B12 in mushrooms has been controversial. A recent study of vitamin B12 concentration in *Agaricus bisporus* reported higher concentration of vitamin B12 in the cap and stalk [28].

Vitamin E and vitamin D were present in more or less equal amounts in the both the species (Table 2). The highest level of vitamin D was produced in *Lentinus edodes*, whose spore-producing lamellae were exposed to the sun. Dried mushrooms also elicited vitamin D production subsequent to sunlight exposure. Vitamin D is also an important factor for immune function and has been identified as a major mitigating factor in many diseases, So the sun-light activated biosynthesis of vitamin D from ergosterols with in mushrooms has substantial implications for the mushroom industry.

The locally grown wild edible mushroom *T. heimii* and *T. microcarpus* included in the present study contained a relatively high amount of ascorbic acid (vitamin C) which varied from 0.056 to 0.047 mg/g dry weight (Table 2). In *Agaricus bisporus* vitamin C, folic acid, thiamin, riboflavin and niacin, the mean content were 6.75-3.97, 0.09-0.08, 0.085-0.09, 0.27-0.29 and 3.62-2.94 mg/kg, respectively [29]. *Pleurotus ostreatus* has been reported to certain higher amount of vitamin C followed by niacin and riboflavin [30].

The mushrooms investigated in this study found to be a good source of vitamins with low fat content. Hence, *T. heimii* and *T. microcarpus* is comparable to other edible mushrooms *A. bisporus*, *P. ostratus*, *L. edodes* cited in literature for its desirable composition and constitutes one of the functional, healthful foods. Besides high content of the unsaturated fatty acid, the oleic acid that has been implicated in the cardiovascular disease with a cholesterol-lowering ability makes it ideal for induction in the diet of the people with hypercholesterolemia.

CONCLUSION

This discussion suggests that the potentiality of mushroom cultivation could be a possible offer to alternate food and develop the life style of the people. They are good source of high quality fibers and low caloric food. From this analysis it can be concluded that these edible mushrooms *T. heimii* and *T. microcarpus* hold tremendous promise in addressing fatty acids and vitamins deficits prevalent in the diet of low income families in this district. Thus mushroom is an ideal food supplement, especially in our densely populated country. The findings in this research indicates that we might use these mushrooms as an inexpensive source to be used in dried form in some products such as instant soups and in producing fabricated snacks.

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