Preparation and pharmacognostic evaluation of Ashwagandha Mashi

Sonali A Barke, Sandesh R Wayal, Mukta M Abhyankar and Dr. RY Patil

Abstract
Ashwagandha is a popular medicinal herb of Ayurveda and it’s a common ingredient of polyherbal or herbo-mineral formulations used for health, vitality, longevity and rejuvenation. Mashi is an Ayurvedic dosage form in which bulk of raw material is reduced to greater extent by application of certain quantum of energy due to which hidden chemical constituents become prominent or new chemical moieties are formed. Mashi of powdered Ashwagandha roots were prepared by Bahir-Dhum and Anter-Dhum Paddhati. The powdered Ashwagandha roots and its Mashi were examined comparatively for macroscopical, microscopical and physicochemical properties. The phytochemical constituents of Ashwagandha roots and its Mashi were extracted by soxhlet and decoction using ethanol and water solvent respectively. The extracts were screened for phytochemical constituents and evaluated for inorganic metal content. The results of evaluations were reveals that, Ashwagandha Mashi can effectively use as solid Ayurvedic dosage form as compared to powdered form of Ashwagandha roots.

Keywords: Mashi, Ashwagandha Mashi, Withania somnifera L., Anter-Dhum Paddhati, Bahir-Dhum Paddhati

Introduction
As Ayurveda offers the knowledge of natural herbal drugs, these natural herbs constitute a major part in all the traditional systems of medicine. The plants from different categories have been used for preparation of medicine since from ancient time because they are easily accessible and inexpensive. Plants are considered to be medicinal if they possess pharmacological activities of possible therapeutic use. These activities are often known as a result of millennia of trial and error, but they have to be carefully investigated if we wish to develop new drugs that meet the criteria of modern treatment [1].

Ashwagandha (Withania somnifera L.), a popular medicinal herb of Ayurveda is used for health, vitality, longevity and rejuvenation properties. It is a common ingredient of polyherbal or herbo mineral formulations used for preventive or therapeutic use [2]. The increasing demand of Ashwagandha as a medicine motivate to us for their study of pharmacognostic properties. In present work, the preparation of Ayurvedic dosage form i.e. Mashi would be confront for innovation.

Mashi is the Ayurvedic dosage form in which bulk of raw material is reduced to a greater extent by application of certain quantum of energy. Due to this treatment hidden chemical constituents become prominent and/or new chemical moieties are formed which are therapeutically active. The black color of Mashi indicates high percentage of carbon and oxides. Non-specific odor and charcoal like taste may be attributed to oxides, inorganic elements and carbon. Thus without application of any costly method for extraction using organic solvents, we can get therapeutic active organic and inorganic chemical constituents in the form of black mass known as Mashi [3]. Comprehensive literature study revealed strong phytochemical & pharmacological potential of the plant Withania somnifera L. [4]. Therefore it would be worthwhile to prepare Ayurvedic dosage form i.e. Mashi from the roots of Withania somnifera L.

The aim of the present work is preparation of Mashi by different techniques as well as qualitative and quantitative assessment of prepared Mashi. The present study is an attempt of providing scientific traditional claims about the Withania somnifera L. mentioned in Ayurvedic text for development of new dosage form of Mashi.
Materials and Methods

Procurement of Ashwagandha Roots

The dried mature roots of Ashwagandha (Withania somnifera L.) were procured from Local market of Pune.

Authentication, Size Reduction and Storage of the Plant Material

The procured plant material was authenticated by A. S. Upadhye, Scientist, Plant Drug Authentication Service, Botany Group, Plant Science Division, Agharkar Research Institute, Pune (Auth.12-102) by comparing with voucher specimen. Then procured root drugs were thoroughly cleaned and air-dried. It was then homogenized to fine powder and stored in air-tight bottles separately for further use.

