STANDARDIZATION, PREPARATION AND EVALUATION OF AN AYURVEDIC POLYHERBAL FORMULATION IN CAPSULE DOSAGE FORM SUITABLE FOR USE IN CLINICAL TRIALS

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ARTICLE INFO

Article history
Received 14/10/2014
Available online
30/10/2014

Keywords
Standardization,
Ayurvedic Formulation,
Elemental Analysis.

ABSTRACT

Application of modern scientific tools and techniques is important for the quality evaluation and standardization of polyherbal formulations. Herbal formulations that are being prepared on traditional methods lack quality and batch-to-batch consistency. The present study aimed to develop an ayurvedic polyherbal extract into a capsule dosage form, evaluating its physiochemical, phytochemical, formulation parameters and setting up its quality control standards. Hard gelatin capsules for oral administration were prepared by lyophilizing the traditional liquid dosage form of the standardized extract. The formulation was then characterized as per pharmacopoeial standards. The variation of weight among the capsules was least, which showed a good ratio of excipients in the formulation with dissolution of 92%. The current work revealed that encapsulation of the lyophilized extract resulted in the concealing of bitter taste. It can be concluded that these parameters can be conveniently used to check the quality control of various herbal formulations thereby paving a way for their pharmacological activities.

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INTRODUCTION

Ayurveda is one of the oldest Indian systems of medicine that has been well recognized and practiced since 1500 B.C [1]. According to World Health Organizations’ (WHO) estimate, around 65% of the people in India use Ayurvedic medicine as an alternative treatment of various ailments [2]. The importance of medicinal plants is appreciated worldwide, in fact in United States herbal medicine represents a market of approximately $180 billion [3]. This growing popularity and recognition of Ayurveda worldwide, raises the concern regarding the quality assurance, toxicity, formulations, interactions, safety and efficacy of the ayurvedic medicines. Plant based medicines have poor quality control and high content variability due to the inconsistency in the method of preparation, poor quality of traditional dosage forms and the discrepancy in the phytochemical constituents [4]. For instance, in the traditional preparations, many plants are proposed to be effective if they are given in decoction form, which may lead to variations in dose each time a treatment is prepared.

Due to poor quality control, it is not possible for most of the herbal drugs to prove their effectiveness in clinical practice. Additionally, the practical problems associated with the preparation, storage, unpleasant taste and odor acts as an obstacle and interferes with the pharmacological activity of these traditional dosage forms. Considering the clinical use of these drugs, the focus has been shifted to the ease of medication rather than the traditional dosage form, which reduces the patient compliance [5].

In our previous study a combination (Diabcap) extracted from Berberis aristata, Cyperus rotundus, Cedrus deodara, Emblica officinalis, Terminalia chebula and Terminalia bellerica has shown a good antihyperglycaemic activity in vivo [6]. Ethno medically, the preparation was prescribed for diabetes mellitus in the form of decoction [7]. This traditional liquid dosage form has several disadvantages like shelf life of decoction as per literature is three hours only [8] making it prone to physical, chemical and microbiological instability and it is also bitter in taste. Development of this preparation into a suitable drug delivery system in the form of capsule was sought to be of appropriate pharmacopoeial quality and would have similar release profiles of the actives as that of the traditional dosage form.

The present investigation was carried out to standardize the extract and formulate a suitable dosage form (capsule dosage form).

MATERIALS AND METHODS

Plant materials

The fruits of Emblica officinalis, Terminalia chebula, Terminalia bellerica, rhizome of Cyperus rotundus were collected from the farm of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow. Heartwood of Cedrus deodara and roots of Berberis aristata, collected locally and all the plant material were authenticated by department of Botany CSIR-CIMAP, Lucknow. Specimen of the plants collected were preserved in the Herbal Medicinal Products Department, CSIR-CIMAP, Lucknow (Specimen #: HMPD/2010Di-201; HMPD/2010Di-202; HMPD/2010Di-203; HMPD/2010Di-204; HMPD/2010Di-205; HMPD/2010Di-206).

