Research Article

# Evaluation of Metal Concentration, FT-IR Studies and Antifungal Screening of *Calocybe indica* – A Fruiting Mushroom

**Sharmila.S*, Ramya.E.K, Mownika.S**

1,2,3PG and Research Department of Botany, Vellalar College for Women (Autonomous), Thindal, Erode - 638012, Tamil Nadu, India.

***Corresponding author's E-mail:** drsharmilas@yahoo.com

**Received:** 05-05-2019; **Revised:** 22-06-2019; **Accepted:** 01-07-2019.

**ABSTRACT**

This investigation was designed for the purpose of phytopharmacognostical, metal concentrations, FT-IR group identification, total phenolics and flavonoid content and antifungal studies of *Calocybe indica,* a healthy and essential medicinal mushroom. Concentrations of four heavy metals (Pb, Cd, Cr, Ni) and two minor elements (Zn, Cu) are determined. In the study of *Calocybe indica*, Zn and Cu concentrations are found in a high level. The phytochemical screening of the edible mushroom was powdered with various solvents showed the presence of proteins and amino acids, alkaloid, flavonoid, anthraquinone, quinone and coumarin. Total phenol content was calculated and expressed as gallic acid equivalent in the series of 4.4 ± 0.54 mg/g. The total flavonoid content was 3.5 ± 0.35. FT-IR analysis of the ethanolic extract given the major peak observed was at wave number 3444.87cm-1 that indicates the presence of C-O (alcohol), C-O (ester), C=C (aromatic), N-O (nitro compounds), N-H (amide), C=C (alkene), C=O (carbonyl), C=C (alkyne), O-H (acid), C-H (alkane) and N-H (amine) groups. The results showed that the antifungal response shown by the ethanol extract of C. indica exhibited variable degree of antifungal activity against the tested fungus *Aspergillus niger*.

**Keywords:** *Calocybe indica*, FT-IR, ethanol, antifungal activity.

## INTRODUCTION

Currently the global population is 7.7 billion and in future it is growing at a faster rate. By the year 2050 the overall population is predictable to attain 9 billion and it could be 20 billion in the year of 2100 [1]. Deficiency of food and retreating value of human vigor will be growing concern because of the population raise and urbanization, with a related decline in arable land. Lignocellulolytic agricultural and forest residues are converted into protein-rich mushrooms. It is one of the most economically feasible and sustainable biotechnology processes to deal with world food demand, particularly protein demand[2]. Mushroom production is the top biotechnology process for integrated agro-waste management in rural areas. As a fundamental part of secondary agriculture, mushroom cultivation helps to produce sustainable rural employment, and to addressing protein malnutrition. Indian mushroom industry is witnessing a tremendous alteration in recent years with respect to the types and strains cultivated[3]. Edible fungi are utilize by human nutritional needs and it has been a universal denominator in the history of mankind [4,5].

In the food and chemical background, one of the edible mushroom *Calocybe indica* has been selected for phytochemical, metal and anti-fungal studies. In the eastern Indian state of West Bengal, *Calocybe indica* was first identified and it can be cultivated on a wide variety of substrates. For several decades, West Bengal people (Eastern Indian state) have collected *Calocybe indica* mushrooms and sold in local markets. It is a milky white color and robust nature are appealing to consumers[6]. The milky white mushroom variety (*Calocybe indica* P&C var. APK2) was released from Tamil Nadu Agricultural University, Coimbatore, India during 1998. Over a decade, commercial production of this mushroom variety has assumed greater impetus in India, uplifting rural livelihood[7].

Heavy metal concentrations in mushrooms are significantly higher than agricultural crop plants, fruits and vegetables [8,9,10]. For example, radioactive heavy metals in fruit bodies of edible mushrooms were previously reported in the 1960s [11]. Several things may affect the accumulation and concentration of trace elements and heavy metals in mushrooms. Fourier transform infrared spectroscopy (FT-IR) is a powerful technique that can provide information on molecular structure. Both qualitative and quantitative activities can be obtained using FT-IR spectroscopy. A number of organic compounds and functional groups can be identified by their wave number of bands, and the absorption intensity can be used for the computation of their relative concentration.

