Evaluation of Ayurvedic compound formulations 6- Panchkola Churna

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Received 30 June 2016, revised 06 December 2016

Increasing public interest and acceptance of herbal drugs and natural therapies in both developing and developed countries has given rise to various forms of adulteration and substitution in the herbal formulations leading to consumers and manufacturers disappointment and in some instances fatal consequences. Standardization and quality evaluation is the process of preparing a set of standards or identical characteristics, constant parameters, definitive values that carry an assurance of quality, efficacy, safety and reproducibility of any single or compound herbal formulation. In the continuation of earlier studies on standardization of Ayurvedic herbal formulations four samples of Panchkola Churna, procured from different Ayurvedic pharmacies, were subjected to physicochemical analysis, HPTLC fingerprinting and botanical characterization, and compared using authentic ingredients as reference. The studies suggest that these parameters may, together, be used for quality evaluation and standardization of compound formulations and maintaining the quality, purity and efficacy of the studied formulation.

Keywords - Ayurveda, Panchkola Churna, Compound formulation, Quality control.

IPC Int. Cl.: A61B 5/117, G06K 9/00, A61K, A01D 20/00

In India the knowledge of traditional herbal medicines synonyms with its rich cultural heritage and first found its mention in Vedic literature, particularly the Rigveda, Charak samhita and Susruta samhita. Over the past few decades, there has been increasing public interest and acceptance of traditional knowledge and use of medicinal plants has been widely acknowledged in developing and developed countries as well as across the world. About 80 % of the world’s population, especially in the developing countries, uses herbal medicine as their source of primary healthcare1,2. There is no doubt that traditional medicines are making a dramatic revival in International as well as in National markets. In India, people have immense faith in different indigenous systems of medicines, especially in Ayurveda3. This resurgence of interest in herbal medicines has led to an increase in their demand leading to a decline in their quality, primarily due to lack of adequate regulations pertaining to drugs. One of the major problems faced by the herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations. Thus an adequate attention is urgently required to regulate proper standardization and quality control in the herbal medicines of Indian indigenous systems (Ayurveda, Siddha & Unani). In India, the Ministry of AYUSH, Government of India, earlier launched a central scheme to develop standard operating procedures for the manufacturing process and to develop pharmacopeial standards for Ayurvedic preparations. Earlier studies in this area that have led to the development of analytical protocols, both for single herbal drugs4,5 as well as for compound herbal formulations6-7, that can be used in the routine standardization of Ayurvedic drugs and formulations. The present study reports the standardization methods for evaluation of another Ayurvedic compound formulation, viz. Panchkola Churna. As prescribed in the Ayurvedic Formulary II8 it is used generally to cure Grahani Rog. Panchkola Churna consists of a moderately fine powder of equal parts of Piper longum L. Fruit (Pippali), Piper longum L. Root (Pippalimula), Piper retrofractum Vahl. Fruit (Chavya), Plumbago indica L. Root (Chitrak) and Zingiber officinale Rosc. Rhizome (Shunthi).

Materials and methods

Samples of Panchkola Churna, one batch each, prepared by 4 different Ayurvedic pharmacies of India (Almora, Jaipur, Jamnagar and Patiala) were procured. Authentic samples of P. longum Fruit

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(Pippali), *P. longum* Root (*Pippali mula*), *P. retrofractum* Fruit (*Chavya*), *P. indica* Root (*Chitrak*) and *Z. officinale* Rhizome (*Shunthi*) were procured from Lucknow herbal drug market, authenticated by Dr Bhaskar Dutt, Taxonomist, NBRI. They were deposited (Specimen numbers NBR/PH/2739 to NBR/PH/2743, respectively) in the departmental herbal drug museum of the Pharmacognosy Division, NBRI, Lucknow, India for future reference and used as standard. Organoleptic characters and particle size of all the samples were recorded. Quantitative analysis for total ash, acid insoluble ash, and sulphated ash, successive Soxhlet extractives in n-hexane, alcohol and water, loss on drying at 105 °C and pH of filtrate of 10 % w/v aqueous solution were carried out in triplicate in all 4 samples of *Panchkola Churna*, according to the standard methods prescribed in Indian Pharmacopoeia. Total percentage of tannins was also determined in all the samples.

