RESEARCH ARTICLE

QUALITATIVE PHYTOCHEMICAL SCREENING OF VARIOUS SOLVENT EXTRACTS OF CALOCYBE INDICA, MILKY MUSHROOM

N.K. Sankaranarayanan¹, S. Krishna Kumari¹ and S. Kathiravan²

1. Department of Biochemistry, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India.
2. Department of Biochemistry, Dr.N.G.P Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India.

Abstract

The present study investigates the presence of phytochemical compounds in the cold & hot aqueous extract, ethanol extract and methanol extract of Calocybe indica, milky mushroom. The mushroom spawn was prepared from the procured culture with the white sorghum grains as the substrate and well matured spawn were used for the production of mushrooms. The paddy straw was used as a substrate for the growth of the mushrooms. The matured spawn was inoculated in the processed substrate and maintained with the optimum conditions. The well grown mushrooms were harvested and shade dried. The dried mushrooms were powdered and cold & hot aqueous extract, ethanol extract and methanol extract was prepared and used for the qualitative phytochemical analysis. The phytochemicals such as carbohydrates, proteins, alkaloids, flavonoids, phenols, saponins etc. were screened for their presence and the results shown that the four different extracts of Calocybe indica, milky mushroom possess various phytochemicals at considerable proportion which can be said as a source of phytochemicals for various pharmaceutical applications.

Introduction:

Knowledges about the bioactive compositions and nutritional values of different mushrooms have been increased massively significant in the past several years, from which the mushrooms were defined as a potential functional food with the high content of dietary fiber and low level of fat. Moreover, the high quality of proteins including most of the essential amino acids were also found in mushrooms, as well as the numerous vitamins and mineral substances. Mushrooms are thought to exert many pharmacological functions such as antitumor, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolemic, antihypertensive, antiplatelet-aggregating, antihyperglycemic, antimicrobial and other activities.

The therapeutic and nutraceutical exploration of mushrooms gives the impression that they are the next generation food, not only in providing quality protein but also in curing deadly diseases like cancers, tumors and nervous disorders. There is a huge challenge to better exploit this wonderful gift of nature and bring it forward from the farm to fork level. Mushrooms from last many decades are not only being consumed as food but also utilized as major bioactive component in making of dietary supplements to improve the quality of human life. This reflects that...
the mushroom extracts are target specific and have been regularly tested in the clinical trials by the scientists and also by the pharmaceutical companies for developing functional foods. Because making such products from the fruit bodies, mycelia or its extracts have pharmacological benefits helping in the early intervention of sub-healthy states in humans which prevents the consequences of life threatening diseases. Edible mushrooms are cultured in media that usually consist of lignocellulosic agricultural wastes such as crop straw. The organic solid waste remaining after cultivation of edible mushrooms is the spent mushroom substrate (SMS). SMS can be used as compost, as animal feed, to promote health of animals, and to produce packaging and construction materials, biofuels, and enzymes. Hence, the mushroom cultivation is a value added process to convert these materials and represents one of the most efficient biological ways by which these residues can be recycled.

*C. indica* is a lignocellulolytic mushroom, which requires a temperature of 30–35°C and relative humidity of 80 to 90 % for good growth. Therefore, it is an ideal candidate for hot weather cultivation when no other mushroom excepting *Volvariella* species can grow. It has a robust sporophore, attractive colour, sustainable yield, delicious taste, unique texture and excellent shelf life as compared to oyster or button mushroom. In nature, milky white mushrooms are seen grown on humus rich soil in agricultural fields or along the roadside in tropical and subtropical parts of India, especially in the plains of Tamil Nadu (South Indian State) and in Rajasthan (located in the western edge of India). Milky white mushrooms are highly suitable for commercial production in humid tropical and subtropical regions of the world. The six major constituents of mushrooms are water, proteins, carbohydrates, dietary fiber, fat, and ash.

The present study was designed to analyse the presence of various metabolites in the aqueous extract of *C. indica* in a qualitative manner.

**Materials and Methods:**

**Mushroom culture:**
The culture of *C. indica* was procured from Vijaya Mushrooms, Coimbatore. The species was sub cultured and maintained in Potato dextrose agar medium at room temperature as slants and in petriplates.

