Development of Fingerprinting Methods of Balacaturbhadrika Churna: An Ayurvedic Formulation

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ABSTRACT

Balacaturbhadrika churna is an Ayurvedic formulation widely used in diarrhoea, fever, cough and asthma. The present article is an attempt to establish the scientific basis of one of the reputed Ayurvedic formulation. Investigations were carried out to study the physicochemical, phytochemical and spectrophotometric analysis of formulation. The values of percent loss on drying, angle of repose, Hausner ratio, Carr’s index of the lab formulation were calculated as 6.84 ± 0.224, 27.36, 1.25 and 20 respectively, which indicates the moisture contents of formulation is within the range and depict good flow characteristics. Total ash, acid insoluble ash and water soluble ash were found 8.148 ± 0.337, 3.281 ± 0.286, and 45.602 ± 0.414 respectively; the value of total ash indicates the inorganic contents of formulation are below the standard limits, above stated results were also compared with marketed formulation. Alcoholic and aqueous extracts of formulations and ingredients were prepared and evaluated for phytochemical analysis and the results of extractive values shows higher alcoholic extractive value (39.294±2.226) of formulation depict that alcohol is a better solvent for extraction. Three laboratory batches of formulation and Piper longum powder were estimated for their piperine content against standard piperine solution on double beam UV-Visible spectrophotometer at λ max 342.5 nm.

Key words: Balacaturbhadrika churna, physicochemical properties, phytochemical properties, spectrophotometric analysis.

INTRODUCTION

In the past decade, there has been renewed attention and interest in the use of traditional medicine (Ayurveda, Yoga, Naturopathy, Unani, Siddha and Homeopathy) in India and globally. Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain [1]. The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effects of the phytochemicals in them [2-3]. The utilization of plants for medicinal purposes in India has long history, and the proportion of medicinal plants is the highest proportion of plants known for their medicinal purposes in any country of the world for the existing flora of the respective country. Medicinal plants are essential natural resource which constitutes one of the potential sources of new products and bioactive compounds for drug development [4]. It is estimated that 80% of the population in rural India use medicinal plants to meet primary health care needs [5]. Continuous erosion in the traditional knowledge of many valuable plants for medicine in the past and the renewal interest currently, the need existed to review the valuable knowledge with the expectation of developing the medicinal plants sector [6]. Under the parasol of traditional medicine systems the Ayurvedic system of medicine also gaining global acceptance due to its amazing clinical efficiency. While Ayurvedic systems of medicines have long been used, there is negligible documented evidence regarding its safety and effectiveness. The lack of evaluation has, in turn, slowed down the development of regulations and legislation. Recently Good Manufacturing Practices (GMP) rules for Ayurvedic medicines to ensure the quality of the manufactured drugs and gain credibility to make them acceptable globally. The Drugs and Cosmetic Act 1940 controls the standards of manufacturing, sale and distribution of Ayurvedic drugs [7]. Balacaturbhadrika churna is a fine powder form, which is widely used in Diarrhoea, Fever, Cough and Asthma at dose of 500 mg to 1 gm/day [8]. It is composed of Ghana (musta), Krsna (pippali), Aruna (ativisa) and Sringi (karkatasringi). All the ingredients are firstly powdered separately and mixed together.

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In the present study physicochemical, phytochemical and spectrophotometric evaluation of the Ayurvedic formulation Balacaturbhadrika churna and its ingredients has been carried out because these evaluations are amazingly uncharted till date and determination of these parameters are incredibly essential to assure the quality, safety and efficacy of this formulation.

MATERIALS AND METHODS

Materials

All the plant materials such as Ghana (*Cyperus rotundus*), Pippali (*Piper longum*), Ativisa (*Aconitum heterophy*) and Sringi (*Pistacia integerrima*) were purchased from the local market of Raipur, C.G. and identified morphologically and microscopically and compared with standard pharmacopoeial monograph. All the reagents and solvents used were of analytical grade. The ash values, extractive values with various reagents and were determined as per the WHO guidelines [9].

Preparation of formulation

Formulation was strictly prepared as prescribed in the official book of Ayurvedic Formulary of India (2003) [8]. 50 grams of each ingredient which includes Ghana (musta), Krsna (pippali), Aruna (ativisa) and Sringi (karkatasringi) were taken. All the ingredients were weighed accurately and made fine powder by passing through sieve no. 80. Fine powders were mixed geometrically in plastic tray and packed in plastic container.