In recent years, a commercial antimicrobial drug has developed by multiple drug resistance in human pathogenic microorganisms commonly used in the treatment of infectious diseases. This condition has required scientists to explore for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents [12]. Anti-microbial drugs have long been used for prophylactic and therapeutic purposes. Resistance of microorganisms to antibiotics has created an massive clinical problem in the treatment of transmittable diseases. The present cram has been initiated with a view

to establish pharmacopoeial studies and screen the antifungal activity of edible mushroom *Calocybe indica*.

## MATERIALS AND METHODS

### Collection and preparation of fruiting body

Fruiting bodies of edible milky mushrooms were collected commercially from the local market. The mushrooms were collected in their fruiting seasons from the natural habitat. The specimen was recognized with the help of standard literatures [13]. The collected plant specimens were pressed correctly following the method [14]. Dried specimens were sterilized with 0.1% HgCl2 dissolved in absolute alcohol and mounted with glue on standard herbarium sheet (42 x 28 cm). The herbaria were deposited in Department of Botany, Vellalar College for Woman, Thindal, Erode. Photographs were also taken to supplement the herbarium. The collected mushrooms were authenticated by the Department of Pathology, Tamil Nadu Agricultural University, Coimbatore. This project work was carried over in the Department of Botany, Vellalar College for Women during the year 2016-2017. Macroscopic characters of *Calocybe indica* were studied.

### Pharmacognostical studies

#### *Macroscopical analysis*

The morphological characters of the fruiting body such as colour, surface texture, taste and odour were examined[15,16].

#### *Shade drying and powdering of fruiting body*

Freshly collected edible fruiting bodies of mushrooms were cleaned to remove adhering dust and then dried in hot air oven at 370C for three days. Dried samples were packed into an air tight container to protect it from humidity. The plant materials were oven dried and it was mechanically ground to coarse powder and passed through a Willy Mill to get 60-Mesh size and used for physicochemical, phytochemical and fluorescence analysis. Samples were stored in the plastic containers which are maintained at room temperature until analysis[17].

#### *Soxhlet extraction*

The air-dried fruiting mushroom bodies of the samples (10 g) were extracted successively by using a Soxhlet extractor for 5 hrs with petroleum ether, ethanol and water with increasing order of polarity, finally filtered. Before extraction of the next solvent, every time the powdered material was dried in a hot air oven at 40°C to dryness and kept in the dark at ±4°C until tested. Finally, the material was macerated using hot water with stirring for 16 hrs and then water extract was filtered. The different solvent extracts were concentrated, vacuum dried and weighed. The percentage yields were expressed in terms of air dried sample. The macerated extracts were dried over anhydrous sodium sulfate and stored in sealed in refrigerator (5-8°C) until analysis[19]. Different dilutions of the extracts will be prepared in DMSO (Di Methyl Sulphoxide). The extracts were determined to physico-chemical, phytochemical and antifungal activity.

### Physico-chemical studies

The shade dried mushrooms are examined for this current investigation with standard protocols.

### Organoleptic characters of edible mushroom powder and the extract

The organoleptic evaluation of *Calocybe indica* mushroom powder and their extracts, the parameters such as texture, colour, odour and taste were established[15]

### Behaviour of edible mushroom powder with different chemical reagents

The behaviour of edible mushroom powder treated with different chemical reagents such as concentrated HCl, concentrated $H_2SO_4$, acetic acid, ethanol, methanol, acetone, petroleum ether, benzene, $HNO_3$ and aqueous solution was observed[18].

### Fluorescence analysis

Fluorescence analysis treated with different chemical reagents like Hager`s, Mayer's, Dragendroff's, Iodine solution, Acetone, 50% $HNO_3$, Sodium nitroprusside, $FeCl_3$ and Methanol was observed under day light and UV light at long (365 nm) and short (254 nm) [18].

### Determination of moisture content (Loss on drying)

Two gram of the mushroom powder was taken in a tarred weighing bottle and weighed perfectly. The weighed powder was dried at 105°C for 5 hrs and allowed to cool in a desiccators and re-weighed. The drying was continued at 150°C and weighed at 1 h interval. When the weight of the sample became constant, the loss in weight and the percentage of loss on drying were calculated [19].

### Analysis of heavy metals and micro elements

Heavy metal analysis in edible mushroom powder was determined by acid digestion and subsequent quantification by Inductively Coupled Plasma / Optical emission Spectrometer (ICP – OES). The acid digestion would help in removing the organic matter as well as in releasing the metals from particulates. Heavy metal and micro element analysis to estimate lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), zinc (Zn) and copper (Cu)[20].

