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RESEARCH ARTICLE

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

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Abstract

The present study investigates the presence of phytochemical compounds in the cold & hot aqueous extract, ethanol extract and methanol extract of *Calocybeindica*, 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 *Calocybeindica*, milky mushroom possess various phytochemicals at considerable proportion which can be said as a source of phytochemicals for various pharmaceutical applications.

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Introduction:-

Knowledge 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 substances¹. 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^{2,3}.

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 disorders⁴. 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

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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 diseases⁵.

Edible mushrooms are cultured in media that usually consist of lignocellulosic agricultural wastes such as crop straw⁶. The organic solid waste remaining after cultivation of edible mushrooms is the spent mushroom substrate (SMS)⁷. SMS can be used as compost, as animal feed, to promote health of animals, and to produce packaging and construction materials, biofuels, and enzymes⁸. 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 recycled⁹.

Calocybeindica 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 mushroom¹⁰. 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)¹¹. Milky white mushrooms are highly suitable for commercial production in humid tropical and subtropical regions of the world¹². The six major constituents of mushrooms are water, proteins, carbohydrates, dietary fiber, fat, and ash¹³.

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

Materials and Methods:-

Mushroom culture:

The culture of *Calocybeindica* 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 petriplates¹⁴.

Mushroom spawn production¹⁵:

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 11inch 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 Technology¹⁵

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 analysis¹⁶:

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, anthroquinone, 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	Flavanoids				
	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	-	-	-	-

15	Gum and mucilages				
	Swelling test	+	+	-	-
16	Carbohydrates				
	a) Benedict's test	-	++	++	++
	b) Barford's test	-	-	-	-
	c) Fehling's test	-	++	+++	++
17	Proteins				
	a) Biuret test	+	++	+	++
	b) Ninhydrin test	+	+++	++	++
18	Steroids				
	Salkowski test	++	+	+	+

Table 1:- Qualitative phytochemical analysis of the various solvent extracts of *Calocybeindica*.

(+)-indicates the presence of the phytochemical; (-) – indicates the absence of the phytochemical

(+)- Less presence; (++) – moderate presence; (+++)- more presence

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 pharmaceutical 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 diseases¹⁷. Phenolics have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic, and antiinflammatory activities^{18,19}. 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 bacteria²⁰. Saponins is very important in the treatment of cough and in the controlling of soreness of the upper respiratory region. Furthermore, plant based saponins serve as heart tonic naturally and have been documented to prevent diabetics and hinder the growth of fungi²¹. 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 species²². 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 and are known probable inhibitors of cell propagation in several carcinoma cell shapes²³. Controlled intake of cardiac glycosides, helps in arresting cardiac arrhythmia and give strength to a weak heart, thus helping the heart perform more efficiently²⁴.

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.

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