Preparation of dried aqueous extract and standardization

The plant materials were separated from earthy and other foreign material; shade dried and powdered using a mill. Polyherbal mixture was prepared using the above mentioned six ingredients in equal ratio [7]. The extraction procedure resembled the method mentioned in the literature for the preparation of traditional dosage form. 100 g of polyherbal mixture was taken in 800ml water and a decoction was prepared as per Sarangdhar Samhita [9]. Decoction was filtered through muslin cloth to obtain 200 ml of aqueous extract, concentrated under vacuum using rotatory evaporator (Buchi) and lyophilized using lyophilizer (Labconco, Biogentek BG Pvt. Ltd.) at -80°C for 48 hours in order to remove the water content and obtain the extract in dry solid form. The extract was then standardized as per WHO guidelines of quality standardization [10] and Ayurvedic Pharmacopoeia of India (API) [11].

Phytochemical screening of freeze-dried aqueous extract

Phytochemical composition in the lyophilized powder was estimated following the standard methods [12]. Alkaloids were determined with Dragendorff’s reagent, cardiac glycosides with Liebermann’s test, flavonoids with magnesium and hydrochloric acid, tannins with ferric chloride, terpenoids with chloroform and sulphuric acid, steroids with Liebermann-Burchard reaction.

Physicochemical properties

Particle size distribution

The size distribution of the polyherbal mixture was measured with the help of Mastersizer (Malvern Instruments Ltd., Malvern, UK) by Laser diffraction method [13].

Bulk Density (D0)

25 g of accurately weighed powder was poured into a graduated cylinder, powder bed was made uniform without disturbing the cylinder and the volume was measured directly from the graduation mark on the cylinder as ml [14]. The volume measure was called as bulk volume and bulk density is calculated as

\[
\text{Bulk Density (D0)} = \frac{\text{weight of powder}}{\text{Bulk volume}}
\]

Tap Density (Df)

After measuring D0 same cylinder was set to measure tap density [14]. The cylinder was tapped with 100 tap drop/minute and operated for 500 taps. Volume was noted as V_a, tapping was done again for 750 times and final volume was noted as V_b. The
difference between $V_a$ and $V_b$ was calculated and when it was found to be not more than 2 %, then $V_b$ was considered as final tapped volume and tapped density was calculated using the following formula

$$\text{Tapped density} = \frac{\text{weight of powder}}{\text{Tapped volume}}$$

**Angle of repose**

To determine the angle of repose the powder was passed through the walls of a funnel, fixed at a definite position. The powder was poured till the upper tip of the pile surface touched the lower most end of the funnel and the angle of repose was calculated [14],

$$\theta = \tan^{-1}\left(\frac{h}{r}\right)$$

Where $\theta$ is the angle of repose, $h$ is the height in cm and $r$ is the radius in cm.

**Haussner’s ratio and Carr’s index**

Haussner’s ratio and carr’s index was also calculated with the help of bulk density and tap density using the following formulae [14]

$$\text{haussner's ratio} = \frac{D_f}{D_0}$$

$$\text{carr's index} = \frac{D_f - D_0}{D_f} \times 100$$

**Heavy metal analysis**

Heavy metals were analyzed in the formulation by means of Inductively Coupled Plasma-Mass spectrophotometer [15]. 2 g of the powdered sample was digested with nitric acid (HNO3) and perchloric acid (HClO4) in 1:3 ratio. 0.2 g of digested sample was dissolved in 50 ml of distilled water and analyzed on Inductively Coupled Plasma-Mass (ICP) spectrophotometer (Perkin Elmer Optima 5300V).

**Microbial contamination test**

Microbial tests were carried out to estimate the number of viable aerobic microorganisms present in the polyherbal formulation [16] which includes *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, yeasts and moulds, total microbial plate count.

**Formulation of hard gelatin capsules**

The development of the formulation for the present study was based on a trial and error method. The yield of lyophilized extract obtained from the traditional liquid dosage form was used to calculate the amount of active ingredient to be incorporated in the capsule. The prescribed maximum recommended dose of decoction is up to 80 ml [17,18]. Lyophilizing the filtrate of 80 ml decoction produced approximately 3 g of dried aqueous extract. As a result 3 g was decided as the maximum single dose. For patient compliance, this 3 g was divided into three doses of 500 mg capsule (2 capsules ter in die). Since prescription is usually based on the individuals’ respond, therefore 500 mg could be considered as a starting dose in clinical study.