Preparation of Ashwagandha Mashi by Applying Different Methods

Ashwagandha (Withania somnifera L.) Mashi was prepared by two different methods mentioned in Ayurvedic literature viz. Bahir-Dhum paddhati (External heat method), Anter-Dhum paddhati (Internal heat method) [13]. In Bahir-Dhum paddhati, sufficiently weighed quantity of dried powder of Withania somnifera L. roots were transferred in an earthen vessel and heat was supplied externally with continuous stirring. The heating was continued until whole material turns black and the black smoke was completely ceased. Whereas in Anter-Dhum paddhati, powdered Withania somnifera L. roots was placed in between two sharav samput earthen vessels, which was then sealed by multani mattie. The vessel was subjected to puta in kund filled with cow dung cakes and then fired. The sharav samput was taken out of kunda after swangsheet (cooling by natural means). The Mashi obtained by both methods were collected, triturated separately into a fine powder and packed in air tight containers for further study.

Pharmacognostic Study of Ashwagandha Roots and Mashi

The standardization of roots of Withania somnifera L. was carried out by macroscopical and microscopical characteristic studies [6, 7] (Table No. 1, 2 and Figure No. 1, 2). The powdered Withania somnifera L. roots and Mashi were examined for organoleptic and physicochemical (bulk and tap density, Carr’s index, specific gravity, ash values, moisture content, foreign organic matters, microbial contamination, extractive values) parameters. The experiments of physicochemical evaluation were carried out in triplicate and the results were presented as average ± Standard Deviation (S.D.) in Table No. 3. The procedures for Pharmacognostic evaluation were taken from standard reference books of pharmacy [8, 9].

Extraction and Isolation of Active Constituents of Ashwagandha Roots and Mashi

The active constituents of powdered Withania somnifera L. roots and Mashi were extracted with ethanol by continuous soxhlet extraction process for 12-15 hours and with water by decoction [10, 11]. After complete extraction solvents were distilled off and residual extract was kept in desiccators for complete removal of the solvents. The dried extracts were stored in appropriate container for further evaluation.

Preliminary Phytochemical Evaluation of Extracts of Ashwagandha Roots and Mashi

The extracts thus prepared were then subjected to preliminary phytochemical screening for the detection of major chemical groups [12, 13]. The results of preliminary phytochemical evaluation are presented in Table No. 4.

Determination of Total Inorganic Metal Content of Extracts of Ashwagandha Roots and Mashi

The prepared ethanolic extracts of Withania somnifera L. roots and Mashi were subjected to inorganic metal content analysis by using Flame Photometer (Systronics Model 128) [14]. The experiment was carried out in triplicate and the results were presented as average ± Standard Deviation (S.D.) in the Table No. 5.

Results and Discussions

The macroscopic and microscopic evaluations provides simplest and quickest means to established the identity and purity and there by ensure quality of drug material [15]. The result of the macroscopic evaluations of Withania somnifera L. roots are presented in Table No. 1

Table 1: Macroscopic Characteristics of Withania somnifera L. Roots

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Shape</td>
<td>Straight and un-branched, conical or cylindrical</td>
</tr>
<tr>
<td>2</td>
<td>Size</td>
<td>10-17 cm (Length), 6-12 mm (Width)</td>
</tr>
<tr>
<td>3</td>
<td>Surface</td>
<td>Smooth on outer surface with Longitudinal wrinkles</td>
</tr>
<tr>
<td>4</td>
<td>Fractures</td>
<td>Short and uneven fractures</td>
</tr>
<tr>
<td>5</td>
<td>Extra Characteristics</td>
<td>Creamy center with soft and solid mass</td>
</tr>
</tbody>
</table>

The fine transverse section of root of Withania somnifera L. was taken (Figure No. 1) and it was studied for special microscopical identification characteristics.

Fig 1: Microscopical Characteristics of Withania somnifera L. root

The few layers of stratified, non-lignified yellowish parenchymatous cell were identified on outer side which was followed by 2-4 diffused rows of cork cambium. The layers of slightly flattened parenchymatous cells of cortex were observed with full of starch grains (Blue colour with iodine). Secondary phloem was found immediately after cortex consists of isodiametric parenchymatous cells with intracellular spaces, sieve tubes, companion cells. The lignified fibers (Pink colour with phloroglucinol and HCl), medullary rays and parenchymatous were found to be present in secondary xylem. Starch grains were abundantly present in parenchymatous cells.

