Original Research Article

COMPARATIVE STUDIES ON NUTRITIONAL AND PHYTOCHEMICAL COMPOSITION OF THREE MILKY MUSHROOM VARIETIES

Abstract:

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 *C. indica* (17 gm, 7 gm) and followed by APK-2(11g and 1 g) and *C.gambosa (*10g and 3 g) repectively. The secondary metabolites phytochemicals like phenols and tannins were highest content in *C. indica* (4.5 gm,2.8 gm) followed by *C. gambosa* (4 gm, 1.6 gm) and next followed by APK-2 (3.5 gm, 1.4 gm). Flavonoids and alkaloids with highest content in *C. indica* (1.5 gm, 2.4 gm) followed by APK-2 (1.5gm, 1.8 gm) and next followed by *C. gambosa* (1gm, 1.2 gm).

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

Introduction:

Calocybe indica is a tropical edible mushroom of Indian origin and can be cultivated in higher temperatures and humidity areas (Purkayastha & Chandra, 1974). *C. indica* commonly known as the milky mushroom was commercialized as a new variety *C. indica* var. APK- 2 from the Tamil Nadu Agricultural University (TNAU), Coimbatore.

C. indica is rich in protein, lipids, mineral, fiber, carbohydrates and is abundant with essential amino acids (Alam *et al.*, 2008). The mushroom *Calocybe indica*, commonly known as milky mushroom is a tropical edible fungus with great demand for its healthy, highly nutritive, fleshy, tasty, attractively designed and bright white coloured structure.

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). (Nahu Alam *et al.*, 2018).

Materials and methods:

Collection of materials:

The three Milky Mushroom varieties such as *Calocybe indica*, *Calocybe gambosa* and APK-2 were collected from different areas.Mushrooms are oven dried at 40ºc for 4-5 days and ground it into a powder. The powdered mushroom was used for estimation.

Estimation of proteins by Lowry's method:

Working standard were prepared by diluting10 ml of stock solution .0.2,0.4,0.6,0.8,1ml of the working standard, pipette 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.

Estimation of carbohydrates by Phenol Sulphuric Acid method:

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.

Estimation of phenols by Spectrophotometric method (Brunner, 1984).

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 methanol was added and repeat this thrice and supernatant was collected 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 standards 0.1, 0.2. 0.3, 0.4, 0.5 were added with 0.5 ml of Folin ciocalteau reagent and then add Sulphuric acid and then incubated at 37 ^{o}C for 1 hr and wavelength was measured at 760 nm.

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

0.5, 1.0, 1.5, 2.0 working standards were taken and make up the volume to 5 ml with distilled water and add sodium nitrite (0.3 ml), incubate for 6 mins at 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.

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 ^{o}c for 30 mins. The weight of alkaloids was determined using the formula:

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

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

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.

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 highest protein and carbohydrate content was observed in *Calocybe indica* (17g, 7 g) followed by APK-2 (11g and 1 g) and *C. gambosa* (10g and 3 g) and highest fats in APK-2 (3.13 g), followed by *C. gambosa* (1.05g) and followed by *C. indica* (0.63 g) 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 proteins, carbohydrates, fats, phenols, flavonoid, alkaloids and tannin in varying concentrations. Highest phenol content was recorded in *C indica* of 4.5 gm, closely followed by *C. gambosa* (4 gm) and APK-2 (3.5 gm). Highest flavonoid content was recorded in *C indica* and APK-2 of 1.5 gm and least was recorded in *C. gambosa* of 1gm. Highest content of alkaloid was recorded in *C indica* of 2.4 gm, followed by APK-2 (1.8gm) and *C. gambosa* (1.2 gm). Highest tannin content was recorded in *C indica* of 2.8 gm, followed by *C gambosa* (1.6 gm) and APK-2 (1.4 gm).

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.,* 2014 , who reported that out of all the phytochemicals, phenol content was highest in edible mushrooms.

