STANDARDIZATION OF POLYHERBAL FORMULATION – ARSHONYT FORTE

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ABSTRACT

Indian system of Medicine comprises of Ayurveda, Unani, and Siddha. In all the systems, maximum drugs are made up of poly-herbs. The World health organization (WHO) had given a detailed protocol for standardization of herbal drugs which mostly consists of single herbs. A detailed protocol is given by the WHO to avoid any adulteration in the formulation and to maintain its quality, safety and efficacy. Objective of this work was to standardize a polyherbal formulation available in the market for quality and efficacy. Arshonyt forte formulation was selected for carrying out standardization, it is a polyherbal drug comprising of complex mixture of different herbal substances. A pack of 80 tablets of Arshonyt forte 650 mg had been taken from Charak Pharma Himachal Pradesh outlet; batch no.AR 055 Exp.03/2012. Arshonyt forte is a mixture of 5 herbs. The formulation was subjected to preliminary phytochemical test, colour test for pesticides, colour test for heavy metals, estimation of active constituents by UV spectrophotometer, chromatographic studies like TLC, HPTLC, HPLC and microbial load test. The results obtained indicated proper extraction of polyherbal drugs which yields some active constituents which are identified by high performance thin layer chromatography, high performance liquid chromatography, ultraviolet spectroscopy determination.

KEYWORDS: Polyherbal formulation, Standardization, Arshonyt Forte
INTRODUCTION

Medicinal plants constitute a source of raw material for both traditional systems of medicine (e.g. Ayurvedic, Chinese, Unani, Homeopathy, and Siddha) and modern medicine. Nowadays, plant materials are employed throughout the industrialized and developing world as home remedies, over-the-counter drugs, and ingredients for the pharmaceutical industry. As such, they represent a substantial proportion of the global drug market (Bhanu et al., 2005; Bhutani K, 2003). Most rural populations, especially in the developing world, depend on medicinal herbs as their main source of primary healthcare. Although most medicinal herbs are not in their natural state, fit for administration, preparations suitable for administration are made according to pharmacopoeial directions. So standardization of poly-herbal formulation is necessary. It involves adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity by adding excipients or by mixing herbal drugs or herbal drug preparations. Botanical extracts made directly from crude plant material show substantial variation in composition, quality, and therapeutic effects. Standardization is done by determining the extractive value, ash value, heavy metal content, pesticide residue, microbial contamination and active content by chromatographic methods (Mukherjee et al., 1998; Mukherjee PK, 2008). Standardized extracts are high quality extracts containing consistent levels of specified compounds, and they are subjected to rigorous quality controls during all phases of the growing, harvesting, and manufacturing processes (Gokhale & Surana, 2006; Kokate et al., 2005; Lazarowych & Pekos, 1998). So Objective of this work was to standardize a polyherbal formulation (Arshonyt Forte), available in the market for quality and efficacy.

MATERIALS AND METHODS

A pack of 80 tablets of Arshonyt forte 650 mg had been taken from Charak Pharma, Himachal Pradesh outlet; batch no. AR 055 Exp.03/2012. Arshonyt forte is a mixture of 5 herbal drugs. Arshonyt forte is a mixture of the following 5 polyherbal materials.

Cyamopsis tetragonoloba (L.) Taub Acacia catechu (L.f.) Willd. Aloe vera (L.) Burm.f. Terminalia chebula Retz. Plumbago zeylanica L.

Steps for Standardization of herbal medicine

Step 1: Preliminary phytochemical test.

Hydro alcoholic solution was used for extraction of various constituents from Arshonyt forte sample powder. Then test for identification of alkaloids, flavonoids, tannins, saponin were carried out.

Step 2: Extraction and authentication of the active therapeutic constituent from the extract.

Isolation of total glycoside contents: Weight of 5 g of the Arshonyt forte sample powder was taken into 100 ml volumetric flask and was made acidic with dilute HCl (5%). Then we took the sample in separating funnel with chloroform and then chloroform layer was separated and evaporated on water bath. The residue was collected and was used for further studies.

Isolation of total tannin contents: Extract of 400 mg was weighed accurately in 100 ml volumetric flask. 50 ml of hot water was added to it, pH was above 7, the temperature was maintained at above 40°C and it was shaken well. The aqueous fraction was used for the estimation of tannin content.

Step 3: Estimation of active chemical constituents by UV spectrophotometer (Rubesh et al., 2010).

Standard solution: 10 mg of all standard was dissolved in 100 ml of methanol to give 100 µg/ml.
Test solution: 100 mg of all test extract prepared as mentioned earlier was dissolved in
100 ml of methanol. From that 10 ml was taken and diluted up to 100 ml with methanol to give 100 µg/ml.

Preparation of calibration curve:

1. For Estimation of total tannin: concentrations of 10, 20, 30, 40, 50 ppm were prepared.
2. For Estimation of total polyphenolics: concentrations of 20, 40, 60, 80, 100 ppm were prepared.
3. For Estimation of total saponin: concentrations of 25, 50, 75, 100, 125, 150, 200, 250 ppm were prepared.

