ANTIBACTERIAL POTENTIAL OF FRUITING BODY OF 
CALOCYBE INDICA EXTRACTS (APK2)

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ABSTRACT
Antibacterial activity of methanol and acetone extract of Calocybe indica were determined in vitro against two pathogenic bacteria Escherichia coli, Staphylococcus aureus and following agar well diffusion method using different concentration (25, 50, 75 and 100%). Methanol and acetone extract showed potent antibacterial activity against tested bacteria. Methanol extract showed maximum inhibitory effect against growth of each of the test bacterium. There is a need for further studies to isolated and characterize the antibacterial moieties in this fungus for practical disease control measures.

KEYWORDS: Antibacterial, Calocybe indica, Staphylococcus aureus, Escherichia coli, Methanol.

1. INTRODUCTION
The mushroom comprises a large heterogeneous group having various shape, size and colours. All are quite different in characters, appearance and edibility, white milky mushroom (Calocybe indica) is an edible mushroom having white sporophore, large sized fruit bodies and delicious flavor. It is a high temperature tolerant mushroom that can grow at temperature ranges of 25⁰C -35⁰C. It has moderate protein content and has a good biological efficiency (60 -70 %) under optimum conditions. Their sporophores (fruit bodies) have long shelf life. Milky mushroom is robust, fleshy, umbrella like structure and milky white in color even after flattening (Chadha and Sharma, 1995). It grows in nature on soil under the pond or in forests.

Mushroom "nutriceuticals" are bioactive compounds extracted and have nutritional and medicinal features that may be used in the prevention and treatment of diseases.
increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microorganisms hassled to the screening of several medicinal plants for their potential antimicrobial activity. Mushroom species possess antagonistic effects against bacteria, fungi, viruses and cancer. Hence, the present study was carried out to test the antibacterial activity of the extract from two different mushrooms against *E. coli*.

In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources which are the good sources of novel antimicrobial chemotherapeutic agent (Karaman *et al*., 2003). Researchers show antimicrobial activity of several mushrooms (Gezer *et al*., 2006; Mercan *et al*., Turkoglu, *et al*., 2007). Extracts from fruiting bodies and the mycelia of various mushrooms have been reported for antimicrobial activity against wide range of infectious bacteria (Hirasawa, *et al*., 1999; Dulger *et al*., 2002).

Thus, the present study focused on evaluation of antibacterial activities of methanol and acetone extracts of *Calocybe indica* using agar well diffusion method against two clinical isolated *Echerichia coli* and *Staphylococcus aureus*.

2. MATERIALS AND METHODS

The fruiting bodies of *Calocybe indica* produced from CAS marine biology, Parangipettnai and two test bacterial pathogen (*Escherichia coli* and *Staphylococcus aureus*). Pathogenic strains of bacteria were procured from Raja Muthiaaya Medical College, Annamalai University, Tamil Nadu, India.

2.1. Isolation of pure culture of *Calocybe indica*

The cultures were raised from the stipe and stroma portion of healthy, showed dried and fresh specimen. The specimen was first washed with distilled water and then the tissue from the stipe and stroma portion were cut with the help of a sterilized blade. The bits of tissue (2-3 mm) were taken up with a sterilized forceps. Now the tissue was placed on filter paper to remove the excess moisture. The small bit of *Calocybe* tissues was then transferred aseptically into the Petri plates containing Potato Dextrose Agar (PDA) medium with the help of a sterilized forceps (Chandra *et al*., 2012; Pala *et al*., 2013). These were then incubated at 250°C for at least 8-10 days and observed regularly for appearance of culture. The actively growing mycelial colonies were sub cultured to obtain pure cultures.
2.2. Preparation of crude mushrooms extract

The fresh fruiting bodies were dried in shade conditions and the dried material (20 g) was pulverized in a blender to get a coarse powder and soaked separately in 150ml of methanol and acetone in Erlenmeyer flask for methanol and acetone extracts. The flasks were covered with aluminum foil and allowed to stand for 7 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator (Jonathan and Fasidi, 2003; Balakumar et al., 2011). The extracts were collected and stock solution of conc.10 mg/ml was prepared.

2.3. Screening of extracts of Calocybe indica for antibacterial activity

Screening of mushroom extracts (methanol and acetone) of Calocybe indica was done using agar well diffusion method. Muller Hinton agar plates were used throughout the investigation. The medium was autoclaved at 121.6°C for 30 minutes and poured into Petri plates. Bacteria were grown in nutrient broth for 24 hours. A 100l of bacterial suspension was spread on each nutrient agar plates. Five agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 25%, 50%, 75% and 100% concentration of prepared extracts of Calocybe indica. The control well containing pure solvent only. The plates were incubated at 37 ± 2°C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all three replicates and the average values were tabulated. Percentage inhibition of growth of bacterial microorganisms was calculated after subtracting control from the values of inhibition diameter using control as standard (Hemashenpagam and Selvaraj, 2010).

