International Journal of Environment and Climate Change

Volume 13, Issue 10, Page 3164-3169, 2023; Article no.IJECC.103493
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Comparative Studies on Nutritional and Phytochemical Composition of Three Milky Mushroom Varieties

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI:10.9734/IJECC/2023/v13i102985

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/103493

Original Research Article

Received: 24/06/2023
Accepted: 28/08/2023
Published: 13/09/2023

ABSTRACT

Milky mushroom (*Calocybe indica* P&C) is a tropical potentially new species gaining popularity and demand by the mushroom growers especially in South India.As biochemical composition of milky mushrooms are influenced by the growing environmental conditions, research pertaining to these aspects is very meagre and also to compare the nutritional and biochemical composition of three milky mushroom species so that the best species can be selected by the growers, hence the present research work has been taken up to estimate the concentration of proteins, carbohydrates and phytochemical compounds like phenols, flavonoids, alkaloids and tannins in three varieties of milky mushrooms namely *Calocybe indica*, *Calocybe gambosa* and APK-2 in Mushroom Cultivation Scheme, Department of Plant Pathology, Hyderabad. The results revealed the presence of highest protein and carbohydrates content in *Calocybe indica* (15.9g, 2.7g) followed by APK-2 (11.2g and 2.1g) and *C. gambosa* (10.0g and 1.6g) and highest fats in APK-2 (2.0g), followed by *C.*

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gambosa(1.4g) and least in *C. indica* (1.3g) respectively. The secondary metabolites phytochemicals like phenols and tannins were highest content in *C. indica* (2.3 gm, 1.9mg) followed by *C*. *gambosa* (2.2gm, 1.6 mg) and next followed by APK-2 (2.1 gm, 1.5mg). Highest flavonoid content was recorded in *C. indica* and APK-2 of 1.5mg and least was recorded in *C. gambosa* of 1.4mg. Highest content of alkaloid was recorded in *C. indica* of 1.8mg, followed by APK-2 (1.6mg) and *C. gambosa* (1.5mg).

Keywords: Milky mushroom; phytochemicals; proteins; carbohydrates; fats, phenols; alkaloids; flavonoids; tannins; wavelength.

1. INTRODUCTION

"Among the cultivated mushrooms, the milky mushroom (*Calocybe indica* P&C) is a potentially new species to the world mushroom growers. It is a robust, fleshy, milky white, umbrella like mushroom, which resembles button mushroom. This mushroom was first reported from West Bengal, India" [1]. (Purkyastha, 1984-85, Pandey and Tewari, 1993). It is also known as "Dudh Chatta" because of its milky white appearance and large sized sporophores or as white summer mushroom because of its tropical nature. "It is more attractive with excellent shelf- life, grows on several agricultural wastes and on wide range of temperatures" (Krishna moorthy, 1995).

"Natural occurrence of milky mushroom in plains of Tamil Nadu and Rajasthan has also been reported" (Doshi *et. al,* 1989; Krishnamoorthy, 1995). These mushrooms grow every year between the months of May and August, which normally coincides with sufficient showers after a prolonged dry spell, *C. indica* is mainly a grassland species, saprophytic in nature and sometimes ectomycorrhiza.

Artificial cultivation started as early as 1976 in the state of West Bengal, now, this mushroom has gained popularity in the states of Karnataka, Tamil Nadu, Kerala and Andhra Pradesh. At present efforts are on to popularize milky mushroom in India like paddy mushroom and oyster mushroom. Krishnamoorthy 1997 rediscovered the fungus from Tamil Nadu, India and standardised the commercial production techniques for the first time in the world to popularize milky mushroom in India (Anurag, 2017).

"Among the new mushrooms, milky mushroom is fourth most largely growing mushroom gaining popularity recently in many parts of the country particularly in southern parts of India. Due to their high content of vitamin, protein and minerals, mushrooms are considered as "Poor man's proteins. Mushrooms have good nutritional value particularly as a source of protein that can enrich human diets, especially in some developing countries where animal protein may not be available and are expensive" (Pandey, 2004).

"C. indica is rich in protein, lipids, mineral, fiber, carbohydrates and is abundant with essential amino acids" [2]. Mirunalini *et al*, [3] and Babu and Rao [4] have reported "*in vitro* antioxidant activities *of C. indica* extracts. The results showed higher DPPH scavenging activity, reducing power, chelation, and hydrogen peroxide scavenging activity in *C. indica* compared to *Agaricus bisporus*. Interestingly, the stipe of *C. indica* exhibited more chelation, hydrogen peroxide scavenging activity, flavonoid and total phenolic contents as compared to its cap". "The phytochemical compounds such as alkaloids, saponin, tannin, phenol, anthraquinones, flavonoids, glycosides, steroids, terpenes, and chalcones are present in mushrooms. Qualitative phytochemical analysis confirmed that both the *P. florida* and *C. indica* possess the presence of active compounds like phenols, flavonoids, saponins, and tannins. The recorded total phenol 38.06±10 .09 in *P. florida* and 30.72±2.48 in *C. indica* and total flavonoid 1.35±0.34 in *P. florida* and 0.80 ±0.04 in *C. indica* were reported earlier" (Prabhu M. et al., 2014).

