Pharmacognostical, Physicochemical and Phytochemical Studies of Some Marketed Samples of Roots used in Ayurvedic Medicines

Amrita Mishra*, Arun K Mishra1, Ashoke K Ghosh1, Shivesh Jha2

1Department of Pharmacognosy, College of Pharmacy, IFTM, Moradabad India-244001.
2Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi, India-835215

ABSTRACT

Background: Kuth (Saussurea lappa), Nisotha (Operculina turpethum), Akarakara (Anacyclus pyrethrin) and Chitrak (Plumbago zeylanicum) are some common plants used in Ayurvedic system of medicines and herbal drugs. Objective: The objective of the present study is to evaluate the quality of the samples of same plant marketed into different area, on the standardization parameters given in Ayurvedic pharmacopoeia. Methods: Three different marketed samples of these roots were subjected to pharmacognostic, physicochemical and phytochemical analysis and results were compared to the standards given in Ayurvedic pharmacopoeia. Results: Variations were found in physicochemical and phytochemical parameters of two samples in each case of Saussurea lappa, Operculina turpethum, Anacyclus pyrethrin and of one sample in case of Plumbago zeylanicum. Pharmacognostical parameters were found same as given in Ayurvedic pharmacopoeia for all the samples. Conclusions: The outcome of the study suggested that there is a lot of difference in the quality of the same drug marketed in different parts of country which may also cause variation in products prepared from them. These findings may be very useful for the identification of the species which may be useful to pharmaceutical industries for the quality control of the commercial samples.

Keywords: Ayurvedic Pharmacopoeia, Commercial sample, Quality control.

INTRODUCTION

Kuth (Saussurea lappa), Nisotha (Operculina turpethum), Akarakara (Anacyclus pyrethrin) and Chitrak (Plumbago zeylanicum) are some common plants used in Ayurvedic system of medicines and herbal drugs.[1,2,3,4] Kuth is used for anti-inflammatory, anti-ulcer, anticancer and hepatoprotective activities; Nisotha is an important ingredient of Ayurvedic formulation viz. Avipattikara churna used for the treatment of gastric ulcer, gastrointestinal related disturbances and also used in treatment of piles, tumors and jaundice.[5,6,7] It also reported to have hepatoprotective and antimicrobial activities.[8,9] Akarakara plant is used in traditional system of medicine as a tonic to the nervous system and also reported to have antibacterial, antidepressant and anti-inflammatory activities.[10] Chitrak is used against a number of ailments including skin diseases, diarrhea and leprosy. It also possesses antibacterial, antifungal, anti-carcinogenic, antitumor properties.[11] These plant drugs are available in local market of many cities in Uttar Pradesh, from where they are utilized by local people as home remedies and by small scale Ayurvedic drug manufacturers. These Plant materials may vary in their quality and therefore in its therapeutic effect according to different places of collection, with different times in a year for collection, with collection at the same time and places but in different years and with different environmental factors surrounding the cultivation of a particular medicinal plant.[12] This difference may cause batch to batch variation or also may cause city to city variation in quality, safety and efficacy of same formulation. The objective of the present study is to evaluate the quality of the samples of same plant marketed into different area, on the standardization parameters given in Ayurvedic pharmacopoeia.
MATERIALS AND METHODS

Sample collection
The samples of all four drugs were purchased from three different locations and labeled properly as per [table 1].

Authentication
Samples were authenticated by Dr. N. K. Dubey, Professor, Department of Botany, BHU, Varanasi and a voucher specimen is preserved in herbarium section for future reference.

Organoleptic properties
Organoleptic properties were evaluated including appearance, size, color, taste and odour following the method described by Wallis et. al, 1989.[13] For determining the odor of an innocuous material, small portion of the sample was placed in the beaker of suitable size, and examined by slow and repeated inhalation of the air over the material. If no distinct odor was perceptible, the sample was crushed between the thumb and index finger, between the palms of the hands, using gentle pressure or if the material was known to be dangerous, by other suitable means such as pouring a small quantity of boiling water onto the crushed sample placed in a beaker. First, the strength of the odor was determined (none, weak, distinct, strong) and then the odor sensation (aromatic, fruity, musty, moldy, rancid, etc.) was studied. Taste was distinctively classified as aromatic, pungent, sweet, sour, astringent, mucilaginous, or bitter.

