PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF WILD EDIBLE MUSHROOMS COLLECTED FROM MAHAL FOREST OF DANG DISTRICT, GUJARAT, INDIA

H.A.Modi*, Sanjay Parihar, E.A. Pithawala and N.K. Jain

Department of Life Sciences, School of Sciences, Gujarat University, Ahmedabad-380009

ABSTRACT

Pleurotus ostreatus, Agaricus bisporus, Coprinus comatus and Volvareilla volvacea, wild edible macrofungi were screened for preliminary phytochemical analysis and evaluate in vitro antimicrobial potential of various solvent extracts. The phytochemical screening was performed by modified Harborne, and Adebayo & Ishola method and antimicrobial activity against human pathogens was evaluated by agar well diffusion method. The phytochemical analysis reveals that the extracts were rich source of phytoconstituents containing carbohydrate, saponins, phenols, steroids, glycosides, terpenoids, flavanoids and Anthraquinones. Methanol extracts exhibit higher solubility for more active antimicrobial and phytochemical constituents, consequently displaying the highest antimicrobial activity. The extract could be potential source of new antimicrobial agents and scientifically validates the use of the macrofungi in traditional medicine.

Key words – Edible mushroom, Agar well diffusion assay, Phytochemicals, Antibacterial activity.

INTRODUCTION

Fungi are a distinct group of organisms which include species with large and visible fruiting bodies called as macrofungi. Macromycetes arranged in the phylum Basidiomycota and some of them in the Ascomycota are known as the higher fungi (Moradali et al., 2007, Sicoli et al., 2005). Edible mushrooms are important source of biologically active compounds (Rajewska and Balasinska 2004). Infectious diseases account for a high section of health problems in most of the developing countries. Although several antimicrobial agents have been
synthesized chemically, but an random use of these commercial antimicrobial drugs has led to the development of resistance to the existing antibiotics by the microorganisms (Raghunath, 2008). Traditional medicines are used for primary health care of the poor in developing countries.

These Edible mushrooms *Pleurotus ostreatus*, *Agaricus bisporus*, *Coprinus comatus* and *Volvareilla volvacea* most accepted in the market which is recognized not only to its nutritional value but also to possible potential for therapeutic applications. Edible mushrooms are used medicinally for diseases involving depressed immune function, cancer, allergies, fungal infection, frequent flu and colds, bronchial inflammation, heart disease, hypertension, infectious disease, diabetes and hepatitis (Yaswant P. et al., 2012). Most attention has been paid to the investigation of natural drugs from various edible mushrooms.

**MATERIALS AND METHODS**

**Collection site**
Fresh wild edible mushrooms *Pleurotus ostreatus*, *Agaricus bisporus*, *Coprinus comatus* and *Volvareilla volvacea* were collected from Mahal forest area of the Dang district, Gujarat, India. The longitude and latitude of the research region is 20.91°N, 73.663°E.

**Preparation of extractions**
After collection, the mushrooms samples were wrapped in newspaper and stored in moisture free open places. They were air-dried in shade that took 15 days or more. Identified samples were collected and cleaned by rubbing, scraping and brushing. The removal of all foreign matters was confirmed. Thereafter they were cut in small pieces of around 2 to 3 cm across using a machete. Then they were ground using metal mortar and pestle. The smashed pieces were further dried at 45 °C for an hour and immediately powdered in a grinder. The powder was collected and ground again at the end.

Preparation of extract was carried out as reported by Pooja shah et al., 2014 .The mushroom powder was weighed accurately to 1 g and the same was filled in a thimble and placed in the central assembly of the Reflux apparatus with measured 50 ml methanol and 50 ml of distilled water separately to form extracts in methanol and water respectively. The extraction was done in this apparatus at 100°C for 6 h. After the completion of extraction, the supernatant was filtered through Whatman No. 1 filter paper. All solvent extracted fractions were evaporated to dryness to obtain residues. The extracts were stored at 4°C in air tight containers.
Preliminary phytochemical screening (Harborn, 1998; Adebayo and Ishola, 2009)
The freshly prepared extracts were subjected to standard phytochemical analysis to ensure the presence of following phytoconstituents.

