A REVIEW OF PHYTOCHEMICAL SCREENING AND PHARMACOGNOSTIC STUDY OF PLEUROTUS FLORIDA.

Chinchu Sara George*, Honey Haridas, Ashly Jose, Devika Krishnan, Jayachandran T. P.

*Correspondence for Author
Chinchu Sara George
M. Pharm, Pharmacology, DPS, RIMSR, Mahatma Gandhi University, Puthupally, Kerala, India.

ABSTRACT

Mushrooms are widely used as food supplement not only due to their nutritional values but also their medicinal values. Phytochemistry explains that all plants produce chemical compounds as a part of their normal metabolic activity. Phytochemicals can be extensively divided into: a) Primary metabolites such as sugars and fats, which are found in all plants. b) Secondary metabolites-compounds which are found in smaller range of plants. It is the secondary metabolites in plants that can have therapeutic actions in humans and which can be refined to produce drugs. The aim of this work was to carry out the phytochemical screening of aqueous extract to identify the chemical constituents present and to carry out pharmacognostic studies so as to determine the level of adulterants, moisture content, tannins, extractive value using various solvents and analyze the presence of phytochemical constituents in extract of Pleurotus florida due to which they possess different pharmacological activities and also with a goal of increasing awareness of the beneficial effects of edible mushrooms. Aqueous extract of Pleurotus florida fruiting bodies was prepared by decoction in hot water. This was further studied for their Organoleptic, physiochemical, phytochemical, powder characteristics. Less ash value showed a minimum level of contamination, extractive value indicated the level of adulteration, Average particle size was determined by sieving method, phytochemical screening was performed using standard chemical reagents and presence of carbohydrates, alkaloids, steroids, flavanoids, tannins and phenols were confirmed.
KEYWORDS: *Pleurotus florida*, pharmacognostic study, secondary metabolites.

INTRODUCTION
Mushrooms have been used from time immemorial for their medicinal and nutritional values. Their secondary metabolites are refined which produce therapeutic activities in plants. An edible mushroom *Pleurotus florida* has been used here. Aqueous extract of *Pleurotus florida* fruiting bodies was prepared by decoction in hot water. Their Organoleptic, physiochemical, phytochemical, powder characteristics were studied.

METHODOLOGY

**Preparation of the extract**[1]: 10 gram of fruiting bodies of *Pleurotus florida* was treated with 100ml hot water and heated at 100 degree Celsius for 3 hours, filtered and filtrate evaporated to dryness.

1. **Organoleptic properties**[2]
   Colour: Cream
   Texture: Soft
   Odour: Pungent
   Taste: Bland

2. **Phytochemical screening**[3,4]
   Phytochemical screening for the identification of various phytoconstituents such as alkaloids, carbohydrates, steroids, cardiac glycosides, flavonoids, carbohydrates, amino acids, phenolics, naphthoquinones and tannins according to standard methods were performed.

   **Test for carbohydrate**
   - Molisch test: A small quantity of the extracts was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch’s reagent and formation of brick red colour confirmed the presence of reducing sugar.
   - Fehling’s test: Equal volume of Fehling A (coppersulphate in distilled water) and Fehling B (potassium tartrate and sodium hydroxide in distilled water) reagents were mixed with few drops of crude extract is added and boiled, a brick red precipitate of cuprous oxide forms, if reducing sugar are present.
**Test for glycosides**
- Borntrager’s test: 200 mg crude extract was mixed with 2 ml of dilute sulphuric acid and 2 ml of 5% aqueous ferric chloride solution, boiled for 5 minutes which lead to oxidation to anthraquinones, indicating the presence of glycosides.

**Test for Alkaloids**
- Mayer’s test: Crude extract was mixed with Mayer’s reagent (potassium mercuric iodide solution). Cream colour precipitate was formed, indicating the presence of alkaloids.
- Dragendorff’s test: Crude extract was mixed with Dragendorff’s reagent (potassium bismuth iodide solution). Reddish brown precipitate was formed which suggested the presence of alkaloids.
- Wagner’s test: Filtrates were treated with Wagner’s reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

**Test for Flavanoids**
- Alkaline reagent test: Crude extract was mixed with few drops of sodium hydroxide solution. An intense yellow colour was formed. Yellow colour turned to colorless on addition of few drops of diluted acid, marked the presence of flavanoids.
- Lead acetate test: To a solution of 0.5 g extract in water, about 1 ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

**Test for Saponins**
- Froth test: 0.5g extracts were dissolved in 10ml of distilled water for about 30 seconds. The test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over 30 minutes period of time. If a “honeycomb” froth above the surface of liquid persists after 30 minutes the sample is suspected to contains saponin.

**Test for Tannins**
- Ferric chloride test: Crude extract was mixed with ferric chloride. Blue green colour appeared, suggested the presence of tannins.
3. Physico-chemical studies\[5\]

Determination of ash value

Determination of total ash: 2-4g of the sample was weighed in a crucible and was spread evenly and ignited slightly increasing the temperature to 500-600 degree Celsius until it turns white, it was cooled in a dessicator and weighed.