Microscopic analysis of the samples was also carried out. A small quantity representative of the *Cūrnas*, along with the genuine samples, i.e., *Pippali* (Fruit), *Pippali mula* (Root), *Chavya* (Fruit), *Chitrak* (Root) and *Shunthi* (Rhizome), mixed well with water, stained with iodine and mounted in glycerine were used to examine the starch grains and its type. Another small quantity of samples cleared by heating with chloral hydrate and mounted in glycerine was used to identify diagnostic microscopical characters of the ingredients. Further, small quantity of the *Cūrnas* cleared with dilute KOH 5 % were mounted in glycerine. For HPTLC, 2 gm of each sample was extracted with 25 ml of methanol on boiling water bath for 25 min consecutively 3 times using fresh portion of 25 ml methanol, filtered and concentrated. Similarly, methanolic extracts were prepared for all 5 ingredients, i.e., *P. longum* -Fruit, *P. longum* -Root, *P. retrofractum* -Fruit, *P. indica* – Root and *Z. officinale* - Rhizome for use as standard. TLC of the methanolic extracts of all the samples and the reference ingredients was carried out on silica gel 60F 254 precoated plates (0.2 mm thickness; Merck). Camag Linomat IV applicator was used for band application and Desaga Video documentation Unit III was used for documentation of fingerprint profiles. The mobile phase used was Toluene: Ethyl acetate (70:30). The plate was developed over a distance of 9 cm and visualized under UV 254 nm. Spiking with individual ingredients was also done before microscopical and HPTLC studies.

**Results and discussion**

*Panchkola Churna* samples of 4 different manufacturers, PC-1, PC-2, PC-3 and PC-4, were subjected to analysis as above. All the samples were creamish brown powders, odour sweet and pleasant, and taste bitter pungent. Nearly 70.63-89.91 % of all 4 samples passed through 60 mesh SS sieve but only 45.86-49.00 % of the samples passed through 85 mesh sieve. Results of loss on drying at 105 °C, pH of 10 % w/v aqueous solution, ash values, successive Soxhlet extractives and tannins were calculated and are given in Table 1. It was observed that although

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PC-1</th>
<th>PC-2</th>
<th>PC-3</th>
<th>PC-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Creamish brown powder, odour sweet pleasant, taste bitter pungent</td>
<td>Creamish brown powder, odour sweet pleasant, taste bitter pungent</td>
<td>Creamish brown powder, odour sweet pleasant, taste bitter pungent</td>
<td>Creamish brown powder, odour sweet pleasant, taste bitter pungent</td>
</tr>
<tr>
<td>Particle size (% w/w)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passing through 60 mesh</td>
<td>82.62</td>
<td>70.63</td>
<td>89.91</td>
<td>85.52</td>
</tr>
<tr>
<td>Passing through 85 mesh</td>
<td>45.86</td>
<td>48.88</td>
<td>49.00</td>
<td>48.00</td>
</tr>
<tr>
<td>Ash values (% w/w)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ash</td>
<td>7.42</td>
<td>7.14</td>
<td>9.34</td>
<td>5.81</td>
</tr>
<tr>
<td>Acid insol. ash</td>
<td>0.97</td>
<td>1.22</td>
<td>1.97</td>
<td>0.83</td>
</tr>
<tr>
<td>Sulphated ash</td>
<td>10.48</td>
<td>10.25</td>
<td>12.75</td>
<td>7.86</td>
</tr>
<tr>
<td>Successive Soxhlet extractives (% w/w)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexane</td>
<td>4.38</td>
<td>3.67</td>
<td>4.44</td>
<td>4.68</td>
</tr>
<tr>
<td>Alcohol</td>
<td>7.82</td>
<td>12.59</td>
<td>10.72</td>
<td>16.71</td>
</tr>
<tr>
<td>Water</td>
<td>9.27</td>
<td>6.46</td>
<td>4.71</td>
<td>6.56</td>
</tr>
<tr>
<td>Loss on drying (105 °C) (% w/w)</td>
<td>10.85</td>
<td>6.65</td>
<td>8.28</td>
<td>8.00</td>
</tr>
<tr>
<td>pH (Filtrate of 10 % w/v aqueous solution)</td>
<td>5.37</td>
<td>5.10</td>
<td>5.44</td>
<td>5.24</td>
</tr>
<tr>
<td>Tannin (% w/w)</td>
<td>0.78</td>
<td>0.65</td>
<td>0.52</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*Values are means of three determinations each*
most of the parameters showed similar results. However, there were some variations in the parameters like, alcohol and water soluble extractives. Percentage of alcohol soluble extractive was less, i.e., 7.82% in PC-1 and high 16.71% in PC-4 sample. Likewise the percentage of water soluble extractive was less in PC-3 and high in PC-1. The ash values also varied in the samples analyzed. The results indicate that, these variations may be due to the quality of raw materials used to prepare the sample.