Tests for alkaloids
Mayer’s Test
Take 5 ml of extract, few drops of Mayer’s reagent is added by the side of the test tube. A white creamy precipitate indicates the test as positive.

Wagner’s Test
Take 5 ml of extract, few drops of Wagner’s reagent is added by the side of the test tube. A reddish brown precipitate confirms the test as positive.

Dragendorff’s Test
Take 5 ml of extract, 1 or 2 ml of Dragendorff’s reagent was added. A prominent yellow/orange precipitate indicates the test as positive.

Tests for carbohydrates
Molisch’s test
Take 5ml of extract, two drops of alcoholic solution of α -naphthol is added, the mixture is shaken well and 1 ml of conc. H$_2$SO$_4$ is added slowly along the sides of the test tube and allows standing. A violet ring indicates the presence of carbohydrates.

Fehling’s Test
3 ml of extract is boiled on water bath. To this, 1 ml of Fehling solutions A and B are added. A red precipitate indicates the presence of sugar.

Benedict’s test
To 2 ml of extract, 1 ml of Benedict s reagent is added. The mixture is heated on a boiling water bath for 2 mins. A characteristic colored precipitate indicates the presence.

Barfoed’s Test
To 5 ml of extract, 1 ml of Barfoed's Reagent is added and heated on a boiling water bath for 2 min. Red Precipitate indicates the presence of sugar.
Detection of Glycosides

Keller-Killiani test
To 5 ml of extract, 2 ml glacial acetic acid is added, followed by one drop of 5% FeCl₃. Then conc. H₂SO₄ is added from the side of the test tube. Reddish brown ring appears at the junction of the two liquid layers indicating the presence of cardiac glycosides.

Legal’s Test
To the extract, few drops of 10% NaOH are added to make it alkaline. Then freshly prepared sodium nitroprusside was added to the solution. Presence of blue colouration indicates the presence of glycosides in the extract.

Detection of Proteins and Amino Acids

Millon’s Test
To 2 ml extract, few drops of Millon’s reagent are added. A white precipitate indicates the presence of proteins.

Biuret’s Test
An aliquot of 2 ml of extract is heated with 1 drop of 2% CuSO₄ solution. To this 1 ml of ethanol (95%) is added, followed by excess of KOH Pellets. Pink color in the ethanolic layers indicates the presence of proteins.

Test for detection of flavanoids

Shinoda test (Magnesium Hydrochloride reduction test)
To the test Solution, few fragments of Magnesium ribbon are added and concentrated HCl was added drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

Alkaline reagent test
To the test solution few drops of NaOH solution is added; formation of an intense yellow color, which turns to colorless on addition of few drops of dil. acid, indicates the presence of flavonoids.

Detection of triterpenoids and steroids

Libermann Burchard’s Test
Extract is treated with few drops of acetic anhydride, boiled and cooled. Conc. sulfuric acid was added from the sides of the test tube, shows a brown ring at the junction of two layers
and the upper layer turning green shows the presence of steroids and formation of deep red colour indicated the presence of triterpenoids.

**Salkowski test**
Extract is treated with few drops of conc. sulfuric acid, shaken well and allowed to stand for some time, red color at the lower layer indicates the presence of steroids and formation of yellow colored lower layer indicates the presence of triterpenoids.

**Test for detection of Phenolic compound and tannins**

**Ferric Chloride Test**
To the 5 ml of extract, few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds.

**Lead Acetate Test**
To the 5ml of extract, 4 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

**Test for Saponins by froth test**
5ml of the extract is vigorously shaken with 8 ml of distilled water in a test tube for 30 sec and was left undisturbed for 20 min. Persistent froth indicating the presence of saponins.