"Nutritive value of milky mushroom is comparable with other mushrooms, mature fruiting body of *Calocybe indica* contains higher proteins. It contains all the amino acids, out of which glycine is predominant. It also contain all mineral salts required by human body such as potassium, sodium and phosphorous. Due to alkaline ash and high fibre contain these mushrooms are suitable for people with hyperacidity and constipation. These mushrooms are rich in proteins (20-25%) and fibres (13-24% in dry samples) and contained a lower amount of lipid (4-5%). The carbohydrate contents ranged from 37-48% (on the basis of dry weight)".

(NahuAlam et al., 2018). "*Calocybe indica* comprise of proteins and glucans which showed immunostimulatory effects and also stimulates the activity of natural killer cells which kill cancerous cells directly" [5].

As there is great demand for its highly nutritive, fleshy, tasty, attractively designed and bright white coloured structure among the consumers there is need to compare the nutritional and biochemical composition of three commercially cultivated milky mushrooms to identify nutritionally and pharmaceutically important species, so that growers can opt best species as per their requirement hence the present investigation is taken up.

2. MATERIALS AND METHODS

2.1 Collection of Materials

The three milky mushroom varieties such as *Calocybe indica, Calocybe gambosa* and APK-2 were collected from IIHR, Bangalore, TNAU and college of Agriculture, Vellayani, Kerala.Mushrooms are oven dried at 40ºc for 4-5 days and ground it into a powder. The powdered mushroom was used for estimation.

2.2 Estimation of Proteins by Lowry's Methods

Working standard were prepared by diluting10 ml of stock solution. 0.2,0.4,0.6,0.8,1ml of the working standards were prepared by pipetting out the sample and make up the volume to 1 ml. 5 ml of reagent C is added to each tube by thoroughly mixing the solution. Later, 0.5 ml of reagent D is added to it by mixing well. Observations were recorded at the wave length 660 nm.

2.3 Estimation of Carbohydrates by Phenol Sulphuric Acid Methods

Mushrooms were oven dried at 40ºc for 4-5 days and ground it into a powder. Neutralize with sodium carbonate until effervescence ceases, make up the volume to 100 ml and centrifuge.

Standards 0, 0.2, 0.4, 0.6, 0.8, 1 ml were prepared by making volume upto 1 ml by adding distilled water. To these working standards reagents were added one after another and placed on water bath at 25-30ºc for 20 mins. Observations were recorded at wavelength 490 nm.

The phytochemicals present in three different milky mushroom varieties were estimated by following methods using the powdered sample.

2.4 Estimation of Phenols by Spectrophotometric methods (Brunner, 1984)

2.4.1 Extraction of sample

The total phenolics were determined by using Spectrophotometric method. 1 gm of sample was taken in 100 ml beaker and 15 ml of acidified methanol was added into beaker and kept for shaking for 30 mins. Decant into another beaker. To the residue 15 ml of acidified methanolwas added and repeat this thrice and supernatant wascollected into centrifuge tubes and then they were centrifuged @ 6000 rpm for 15 mins. Filter into 50 ml volumetric flask and make upto mark with acidified methanol. Now this extraction was used for estimation of phenols and flavonoids.

Working standards0.1, 0.2. 0.3, 0.4, 0.5were added with 0.5 ml of Folin ciocalteau reagent and then add Sulphuric acid and then incubated at 37 ºC for 1 hr and wavelength was measured at 760 nm.

2.4.2 Estimation of flavonoids by Aluminium chloride calorimetric method (Chang *et al,* 2002)

0.5, 1.0, 1.5, 2.0 working standardswere taken and make up the volume to 5 ml with distilled water and add sodium nitrite (0.3 ml), incubate for 6 minsat room temperature and then add 0.6 ml of Aluminium chloride and vertex for 10 mins, then add NaOH and wavelength was measure at 413 nm.