The microscopical powdered characteristics of Withania somnifera L. roots were studied. The results of powdered characteristics are presented in Figure No. 2
The prepared Mashi were studied for organoleptic characteristics like colour, odour and taste in comparison with Withania somnifera L. roots. The results of organoleptic evaluations are presented in Table No. 2.

Table 2: Organoleptic Characteristics of Withania somnifera L. Roots and Mashi

<table>
<thead>
<tr>
<th>Organoleptic Parameters</th>
<th>Roots of Withania somnifera L.</th>
<th>Mashi Prepared by Bahir-Dhum Padhati</th>
<th>Mashi Prepared by Anter-Dhum Padhati</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Outer Surface Buff to Grey-Yellow, Inner Surface white in color</td>
<td>Dark Brown to Black</td>
<td>Dark Brown to Black</td>
</tr>
<tr>
<td>Odour</td>
<td>Characteristic</td>
<td>Characteristic</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Taste</td>
<td>Bitter and Acrid</td>
<td>Acrid</td>
<td>Acrid</td>
</tr>
</tbody>
</table>

The physical and chemical quality however plays an equally important role for the manufacturer and processor. The results of physicochemical evaluation of powdered drug of Withania somnifera L. roots and Mashi are presented in Table No. 3.

Table 3: Physicochemical Properties of Powdered Withania somnifera L. roots and Mashi

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Withania somnifera L. Roots (Values in Mean ± S.D)</th>
<th>Withania somnifera L. Mashi (Values in Mean ± S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bahir-Dhum Method</td>
<td>Anter-Dhum Method</td>
</tr>
<tr>
<td>1</td>
<td>Bulk Density (gm/cm³)</td>
<td>1.04 ± 0.0011</td>
<td>1.16 ± 0.0045</td>
</tr>
<tr>
<td>2</td>
<td>Tap Density (gm/cm³)</td>
<td>1.14 ± 0.0015</td>
<td>1.28 ± 0.0011</td>
</tr>
<tr>
<td>3</td>
<td>Carr’s Index (%)</td>
<td>8.77 ± 0.0421</td>
<td>9.37 ± 0.0191</td>
</tr>
<tr>
<td>4</td>
<td>Specific Gravity (gm/ml)</td>
<td>0.457± 0.0945</td>
<td>0.217± 0.0245</td>
</tr>
<tr>
<td>5</td>
<td>Total Ash Value (%w/w)</td>
<td>6.83± 0.0762</td>
<td>7.75± 0.0655</td>
</tr>
<tr>
<td>6</td>
<td>Acid Insoluble Ash Value (%w/w)</td>
<td>0.81± 0.0550</td>
<td>0.91± 0.0645</td>
</tr>
<tr>
<td>7</td>
<td>Water Soluble Ash Value (%w/w)</td>
<td>1.45± 0.0776</td>
<td>2.07± 0.0755</td>
</tr>
<tr>
<td>8</td>
<td>Total Moisture Content(%/w/w)</td>
<td>9.45±0.1704</td>
<td>1.36± 0.1647</td>
</tr>
<tr>
<td>9</td>
<td>Foreign Organic Matters(%/w/w)</td>
<td>1.52±0.0975</td>
<td>1.09±0.02451</td>
</tr>
<tr>
<td>10</td>
<td>Alcohol Soluble Extractive Values (%/w/w)</td>
<td>14.64±0.7726</td>
<td>12.47±0.5587</td>
</tr>
<tr>
<td>11</td>
<td>Water Soluble Extractive Values (%/w/w)</td>
<td>18.96±2.7612</td>
<td>15.24±0.6644</td>
</tr>
<tr>
<td>12</td>
<td>Chloroform Soluble Extractive Values (%/w/w)</td>
<td>2.31±0.1629</td>
<td>1.11±0.1145</td>
</tr>
</tbody>
</table>
The bulk density and tap density were determined to obtained information on powdered drugs compaction. The bulk density of powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of interparticulate void volume. Whereas tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample [10]. The bulk densities of Withania somnifera L. Mashi prepared by Anter-Dhum method (1.14± 0.0040 gm/cm³) and by Bahir-Dhum method (1.16± 0.0045 gm/cm³) as well as tap densities of Withania somnifera L. Mashi prepared by Anter-Dhum method (1.19± 0.0045 gm/cm³) and by Bahir-Dhum method (1.28 ± 0.0011 gm/cm³) were found to be high as compared to the powdered Withania somnifera L. roots bulk density (1.04 ± 0.0011 gm/cm³) and tap density (1.14± 0.0015 gm/cm³).

Carr’s index also called as compressibility index which gives idea about compressibility of flow properties of powdered drug sample [17]. The Carr’s index of powdered Withania somnifera L. root (8.77± 0.0421%), Withania somnifera L. Mashi prepared by Anter-Dhum method (5.20±0.0624%) and Withania somnifera L. Mashi prepared by Bahir-Dhum method (9.37±0.0191%) were found in the range of 5-15% which indicates excellent flow property or compressibility of all forms of powdered drug.

The specific gravity gives knowledge about solid to liquid ratio of drug samples. Appearance and consistency of drugs generally depends upon this attribute [18]. The specific gravity of Withania somnifera L. Mashi prepared by Anter-Dhum method (0.2355± 0.0942 gm/ml) and prepared by Bahir-Dhum method (0.2176± 0.0245 gm/ml) was found to be decreased when compared with the powdered Withania somnifera L. root (0.4573± 0.0945 gm/ml) drug this could be due the fire applied during preparation of Withania somnifera L. Mashi.

The ash values were used to determine the quality and purity of crude drugs in powdered form. The total ash usually consists of carbonates, phosphates, silicates and silica of sodium, potassium, magnesium and calcium which includes both physiological ash and non-physiological ash. Their determination is an indicative of contamination, substitution or adulteration in crude drugs [19]. The concentration of inorganic matters of Withania somnifera L. Mashi prepared by Anter-Dhum method (8.92 ± 0.0567% w/w) was found to be high as compared to the Withania somnifera L. Mashi prepared by Bahir-Dhum method (7.75±0.0655% w/w) and powdered Withania somnifera L. roots (6.83 ± 0.0762% w/w). The acid-insoluble ash value particularly indicates contamination with siliceous materials like earth or sand [20]. The acid insoluble ash of Withania somnifera L. Mashi prepared by Anter-Dhum method (1.92±0.0755% w/w) indicates it was highly contamination with siliceous matter as compared to other forms of drug.

The water soluble ash value was determined to obtained information on either previous extraction of water-soluble salts in the drug or incorrect preparation. [21] The water soluble salts were found to be in greater concentration in Withania somnifera L. Mashi prepared by Anter-Dhum technique (2.27±0.0770% w/w). The water soluble extractive values of powdered Withania somnifera L. roots and its Mashi prepared by Bahir-Dhum techniques was found to be 1.45±0.0776% w/w and 2.07±0.0755% w/w respectively.

Moisture was considered as an inevitable component of crude drugs, which must be eliminated as far as practicable. The preparation of crude drug involves garbling and drying which plays a very important role in the quality as well as the purity of the material. Insufficient drying favors spoilage by moulds and bacteria and enzymatic destruction of active principles [22]. Total Moisture Content of powdered Withania somnifera L. roots was found to be 9.45±0.1704% w/w. The moisture content of Withania somnifera L. Mashi prepared by Anter-Dhum and Bahir-Dhum method was found to be 2.77±0.0957% w/w and 1.36 ± 0.1647% w/w respectively.

Foreign organic matters generally considered as parts other than those names with specified limits or it may be any organism, part or product or an organism other than that name in the definition and description. It may also include mineral admixtures not adhering to the medicinal plant materials e. g. stones, soil, and dust etc. Medicinal plant material should be entirely free from visible signs of any contamination [23]. The values of foreign organic matters of powdered Withania somnifera L. roots (1.52±0.0975% w/w) and its Mashi prepared by Anter-Dhum method (0.97±0.0184% w/w) and Bahir-Dhum method (1.09±0.0245% w/w) indicates minute attention must be given to eliminate foreign matters of Mashi as compared to powdered roots drug.

The extractive values were important to know the nature of chemical constituent and the solubility of the drug in particular solvent [24]. The alcohol and water extractive values of powdered Withania somnifera L. roots (14.64± ±0.7726% w/w and 18.96 ±2.7612% w/w respectively)was found to be more as compared to Withania somnifera L. Mashi. It may be due the loss or decomposition of thermo labile organic and inorganic constituents at particular temperature during process of heating in Anter-Dhum and Bahir-Dhum method of preparation of Mashi. The study of extractive value determination can conclude that alcohol and water can be used as solvent to extract out active constituents from all forms of drugs of Withania somnifera L. rather than use of chloroform (extractive values were found to be less 2.31±0.1629% w/w, 1.98±0.0954% w/w, 1.11±0.1145% w/w for powdered root drug, Mashi prepared by Anter-Dhum and Mashi prepared by Bahir-Dhum respectively).

The chemical compound present in crude drug were responsible the desire therapeutic property. To obtain these therapeutic effects, the plant material should be extracted with suitable solvents to take out the desired components and the resulting principles being employed as therapeutic agents termed as Phyto-constituents. The extraction of phyto-constituents from powdered Withania somnifera L. roots and from its Mashi prepared by Anter-Dhum and Bahir-Dhum methods was carried out by soxhlet extraction technique and by decoction using ethanol and water respectively as solvents on laboratory scale. The solvent extracts thus obtained were used for further evaluation.

The preliminary phytochemical screening was carried out for ethanolic and aqueous extract of Withania somnifera L. Roots & its Mashi. The results of preliminary phytochemical screening are presented in Table No. 4.
The plant species may be considered as a biosynthetic laboratory not only for the chemical compounds e.g. carbohydrates, proteins and fats that can be utilized as a food by the humans and animals, but also for a magnitude of compounds including alkaloids, terpenoids, Flavonoids, glycosides etc. which exerts definite physiological effects [255]. As the herbal drugs contain so many chemical compounds, it was essential to signal out those were responsible for the therapeutic effects called as preliminary phytochemical screening. From the preliminary phytochemical evaluation it was concluded that alkaloids, steroids, saponin glycosides and carbohydrates were present in aqueous and ethanolic extracts of *Withania somnifera* L. roots as well as its Mashi but the intensity of presence of active constituents was found to be high in ethanolic extracts. Therefore ethanolic extracts were selected for further analytical and pharmacological study. The determination of total inorganic metal content of ethanolic extracts of *Withania somnifera* L. roots and Mashi was carried out. The results of Inorganic Metal Content of *Withania somnifera* L. Roots and its Mashi are presented in Table No. 5.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Parameter</th>
<th><em>Withania somnifera</em> L. Roots (Mean % w/w ± S.D)</th>
<th><em>Withania somnifera</em> L. Mashi (Mean % w/w ± S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[Anter-Dhum Method]</td>
<td>[Bahir-Dhum Method]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanolic Extract</td>
<td>Water Extract</td>
</tr>
<tr>
<td>1</td>
<td>Sodium Content</td>
<td>1.152±0.0125</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Potassium Content</td>
<td>2.320±0.019</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 5: Total Inorganic Metal Content of Extract of *Withania somnifera* L. Roots & Mashi
Inorganic elemental content were determined by using flame photometer. The increase in the amount of inorganic elements in *Withania somnifera* L. Mashi prepared by Anter-Dhum method and Bahir-Dhum method was observed as the temperature increases. The “Na” content of *Withania somnifera* L. Mashi prepared by Anter-Dhum method and Bahir-Dhum method was found to be 2.526±0.1254% w/w and 1.957±0.5241% w/w respectively. Whereas “K” of *Withania somnifera* L. Mashi prepared by Anter-Dhum method and Bahir-Dhum method was found to be 4.1637±0.1693% w/w and 4.0123±0.2354% w/w respectively.