Table 1: Location of sample procurement and coding

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Location of Collection</th>
<th>Code for labeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuth (Saussurea lappa)</td>
<td>Dinanath Gola Market, Varanasi</td>
<td>SL1</td>
</tr>
<tr>
<td></td>
<td>Buddha Bazaar, Moradabad</td>
<td>SL2</td>
</tr>
<tr>
<td></td>
<td>Chauk market, Jhansi</td>
<td>SL3</td>
</tr>
<tr>
<td>Nisotha (Operculina turpethum)</td>
<td>Dinanath Gola Market, Varanasi</td>
<td>OT1</td>
</tr>
<tr>
<td></td>
<td>Buddha Bazaar, Moradabad</td>
<td>OT2</td>
</tr>
<tr>
<td></td>
<td>Chauk market, Jhansi</td>
<td>OT3</td>
</tr>
<tr>
<td>Akarakara (Anacyclus pyrethrin)</td>
<td>Dinanath Gola Market, Varanasi</td>
<td>AP1</td>
</tr>
<tr>
<td></td>
<td>Buddha Bazaar, Moradabad</td>
<td>AP2</td>
</tr>
<tr>
<td></td>
<td>Chauk market, Jhansi</td>
<td>AP3</td>
</tr>
<tr>
<td>Chitrak (Plumbago zeylanicum)</td>
<td>Dinanath Gola Market, Varanasi</td>
<td>PZ1</td>
</tr>
<tr>
<td></td>
<td>Buddha Bazaar, Moradabad</td>
<td>PZ2</td>
</tr>
<tr>
<td></td>
<td>Chauk market, Jhansi</td>
<td>PZ3</td>
</tr>
</tbody>
</table>

Microscopic study
All samples were cleaned and boiled separately. Their transverse sections were cut, stained, mounted and observed under microscope.[14]

Physicochemical analysis

Water soluble extractive value
Five gm. of the air-dried, coarsely powdered drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaken frequently during the first 6 hours and allowed to stand for 18 hours. There often filtered rapidly evaporated 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish and was dried at 105 °C and weighed. Percentage of water soluble extractive was calculated with reference to the air dried drugs.

Ethanol soluble extractive value
Five gm. of the air dried coarsely powdered drug was macerated with 100 ml of ethanol of the specified strength in a closed flask for 24 hours, shaken frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter rapidly taking precautions against loss of ethanol, evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and was dried at 105°C, and weighed. Percentage of ethanol soluble extractive was calculated with reference to the air dried drug.

Total Ash value
Accurately 2 g of the air dried crude drug was weighed in a tarred platinum or silica dish and incinerate at a temperature not exceeding 450° until free from carbon and then cold and weighed again. Percentage of ash was calculated with reference to the air-dried drug.

Acid insoluble ash value
Accurately 2 g of air dried crude drug was weighed in a tarred platinum or silica dish and incinerated at temperature not exceeding 450°C until free from carbon and then cold and weighed again. Then the ash was boiled with 25 ml of 2M hydrochloric acid for 5 minutes. The insoluble matter was collected in a Gooch crucible or on an ash less filter paper, washed with hot water, ignited, cold in desiccators and weighed. Percentage of acid insoluble ash was calculated with reference to the air-dried drug.[15]

Qualitative Phytochemical analysis
To detect the presence of various phytoconstituents in these samples, phytochemical investigation was performed.[16,17]

Thin layer chromatography
TLC of the alcoholic extract of OT1, OT2 and OT3 was developed on Silica gel ‘G’ plate using Toluene: Ethylacetate (9:1) as mobile phase. Vanillin-sulphuric acid reagent was
Pharmacognosy Journal | August 2011 | Vol 3 | Issue 24