### Qualitative phytochemical analysis

Qualitative phytochemical analysis, Phytochemical screening of different successive solvent extracts was carried out following the methods [21, 22]. Carbohydrate, protein and amino acids, alkaloids, tannins and phenolic compounds, flavonoids, triterpenoids, steroids, saponins, glycosides, anthraquinone, quinine, coumarin and fixed oil were qualitatively analyzed.

### Quantitative phytochemical studies

Determination of total phenol was followed by the method[23]. The total flavonoid contents were estimated [24].

### FT-IR analysis

Dried powder of plant material was used for FT-IR analysis. 10 g of the dried powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of plant specimen was loaded in FT-IR spectroscope **(**Perkin Elmer Spectrophotometer system) which was used to detect the characteristic peaks of their functional groups between 4000−400 cm −1.

### Assessment of antifungal activity

The antifungal activity of edible milky mushroom extracts (petroleum ether, ethanol and water) were evaluated against important fungal strains *Candida albicans* and *Aspergillus niger* by agar disc diffusion method [25]. Mushroom species have been shown to possess antagonistic effects against bacteria, fungi, viruses and other human pathogens.

### Growth and maintenance of test microorganisms for antifungal studies

Fungus strain viz., *Candida albicans* and *Aspergilus niger* were obtained as pure isolates from Department of Pharmacognosy, KMCH College of Pharmacy, Coimbatore. The fungus strain was maintained at 37°C on SDA.

### Statistical analysis

All the experimental values were reported and expressed as means ± SD of three values.

## RESULTS AND DISCUSSION

### Macroscopical studies

The present macroscopical investigations of *Calocybe indica* revealed that the fruiting body is upto 15 cm in height, differentiated into pileus and cap (Figure 1; Table 1). The stipe is hard, tough and it is supporting the mushroom's cap.

### Organoleptic characters of dry edible mushroom powder

The edible mushroom powder showed milky odour and salty taste. Upon drying and powdering the colour of the powder changed from whitish shade to light brownish yellow as shown in Table 2. The organoleptic characters such as colour, consistency and odour were noted in the petroleum ether, ethanol and water extracts of *Calocybe indica* (Table 3).

### Behaviour of plant powder with different chemical reagents

The behaviour of mushroom powder with various reagents were observed and presented in Table 4. Yellowish brown to light brown was noted in the powder with different chemical reagents. Its milky white color and robust nature are appealing to consumers [26].

### Fluorescence behaviour of mushroom powder with different chemical reagents

Fluorescence behaviour of the powdered mushroom of *Calocybe indica* after treating with different chemical reagents was observed in day light as well as under UV light at 365 nm and 254 nm and the observations are presented in Table 5. Plant powder as such showed yellowish brown to yellowish white in visible light. Light greyish brown to light yellow colour changes was noted in UV light at 365 nm. Greenish brown to light green changes was seen in UV light at 254 nm.

### Determination of moisture content, heavy metals and micro elements

The moisture content of *Calocybe indica* were analyzed and presented in Table 6. The results indicate that the sample contained 79% of moisture content. Micro element, zinc was the most abundant element with a concentration value of 49.73±1.11 mg kg_1 dry weight. This is followed by Cu (24.59±0.07) respectively. In the case of heavy metals, amounts of Cr and Ni were too close to each other and showed the highest concentrations for this mushroom. Additionally, amount of Pb was determined as 2.63±0.04 mg kg-1.

### Phyto chemical analysis.

#### *Successive solvent extraction*

### Percentage yield

The air dried, powdered sample was extracted with different solvents for the phytochemical and pharmacological studies. The yield of different solvent extracts during successive solvent extraction was calculated and presented in Table 7. The percent yield was maximum in Ethanol extract (8.40±0.30 %) followed by water extract (5.70±1.04 %) and petroleum ether extract (5.50±0.20 %).

### Qualitative phytochemical evaluation

The results of the preliminary phytochemical screening of the edible mushroom powder with different solvents showed the presence of various phytochemicals (Table 8). The petroleum ether extract revealed the presence of proteins, amino acids, alkaloid, flavonoid, anthraquinone, quinone and coumarin. The ethanolic leaf extract revealed the presence of proteins and amino acids, alkaloid, tannin and phenolic compound, flavonoid, anthraquinone, quinone and coumarin and the same extracts showed negative response for carbohydrate, triterpenoid, steroid, saponin, glycoside and fixed oil [27].

### Quantitative phytochemical evaluation

#### *Phenol and flavonoid*

Total phenol content of ethanolic extract of *Calocybe indica* was studied and expressed as gallic acid equivalent. As shown in Table 9, the total phenol content of ethanolic extract was 4.4 ± 0.54 mg/g. Total flavonoid content was

studied and expressed as quercetin equivalent. The total flavonoid content was 3.5 ± 0.35.

**FT-IR analysis**

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FT-IR spectroscopy studies of *Calocybe indica* gave the following characteristic absorption peaks as shown in Table 10 and Fig. 2. The results of FTIR peak value and functional groups were represented in Fig-1. From the FT-IR spectral data, C-O (alcohol), C-O (ester), C=C (aromatic), N-O (nitro compounds), N-H (amide), C=C (alkene), C=O (carbonyl), C≡C (alkyne), O-H (acid), C-H (alkane) and N-H (amine) were identified. The functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of various functional groups of different compounds. The ethanol solvent had its respective functional group like Alkenes (C-H Rocking phosphates and nitrate), Amine (N-H Bending), Carboxyl group (C=O str), Alkenes (C-H str), Hydroxyl (O-H str) and Amine (N-H str).

**Antifungal activity**

***Disc Diffusion Method***

The antifungal activity of petroleum ether, ethanol and water extracts of *Calocybe indica* were invertigated by using the disc diffusion method and results were tabulated in table form (Table 11). The results of the antifungal response shown by the ethanol extract of *Calocybe indica* exhibited variable degree of antifungal activity against the tested fungus *Aspergillus niger* (Figure 3). However, the result indicates that the extracts prepared in ethanol solvent consistently displayed better antifungal activity. The water extract exhibited different levels of antifungal activity against *Candida albicans* (10.36±4.89) and *Aspergillus niger* (18.10±7.11). Petroleum ether and ethanolic extract against *Candida albicans* does not produced any inhibitory zone.

Bio-active compounds contribution established physiological benefits for consumers have been identified in plants and plant based food products [28]. Bio-active compounds such as phenolic compounds epicatechin, catechin and rutin were detected and quantified. Amines, β-carotene, quinones, cerebrosides, catechols, isoflavones, triacylglycerols, sesquiterpenes, steroids, organic germanium and selenium have been implicated in offering health benefits over and above basic nutritional requirements [29]. These compounds among many others are used as ingredients in the manufacture of functional foods and nutraceuticals.

The various extract of mushroom species *Pleurotus florida* and *Calocybe indica* possessed antimicrobial properties against antibiotic resistant human pathogens similar to that of the commercially available antibiotics. However, the present result indicates that the extracts prepared in ethanol solvent consistently displayed better antifungal activity. The water extract exhibited different levels of antifungal activity against *Candida albicans* (10.36±4.89) and *Aspergillus niger* (18.10±7.11). Petroleum ether and ethanolic extract against *Candida albicans* does not produced any inhibitory zone (Figure 3). The medicinal properties of these mushrooms can be exploited to formulate drugs for several disease caused by antibiotic resistant pathogenic microorganisms.

**Figure 1:** Fruiting body of edible mushroom - *Calocybe indica* P&C

**Table 1:** Macroscopic analysis of edible mushroom *Calocybe indica*

| S.No. | Macroscopic characters observed |
|---|---|
| 1. | **The cap:**<br>Height : Up to15cm in diameter<br>Surface shape: Conical, flat or even spherical<br>Texture : Smooth<br>Taste : Delicious, salty<br>Odour : Characteristic<br>Colour : White |
| 2. | **The Gills:**<br>Surface: Wrinkled or veined<br>Texture: Smooth<br>Taste : Delicious, salty<br>Odour: Milky<br>Colour: White |
| 3. | **Stipe** : Hard, tough, axis supporting the mushroom's cap |
| 4. | **Hypha :**<br>Microscopic filament, often white, that draws water and the organic matter necessary for mushroom development (2.2 cm) |
| 5. | **Mycelium :**<br>Tangle of hyphae created through spore germination, from which the above ground part of the mushroom develops |