**Mushroom spawn production:**
The mushroom spawn was prepared on white sorghum grain. The mature grain procured from local market was well cleaned and boiled in water for 30 min. The boiled grain was mixed with 2% calcium carbonate. 300g of calcium carbonate mixed grain was filled in polypropylene bags of size 11 inch x 5 inch and sterilized for 15 psi for one hour. The sterilized bags were cooled to room temperature and inoculated with the mushroom culture maintained in slants. The culture inoculated bags were kept undisturbed at room temperature for mycelium running. After mycelium running, matured spawn after 30 days were used for preparation of mushroom beds.

**Mushroom Cultivation Technology:**
The well matured paddy straws were cut into 3- 5 cm in length and soaked in water overnight. The soaked paddy straw was washed and sterilized for 45 min in steam and shade dried. The shade dried paddy straw was filled in polypropylene bags with the matured mushroom spawn in alternate layers. The filled bags were hanged in the mushroom cultivation chamber for spawn running and growth of mushrooms. The temperature of 25 - 30°C and humidity of 80% is maintained in the chamber. After the complete mycelium spreading, the beds were cut into the two equal halves and casing soil prepared was applied on the beds for thickness of 2.5 cm and the beds were placed in the underground chamber for the growth of mushrooms. The temperature in the underground chamber was maintained at a range of 30 - 35 °C and a humidity of 80 - 85 %. Water is sprayed on the beds at periodic intervals. The well grown mushrooms were harvested and processed for further analysis.

**Preparation of the extract:**
The shade dried mushrooms were grinded to a coarse fine powder and stored for further use. Various solvent extracts such as cold& hot aqueous extract, ethanol extract and methanol extract was prepared by following the standardized procedure and used for the qualitative analysis of the various metabolites present in each solvent extract.
Qualitative phytochemical analysis:
The cold&hot aqueous extracts, ethanol and methanol extracts were subjected to qualitative phytochemical analysis using the standardized procedures. The various metabolites analysed were alkaloids, flavonoids, saponins, tannins, phenols, anthraquinones, coumarins, quinone, thiols, terpenoids, triterpenoids, cardiac glycosides, anthocyanin, gum and mucilages, carbohydrates, proteins and steroids.

Results and Discussion:-
Preparation of mushroom spawn and Mushroom substrate beds:
The mushroom spawn was prepared from the culture of *Calocybeindica* and used for the making of mushroom substrate beds. The well matured spawn was used as inoculum for the preparation of the beds. The well grown mushrooms were harvested, dried and various solvent extracts were prepared and subjected to phytochemical analysis.

Qualitative phytochemical analysis:
The qualitative phytochemical analysis of the cold&hot aqueous extracts, ethanol and methanol extracts revealed the presence of various metabolites such as alkaloids, flavonoids, saponins, tannins, phenol, anthraquinone, carboxylic acids, coumarines, quinone, thiols, terpenoids, triterpenoids, cardiac glycosides, gums and mucilages, carbohydrates and proteins.