Step 4: Estimation and quantification of active constituents from the extract by HPTLC (Sanjeeth et al., 2010).

Standard solution: 2 mg of all standard was dissolved in 500 ml of methanol to give 100 µg/ml.
Test solution: 10 mg of extract was dissolved in 20 ml of methanol to give 500 µg/ml.
Scanning: Absorption mode from 200 to 800 nm.

Step 5: Identification and estimation of active chemical constituents by HPLC.

Standard solution: 10 mg of all standard was dissolved in 100 ml of methanol to give 100 µg/ml.
Test solution: 100 mg of all test was dissolved in 100 ml of methanol. From that 10 ml was taken and diluted up to 100 ml with methanol to give 100 µg/ml.

Step 6: Extraction, Identification and quantification of pesticides from the finished product (Shrikumar et al., 2006).

Extraction of pesticides from material: 5 gm sample was taken in a round bottomed flask and added sodium sulphide with 50 ml n-Hexane. It was refluxed for 1 h. The filtrate was taken in a separating funnel and extracted with 25 ml and 12.5 ml Acetonitrile. The acetonitrile layer was mixed with 250 ml de-mineralized water with 1.5 ml saturated sodium sulphide and again shaken in a separating funnel with n-Hexane layer and evaporated on a water bath. The residue obtained was used for analysis of organochloro, organophosphate and carbamate pesticides.

Step 7: Extraction, Identification and quantification of heavy metals from the finished product.

Extraction of pesticides from material: 5 g sample was taken in a silica crucible and heated to remove the moisture. It was kept in a muffle furnace at 600°C, for 3 h to remove organic material. The crucible was cooled down and was examined for any colour change. The change in colour reveals the presence of copper and zinc. Next the residue was boiled with 10 ml of dilute HCl and filtered. This filtrate may contain metals like arsenic, mercury, lead, cadmium and zinc.

Materials:
1. Copper wire wound tightly around glass rod.
2. Nitric acid 2.5 N.

Procedure for the test of heavy metals: A copper wire was washed with 2.5 N nitric acid and then it was rinsed with 95% ethanol and dried. Then 20 ml of residue was placed and dissolved in water into a small flask to which 4 ml of conc. HCl was added. Then freshly treated copper wires were added to flask. Then the solution was heated for about 1 h. Next the copper wire was removed and examined for any colour change.

Step 8: Microbiological load test of finished product (The Ayurvedic Pharmacopoeia of India, 2006).

Sample of 0.5 g sample was taken and poured onto a nutrient agar media and incubated in appropriate condition for microbial testing.

RESULTS

Results of Preliminary phytochemical test and pharmacognostic investigation are shown below in table I
Table I: Results of preliminary Pharmacognostic investigation

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Tests</th>
<th>Observation</th>
<th>Arshonyt forte tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extractive value</td>
<td>5 g</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>Total ash</td>
<td>0.4 g</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash</td>
<td>10 mg</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash</td>
<td>0.2 g</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>Dragendorff reagent</td>
<td>Reddish brown colour precipitate</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Mayer’s reagent</td>
<td>Cream colour precipitate</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Wagner’s reagent</td>
<td>Reddish brown colour precipitate</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Hager’s reagent</td>
<td>Gives yellow colour precipitate</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Shinoda test</td>
<td>crimson red colour</td>
<td>+</td>
</tr>
</tbody>
</table>

+ indicates Present; − indicates absent

Result for extraction and authentication of the active therapeutic constituent from the extract.

i. For Estimation of total tannin:

Maximum absorbance was found at 775 nm. The equation of calibration curve was found to be \( \text{Abs} = 0.0059x + 0.0032 \) with \( R^2 = 0.9965 \). The percentage estimation of tannic acid is given below in table II.

ii. For Estimation of total polyphenolics:

Maximum absorbance was found at 775 nm. The equation of calibration curve was found to be \( \text{Abs} = 0.0723x + 0.0231 \) with \( R^2 = 0.9984 \). The percentage estimation of gallic acid is given below in table III.

iii. For Estimation of total saponin:

Maximum absorbance found at 775 nm. The equation of calibration curve was found to be \( \text{Abs} = 0.0027x - 0.0049 \) with \( R^2 = 0.9973 \). The percentage estimation of saponin is given below in table IV.