**Table 2:** Organoleptic characters of *Calocybe indica* dry edible mushroom powder

| S.No. | Characters | Observations |
|---|---|---|
| 1. | Colour | Light brownish yellow |
| 2. | Texture | Coarse powder |
| 3. | Taste | Salty |
| 4. | Odour | Milky odour |

**Table 3:** Organoleptic characters of different extracts of *Calocybe indica*

| Extraction Medium | Colour | Consistency | Odour |
|---|---|---|---|
| Petroleum ether | Yellowish brown | Semi solid | Milky smell |
| Ethanol | Orangish brown | Semi solid | Characteristic smell |
| Water | Brownish white | Semi solid | Milky smell |

**Table 4:** Behaviour of *Calocybe indica* dry edible mushroom powder with different chemical reagents

| S. No. | Powder + Reagents used | Colour of the liquid |
|---|---|---|
| 1. | Powder as such | Yellowish brown |
| 2. | Powder + Concentrated HCl | Brown |
| 3. | Powder + Concentrated $H_2SO_4$ | Dark reddish brown |
| 4. | Powder + Acetic acid | Dark brown |
| 5. | Powder + Ethanol | Brown |
| 6. | Powder + Methanol | Yellowish white |
| 7. | Powder + Acetone | Brown |
| 8. | Powder + Petroleum ether | Brownish white |
| 9. | Powder + Benzene | Light reddish brown |
| 10. | Powder+$HNO_3$ | Dark brown |
| 11. | Powder +Aqueous solution | Light brown |

**Table 5:** Fluorescence analysis of *Calocybe indica* dry edible mushroom powder

| Reagent | Visible | UV | |
|---|---|---|---|
| | | Long (365 nm) | Short (254 nm) |
| Powder as such | Yellowish brown | Light Grayish brown | Greenish brown |
| Hager`s | Reddish yellow | Greenish brown | Yellowish brown |
| Mayer's | Pale yellow | Grayish brown | Greenish yellow |
| Dragendroff's | Light orange | Dark brown | Olive green |
| Iodine solution | Brownish red | Dark chocolate brown | Dark greenish brown |
| Acetone | Pale brown | Light brownish shade | Cream |
| 50 % $HNO_3$ | Pale yellow | Greenish grey | Greenish buff |
| Sodium nitroprusside | Pale yellow | Grayish yellow | Pale yellow |
| $FeCl_3$ | Light orangish yellow | Greenish brown | Light green |
| Methanol | Yellowish white | Light yellow | Light green |

**Table 6:** Physico - chemical and heavy metal analysis of *Calocybe indica* dry edible mushroom powder

| Physico - chemical properties | Values |
|---|---|
| Moisture content (Loss on drying) | 79 % |
| **Heavy Metals** | **Values (ppm)** |
| Pb | 2.63±0.04 |
| Cd | 0.38±0.02 |
| Cr | 5.31±1.05 |
| Ni | 5.86±0.19 |
| **Physico - chemical properties** | **Values** |
| **Microelements** | |
| Zn | 49.73±1.11 |
| Cu | 24.59±0.07 |

**Table 7:** Extractive values of *Calocybe indica* dry edible mushroom powder on successive extraction

| S. No. | Method of extraction | Solvents used | Yield (%) |
|---|---|---|---|
| 1. | Continuous hot percolation using Soxhlet apparatus | Petroleum ether | 5.50±0.20 |
| | | Ethanol | 8.40±0.30 |
| 2. | Hot and cold maceration | Water | 5.70±1.04 |

**Table 8:** Qualitative phytochemical screening of different extracts of *Calocybe indica* dry edible mushroom powder

| Constituents | Petroleum ether | Ethanol | Water |
|---|---|---|---|
| **Test for carbohydrate** | | | |
| Barfoed's Test | - | - | - |
| **Test for proteins and amino acids** | | | |
| Biuret teat: | + | + | - |
| Ninhydrin test: | - | + | - |
| **Test for alkaloid** | | | |
| Mayer's test: | + | + | + |
| Wagner's test: | + | + | - |
| **Test for tannin and phenolic compound** | | | |
| Ferric chloride test: | - | + | - |
| **Test for flavonoid** | | | |
| Ammonium hydroxide Test | + | + | + |
| **Test for triterpenoid** | | | |
| Libernann-Burchard test: | - | - | - |
| **Test for steroid** | | | |
| Salkowskis test: | - | - | - |
| **Test for saponin** | | | |
| Foam formation test: | - | - | - |
| **Test for glycoside** | | | |
| Borntrager's test: | - | - | - |
| **Test for anthraquinone** | + | + | + |
| **Test for quinone** | + | + | + |
| **Test for coumarin** | + | + | + |
| **Test for fixed oil** | - | - | - |