| S.No | Test                      | Cold aqueous | Hot aqueous | Ethanol | Methanol |
|------|---------------------------|--------------|-------------|---------|----------|
| 1    | Alkaloids                 |              |             |         |          |
|      | a) Hager’s test           | ++           | +++         | +++     | +++      |
|      | b) Wagner’s test          | +++          | +           | +       |          |
|      | c) Meyers test            | +++          | ++          | +       | +++      |
| 2    | Flavonoids                |              |             |         |          |
|      | a) Lead acetate test      | ++           | +           | ++      | ++       |
| 3    | Saponins                  |              |             |         |          |
|      | Foam test                 | ++           | +           | ++      | +        |
| 4    | Tannins                   |              |             |         |          |
|      | a) Gelatin test           | ++           | +           | -       | +        |
|      | b) Lead acetate test      | ++           | +           | +       | ++       |
| 5    | Phenols                   |              |             |         |          |
|      | a) Ferric chloride test   | ++           | +           | -       | -        |
|      | b) Lead acetate test      | ++           | +           | ++      | +        |
| 6    | Anthraquinones            |              |             |         |          |
|      | a) Born trager’s test     | ++           | +           | +       | -        |
|      | b) HCL test               | +            | +           |         | -        |
| 7    | Carboxylic acids          |              |             |         |          |
|      | Sodium bicarbonate test   | ++           | +           | -       | +        |
| 8    | Coumarins                 |              |             |         |          |
|      | Sodium hydroxide test     | ++           | +           | +       | +        |
| 9    | Quinones                  |              |             |         |          |
|      | Acid test                 | ++           | +           | +       | ++       |
| 10   | Thiols                    |              |             |         |          |
|      | Sodium nitroprusside test | ++           | ++          | +++     | ++       |
| 11   | Terpenoids                |              |             |         |          |
|      | Salkowski test            | +            | +           | +       | +        |
| 12   | Triterpenoids             |              |             |         |          |
|      | Liebermann-burchard test  | +            | -           | -       | -        |
| 13   | Cardiac glycosides        |              |             |         |          |
|      | a) Keller-kiliani test    | ++           | +           | +       | +        |
|      | b) Legals test            | ++           | +           | +       |          |
| 14   | Anthocyanins              |              |             |         |          |
|      | HCL test                  | -            | -           | -       | -        |
The results recorded in the table 1 showed that most of the phytochemicals screened qualitatively were found to be present in all the four different extracts made. Alkaloids, flavonoids, saponins, tannins, phenols, coumarins, quinones, thiols, terpenoids, cardiac glycosides, proteins and steroids were found to be present in all the four different extracts which showed the abundant presence of the phytochemical in the mushroom. Each test for the phytochemical revealed the less presence, moderate presence and more presence of the compounds which is depicted in the above table. On the whole, the mushroom was found to possess most of the important biochemical metabolites that have therapeutic and pharmaceutic applications. The presence of these phytochemicals can be attributed to the pharmacologic and therapeutic properties of the *Calocybeindica*.

Free radicals cause several disorders, including diabetes, and the agents that scavenge free radicals may have great potential in ameliorating these diseases. Phenolics have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic, and anti-inflammatory activities. Tannins have been documented to retain functional and severe haemostatic characteristics that facilitates the healing of wound and enhance irritated mucus membrane and also impede the development of bacteria. Saponins is very important in the treatment of cough and in the controlling of soreness of the upper respirational region. Furthermore, plant based saponins serve as heart tonic naturally and have been documented to prevent diabetics and hinder the growth of fungi. The importance of flavonoids lies in the fact that it controls and prevent tissue damage due to the presence of triggered, radical or singlet oxygen species. The coumarins are important because of their biotic characteristics. They possess physiologic functions, and inhibits bacterial and cancer growth. Coumarin together with some of its derivatives are known probable inhibitors of cell propagation in several carcinoma cell shapes. Controlled intake of cardiac glycosides, helps in arresting cardiac arrhythmia and give strength to a weak heart, thus helping the heart perform more efficiently.

### Conclusion:

The mushrooms were cultivated and analysed for the presence of various phytochemicals. The present study revealed the presence of various phytochemicals in the four different solvent extracts such as cold aqueous, hot aqueous, ethanol and methanol. Based on the results of the qualitative phytochemical analysis, it is inference that, *Calocybeindica* may harbour several phytochemicals that can be exploited for the various medical and disease treating process through pharmaceutical and therapeutic properties of the compounds.

### Acknowledgement:

Our sincere gratitude to Management of Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India for the financial support offered to carry out the research work.