Table 2: Results of organoleptic properties test

<table>
<thead>
<tr>
<th>Sample</th>
<th>Colour</th>
<th>Odour</th>
<th>Taste</th>
<th>Size and shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL1</td>
<td>Brown</td>
<td>None</td>
<td>Blunt</td>
<td>10-15 cm. long, 1.5 cm broad, cylindrical</td>
</tr>
<tr>
<td>SL2</td>
<td>Brown</td>
<td>None</td>
<td>Blunt</td>
<td>5-8.5 cm. long, 1 cm broad, thick, cylindrical, hard</td>
</tr>
<tr>
<td>SL3</td>
<td>Grayish</td>
<td>None</td>
<td>Blunt</td>
<td>5-10 cm. long, 1.5 cm broad, thick, cylindrical hard</td>
</tr>
<tr>
<td>OT1</td>
<td>Dull grey</td>
<td>None</td>
<td>Acrid</td>
<td>1-7 cm long, 1 cm diameter, cylindrical, longitudinal wrinkles</td>
</tr>
<tr>
<td>OT2</td>
<td>Brown</td>
<td>None</td>
<td>Acrid</td>
<td>1-10 cm long, 1 cm diameter, cylindrical, longitudinal wrinkles, thin rootlets</td>
</tr>
<tr>
<td>OT3</td>
<td>Dull grey</td>
<td>None</td>
<td>Acrid</td>
<td>1-15 cm long, 1 cm diameter, cylindrical, longitudinal wrinkles, thin rootlets</td>
</tr>
<tr>
<td>AP1</td>
<td>Dark brown</td>
<td>None</td>
<td>Pungent</td>
<td>8-10 cm long, tapering, hairy rootlets. Aromatic</td>
</tr>
<tr>
<td>AP2</td>
<td>Dark brown</td>
<td>SlightlyPungent</td>
<td>5-10 cm long, tapering, hairy rootlets, Aromatic</td>
<td></td>
</tr>
<tr>
<td>AP3</td>
<td>Grayish brown</td>
<td>None</td>
<td>Acrid</td>
<td>5-10 cm long, tapering, hairy rootlets. Aromatic</td>
</tr>
<tr>
<td>PZ1</td>
<td>Brown</td>
<td>None</td>
<td>Acrid</td>
<td>10-15 cm long, 1.2 cm dia. cylindrical</td>
</tr>
<tr>
<td>PZ2</td>
<td>Brown</td>
<td>Disagreeable</td>
<td>Acrid</td>
<td>15-25 cm long, 1 cm dia., cylindrical</td>
</tr>
<tr>
<td>PZ3</td>
<td>Brown</td>
<td>None</td>
<td>Acrid</td>
<td>15-20 cm long, 1 cm dia. cylindrical</td>
</tr>
</tbody>
</table>

RESULTS

Organoleptic properties

The results of organoleptic evaluations are presented in [Table 2] and [Figure 1].

Microscopic study

Transverse section of root samples SL1, SL2 and SL3 showed the presence of cork, 3-5 layered wide, secondary phloem consisting of mostly storage parenchyma, modularly rays multi seriate, resin canals throughout as cavities, xylem, fibers, vessels and xylem parenchyma groups were found scattered in the center and inulin was observed in storage parenchyma. Transverse section of root samples OT1, OT2 and OT1 showed thin cork, consisting of 3-5 rows of brown cells, broad cortex consisted of clusters of parenchyma cells and resin canals. It was consisting of continuous circular zone of secondary phloem and dense secondary xylem, cleared radially in to wide four or five fan shaped segments by narrow xylem rays. The wide vessels, calcium oxalate crystals in prisms and rosettes shape were also observed. The starch grains were found scattered in cortex, phloem parenchyma, xylem parenchyma and medullary ray cells.