Determination of ash values

Total ash

4gm of the each powdered material was accurately weighed and placed in a previously ignited and tared silica crucible. The material is spread in an even layer and ignited by gradually increasing the heat to a temperature of 500-600°C until it is white, indicating the absence of carbon. The material is Cooled in a desiccators and weighed. The content of total ash is calculated in mg per gm of air-dried material.

Acid-insoluble ash

To the crucible containing the total ash, 25 ml of hydrochloric acid is added, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass is rinsed with 5 ml of hot water and this liquid is added to the crucible. The insoluble matter is collected on an ash less filter-paper and washed with hot water until the filtrate is neutral. The filter-paper containing the insoluble matter is transferred to the original crucible, dried on a hot-plate and ignited to constant weight. The residue is allowed to cool in a suitable desiccator for 30 minutes, and then weighed without delay. The content of acid-insoluble ash is calculated in mg per gm of air-dried material.

Extractive values

The extractive values were recorded in alcohol and water with a view to study the distribution of various constituents of Balacaturbhadrika churna, and all raw ingredients of formulation. Accurately weighed 4.0g of coarsely powdered air-dried material was placed in a glass-stoppered conical flask and macerated with 100ml of the solvent for 6 hours, shaking frequently, and then allowed to stand for 18 hours. The mixture is filtered rapidly taking care not to lose any solvent. 25 ml of the filtrate is transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. The residue is dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay.

Qualitative phytochemical studies

To detect the presence of various phytoconstituents in formulation as well as in raw materials phytochemical investigations were performed. The tests were performed on alcohol and water extracts. Qualitative phytochemical analyses were done for Balacaturbhadrika churna and all the raw ingredients of formulation [10,11]. Alkaloids, carbohydrates, glycosides, tannins and phenolic compounds, flavonoids, fixed oils, saponins, proteins and amino acids and steroids.

Bulk density

A sample of about 50 cm3 of each powdered ingredients that was previously passed through a U.S. Standard no. 20 sieve is carefully introduced into a 100 ml graduated cylinder. The cylinder is dropped at 2-sec intervals on a hard wooden surface three times from a height of 1 inch. The bulk density is then obtained by dividing the weight of the sample in gm by the final volume in cm3 of the sample contained in the cylinder.

Tap density

A sample of about 50 cm3 of each powdered ingredients that was previously passed through a U.S. Standard no. 20 sieve is carefully introduced into a 100 ml graduated cylinder [12]. The cylinder is dropped at 2-sec intervals on a hard wooden surface hundred times from a height of 1 inch until no further decrease in the volume of powder takes place. The tap density is then obtained by dividing the weight of the sample in gm by the final volume in cm3 of the sample contained in the cylinder.
**Angle of repose**

A glass funnel is held in place with a clamp on ring support over a glass plate. The glass plate is placed on a micro-lab jack. Approximately 100 g of powder is transferred in to the funnel (that was previously passed through a number 10 mesh size), keeping the orifice of funnel blocked by the thumb. As the thumb is removed, the lab –jack is adjusted so as to lower the plate and maintain about 6.4 mm gap between the bottom of funnel stem and top of the powder pile. When the powder is emptied from the funnel, the angle of the heap to the horizontal plane is measured with a protector \(^{[18]}\). Measure the height of the pile \((h)\) and the radius of the base \((r)\) with the ruler. The angle of repose is thus estimated by following formula.

\[
\Phi = \tan^{-1}\left(\frac{h}{r}\right)
\]

**Hausner Ratio**

The Hausner ratio is calculated by the formula given below, where \(\rho_B\) is the freely settled bulk density of the powder, and \(\rho_T\) is the tapped density of the powder \(^{[13]}\).

\[
H = \frac{\rho_T}{\rho_B}
\]

**Carr index**

The Carr index is an indication of the compressibility of a powder. It is calculated by the following formula, where \(\rho_B\) is the freely settled bulk density of the powder, and \(\rho_T\) is the tapped density of the powder \((Gibson, 2001)\).

\[
C = 100 \times \left(1 - \frac{\rho_B}{\rho_T}\right)
\]

**Preparation of calibration curve for Piperine**

Standard solutions of piperine were prepared within the concentration range 2-10 µg/ml in 10 ml volumetric flasks \(^{[13]}\). The absorbance of the piperine solution is measured at 342.5 nm (Figure 1) against ethanol and a calibration curve plotted (Figure 2).