Table II: Total Tannin estimation

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Extract</th>
<th>Absorbance at 775 nm</th>
<th>% Estimation</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>sample</td>
<td>0.041</td>
<td>7.49</td>
<td>7.89 ± 0.42</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.046</td>
<td>8.34</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.043</td>
<td>7.83</td>
<td></td>
</tr>
</tbody>
</table>

Table III: Total polyphenolics estimation

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Extract</th>
<th>Absorbance 765 nm</th>
<th>% Estimation</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>sample</td>
<td>1.384</td>
<td>18.59</td>
<td>18.54 ± 0.072</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.378</td>
<td>18.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.383</td>
<td>18.57</td>
<td></td>
</tr>
</tbody>
</table>
Table IV: Total saponin estimation

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Extract</th>
<th>Absorbance 472 nm</th>
<th>% Estimation</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>sample</td>
<td>0.017</td>
<td>8.11</td>
<td>7.61 ± 0.567</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.014</td>
<td>7.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.016</td>
<td>7.74</td>
<td></td>
</tr>
</tbody>
</table>

Result for extraction and authentication of the active therapeutic constituent from the extract by HPTLC.

Figure 1: Track 1 for Gallic acid
Figure 2: Track 1 for Gallic acid
Figure 3: Track 3 for extract

Table V: HPTLC study on the extract

<table>
<thead>
<tr>
<th>Tr</th>
<th>Pk No.</th>
<th>Rf</th>
<th>AUC</th>
<th>Content in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.72</td>
<td>1544.7</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.26</td>
<td>1274.1</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.02</td>
<td>1165.7</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.12</td>
<td>2035.4</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0.24</td>
<td>878.1</td>
<td>29.40 mg</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.26</td>
<td>749.3</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0.73</td>
<td>1753.4</td>
<td>56.75 mg</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.91</td>
<td>2265.7</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>0.97</td>
<td>1842.9</td>
<td>–</td>
</tr>
</tbody>
</table>

The Rf value of standard catechin, gallic acid was found to be 0.72 and 0.26 respectively.

Result for extraction and authentication of the active therapeutic constituent from the extract by HPLC.

Figure 4: Chromatogram for Epicatechin
Figure 5: Chromatogram for Epicatechin
Figure 6: Chromatogram for sample
Result for Colour test for pesticides:

Table VI: Qualitative determination of the pesticides

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Test performed</th>
<th>Observations</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Organo chloro</td>
<td>No colour observed</td>
<td>Dichloropropane absent</td>
</tr>
<tr>
<td>2.</td>
<td>Organo phospho</td>
<td>No colour observed</td>
<td>Phosphate absent</td>
</tr>
<tr>
<td>3.</td>
<td>Carbamate</td>
<td>No colour observed</td>
<td>Amide group absent</td>
</tr>
</tbody>
</table>

Result for Extraction, Identification and quantification of heavy metals from the finished product:

No change was observed in the colour of copper wire which revealed that metals are absent in the formulation.

Result for Microbiological load test of finished product:

No evidence of the colony formation and no turbidity in the nutrient broth suggested the absence of microbial load in the sample.

DISCUSSION

Arshonyt forte powder extract shows the presence of Alkaloids, Flavonoids, Tannins, Saponins and Glycosides. The extractive value denotes the presence of active constituents present in formulation. As extractive value of the formulation is more it can help to carry out all tests neatly and contents can be determined. Total ash usually consists of carbonates, phosphates, silicates and silica which include both physiological ash and non-physiological ash. So a lower total ash value indicates minimal presence of the above mentioned contents. Water soluble ash is that part of total ash content which is soluble in water. It is good indicator of either previous extraction of the water soluble salts in the drug or incorrect preparation. The value for water soluble ash revealed that the formulation is free from other foreign matter.

Tannins and polyphenolics are complex substances. The relatively fair amount of tannins, polyphenolics and saponins in formulation indicates presence of higher amount of active constituents which helps in playing a good therapeutic activity of formulation.

Content of catechin and gallic acid from extract was found to be 56.75 mg and 29.40 mg in sample extract respectively by HPTLC.

In HPLC determination, the RT value of sample gallic acid, epicatechin, lupeol was found to be 3.3667, 2.55, 6.66 and peak area of
sample gallic acid, epicatechin, lupeol 557.679, 1809.335, 1340.61. Content of gallic acid, epicatechin, lupeol was determined to be 21.25 mg, 18.25 mg and 100 mg in sample extract respectively.

Organo-chloro pesticides like DDT cause poisoning and potential hazards to animal and human beings. Aldrin, dieldrin and endrin are considered to be compounds that cause poisoning. Organo-phosphorous compounds are potent cholinesterase inhibitors and can be very toxic. It also acts on CNS and causes depression. So absence of pesticide in the formulation indicates its safety.

Presence of metal in formulation causes severe diseases on consumption. As due to negative results for colour test of metal, it indicates that the formulation is suitable for consumption (Table VI). The presence of microbes like salmonella and pseudomonas causes serious infections which can enter through oral and ophthalmic formulations. So absence of microbes in formulation indicates the safety of formulation.

CONCLUSION

It can be concluded that the marketed formulation (Arshonyt forte tablet) has been standardized by intervention of modern quality control measures. Pharmacognostic characters established for the raw materials could be employed as quality control standards for evaluating its identity and can be used for routine analysis. The results obtained could be used to set new Pharmacopoeial limits for optimal efficacy of the medicine.

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REFERENCES:


