Physico-Chemical Standardization Of Brahmi Vati
An Ayurvedic Polyherbomineral Formulation

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ABSTRACT
Brahmi vati is an ayurvedic polyherbomineral formulation commonly used as memory enhancer and as a anti epileptic agent. The standardization (Physico-chemical) was carried out according to the parameters laid down by the department of ISM & H. The physico-chemical parameters like ash values, extractive values of different solvent system, loss on drying, fluorescence and sieve analysis were done. Qualitative analysis of different phytochemicals present in the formulation and TLC profiles of the formulation in various solvent system were also recorded. In this study an effort was put to fix the standards for commonly used polyherbomineral formulation, which can be utilised in quality control of the formulation.

INTRODUCTION
Indian system of medicine (Ayurveda, Siddha, Unani, Yoga and Naturopathy) and Homeopathy offers a range of safe, cost effective, preventive and curative therapies, which could be very useful in reaching the goal of ‘Health for All’[1]. Ayurvedic physicians and others are prescribing the Brahmi vati for various purposes since long time. Brahmi Vati (BV) is a poly herbo-mineral formulation containing 37 various plant constituents including Brahmi as the main constituents. It is an official monograph in

KEYWORDS
Ayurvedic medicine;
Brahmi vati;
Phytochemicals analysis;
TLC profiles;
Medicinal plants;
Standardization
Full Paper

Ayurvedic Pharmacopoeia of India (AFI) published by Govt. of India Ministry of Family Welfare, (Dept. of Health) New Delhi. It is official monograph of Ayurvedic Formulary of India (AFI) published by Ministry of Health and Family Welfare, Govt. of India. Proper identification and standardization is vital for the proper and rational use of plant medicines, which have played and must continue to play a major role in world health care. Standardization of Guggal (Commiphora mukul) manufactured by different Ayurvedic company was carried out by Agrawal et al. and they have showed different physico-chemical values showing that either the constituents added are different or are collected at different seasons. Similarly Agrawal et al. standardized Kumari Asava purchased from different sources, and it showed variable results. Since no physico-chemical standards are available to assess the quality of the formulation, the present study is designed to fix the various physicochemical and pharmacognostical standards to assess the quality of the formulation, which can be used as quality control tools.

MATERIALS AND METHODS

Drug Sample

The sample BV used for standardization was sent by the Ministry of Health and Family Welfare Govt of India (Dept. of ISM&H) under the project to the college and the same sample was used for all studies.

Physical parameters

1. Ash value

Various values were used for standardization viz. Total Ash Value, Acid Insoluble Ash Value, Sulphated Ash Value & Water Soluble Ash Value. This test is designed to measure the amount of material remaining after ignition. “Physiological Ash” is derived from the plant tissue itself and “Non Physiological Ash” is the residue after ignition of the extraneous matter (e.g. sand and soil) adhering determines both kinds of ashes and is referred as the “Total Ash” test. Total ash usually consists of carbonates, Phosphates, silicates and silica. Acid Insoluble ash is the residue obtained after boiling the total ash with dilute HCl and igniting the washed insoluble matter left on the filter. This determination measures the presence of silica, especially sand and siliceous earth. Water Soluble ash is the calculated difference in weight between the total ash and the residue remaining after treatment of the total ash with water. To get a more consistent ash the EP and BP use a sulphated ash, which involves treatment of the drug with dilute sulphuric acid before ignition. In this all oxides and carbonates are converted to Sulphates and the ignition is carried out at a higher temperature.

2. Extractive values

The determination of solvent soluble extractive is used as a means of evaluating drugs when the constituents are not readily estimated by other means. Extractive values were done by the cold maceration method. Air-dried weighed material was macerated with 100 ml of specified solvent for about 6 hrs, and allowed to stand for 18 hrs. 25 ml of rapidly filtered filtrate was transferred to a tarred flat bottom disk and evaporated to dryness on a water bath. It was dried at 105°C for 6 hrs cooled in a desiccators for 30 minutes and weight was calculated in percentage of extract with reference to the air dried material.

3. Loss on drying

The LOD measures the volatile substances present in the formulation and it is the index for volatile oils and moistures. The samples were kept in dried petridishes and accurately weighed. The sample was distributed as evenly to a depth not exceeding 10 mm. the samples were dried in an oven at a temperature of 105°C, until constant weight of sample was obtained. After drying was completed the petridishes were allowed to cool at room temperature in a desiccators and weighed for LOD calculation.

4. Fluoresce analysis

The fluorescence analyses were done under ordinary daylight and UV light by treating the powder with various reagents like 1N NaOH, 50% H2SO4, 1N HCL and 50% HNO3.

5. Sieve analysis

To assess the particle size of the formulation the sample was sieved using different mesh from number 44 to 120 and % of sample sieved was reported.
6. Phytochemical analysis

The preliminary phytochemical screening was carried out using alcoholic & aqueous extracts of the formulation for different chemical constituents. The qualitative chemical test gives the general idea regarding the nature of chemical constituents of the crude drugs.

7. TLC Profiles

TLC profiles were done for different extracts of the formulation and mobile phases were fixed. The extract was prepared by heating 5 gm coarse powder in 50 ml of solvent on water bath in a stoppered flask for about 30-60 minutes and contents were filtered and used.