2.4.3 Estimation of alkaloids by Gravimetric method (Habrone, 1984)

Weigh 5 g of ground sample and to this add 50 ml of 10% acetic acid in ethanol, filtered and to this add concentrated NH_4OH drop-wise. The precipitate was filtered with filter paper and dried in oven at 60 ºc for 30 mins. The weight of alkaloids was determined using the formula:

$$\% \text{ Alkaloids} = W_2 - W_1 / W \times 100$$

Where

W = Weight of sample
W_1= Weight of empty filter
W_2= Weight of paper plus precipitate

2.5 Estimation of Tannins

Weigh 0.5 gm of powdered mushroom and transferred to 250 ml conical flask. To this add 75 ml of water and heat it for 30 mins. Centrifuge at 2000 rpm for 20 mins and collect the supernatant in 100 ml volumetric flask and make up the volume. T0 1ml of the mushroom extract add 5 ml of Folin Denis reagent ,10 ml of of sodium carbonate solution and dilute to 100 ml with water. Shake well and the wavelength at 700 nm was recorded after 30 min.

The replicated data recorded from the biochemical compounds analysis was statistically analysed using CRD as per the procedures suggested by Gomez and Gomez [6].

3. RESULTS AND DISCUSSION

Different nutritional and phytochemical parameters were measured for three different varieties of milky mushrooms which were collected from different regions of India with varied level of concentration which are given in (Table 1).Three Milky mushroom varieties were oven dried at 40 c for 3 days, powdered and estimation of biochemical compounds like proteins, carbohydrates, fats and phytochemicals such as phenols, flavonoids, alkaloids and tannins was done.

In the present investigation there is significant difference among three milky mushrooms varieties highest protein and carbohydrate content was observed in *Calocybe indica* (15.9g, 2.7g) followed by APK-2 (11.2g and 2.1g) and *C. gambosa* (10.0g and 1.6g) and highest fats in APK-2 (2.0g), followed by *C. gambosa*(1.4g) and least in *C. indica* (1.3g) respectively.

The results of the quantitative analysis of secondary metabolites of the three edible milky mushroom varieties like *Calocybe indica, Calocybe gambos*a and APK-2 revealed the presence of phenols, flavonoid, alkaloids and tannin in varying concentrations and are statistically significant. Highest phenol content was recorded in *C. indica* of 2.3 gm, closely followed by *C. gambosa* (2.2gm) and APK-2 (2.1gm). Highest flavonoid content was recorded in *C indica* and APK-2 of 1.5 mg and least was recorded in *C. gambosa* of 1.4mg. Highest content of alkaloid was recorded in *C indica* of 1.8mg, followed by APK-2 (1.6mg) and *C. gambosa* (1.5mg). Highest tannin content was recorded in *C. indica* of 1.9mg, followed by *C. gambosa* (1.6 mg) and APK-2 (1.5mg).

The results were on par with the findings of Nuhu Alam et al., (2018) who reported that the protein, lipid, fiber and carbohydrate contents in 100 gm of dried *P. sajor- caju* were 23-26g, 4.2-4.6g, 22-23 g, and 37-41.5 g respectively. Similar findings of Udu- Ibiam *et al,* [7] who reported that out of all the phytochemicals, henol content was highest in edible mushrooms.

Ajiboye and his co- workers [8] studied the presence of secondary metabolites and the results revealed that among nine compounds recorded, saponin content was highest (0.563 ± 0.20%) and alkaloid was (0.234 ± 0.10%).

Table 1. The concentrations of biochemical compounds like proteins, carbohydrates and fats present in milky mushrooms i.e.,*Calocybe indica, Calocybe gambosa* and APK-2

Milky mushroom variety	Biochemical compounds						
	Proteins (g/100g)	Carbohydrates (g/100g)	Fats (g/100g)	Flavonoids mg/100g)	Alkaloids (mg/100g)	Tannins (mg/100g)	Phenols (g/100g)
Calocybe indica	15.857	2.697	1.306	1.563	1.803	1.935	2.292
C. gambosa	10.000	1.598	1.411	1.433	1.485	1.595	2.230
APK2	11.214	2.066	2.008	1.547	1.647	1.538	2.118
CD (5%)	0.990	0.150	0.018	0.049	0.026	0.018	0.033
SE(m)	0.331	0.050	0.006	0.017	0.009	0.006	0.011
CV	7.082	6.271	0.999	2.884	1.395	0.944	1.334

Sitati and his co-workers [9] studied qualitative phytochemical profile of the mushroom *Termitomyces striatus* and reported that *T. striatus* extracts are a promising source of novel antimicrobial and antifungal agents.

Meghna *et a*l [10] recorded the crude protein content of *Calocybe indica* (2.09 g/100 g) which is slightly lower than other mushrooms, the highest carbohydrate content both in the fresh (6.8 ± 0.5 g/100 g) and dried (48.5 ± 2.4 g/100 g) form in comparison with *Pleurotus ostreatus*, *Pleurotus sajor caju*, *Pleurotus florida.* They recorded Linoleic acid and elaidic acid as the most abundant fatty acids in *Calocybe indica*.