**Test for Anthraquinones** *(Adebayo et al., 2012)*
2ml of the extracts is shaken with 10 ml of benzene. The solution is filtered and 5 ml of 10% NH₄OH solution is added to the filtrate. A pink, red or violet colour in the ammonical (lower) phase indicates the presence of anthraquinones.

**Antibacterial activity of Mushrooms**

**Test Bacteria used:** A Total of 4 bacterial species were tested. The Gram positive species were *Staphylococcus aureus* MTCC-96 and *Bacillus cereus* MTCC-430 and Gram negative species were *Escherichia coli* MTCC-425 and *Salmonella typhi* MTCC-733.

**Agar well diffusion assay**
Agar well diffusion technique as described by Adeniyi et al.1996 was adopted for the study. Mueller Hinton agar plates, were inoculated with 0.1 ml of an overnight broth culture of each bacterial isolate (Equivalent to 3 x 10⁷ cfu/ml) MF (Mcfarland standard) in sterile Petri-dish. The seeded plates were rocked for uniform distribution of isolates and allowed to set. Holes
were bored on the plates by using standard sterile cork borer of 6 mm diameter and equal volumes of the extracts (100µl) were transferred into the well with the aid of micropipette. The experiments were carried out in triplicate. The plates were allowed to stand for one hour at room temperature to allow proper diffusion of the extracts. The plates were incubated at 37°C for 24 h until marked decline in the potency of the extracts to inhibit the growth of the test isolates was observed. Zones of inhibitions were measured in millimeter (mm) and the average values were calculated and recorded. For control studies antibiotic streptomycin was used.

Statistical analysis
Data were averages of three results ± Standard Deviations (SD) by using Microsoft Excel.

RESULTS AND DISCUSSION
Preliminary phytoconstituents analysis
The test samples were successively extracted using reflux apparatus of various solvents at 60°C. The results of phytochemical analysis reported in Table 1.

<table>
<thead>
<tr>
<th>Phyto-chemical (Phyto-constituents)</th>
<th>Biochemical Test</th>
<th>Pleurotus ostreatus</th>
<th>Agaricus bisporus</th>
<th>Coprinus comatus</th>
<th>Volvareilla volvacea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer’s Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner’s Test</td>
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<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s Test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Molisch’s Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehling’s Test</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Benedict’s Test</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Barfoed’s Test</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Legal’s Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td></td>
<td>Keller-kiliiani Test</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Protein and amino acids</td>
<td>Millon’s Test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>Biurret test</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>Shinoda Test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Alkaline reagent</td>
<td>+</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>Libermaan-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Burchard’s Test</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Libermaan-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
The phytochemical analysis of edible mushrooms *Pleurotus ostreatus*, *Agaricus bisporus*, *Coprinus comatus* and *Volvareilla volvacea* disclosed the presence of major phytoconstituents viz., alkaloids, saponins, steroids, phenols, glycosides, terpenoids and flavonoids. Among the two solvents used for extraction, methanol extract showed more number of phytoconstituents as compared to aqueous.

Bioactive compounds found in edible mushroom are known to play a vital role in promoting health. The presence of essential nutrients and minerals in the wild edible mushroom imply they could be utilized to improve health (Ogbe and Obeka, 2013).

The absence of anthraquinones in *H. erinaceus*, *A. cularia* and *P. ostreatus* correlates with that of previous reported literature (Wandati *et al.*, 2013). These phytoconstituents play a vital role in medicinal properties of plants. Saponins for instant comprise a large family of structurally related compounds containing a steroids or triterpenoid. They are reported to have a wide range of pharmaceutical properties, such as anti inflammatory and anti-diabetic effects. Thus these mushrooms can be used in the management of diabetes and inflammation related diseases. Terpenoids have been reported to show a wide range of pharmacological benefits that include anti-malarial, anti inflammatory and anti- cancer effects among others. Phenolic compound are antioxidant and exhibit a wide range of spectrum medicinal properties such as anti cancer and anti inflammatory. These mushrooms can be therefore being harnessed in the management of oxidative stress induced disease since phenol and flavanoids have been shown to posses various antioxidant functions (Hamzah *et al.*, 2013).