### References:

1. Thatoi, H, Singdevsachan S.K., Diversity, nutritional composition and medicinal potential of Indian mushrooms: a review, African Journal of Biotechnology, 2014; 3, 523–545.
2. Lindequist, U. The merit of medicinal mushrooms from a pharmaceutical point of view. International Journal of Medicinal Mushrooms, 2013; 15, 517–523.
3. Paterson, R.R., Lima, N. Biomedical effects of mushrooms with emphasis on pure compounds. Biomedical Journal, 2014; 37, 357–368.
4. Himanshi Rathore, Shalinee Prasad, Satyawati Sharma. Mushroom nutraceuticals for improved nutrition and better human health: A review. Nutrition, 2017; 5, 35–46.
5. Prasad S, Rathore H, Sharma S, Yadav A.S., Medicinal mushrooms as a source of novel functional food, International Journal of Food Science, Nutrition and Dietetics, 2015; 04 (5), 221–225.
6. Huang, L., Sun, N., Ban, L., Wang, Y., Yang, H. Ability of different edible fungi to degrade crop straw. AMB Express, 2019; 9 (1), 0–5.
7. Gao, W., Liang, J., Pizzul, L., Feng, X.M., Zhang, K., Castillo, M., del, P. Evaluation of spent mushroom substrate as substitute of peat in Chinese beds. International Biodeterioration & Biodegradation, 2015; 98, 107–112.
8. Grimm, D., Wosten, H.A.B. Mushroom cultivation in the circular economy. Applied Microbiology and Biotechnology, 2007; 102 (18), 7795–7803.
9. Elenwo, E.N., Okere, S.E. Waste Re-cycling using edible mushroom cultivation. Journal of Applied Sciences and Environmental Management, 2007; 11 (3), 153–156.
10. Amin R, Khair A, Alam N, Lee T S. Effect of different substrates and casing materials on the growth and yield of *Calocybe indica*. Mycobiology, 2010; 38:
11. Purkayastha RP. Cultivation of *Calocybe indica* (P&C). Indian Journal of Mushrooms. 1984-1985:10–17.
12. Navathe S, Borkar PG, Kadam JJ. Cultivation of *Calocybe indica* (P & C) in Konkan region of Maharashtra, India. World Journal of Agricultural Research, 2014;2:187–191.
13. Reis FS, Barros L, Martins A, Ferreira IC. Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms an inter-species comparative study. Food and Chemical Toxicology, 2012;50:191–197.
14. Sivaprakasam, K., Kandasawmy, T.K. Waste material for the cultivation of *Pleurotussajor-caju*. Mushroom Journal, 1981; 101, 178–179.
15. Kathiravan Subramanian and Krishnakumari Shanmugasundaram. Optimization of casing process for enhanced bioefficiency of *Calocybe indica*, an indigenous tropical edible mushroom. International Journal of Recent Scientific Research, 2015; 6 (2), 2594–2598.
16. Paech, D and Tracey, M.V. In Modern methods of plant analysis, 1955; 6:373-374.
17. Wilson RL. Free radicals and tissue damage, mechanistic evidence from radiation studies. In: Biochemical Mechanisms of Liver Injury. Academic Press, New York, 1998; 123.
18. Saidu AN, Mann A, Onuegbu CD. Pytochemical screening and hypoglycemic effect of aqueous *Blighiasapidar* root bark extract on normoglycemic albino rats. British Journal of Pharmaceutical and Medical Research, 2012; 2: 89-97.
19. Sasikumar JM, Maheshu V, Aseervatham GSB, Darsini DTP. *In vitro* antioxidant activity of *Hedyotiscorymbosa* (L) Lam aerial plants. Indian Journal of Biochemistry and Biophysics, 2010; 47: 49-52.
20. El-Kamali HH, Elshikh AA. Preliminary phytochemical screening of plants species used in ethnoveterinary in Khartoum State, Sudan. Advances in Life Sciences. 2015;5:48-52.
21. Kamel JM. An extract of the mesocarps of fruits of *Balaniteaegyptiaca* exhibited a prominent anti-diabetic properties in mice. Chemistry Pharmacology Bulletin. 1991;39:1229-1233.
22. Rice ECA, Packer L. Flavonoids in health and disease, Marcel Dekkar, New York. 1998; 25.
23. Jain PK, Himanshu JH. Coumarin: Chemical and Pharmacological Profile. Journal of Applied Pharmaceutical Science. 2012;2:236-240.
24. Denwick PM. Natural products: A biosynthetic approach. 2nd Edition, Willey and Sons, Ltd. 2002;241-243.