Microscopic examinations were also carried out to see the presence of the different ingredients in all 4 samples of Panchkola Churna (Fig. 1 a&b. Perisperm cells, large, polygonal, isolated or in groups of 2 or 3, filled with simple and compound starch grains measuring 2-5 µ in diameter, these cells look like a large rhomboidal crystals of calcium oxalate due to the deposition of calcium oxalate on boundaries which dissolve with conc. HCl and 60% H₂SO₄ (w/w), the boundaries treated with H₂SO₄ on standing replaced by needles of calcium oxalate, presence of stone cells measuring 130-190 µ in dia. with broad lumen in groups of 2-8 (Pippali); groups of angular 45-100 µ long, non-lignified, thick walled, pitted parenchymatous cells, patches of polygonal to tangentially elongated, pitted parenchymatous cells, pits minute cells, filled with starch grains at some places; starch grains simple, oval to rod shaped, 15 – 45 µ in dia., hilum eccentric and crescent, patches of light brown polygonal, suberized cork cells; rosette and prismatic crystals of calcium oxalate measuring 90-120 µ in dia.; fragments of pitted vessels. (Chitrak). fragments of suberized cork cells, penta to hexagonal in shape, group of parenchymatous cells, densely filled with starch grains, isolated starch grains, simple, measuring 15-70 µ in dia., hilum eccentric, lamellae distinct, yellow coloured oleo resin cells, which turns yellowish brown with dilute potassium hydroxide solution, fragments of vessels 25-70 µ broad having scalar form and reticulate secondary wall thickening, fibres, non-lignified, sepatate, some of them dentate, 30-50 µ broad, unicellular, trichomes measuring 45-70 µ in length (Shunthi); fragments of parenchyma filled with starch grains, scattered starch grains, simple or compound measuring 5-12 µ in dia., hilum and lamellae not distinct; stone cells, oval, elongated with broad lumen varying in size measuring 50-100 µ in dia., groups of non-lignified thick walled pitted parenchymatous cells measuring 50-60 µ, oil globules which turns red with
Sudan red III, fragments of fibres and vessels, either in group or isolated, vessels with spiral and scalariform secondary wall thickening, 45-70 µ broad, fibres simple, 10-40 µm broad, lignified which turns red when treated with phloroglucinol followed by conc. HCl (Pippali mula); large polygonal cells, isolated or in groups of 2-3 cells, consisting network of circular septum present giving the view of parenchymatous cells (Perisperm cells), these polygonal cells (Perisperm cells) look like a large rhomboidal crystals of calcium oxalate of due to deposition of calcium oxalate on boundaries which dissolved with conc. HCl and 60 % H₂SO₄ (w/w), the sulphuric acid soluble powder on standing replaced by needles of calcium sulphate, filled with very minute starch grains, simple or compound measuring 2-5 µ in dia., stone cells in groups of 5-20, measuring 40-90 µ in width, pitted and brown in colour (Chavya).

TLC of the methanolic extracts of all the samples and the reference ingredients was carried out on silica gel plates (Fig. 2). Several bands were observed, some of which were specific to the individual ingredients. These bands were chosen to identify the presence or absence of the individual ingredients in each of the four samples. It was observed that all these identifying bands were present in all the Cūrṇa samples thereby indicating the presence of all the five ingredients in each of them. Table 2 enlists the identifying bands of the individual ingredients along with their R_f values.

It was observed that the microscopic observations also indicated the presence of all the ingredients in all the four samples thereby supporting the HPTLC results. Thus, the HPTLC findings and the microscopical examinations both revealed that *Piper longum*-Fruit, *Piper longum*-Root, *Piper retrofractum*-Fruit, *Plumbago indica*–Root and *Zingiber officinale* - Rhizome had been used in the manufacturing of PC-1, PC-2, PC-3 and PC-4.

**Conclusion**

In the current studied Ayurvedic formulation *Panchkola churna* the detailed botanical standards (macroscopical & microscopical) and the distinguishing bands in the HPTLC profiles are very important parameters for monitoring the quality of the compound formulation as well as for establishing whether all the required ingredients are present in them. The development of reliable quality protocols for Ayurvedic formulations is of utmost importance for regular authentication of the prepared batches and also to check the batch to batch variations. Hence, the
physiochemical parameters, quantitative analysis, HPTLC fingerprint profiles and the microscopic characteristics together may be used for quality evaluation and the standardization of compound formulations and maintaining their quality, purity and efficacy. As India is one of the major emerging countries where single herbal drug and Ayurvedic formulations are widely used, it can play an important and lead role in production of standardized, therapeutically effective Ayurvedic formulations.

Acknowledgement

The authors are thankful to the Director, NBRI, for providing the research facilities and are thankful to Ministry of AYUSH, Government of India, for financial support and for providing different samples from its pharmacies.

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