**Antibacterial activity of Mushrooms**

Various extracts of edible mushrooms showed antimicrobial activity against certain tested bacteria. *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* were
found to be inhibited by the various extracts of wild edible mushrooms as shown in Table 2 and 3.

Table 2 - Antibacterial activity of methanolic extract

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Mean value of Inhibition zone (mm) of methanolic extract</th>
<th>Antibiotic drug (as control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pleurotus ostreatus</td>
<td>Agaricus bisporus</td>
</tr>
<tr>
<td><em>Salmonella typhi</em> MTCC-733</td>
<td>19±1.09</td>
<td>17± 1.00</td>
</tr>
<tr>
<td><em>E. coli</em> MTCC-425</td>
<td>14±0.10</td>
<td>11±0.70</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> MTCC-430</td>
<td>12±1.10</td>
<td>21±0.01</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> MTCC-96</td>
<td>17± 0.50</td>
<td>23±1.50</td>
</tr>
</tbody>
</table>

Note **Measurement taken including the 6 mm diameter of the hole
Mean of zone of inhibition with S.D using Microsoft windows excel

Table 3- Antibacterial activity of aqueous extract

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Mean value of Inhibition zone (mm) of Aqueous extract</th>
<th>Antibiotic drug (as control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pleurotus ostreatus</td>
<td>Agaricus bisporus</td>
</tr>
<tr>
<td><em>Salmonella typhi</em> MTCC-733</td>
<td>16±0.23</td>
<td>11±1.50</td>
</tr>
<tr>
<td><em>E. coli</em> MTCC-425</td>
<td>13±0.30</td>
<td>07±0.50</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> MTCC-430</td>
<td>09± 1.09</td>
<td>18±2.30</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> MTCC-96</td>
<td>11±0.50</td>
<td>15±0.40</td>
</tr>
</tbody>
</table>

Note **Measurement taken including the 6 mm diameter of the hole
Mean of zone of inhibition with S.D using Microsoft windows excel

The presence of important phytoconstituents like carbohydrates, glycosides, triterpenoids, phenolic compounds and tannins could be responsible for the antibacterial properties. The extracts could inhibit Gram positive as well as Gram negative bacteria indicating that the
active ingredients are broad spectrum compounds. The research needs to be done in order to recognize the actual phytoconstituents responsible for the antibacterial activity before being used for the development of any drugs.

This present study reveals that the antibacterial activity out of the two extracts of Pleurotus ostreatus, Agaricus bisporus, Coprinus comatus and Volvareilla volvacea, methanolic extracts of wild edible mushrooms show highest zone of inhibition against Gram positive bacteria and Gram negative bacteria. This study justifies the claimed uses of Pleurotus ostreatus, Agaricus bisporus, Coprinus comatus and Volvareilla volvacea, in the traditional system of medicine to treat various infectious disease caused by microbes. In similar studies, it has been reported that the extracts of various mushrooms inhibit the growth of microorganisms at different ratios (Iwalokun et al., 2007; Jagadish et al., 2008).

These observations are in accordance with the findings of Nehra et al., 2012 who further validated the antimicrobial potential of P. ostreatus and found that “organic solvents consistently displayed better antimicrobial activity than that of the aqueous extract”.

CONCLUSION
Based on the results obtained from the present study, it can be concluded that the methanolic and aqueous extracts of mushroom can be successfully applied in the development of more potent and efficient antimicrobial agents. The results of preliminary phytochemical analysis are in agreement with the reports of other workers. Further work is therefore under progress to identify the bioactive principles and elucidate their mechanism of action to scavenge the free radicals. This study is strongly suggestive that wild edible mushrooms can be used as antibacterial agent in the development of new drug for the therapy of urinary tract infections which is caused by bacterial pathogens and harmful activity of excess free radicals in humans.

REFERENCES


