PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF MADHUCA ASWAGANDHADI CHOORNA - AN AYURVEDIC POLYHERBAL FORMULATION

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ABSTRACT

There are many traditional systems of medicine in the world, each with different associated philosophies and cultural origins. Ayurveda is one of the traditional medicinal systems of Indian. Due to the scientific advancement today, more and more pharmacologically active ingredients of the Ayurvedic medicines as well as their usefulness in drug therapy have been identified. Basically, it is the phytochemical constituent in the herbs which lead to the desired healing effect, such as saponins, tannins, alkaloids, alkenyl phenols, flavonoids, terpenoids, phorbol esters and sesquiterpenes lactones. A single herb may even contain more than one of the aforementioned phytochemical constituents, which works synergistically with each other in producing pharmacological action. In Ayurveda, single or multiple herbs (polyherbal) are used for the treatment. In this formulation of polyherbal formulation of Mwdhuka Aswagandhadi Choorna (Received from Kerala Ayurveda Limited, Athani, Ernakulam), Glycyrrhiza glabra L., Withania somnifera Dun., and Triphala are used. Preliminary quantitative analysis of the drug showed the presence of the alkaloids, proteins, tannins, phenolic compounds, athraquinon and saponin glycosides and carbohydrates. High extractive value indicates absence of exhausted materials, adulteration or incorrect processing during drying or storage of formulation. A low
ash value is indicative of absence of contamination, substitution, adulteration or carelessness in preparing the formulation. The microscopic analysis of the drug showed the presence of disturbed pitted vessels, disturbed walls of fibres, starch grains, scleroids, in Yastimadhu; stone cells, scleroids, tannin in Bibheetaki; tannin, pitted stone cells in Haritaki; scleroids in Amlaki.

**KEYWORDS:** Polyherbal, Madhuka aswagandhadi choorna, phytochemical, pharmacological evaluation.

**METHODOLOGY**

1. **Organoleptic properties**
   Colour: Light Brown.
   Odour: odourless.
   Taste: Sweet and Astringent.
   Texture: Soft.
   Touch: Smooth.

2. **Phytochemical screening**\(^{[1,2]}\)
   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 1ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

**Test for Proteins**

- **Millions test**
  3ml extracts were dissolved in 10ml of millions reagent. White precipitate is formed which on warming the white precipitate dissolves giving red coloured solution.

- **Biurette test**
  3ml of the extract was mixed with 4% sodium hydroxide and few drops of 1% copper sulphate solution were added, violet or pink colour not appeared. To 3ml of extract few drops
of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple colour. On addition of alkali, it becomes dark violet.

**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$^{[3,5]}$

**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 = \[
\frac{\text{Initial weight taken x 100}}{\text{Weight of ash}}
\]

**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.

Sieve size/powder fineness: 75 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[^5]
100 gram of silica gel G was dissolved in sufficient amount of water and was coated on the glass plate. Solvent system chosen was toluene: ethyl acetate: acetic acid (7:2:1) ethanolic extract was dissolved in sufficient ethanol to make up a concentration of 1mg/ml. The spots were made 1 cm from the bottom of the glass slide. The glass plate was kept in to the chamber after chamber saturation and allowed to run 2-3rd of the glassplate. Rf value was calculated.

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

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>PROTEINS</td>
<td>MILLIONS TEST</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>BIURETTE TEST</td>
<td>+</td>
</tr>
<tr>
<td>TANNINS</td>
<td>FERRIC CHLORIDE TEST</td>
<td>+</td>
</tr>
<tr>
<td>FLAVANOIDIS</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=</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>1000</td>
<td>9.3502</td>
<td>12.485</td>
<td>12.485</td>
<td>855</td>
<td>7994.421</td>
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<tr>
<td>22</td>
<td>710</td>
<td>5.1700</td>
<td>6.903</td>
<td>19.388</td>
<td>532.5</td>
<td>2753.025</td>
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</tr>
<tr>
<td>44</td>
<td>355</td>
<td>21.6305</td>
<td>28.883</td>
<td>48.271</td>
<td>252.5</td>
<td>5461.701</td>
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</tr>
<tr>
<td>100</td>
<td>150</td>
<td>23.2204</td>
<td>31.006</td>
<td>79.277</td>
<td>75</td>
<td>1741.53</td>
<td></td>
</tr>
<tr>
<td>Fine powder</td>
<td></td>
<td>15.520</td>
<td>20.723</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>239.3423</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average particle size=$\frac{\sum nd}{\sum n} = 239.3423g.$

RESULT OF THIN LAYER CHROMATOGRAPHY

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Ethanol extract toluene: ethyl acetate:acetic acid(7:2:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance travelled by the spot 1</td>
<td>3.4cm</td>
</tr>
<tr>
<td>Distance travelled by spot 2</td>
<td>2.5cm</td>
</tr>
<tr>
<td>Distance travelled by the solvent front</td>
<td>4.4 cm</td>
</tr>
<tr>
<td>Rf value of spot 1</td>
<td>0.77</td>
</tr>
<tr>
<td>Rf value of spot 2</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Photography of ethanolic extract of the polyherbal formulation of madhuka aswagandhadi choorna.
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 10.26%.
- The alcohol soluble extractive value was found to be 14.04%.
- Average particle size= 239.3423g.
- Water soluble ash value= 7.97.
- Acid insoluble ash value= 7.58.
- Rf value of spot 1 = 0.77.
- Rf value of spot 2 = 0.56.

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 spot 1 & 2 stand within the range of standard Rf values of flavonoids and phenolic compounds.

REFERENCE