**Conclusion**

In present study, the standardization of Ashwagandha roots was carried out by macroscopic, microscopic, powdered characteristic and chemical study which revealed that, the species selected for study was correct for further utilization. The organoleptic evaluation of *Withania somnifera* L. roots and its Mashi prepared by Bahir-Dhum and Anter-Dhum paddhati was carried out to obtain primary information on quality of drugs and it was found to be satisfactory. The information on quality and purity of both *Withania somnifera* L. powdered drug and its Mashi was obtained by its physico-chemical evaluation. The increased value of bulk and tap density of Anter-Dhum and Bahir-Dhum Mashi indicates increased capacity for powder compaction. Even though Car’s index of prepared Mashi was found to be decreased, their values denote that, they can be used for preparation of solid dosage form with an ease. The decreased in specific gravity value of Ashwagandha Mashi, due to the procedure used during preparation, increases their stability. The slight increase of ash values of Ashwagandha Mashi were indicates its beneficiary herbo-mineral values as compared to powered Ashwagandha roots. The Mashi prepared by Anter-Dhum Paddhati was found to be contaminated with sand siliceous earth matter. The water soluble ash values indicate that Anter-Dhum Mashi contains water soluble salts in greater extent. The heating process used in preparation of Mashi decreases moisture content and therefore increases their stability. The minute attention was found to be required to eliminate foreign matters of prepared Mashi. The extractive values indicate that the decomposition of thermo labile constituents was occurred. The phytoconstituents like alkaloids, steroids, saponin glycosides and carbohydrates can be extracted from powdered Ashwagandha roots and it’s Mashi by soxhlet extraction technique using polar solvents such as ethanol and water. The inorganic metal content analysis showed that inorganic content in Ashwagandha Mashi was increased which would be beneficial for its therapeutic properties.

The extraction of phyto-constituents from powdered *Withania somnifera* L. roots and from its Mashi prepared by Anter-Dhum and Bahir-Dhum methods was carried out by soxhlet extraction technique and by decoction using ethanol and water respectively as solvents on laboratory scale. The preliminary phytochemical screening was carried out for ethanolic and aqueous extract of *Withania somnifera* L. Roots & its Mashi then it was concluded that alkaloids, steroids, saponin glycosides and carbohydrates were present in aqueous and ethanolic extracts of *Withania somnifera* L. roots as well as its Mashi but the intensity of presence of active constituents was found to be high in ethanolic extracts. Therefore ethanol as a solvent can be used for further study. Inorganic elemental content were detemined by using flame photometer and it was found that the “Na” & “K” content is greater in Mashi prepared by Anter-Dhum method as compared to Bahir-Dhum method. Also it was observed that all the values are higher in Mashi form than crude drug. Because there was approximately 50-60% reduction in weight leads to increase in organic and inorganic constituents present in drug. This helps in standardization of drug along with its quality and purity. Also it was observed that the Total ash, Acid insoluble ash and Water soluble ash values are more by Anter-Dhum method Mashi as compared to Bahir-Dhum method Mashi and *Withania somnifera* L. drug. This was due to the loss of organic matter from *Withania somnifera* L. Mashi formulation by thermal degradation. Inorganic constituents from *Withania somnifera* L. Mashi were not affected qualitatively, as there was no thermal degradation of inorganic constituents takes place. Also it was found that the percentage of inorganic element was greater in *Withania somnifera* L. Mashi than the original crude drug *W. somnifera* L. Increase in inorganic radical helps *Withania somnifera* L. Mashi medicinally active especially, in the diseases where inorganic constituents play a key role in pharmacological activities.

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**References**