Percentage of growth inhibition= (Control-Test/Control) x100

Control=average diameter of bacterial colony in control.
Test=average diameter of bacterial colony in treatment sets (Kannan et al., 2009).

3. RESULTS AND DISCUSSION

3.1. Morphological and mycelial characteristics

The fruiting bodies of Calocybe indica were white in colour (Fig.1A). Spores were oval to round having brown colour spore print (Fig. 1C). Mycelial growth of Calocybe indica Imbach was longitudinally radial, aerial initially, creamish white, becoming densely matted and cottony in texture (Fig. 1B).
3.2. Antibacterial activity Calocybe indica of against S. aureus and E. coli

The methanol and acetone extracts of Calocybe indica were screened against S. aureus and E. coli. Stock solutions were prepared by making a concentration of 10 mg/ml, other concentrations were prepared by serial dilution of stock solution. The methanol and acetone extracts of Calocybe indica showed considerable growth inhibition of two test bacteria indifferent concentrations (25%, 50%, 75%, and 100%). The methanol extract of Calocybe indica showed maximum inhibition of 16.67% and 20.00% at 100% concentration of the extract against S. aureus and E. coli respectively (Table 1, Fig. 2A, C) and the acetone extract showed maximum inhibition of 16.67% and 17.77% at 100% concentration against S. aureus and E. coli respectively (Table 2, Fig. 2B, D). It is evident from the results that methanol and acetone extracts of Calocybe indica showed maximum percent inhibition against E. coli and methanol extract was more effective than acetone extract against both the test bacteria.

The results of the present study are in agreement with the work of the earlier workers (Nasim and Ali, 2011; Kamra and Bhatt, 2012), who have also reported strong antibacterial activity of methanol extract of G. lucidum against gram negative bacteria (E. coli) and comparatively less activity against gram-positive (S. aureus) bacteria. Similar trend in antibacterial activity of methanol extract of Lactarius delicious (Sagar and Thakur, 2012), Sparassis crispa (Sagar and Tandon, 2012), Morchellaesculenta (Sagar and Kumari, 2012) and Ganoderma lucidum (Sagar and Kumari, 2012) have been reported against S. aureus and E. coli. Ramesh and Patter (2010) have reported that extract of Clavaria vermicularis and Marasmiu oreadesoffered more inhibition to gram-negativebacteria (E. coli and Pseudomonas aeruginosa) as compared to gram-positive bacteria (Bacillus sutilis and Staphylococcus aureus). Neelam and Singh (2013) also reported the antibacterial potential of ethanol extract of Pleurotus florida and Pleurotus ostreatus. Many pharmaceutical substances with potent and unique health-enhancing properties have been isolated from mushrooms and distributed worldwide. Mushroom based products either from the mycelia or fruiting bodies are consumed in the form of capsules, tablets or extracts (Filipa, et al., 2013. This study has revealed that the edible mushroom Calocybe indica exhibited various levels of antimicrobial activity in different solvents. The bioactive contents of the mushrooms are promising natural antimicrobial agents that can be harvested as potential antibacterial substances.

In present study, we have reported the antibacterial activity of methanol and acetone extract of Calocybe indica against S. aureus and E. coli. So, there is a need for further studies to
isolate and characterize the bioactive compounds present in *Calocybe indica* and these metabolites can be used to develop effective drugs against these human pathogenic bacterial strains.

Table 1 Percent inhibition of growth of *S. aureus* and *E. coli* at different concentration of methanol extract of *C. indica*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration of the methanol extract (%)</th>
<th>Inhibition of growth of the bacteria (%)</th>
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<tr>
<td></td>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>10.00±0.14</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>12.22±0.13</td>
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<tr>
<td>4</td>
<td>75</td>
<td>13.44±0.22</td>
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<tr>
<td>5</td>
<td>100</td>
<td>16.67±0.44</td>
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</tbody>
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Table 2 Present inhibition of growth of *S. aureus* and *E. coli* at different concentration of acetone extract of *C. indica*.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Concentration of the acetone extract (%)</th>
<th>Inhibition of growth of the bacteria (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
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<tr>
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<tr>
<td>5</td>
<td>100</td>
<td>16.67±0.86</td>
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(A) Pure culture of *Calocybe indica* (B) Basidiospores of *Calocybe indica*

Fig. 1 Fruiting bodies of *C. indica*
Fig.2 (A) Inhibition in the growth of *S.aureus* at different concentrations of methanol extract, (B) Acetone extract of *Calocybe indica*. (C) Inhibition in the growth of *E. coli* at different concentrations of methanol extract, (D) Acetone extracts of *Calocybe indica*.

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CONCLUSION
The present study provides standards of *Calocybe indica* powder with the help of techniques and suitble parameters. We have reported the antibacterial activity of method extract of *Calocybe indica* against *S.aureus* and *E.coli*. The bioactive compound used for the metabolites can be used to develop effective drugs against these human pathogenic bacterial strain.

REFERENCE


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