Transverse section of root samples of AP1, AP2 and AP3 showed cork consisting of tabular cells, many of which developed as sclerenchyma, a few sclerenchymatous cells also found scattered in secondary cortex; developed secondary phloem; cambium 2-5 layered; secondary xylem very wide consisting of xylem vessels, tracheids and xylem parenchyma; vessels pitted; medullary rays numerous, running straight, bi to tri and multi seriate; oleo-resinous schizogenous glands found scattered in secondary cortex, secondary phloem and medullary rays.

Transverse section of root samples PZ1, PZ2 and PZ3 showed outer most layer cork with 5-6 rows of light brown cells, rectangular in shape; starch grains compactly packed in the cortex region, phloem well developed with phloem fibers. Groups of phloem fibers were present near the phloem. Cambium single layered, xylem was well developed with xylem vessels. Medullary ray was single to multilayered and loaded with simple to compound starch grains [Figure 2].
Figure 2: Microscopy of Kuth (Saussurea lappa), Nisotha (Operculina turpethum), Akarakara (Anacyclus pyrethrin), Chitrak (Plumbago zeylanicum)
Physicochemical analysis
Upon physicochemical analysis for all the roots samples on several parameters, outcome was matched with standard and presented in Table 3. All the tests were performed in triplicate and result is presented in mean ± SEM.

Qualitative Phytochemical analysis
Upon phytochemical investigation of all the samples, different constituents were reported for all the samples. [Table 5]

Thin layer chromatography analysis
OT 1 showed five spots of violet color appearing at Rf 0.20, 0.40, 0.49, 0.57 and 0.97. OT2 showed seven spots appearing at Rf 0.21, 0.41, 0.48, 0.58, 0.60, 0.62 and 0.96. OT3 showed seven spots appearing at Rf 0.21, 0.41, 0.49, 0.58, 0.60, 0.92 and 0.97. The reported numbers of spots are seven with Rf values 0.21, 0.41, 0.49 (all light violet), 0.58, 0.70, 0.90 and 0.97 (all violet) as per pharmacopoeia [Figure 3].

DISCUSSION
Kuth (Saussurea lappa), Nisotha (Operculina turpethum), Akarakara (Anacyclus pyrethrin) and Chitrak (Plumbago zeylanicum) are some of the very common plants used in Ayurvedic system of medicine. Evaluation of qualitative pharmacognostical parameters, physicochemical parameters and qualitative phytochemical screening can be useful in...
standardization of the marketed samples of these drugs. In the present study, all samples were collected from different parts of Uttar Pradesh, India to evaluate the uniformity of the quality of raw materials used by small scale Ayurvedic drug manufacturers.

The samples were found almost uniform in their organoleptic properties. The variation observed may be due to the difference in storage conditions, collection process and age of plant. The qualitative pharmacognostical parameters were found uniform in all the samples.

The variation was observed in physicochemical properties; SL1, SL3, OT1, OT2, AP1, AP2 and PZ3 showed variation in all physicochemical parameters, from the standard value given in Ayurvedic Pharmacopoeia. The physicochemical parameters like extractive value, ash value indicates the quality and purity of drugs. Extractive values are representative of the presence of the polar or nonpolar extractable compounds in a plant material. The total ash usually consists of carbonates, phosphates, silicates, and silica, which include both physiologic ash and nonphysiologic ash. The variation in these parameters from standard value indicates the low quality of the samples. In qualitative Phytochemical analysis the sample were found uniform but in the thin layer chromatography results, absence of some spots in sample OT1 and OT2 was observed which indicates the absence of a particular group of compound in these samples.

**CONCLUSION**

By the above study, it can concluded that there is absence of uniformity in the quality of same plant material marketed in different area, which can result into variation in quality, safety and efficacy of same formulation manufactured in different area. Therefore emphasis is to be laid on the collection process, sources, storage conditions and standardization of raw materials in order to maintain the quality aspects of the product throughout worldwide.

**ACKNOWLEDGEMENT**

The authors are thankful to Managing Director, IFTM, Moradabad for providing all the laboratory facilities and chemicals to carry out this work.

**REFERENCES**