Determination of acid insoluble ash: 25 ml of dilute hydrochloric acid was added into the total ash, covered with a watch glass and ignited for 5 minutes. It was filtered, washed with hot water until neutral and filter paper was dried and the ash was transferred back to the crucible, dried on a hot plate and was weighed.

Determination of water soluble ash: 25 ml of water was added into the total ash, covered with a watch glass and ignited for 5 minutes. It was filtered, washed with hot water until neutral and filter paper was dried and the ash was transferred back to the crucible, dried on a hot plate and was weighed.

Percentage ash value= \[\frac{\text{Initial weight taken} \times 100}{\text{Weight of ash}}\]

Determination of extractive value

Water soluble extractive value: 5 grams of the coarse drug was macerated with 100ml of water for 24 hours with occasional shaking for the first 6 hours and then left aside for 18 hours. It is filtered taking precautions to avoid loss of solvent. It is evaporated in a flat container at 105 degrees until constant weight. The percentage of water soluble extractive value was calculated with reference to the air dried drug.

Alcohol soluble extractive value: 5 grams of the coarse drug was macerated with 100ml of alcohol for 24 hours with occasional shaking for the first 6 hours and then left aside for 18 hours. It is filtered taking precautions to avoid loss of solvent. It is evaporated in a flat container at 105 degrees until constant weight. The percentage of water soluble extractive value was calculated with reference to the air dried drug.

4. Estimation of tannins.\[6\] The method of Okeke and Elekwa was used here for the determination using 5 g sample shaken with 50ml of water and left to stand for 30 minutes. The solution was filtered and 2ml of the filtrate was introduced into a test tube and 3ml of 0.1 M Ferric chloride and 2ml of potassium ferrocyanide were added. To this 46 ml of water was
added. It was filtered again and 1ml of the filtrate was used to read the absorbance at 710nm within 10 min.

5. Powder analysis

Sieve size/powder fineness: 10 g of the sample was accurately weighed and passed through various sieves numbered 16, 22, 44, 100 and shaken for 20 minutes successively and the powder remaining on each sieve was weighed and average particle size was determined.

6. Thin Layer Chromatography: 100 grams of silica gel G was dissolved in sufficient amount of water and was coated on the glass plate. Solvent system chosen was benzene: methanol: aceton in the ratio 6:2:2. Aqueous extract was dissolved in sufficient water to make up a concentration of 1 mg per ml. Similarly methanolic extract was dissolved in methanol. The spots were made 1 cm from the bottom of the glass slide. The glass plate was kept into the chamber after chamber saturation and allowed to run upto 2-3rd of the glass plate. Rf value was calculated.

\[ R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}} \]

RESULTS

• Preliminary phytochemical screening

Table 1

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Tests</th>
<th>Presence/Absence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Molischs</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehlings</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Dragendorffs Test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagners Test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mayers Test</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>Ferric Chloride Test</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Froth Formation Test</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric Chloride Test</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>Alkaline Test</td>
<td>+</td>
</tr>
</tbody>
</table>

• PARTICLE SIZE

Table 2

<table>
<thead>
<tr>
<th>Sieve size</th>
<th>Pore size</th>
<th>Weight of powder(n)</th>
<th>n/gx100</th>
<th>Cumulative frequency</th>
<th>Mean pore diameter(d)</th>
<th>nd</th>
<th>Total=4</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>1000</td>
<td>3.2785</td>
<td>32.785</td>
<td>32.785</td>
<td>855</td>
<td>2803.175</td>
<td>412.091</td>
</tr>
<tr>
<td>22</td>
<td>710</td>
<td>1.7638</td>
<td>17.638</td>
<td>50.423</td>
<td>532.5</td>
<td>939.2235</td>
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</tr>
<tr>
<td>44</td>
<td>355</td>
<td>1.6820</td>
<td>16.820</td>
<td>67.243</td>
<td>252.5</td>
<td>424.705</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>150</td>
<td>3.2665</td>
<td>32.665</td>
<td>32.665</td>
<td>75</td>
<td>244.9875</td>
<td></td>
</tr>
<tr>
<td>Total=9.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Average particle size=$\sum_{n} n d = 441.2091g$

- **TLC**

  Rf value = $\frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}$

  Rf value of aqueous extract = $\frac{2}{5} = 0.4$

  Rf value of methanolic extract = $\frac{4.5}{5.5} = 0.8181$

**DISCUSSION**

- The aqueous extract showed positive tests for carbohydrates, proteins, tannins, phenols, alkaloids, glycosides indicating their presence.
- The water soluble extractive value was found to be 8.8%
- The alcohol soluble extractive value was found to be 9.5%
- Average particle size = 441.2091g
- Water soluble ash value = 5.49
- Acid insoluble ash value = 3.08
- Rf value of aqueous extract = 0.4
- Rf value of methanolic extract = 0.8181
CONCLUSIONS
Preliminary phytochemical screening showed the presence of active constituents necessary for the pharmacological activity. Pharmacognostic study revealed the Ash values, extractive value and Particle size and helped to determine the average particle size, level of contamination and adulterants. Less extractive value indicates presence of exhausted materials, adulteration. High ash is indicative of contamination, substitution or carelessness in preparing the formulation. Rf value of both the extracts stands within the standard limits.

REFERENCE
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2. Trease and Evans, Pharmacognosy, 425