**RESULTS AND DISCUSSION**

**Physicochemical properties**

Table-I shows the moisture content of Cyperus rotundus, Piper longum, Aconitum heterophy, Pistacia integerrima, lab formulation and marketed formulation were found respectively. The moisture content of formulation was within acceptable range (5-8%) thus implying that the formulation can be stored for a long period and would not easily be attacked by microbes. Physical properties namely tapped density, bulk density, angle of repose, hausner ratio and carr’s index were calculated for Lab formulation, marketed formulation and its raw materials. The value of angle of repose for raw materials Cyperus rotundus, Piper longum, Aconitum heterophy, Pistacia integerrima, lab formulation and marketed formulation were 28.62, 31.28, 29.86, 24.34, 27.36, and 27.45 respectively which shows good flow properties of prepared lab formulation. The flow properties are also confirmed by Hausner’s ratio and Carr’s index (Table-I). Values of Hausner’s ratio less than 1.25 indicate good flow (20% Carr Index) and the value greater then 1.25 indicates poor flow (33% Carr Index) \((Gupta et al 2010)\). Both parameters were determined for prepared Ayurvedic formulation and it was found 1.25 and 20% respectively and indicates good flow characteristics.

**Phytochemical analysis**

Results of the phytochemical screening of the raw materials, lab formulation and marketed formulation of Balacaturbhadrika churna are concluded in Table-II. One notable difference as a result of methods of extraction is the possibility that the alkaloids in Piper longum and Pistacia integerrima are more soluble in ethanol, the reason why the presence of that group was not detectable in the aqueous extract. Furthermore, where more than one test was conducted for the detection of a
Chemical group such as the alkaloids, no differences in the results were observed for the different tests.

Out of the nine phytochemical groups investigated, seven namely carbohydrate, glycosides, tannins, flavonoids, fixed oil and proteins were detected in the ethanolic extract of lab and marketed formulations however the aqueous extracts of both formulations shows the presence of saponins with previously stated seven phytochemical groups. Steroids were absent in all the ingredients and formulations for both methods of extraction.

**Determination of Ash value**

Total ash value of *Cyperus rotundus, Piper longum, Aconitum heterophy, Pistacia integerrima*, lab formulation and marketed formulation were 7.346±0.346, 5.032±0.624, 2.981±0.243, 4.621±0.334, 8.148±0.337 and 19.633±0.552 respectively (Table-III). The value of total ash in marketed formulation is comparatively high in comparison to lab formulation may be because of the higher amounts of inorganic components present in marketed formulation. Acid-insoluble ash value of prepared lab formulations were 3.281 ± 0.286 and 5.041 ± 0.368 for lab and marketed formulation respectively shows that a small amount of the inorganic component is insoluble in acid it indicates adulteration of raw ingredients by substance like silica, rice husk is very less in both formulation. Low acid-insoluble ash value may also affect amount of the component absorbed in the gastrointestinal canal when taken orally.

**Determination of extractive values**

Alcohol-soluble and water soluble extractive values of ingredients and formulation are depicted in Table-IV, which shows 39.294 ± 2.226 and 30.662 ± 0.472 alcohol-soluble extractive value for lab and marketed formulation respectively which is higher than water soluble extractive value of both chemical group such as the alkaloids, no differences in the results were observed for the different tests.

### Table 1: Physicochemical parameters of Balacaturbhadrika churna and its raw material

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Name</th>
<th>% LOD</th>
<th>Tap density</th>
<th>Bulk density</th>
<th>Angle of repose</th>
<th>Hausner ratio</th>
<th>Carr’s index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CR</td>
<td>6.32±0.268</td>
<td>0.62</td>
<td>0.50</td>
<td>28.62</td>
<td>1.24</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>PL</td>
<td>7.78±0.642</td>
<td>0.66</td>
<td>0.52</td>
<td>31.28</td>
<td>1.26</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>AH</td>
<td>5.29±0.382</td>
<td>0.60</td>
<td>0.50</td>
<td>29.86</td>
<td>1.20</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>PI</td>
<td>8.02±0.196</td>
<td>0.52</td>
<td>0.41</td>
<td>24.34</td>
<td>1.26</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>LF</td>
<td>6.84±0.224</td>
<td>0.50</td>
<td>0.40</td>
<td>27.36</td>
<td>1.25</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>MF</td>
<td>5.88±0.292</td>
<td>0.62</td>
<td>0.52</td>
<td>27.45</td>
<td>1.19</td>
<td>16</td>
</tr>
</tbody>
</table>