Based on the above findings it was decided that 500 mg capsules were prepared by mixing the lyophilized aqueous extract with Avicel PH 101 in geometrical manner followed by the addition of required quantity of Aerosil® 200 and magnesium stearate (passed through sieve no. 80). The mixture was blend and mixed thoroughly. The quantity of excipients selected was based on the preformulation study and dosage calculation. The selected formula was encapsulated in hard vegetarian capsule shell (0 size Vcaps, Capsugel) using manually operated capsule filling machine to obtain the final formulation.

**Characterization of prepared polyherbal capsule**

The developed polyherbal capsules were characterized for the following physiochemical parameters.

**Weight variation test**

Test for uniformity of weight was performed as per Indian pharmacopoeia (IP), 2007 [19]. Randomly selected 20 capsules were weighed (individually and together) in a single pan balance. The average weight, variation in the individual capsule and the standard deviation was calculated. IP limit for weight variation in case of capsule weighting more than 300 mg is ± 5%.

$$\text{weight variation} = \frac{\text{Weight of capsule} - \text{Average weight}}{\text{average weight of capsule}} \times 100$$
**Moisture analysis**

The capsule blend was weighed, kept in an oven at 105°C and equilibrated. It was reweighed again till three constant readings using moisture balance and moisture content was measured gravimetrically [20].

**pH of polyherbal formulation**

pH of 1% solution of the formulation was determined by means of a digital pH meter (Mettlertedol) [19].

**Drug content**

Test for drug content was carried out as per IP [19]. Twenty capsules were taken and emptied their content in a mortar and pestle. In a volumetric flask, 500 mg of powder was taken and diluted with phosphate buffer (pH 6.8). The absorbance of the solution was measured at 213 nm (λ max of the formulation calculated by scanning the different concentration of it in phosphate buffer ph 6.8 within a range of 200 to 400 nm [21]) using UV/visible spectrophotometer (Shimadzu 1601 UV–VIS Spectrophotometer, Japan). The amount of drug present in individual capsule was then estimated using standard calibration curve.

**Dissolution study**

Dissolution profile of capsule formulation containing lyophilized polyherbal extract was determined according to USP type-I dissolution tester apparatus (rotating basket) (Electro lab dissolution tester, Electro lab, India). An accurately weighed amount of capsule was placed in USP dissolution basket rotated at 100 ± 5 rpm using phosphate-buffered saline (PBS) (pH = 6.8) as a dissolution medium and temperature was adjusted to 37°C ± 0.5. 3 ml aliquot of sample was withdrawn at regular time intervals (0, 30, 45, 60, 90 and 120 min) diluted and assayed spectrophotometrically at 213 nm. Meanwhile an equal volume of PBS was added to maintain the constant volume. The cumulative % release was calculated for the formulation from previously constructed calibration curve.

**RESULTS AND DISCUSSION**

The most important part of any formulation is standardization which ensures the quality, safety and reproducibility [22]. It involves the complete process of bio-prospection right from the collection of raw material to development of finished product [23]. In the present study, standardized polyherbal mixture was formulated in hard gelatin capsule to replace the traditional liquid dosage form. Quantitative analysis of the lyophilized extract viz. organoleptic properties, extractive values (water soluble and methanol soluble), total ash, and acid-insoluble ash, moisture content, HPLC fingerprint profile were mentioned before in a previous publication [6]. The lyophilised powder was dark brown in colour with a characteristic odour and taste. The particle size distribution by volume is shown in table 1. The z average of the extract was 1024.4 nm with a polydispersity of 0.21.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Characteristics</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Physical appearance</td>
<td>Dark brown powder</td>
</tr>
<tr>
<td>2</td>
<td>Solubility</td>
<td>Water</td>
</tr>
<tr>
<td>3</td>
<td>PH (1% w/v)</td>
<td>3.4 ± 0.04</td>
</tr>
<tr>
<td>4</td>
<td>z-average (nm)</td>
<td>1024.4 ± 20</td>
</tr>
<tr>
<td>5</td>
<td>polydispersity index</td>
<td>1.34 ± 0.21</td>
</tr>
<tr>
<td>6</td>
<td>Bulk density</td>
<td>0.55 ± 0.03</td>
</tr>
<tr>
<td>7</td>
<td>Tap density</td>
<td>0.68 ± 0.01</td>
</tr>
<tr>
<td>8</td>
<td>Compressibility index</td>
<td>19.41 ± 2.95</td>
</tr>
<tr>
<td>9</td>
<td>Hausner’s ratio</td>
<td>1.24 ± 0.08</td>
</tr>
<tr>
<td>10</td>
<td>Angle of repose</td>
<td>31.2 ± 0.75</td>
</tr>
<tr>
<td>11</td>
<td>Microbiological limits</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total plate count</td>
<td>&lt;100 cfu/g</td>
</tr>
<tr>
<td></td>
<td>Yeast/molds</td>
<td>&lt;100 cfu/g</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>Negative</td>
</tr>
</tbody>
</table>