Mushroom extracts have a high concentration of phenolic compounds that are mainly composed of one or more aromatic rings including one or more hydroxyl groups and can serve as hydrogen donors or electron donors, and possess metal ion-chelating characteristics [11-13].

The results of the phytochemical analysis of varieties of milky mushrooms revealed the presence of phenols, alkaloids, flavonoids, tannins, proteins, carbohydrates, though in various concentrations. These phytochemicals play a vital role in the medicinal properties of many plants.

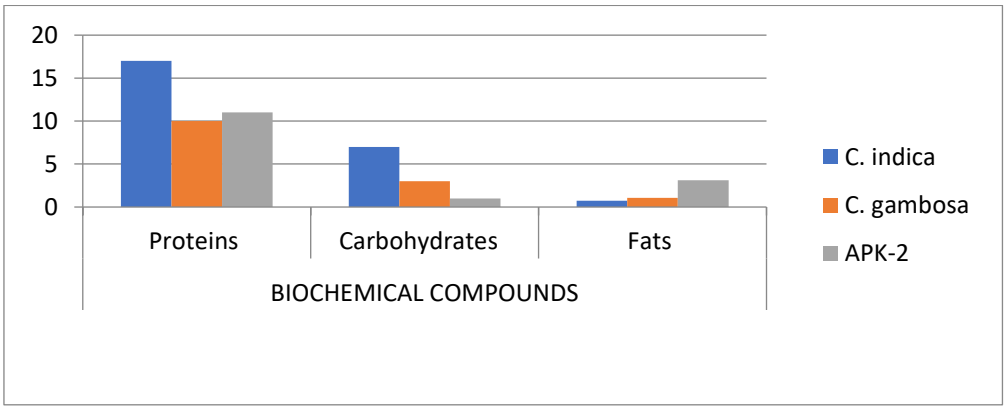

Fig. 1. The concentrations of biochemical compounds like proteins, carbohydrates and fats present in milky mushrooms i.e., *Calocybe indica, Calocybe gambosa* and APK-2

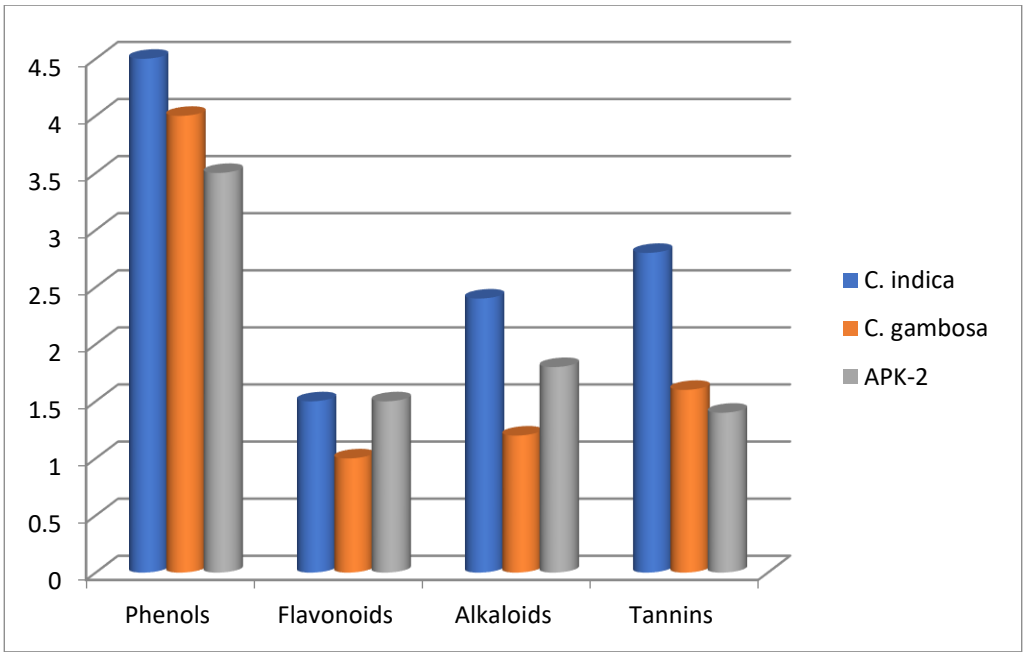

Fig. 2.The concentrations of phenols, alkaloids, flavonoids, tannins, present in milky mushrooms i.e., *Calocybe indica, Calocybe gambosa* and APK-2

4. CONCLUSION

This study has further elaborated the knowledge of medicinal and health benefits of mushrooms. The presence of the phytochemicals in the tested materials could possibly account for these benefits. According to this investigation, highest concentration of proteins, carbohydrates, phenols, flavonoids, alkaloids and tannins were recorded in *Calocybe indica* compared to others which may be due to genetical makeup and due to environmental conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/103493