CR (*Cyperus rotundus*), PL (*Piper longum*), AH (*Aconitum heterophy*), PI (*Pistacia integerrima*), LF (lab formulation), MF (Marketed formulation)

### Table 2: Phytochemical characterization of ethanolic and aqueous extracts of Balacaturbhadrika churna and its raw materials

<table>
<thead>
<tr>
<th>Test</th>
<th>CR</th>
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<th>AH</th>
<th>PI</th>
<th>LF</th>
<th>MF</th>
<th>CR</th>
<th>PL</th>
<th>AH</th>
<th>PI</th>
<th>LF</th>
<th>MF</th>
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<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Tannins and phenolic compounds</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Flavonoids</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>+</td>
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<td>Fixed oil</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteins and amino acids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Steroids</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

CR (*Cyperus rotundus*), PL (*Piper longum*), AH (*Aconitum heterophy*), PI (*Pistacia integerrima*), LF (lab formulation), MF (Marketed formulation)

### Table 3: Percentage ash value of Balacaturbhadrika churna and its raw materials

<table>
<thead>
<tr>
<th>SN</th>
<th>Name</th>
<th>Total ash (% w/w)</th>
<th>Acid insoluble ash (% w/w)</th>
<th>Water soluble ash (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CR</td>
<td>7.346 ± 0.346</td>
<td>0.756 ± 0.031</td>
<td>13.431 ± 0.387</td>
</tr>
<tr>
<td>2</td>
<td>PL</td>
<td>5.032 ± 0.624</td>
<td>1.302 ± 0.346</td>
<td>23.163 ± 0.736</td>
</tr>
<tr>
<td>3</td>
<td>AH</td>
<td>2.981 ± 0.243</td>
<td>1.324 ± 0.078</td>
<td>38.263 ± 0.642</td>
</tr>
<tr>
<td>4</td>
<td>PI</td>
<td>4.621 ± 0.334</td>
<td>2.418 ± 0.249</td>
<td>18.725 ± 0.354</td>
</tr>
<tr>
<td>5</td>
<td>LF</td>
<td>8.148 ± 0.337</td>
<td>3.281 ± 0.286</td>
<td>45.602 ± 0.414</td>
</tr>
<tr>
<td>6</td>
<td>MF</td>
<td>19.633 ± 0.552</td>
<td>5.041 ± 0.368</td>
<td>51.403 ± 0.223</td>
</tr>
</tbody>
</table>

CR (*Cyperus rotundus*), PL (*Piper longum*), AH (*Aconitum heterophy*), PI (*Pistacia integerrima*), LF (lab formulation), MF (Marketed formulation)
formulations. Higher ethanol-soluble extractive value implies that ethanol is a better solvent of extraction for the formulation than water.

**Spectrophotometric analysis of piperine**

The determination of formulations was carried out through UV spectrophotometer at 342.5 nm for piperine. The absorbance characteristics show that piperine follow Beer Lambert’s law within the concentration range 2-10 µg/ml at the λ-max of 342.5 nm. The estimation of piperine content of the balchaturbhadrika churna and powder of Piper longum (Pippali) was carried out separately. The concentration of piperine content in raw material was found to be 0.695 ± 0.012 % w/w in Piper longum. The content of piperine in different batches of balchaturbhadrika churna was found to be 0.392 ± 0.030 %, 0.363 ± 0.001 %, 0.382 ± 0.004 % and 0.233 ± 0.002 % w/w respectively for lab formulation (LF-1, LF-2, LF-3) and marketed formulation (MF) (Table-V). The developed method was found to be reliable, accurate, precise and sensitive.

**CONCLUSION**

WHO has emphasized the need to ensure quality control of Ayurvedic formulations by using modern technique and by applying suitable parameters and standards (WHO, 2007). It is the cardinal responsibility of the regulatory authorities to ensure that the consumers get the medication, with purity, safety, potency and efficacy. As prescribed by the WHO, evaluations of physicochemical and phytochemical properties are essential to standardize the different Ayurvedic formulations. In this connection authors investigated the stated parameters of an Ayurvedic formulation Balacaturbhadrika churna, which is amazingly unexplored till date. At the same time widely used by the ayurvedic medical practitioner for the treatment of various abdominal disorders. These explorations definitely help to set a standard of this traditional medicine.

**REFERENCES**