Values are given in mean ± standard deviation.
Table 2: Elemental analysis in polyherbal aqueous extract.

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>188.979</td>
<td>0.02</td>
</tr>
<tr>
<td>Cd</td>
<td>228.802</td>
<td>0.49</td>
</tr>
<tr>
<td>Cr</td>
<td>267.716</td>
<td>0.02</td>
</tr>
<tr>
<td>Cu</td>
<td>327.393</td>
<td>0.005</td>
</tr>
<tr>
<td>Hg</td>
<td>194.168</td>
<td>0.01</td>
</tr>
<tr>
<td>Mg</td>
<td>285.213</td>
<td>0.61</td>
</tr>
<tr>
<td>Mn</td>
<td>257.372</td>
<td>0.09</td>
</tr>
<tr>
<td>Mo</td>
<td>202.031</td>
<td>0.02</td>
</tr>
<tr>
<td>Ni</td>
<td>231.604</td>
<td>0.04</td>
</tr>
<tr>
<td>Pd</td>
<td>220.353</td>
<td>0.06</td>
</tr>
<tr>
<td>Sr</td>
<td>407.771</td>
<td>0.09</td>
</tr>
<tr>
<td>Zn</td>
<td>206.2</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 3: Phytochemical analysis of polyherbal aqueous extract.

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Phytoconstituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic compounds</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides:</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoid</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>-</td>
</tr>
</tbody>
</table>

The dose prescription in Ayurveda depends on the *AyukKalBal* i.e age, season and capacity of the patient. Considering the patient acceptability and convenience it was put into capsule of size 0 (500 mg) which can be used as a starting dose [7]. The prepared capsules were subjected to characterization as per IP. The results are shown in Table 4 for various parameters, all of which were found within the limits as per IP. In vitro release study of the developed formulation was studied and presented in Figure 1. The release rate of the polyherbal formulation showed a maximum cumulative release of 92% at the end of the study.

Table 4: Characterization parameters for polyherbal capsule.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Data obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight variation test (n=20)</td>
<td>Within I.P. Limit</td>
</tr>
<tr>
<td>Moisture analysis (n=20)</td>
<td>3.98 ± 0.5</td>
</tr>
<tr>
<td>pH (n=20)</td>
<td>3.5 ± 0.02</td>
</tr>
<tr>
<td>Drug content uniformity (n=20)</td>
<td>Within I.P. Limit</td>
</tr>
</tbody>
</table>

![Graph showing the release rate of the polyherbal formulation](image-url)
CONCLUSION

Ayurveda imbibles various potent therapeutic approaches in its vast literature that have become increasingly popular worldwide. However, clinical trials proving the safety and efficacy of the traditional formulations are lacking mainly due to the absence of good quality dosage forms. Advancement in the science has given modern tools and techniques by which drawbacks of traditional dosage form like poor solubility, low absorption, poor patient compliance can be reduced and render the clinical trial of traditional formulations. In the present study, the development of polyherbal capsules incorporating the herbs in standardized form provides an opportunity to validate its traditional claim regarding its therapeutic efficacy.

The standardization of the polyherbal plant extract and formulation development approach is likely to be helpful for quality control, mechanistic study and clinical research as it can be produced and managed as a consistent product. Conclusively, the present study exhibited the successful development of polyherbal formulation. Thus, similar approach can be adopted to formulate patient friendly dosage form of various aqueous based medicinal preparations.

Recommended study: Pharmacokinetic study is still warranted in the present study.

ACKNOWLEDGEMENT

The authors are thankful to the Director, CSIR-CIMAP for providing the formulation in standardized form.

Authors’ statement

The authors declare that there is no conflict of interests

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