Ajiboye and his co- workers (2013) 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	**Carbohydrates**	**Fats**	**Phenols**	**Flavonoids**	**Alkaloids**	**Tannins**
C. indica	17	7	0.73	4.5	1.5	2.4	2.8
C. gambosa	10	3	1.05	4	1	1.2	1.6
APK-2	11	1	3.13	3.5	1.5	1.8	1.4

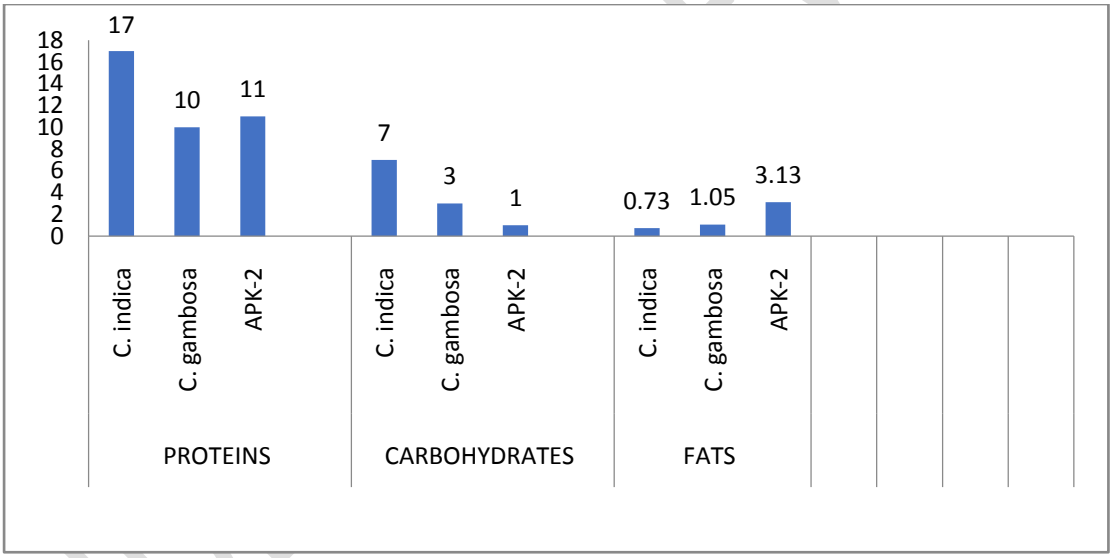

Fig 1: The concentrations of biochemical compounds like proteins, carbohydrates and fats present in milky mushrooms i.e., *Calocybe indica, Calocybe gambosa* and APK-2

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.

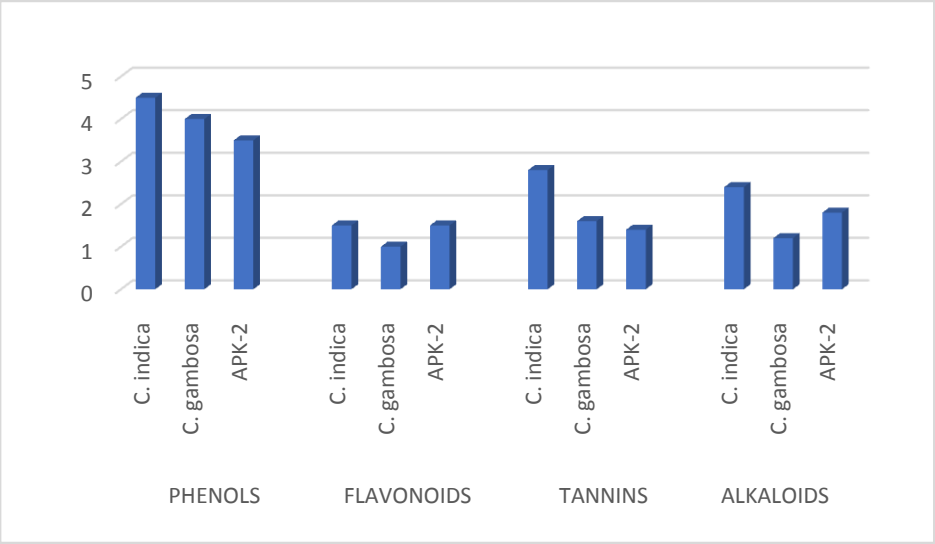

Fig 2: The concentrations of phenols, alkaloids, flavonoids, tannins, present in milky mushrooms i.e., *Calocybe indica, Calocybe gambosa* and APK-2

Conclusion:

This study has further elaborated that knowledge of medicinal and health benefits of mushrooms. The presence of the phytochemicals in the tested materials could possibly account for theses benefits. According to this investigation, highest concentration of proteins, carbohydrates, phenols, flavonoids, alkaloids and tannins were recorded in *Calocybe indica*

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