**Table 9:** Estimation of total phenol and total flavonoid content of ethanolic extract of *Calocybe indica* dry edible mushroom powder

| S. No. | Extraction Medium | Phenol (Gallic acid equivalent/mg) # | Total flavonoid (Quercetin equivalent/mg) # |
|---|---|---|---|
| 1. | Ethanol | 4.4 ± 0.54 | 3.5 ± 0.35 |

**Figure 2:** FT-IR spectra of ethanolic extract of *Calocybe indica*

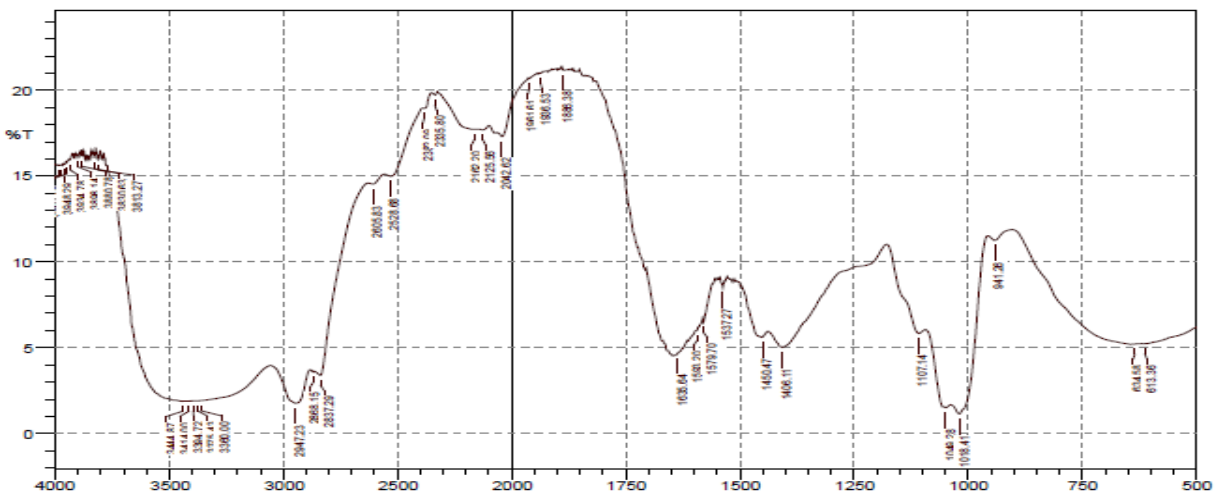


**Table 10:** FTIR Spectroscopic data of ethanolic extract of *Calocybe indica*

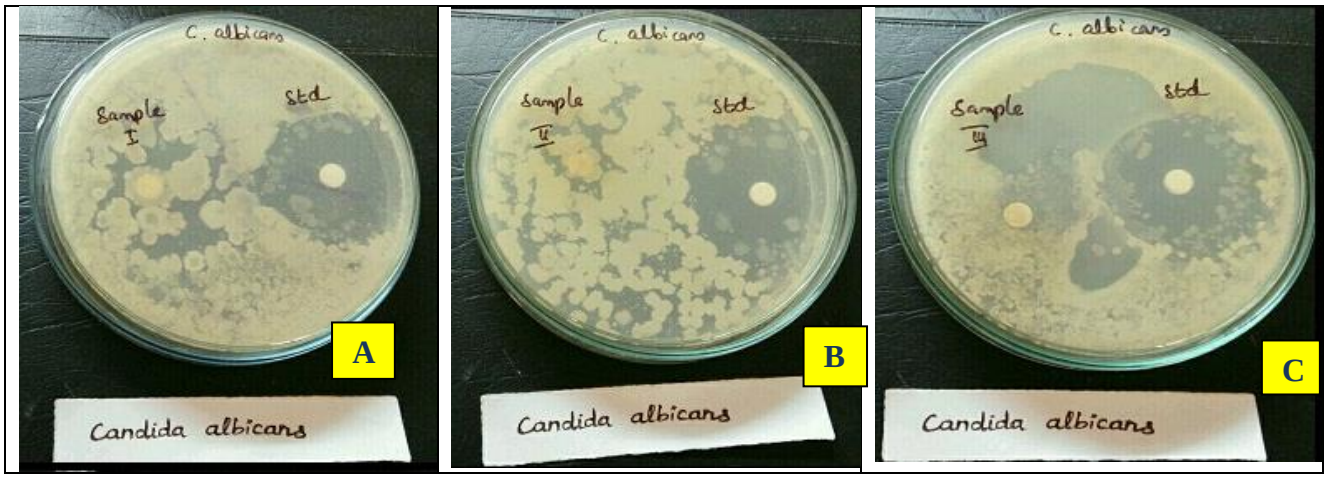
| S.No. | Name of the extract | Peak Area | Functional group | Types of vibration |
|---|---|---|---|---|
| 1. | Ethanol extract | 613.36 | sulfates | |
| 2. | | 634.58 | sulfates | |
| 3. | | 941.26 | C-O (alcohol) | Stretching |
| 4. | | 1018.41, 1049.28 | C-O (ester) | Stretching |
| 5. | | 1107.14 | | Sulfates |
| 6. | | 1406.11 | | Carbonates |
| 7. | | 1450.47 | C=C (aromatic) | Stretching |
| 8. | | 1537.27 | N-O (nitro compounds) | Stretching |
| 9. | | 1579.7, 1593.2 | N-H (amide) | Bending |
| 10. | | 1635.64 | C=C (alkene) | Stretching |
| 11. | | 1886.38 | C=O (carbonyl) | Stretching |
| 12. | | 2042.62 | | |
| 13. | | 2125.56, 2162.2, 2335.8, 2382.09 | C≡C (alkyne) | Stretching |
| 14. | | 2605.83, 2837.29 | O-H (acid) | Stretching |
| 15. | | 2868.15, 2947.23 | C-H (alkane) | Stretching |
| 16. | | 3360, 3375.43, 3394.72, 3414, 3444.87 | N-H (amine) | Stretching |

**Table 11:** Antifungal activity of different extracts of *Calocybe indica* dry edible mushroom powder

| S.No. | Name of the Organism | Zone of inhibition (Mm) | | | |