Silica gel-G was used as a stationary phase, fine slurry was prepared and spread on a clean glass plates. Thickness of silica gel was about 0.5 mm. the prepared plates were air dried at room temperature. The air-dried plates were kept in an oven at 110°C for an hour and were stored in a desiccators subsequently. Placing strip of filter paper on three sidewalls of the chamber saturated the chromatographic chamber. The dissolved fractions were spotted on the plates with a fine capillary tube and then allowed to air dry. The spotted plates were kept for 30 minutes in the saturated chromatographic chambers containing pure/mixture of solvent system, which were covered with greases glass plates. Optimization of the mobile phase was done by choosing different solvent system combination. The air-dried plates were viewed in UV chamber to look for the fluorescent spots if any. The plates were then exposed to iodine vapors for 5 minutes and the spots were recorded. The plates were also sprayed with spray reagent 5 v/v ethonolic sulphuric acid and then kept in oven at temperature of 105°C for about 30 minutes and the spots were noted.

RESULTS AND DISCUSSION

Various physical parameters were used for standardization viz. Total Ash Value, Acid insoluble Ash Value, Sulphated Ash Value, Water soluble Ash Value, Water soluble extractive value, alcohol soluble extractive value, petroleum ether extractive value, acetone extractive value, chloroform extractive value according to the method given in IP 1985. The values are as shown in TABLE 1.

LOD measures the volatile substances present in the formulation and it is the index for volatile oils and moistures. (TABLE 1)

Many herbs fluoresce when cut surface or powder is exposed to UV light. Fluorescence can also occur on treatment with certain reagents. This can be useful in certain cases for identification of medicinal plants. The Bahmi vati shows different colour with different reagents as shown in TABLE 2.

The aqueous and alcoholic extracts of Brahmi Vati were showed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds & protein & amino acids. Sterols, fixed & volatile oils, saponins and acidic compounds were absent.

The TLC is a simple, useful and easy method for detection of adulteration and identification of the quality of the sample by comparing the number, Rf value and intensity, of the spot(s) of the sample extract with that of the pure marker compound(s) or by comparing with the standards as given in the Table.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash</td>
<td>27.12%</td>
<td>Water soluble Extractive</td>
<td>31.89</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>3.98</td>
<td>Alcohol soluble Extractive</td>
<td>37.14%</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>14.08%</td>
<td>Petroleum ether soluble Extractive</td>
<td>2.71%</td>
</tr>
<tr>
<td>Sulphated ash</td>
<td>19.43%</td>
<td>Chloroform soluble Extractive</td>
<td>17.29%</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>5.79%</td>
<td>Acetone soluble Extractive</td>
<td>31.20%</td>
</tr>
</tbody>
</table>

TABLE 2: Fluorescence analysis of Brahmi vati

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day Light</th>
<th>UV Light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Powder</td>
<td>Light Brown</td>
<td>Intense brown</td>
</tr>
<tr>
<td>Drug Powder + 1 N NaOH</td>
<td>Light Brown</td>
<td>Dark Brown</td>
</tr>
<tr>
<td>Drug Powder + 50% H2SO4</td>
<td>Opaque Brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Drug Powder + 1 N HCL</td>
<td>Light brown</td>
<td>Intense Brown</td>
</tr>
<tr>
<td>Drug Powder + 50% HNO3</td>
<td>Light Red to Brown</td>
<td>Dark Brown</td>
</tr>
</tbody>
</table>
TABLE 3a: TLC profiles of alcoholic extract of brahmi vati

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>Day Light</th>
<th>UV Fluorescence</th>
<th>Iodine Vapor</th>
<th>5% Ethanolic Sulphuric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform: Toluene (8:2)</td>
<td>No spots were observed</td>
<td>(09) at Rf-0.13,0.15,0.18, 0.20,0.22,0.33, 0.44,0.50, 0.62</td>
<td>(05) at Rf-0.1, 0.15, 0.20, 0.34, 0.61</td>
<td>(04) at Rf-0.41, 0.64 (all are yellowish brown)</td>
</tr>
<tr>
<td>Chloroform: Toluene (9.5:0.5)</td>
<td>No spots were observed</td>
<td>(06) at Rf-0.11,0.32,0.41, (dark blue), 0.58,0.64 (light purple), 0.78 (light blue)</td>
<td>(04) at Rf-0.32, 0.41, 0.56, 0.64, 0.68 (dark brown)</td>
<td>(04) at Rf-0.11, 0.33, 0.41, 0.43, 0.64 (brownish red)</td>
</tr>
</tbody>
</table>

TABLE 3b: TLC profiles of aqueous extract of brahmi vati

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>UV Fluorescence</th>
<th>Iodine Vapor</th>
<th>5% Ethanolic Sulphuric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Butanol:Glacial Acetic Acid: Water (7:2:10)</td>
<td>No spots were observed</td>
<td>(03) at Rf-0.23, 0.67, 0.89 (light blue)</td>
<td>(02) at Rf-0.23, 0.89 (reddish)</td>
</tr>
<tr>
<td>n-Butanol: Glacial Acetic Acid (7.5:2.5)</td>
<td>No spots were observed</td>
<td>(03) at Rf-0.24,0.41, 0.59 (all blue)</td>
<td>(02) at Rf-0.36, 0.41 (brownish)</td>
</tr>
</tbody>
</table>

The results of the TLC performed in various solvent systems using UV fluorescence, Iodine Vapor and 5% Ethanolic Sulphuric acid as detecting agents is summarized in TABLE 3a-3b.

ACKNOWLEDGMENT

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