|---|---|---|---|---|---|
| | | Standard Flucanazole (10µg/Disc) | Samples (100 µg / Disc) | | |
| | | | Petroleum ether | Ethanol | Water |
| 1. | *Candida albicans* | 34.00±9.84 | - | - | 10.36±4.89 |
| 2. | *Aspergillus niger* | 36.00±11.7 | 13.33±5.85 | 20.33±7.09 | 18.10±7.11 |

[#] Values are means of triplicate determinations ± Standard Deviation

**Figure 3:** Antifungal activity of different extracts of *Calocybe indica* against *Candida albicans* strain




.

## CONCLUSION

*Calocybe indica* is a well-known, important medicinal mushroom. But its quality standards are not yet reported. Therefore, the current study was revealed which included macroscopic, physico-chemical, heavy metal and florescent characterisation of dry powder and extracts derived from the fruit bodies of the mushroom. A ethanolic extract of this fungus was prepared for analysis of phenol and flavonoid content. The FT-IR spectroscopy studies of *Calocybe indica* was used to recognize the functional group of the active components based on the peak value in the region of infrared radiation. In the end data depicted in this investigation is quite significant towards future identification and production of antifungal compounds, enzymes etc from the edible mushrooms. Further studies going on to produce these antifungal substances from the various non edible mushrooms are also essential. Commercial making and promoting of these mushrooms create self employment to rural people.

**Acknowledgement:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## REFERENCES

1. Livi Bacci MA, Concise history of world population, 5th ed. Malden (MA): Wiley-Blackwell. 2012.
2. Hawksworth DL, The fungal dimension of biodiversity: magnitude, significance, and conservation, Mycological Research, 1991, 95: 641–655.
3. Krishnamoorthy Akkanna Subbiah, Biodiversity Exploration of Milky Mushroom (*Calocybe indica* P&C) - Concept to Commercialization, Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8), 2014, 490-495.
4. Chang ST, The world mushroom industry: trends and technological development, International Journal of Medicinal Mushrooms, 8, 2006, 297–314.
5. Wakchaure GC, Production and marketing of mushrooms: global and national scenario. In: Singh M, Vijay B, Kamal S, Wakchaure GC, editors. Mushrooms: cultivation, marketing and consumption. Solan (HP): Directorate of Mushroom Research, 2010, 15–22.
6. Vikineswary S, Chang ST, Edible and medicinal mushrooms for sub-health intervention and prevention of lifestyle diseases. Tech Monitor, 2013, 33–43.
7. Krishnamoorthy AS, Muthuswamy MT, Nakkeeran S, Technique for commercial production of milky mushroom *Calocybe indica* P&C, Indian journal of mushrooms, 18, 2000, 19–23.
8. Turkekul I, Elmastas M, Tuzen M, Determination of iron, Manganese, Zinc, Lead and Cadmium in mushroom samples from Tokat, Turkey Food Chemistry, 84, 2004, 389-392.
9. Cocchi L, Vescovi L, Schede della rubrica Funghi – Metalli – Radioattivita. Il Fungo, Associazione Micologica Bresadola. 1997–2005.
10. Cocchi L, Petrini O, Vescovi L, Metalli pesanti isotopi radioattivi nei funghi: aspetti igienico – sanitari. In: Proceedings of the Second International Meeting of Mycotoxicology, 17, 2002, 73–91.
11. Gruter H, Selective accumulation of the fission product 137Cs in fungi. Naturwissenschaften, 51, 1964, 161–162.
12. Karaman I, Sahin F, Gulluce M, Ogutcu H, Hsengu IM, Adiguzel A, Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L, Journal of Ethnopharmacology, 85, 2003, 213-235.
13. Purkayastha RP, Chandra A, New species of edible mushroom from India, Transactions of the British Mycological Society, 62, 1974, 415-418.
14. Jain SK, Rao RR, Hand Book of Field and Herbarium Methods, 1970, New Delhi.
15. Trease GE, Evans WC, In: Pharmacognosy, Balliere Tindall, East bourne, 12th edition, 1983, 46-53.
16. Wallis TE, Text book of Pharmacognosy. CBS Publishers and Distributors, 1985, New Delhi, India, (5th ed.).
17. Harborne JB, Phytochemical methods, Chapman and Hall, London (1st ed.), 1973, 33-35.
18. Kokoshi CJ, Kokoshi RJ, Sharma FJ, Fluorescence of powdered vegetable drugs under UV radiations science, Journal of the American Pharmaceutical Association, 48, 1958, 715-717.
19. Anonymous, Pharmacopoeia of India. Ministry of Health, Govt. of India Publication, 1966 and 1985, New Delhi.
20. Watling L, Amphipoda from the northwestern Atlantic: The genera Jerbarnia, Epimeria, and Harpinia. Sarsia, 66, 1981, 203-211.
21. Horborne JB, In: Phytochemical methods. Chapman and Hall, New York, (2nd ed.), 1984, 44.
22. Kokate CK, Khandelwal KR, Pawar AP, Gohalz SB, Practical Pharmacognosy, Vallabh Prakashan, New Delhi, India, (4th ed.), 1995, 107.
23. Siddhuraju P, Becker K, Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of Drumstick tree (*Moringa oleifera* Lam.) leaves, Journal of Agricultural and Food Chemistry, 51, 2003, 2144-2155.
24. Zhishen J, Mengcheng T, Jianming W, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chemistry, 64, 1999, 555-559.
25. Broekaert WF, Terras FRG, Cammue BPA, Vanderleyden J, An automated quantitative assay for fungal growth inhibition. FEMS Microbiology Letters, 69, 1990, 55-60.
26. Vikineswary S, Chang ST, Edible and Medicinal Mushrooms for Sub-Health Intervention and Prevention of Lifestyle Diseases. Technology Trends. Tech Monitor. 2013, 33 – 43.
27. Sharmila S, Ramya EK, Phytopharmacognostical standardization on Indian Medicinal Plant *Acacia Caesia* (L.) Wild., Research Journal of Pharmaceutical, Biological and Chemical Sciences, 9, 2018, 379-388.
28. Lindequist U, Niedermeyer THJ, Julich WD, The pharmacological potential of mushroom. Evidence-based Complement. Evidence Complemen Altern Med. (ECAM), 2, 2005, 285-299.
29. Ramesh C, Pattar MG, Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of Western Ghats of Karnataka, India, Pharmaceutical Research, 2, 2010, 107–112.

**Source of Support:** Nil, **Conflict of Interest:** None.