PHCOG J: Research Article
Evaluation and Identification of Meda and Mahameda to Common Substitutes and Adulterants In the Crude Drug Markets of India

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ABSTRACT
Meda (Polygonatum verticillatum (L.) Alt.) and Mahameda (Polygonatum cirriformum (Wall.) Royle) are described as Astavargha group of drugs having antioxidanct and anti-ageing effect in ancient texts of ayurveda but the botanical identity of Meda and Mahameda are controversial and different plant species are being used or sold in the name of Meda and Mahameda in different parts of India. In present paper efforts have been made to lay down pharmacopeial characters and to identify botanical identity of market samples by comparing with genuine drugs.

KEYWORDS: Macroscopy, Microscopy, Thin Layer Chromatography, Physico-chemical evaluation.

INTRODUCTION
In Charak samhita Meda and Mahamaeda are components of jivaniyagana while ‘Susruta’ treated them under Kakolyadigana to promote longevity but in modern period, these are considered under Ashwatavarga and are attributed to immunomodulators and tonics. These are widely used in fever; as restorative, galactagogue, blood purifier and spermogenetic.

In ayurveda rhizomes of Polygonatum verticillatum (L.) Alt. (syn: Convallaria verticillata L., syn: Evallaria verticillata Necker) and Polygonatum cirriformum (Wall.) Royle of family Liliaceae are used as Meda and Mahamaeda. However it was observed that in different places, different plant species are being used as Meda and Mahamaeda. Hence there is an urgent need to evaluate the genuine drug and to identify which botanical species are being used / sold as substitute / adulterants.

In this context the detailed pharmacognstical study along with preliminary physicochemical study on roots and rhizomes of P. verticillatum and P. cirriformum have been undertaken in order to establish salient diagnostic features of both the species and efforts have also been made to identify botanical equivalent of commercial samples procured from different crude drug markets of the Country.

MATERIAL AND METHODS
Fresh roots and rhizomes of botanically identified plants of Polygonatum verticillatum and Polygonatum cirriformum were collected from medicinal plants garden of Regional Research Institute (Ayu.), Tarikhet and Indian Medicines Pharmaceutical Corporation Limited, Mohan (Almora). Roots and rhizomes were washed, cut into pieces and preserved in Formal-acetyl-alcohol (FAA) and labeled PV5 and PC5 for pharmacognostical study and some were shade dried and coarse (20 to 30 #) powdered for qualitative tests and Physico-chemical study as per IP/API / WHO guidelines. The physicochemical parameters like total ash, acid insoluble ash, water and alcohol soluble extractives of all the samples were carried out according to IP procedures. The thin layer chromatography of 90 % ethanolic extract of all samples were performed on precoated silica gel 60 F254 aluminum plates and the plates were developed in mobile phase Toluene: Ethyl acetate: Glacial acetic acid (5:6:1:0.3). The developed plates were observed under UV 254 nm and 366 nm and after derivatization in iodine vapours.

Market samples PV1, PV2, PV3, PV4 and PC1, PC2, PC3, PC4 were collected in the name of Meda and Mahamaeda from Lucknow (Uttar Pradesh), Jaipur (Rajasthan), Mandi (Himachal Pradesh), New Delhi, respectively and compared Pharmacognostically as well as phytochemically with authentic drugs.

RESULTS
Pharmacognosy of Polygonatum verticillatum Alt.
Macroscopic Characters
The dried rhizomes branched irregularly, about 5 cm long and 1.5 cm thick, cylindrical – knotty, dorsiventrally flattened with blunt to tapering ends. The outer surface mostly dull reddish-brown to light grayish in colour, marked with numerous encircling leaf scars and show root scars and wiry adventitious roots. Hard in texture, fracture short and horny, internally deep reddish-brown with characteristic odour of burnt sugar and acrid in taste.

Microscopic characters
The T.S of adventitious roots (Figure 1a-b) show a single layered epidermis interrupted by unicellular hairs, the size of the hair varies between 45-355 µm followed by 12-16 layers of varying sizes (75-140-355 µm) at parenchymatous cortex. A few cortical cells contain bundles of raphides of calcium oxalate. The schizogenous mucilage canals lined by epithelium of 5-8 cells and varying in sizes (18-45-66 X 21-54-70) µm also present in the cortex. The endodermis distinct showing well developed thickening on the inner tangential and radial walls, a few thin walled passage cells are also observed.
The pericycle thin walled and encloses within it a polyarch stele. The T.S. of rhizome (Figure 1c-d) shows 6-9 layers of cork cells (12-24-54 X 15-45-60 µm) with lenticels at places. The cork followed by wide parenchymatous ground tissue, the outer region of which shows only large mucilaginous canals (90-120-240 µm). In the middle and central regions, a large number of vascular bundles present. The vascular bundles are mostly of amphivasal type but a few showing typical conjoint collateral arrangement and both type being enclosed by bundle sheath, mucilage canals also distributed in this region. The phloem consists of sieve-tubes, companion cells and phloem parenchyma and xylem mostly consists of tracheids, vessels and a few fibres. The vessels and tracheids greatly vary in shape and sizes mostly showing either peg like outgrowth or irregular lateral projections and scalariform thickening. However, few shows spiral thickening on their walls.

**Pharmacognostical Studies of Polygonatum cirrifolium Royle**

**Macroscopic Characters**
The dried rhizomes branched 3-5 cm long and 0.4-0.7 cm thick, somewhat conical, dorsiventrally flattened showing a distinct groove or furrow on one of the flattened side. Few pieces show fused pear shaped or irregularly oblong rhizomes in pairs. Rough surface, wrinkled with indistinct leaf scars and root scars or wiry adventitious roots as in *P. verticillatum* observed. Grayish-brown in colour and show beak like tapering ends, fracture short exposing deep reddish brown waxy interior, taste sweetish becoming acrid afterwards and burnt sugar odour.

**Microscopic Characters**
The T.S. of adventitious roots (Figure 2a) showed similar structure as in the roots of *P. verticillatum* except absence of the bundles of raphides of calcium oxalate in the cortical cells of size (12-30-45 X 9-21-39 µm) and the meta-xylem at the periphery of pith cell size (5-12-16 X 9-18-21 µm).

Table 1: Observations Physicochemical parameters of powdered samples of Meda

<table>
<thead>
<tr>
<th>Parameters %</th>
<th>PV5 Polygonatum verticillatum</th>
<th>PV2 Polygonatum verticillatum</th>
<th>PV3 Polygonatum verticillatum</th>
<th>PV4 Polygonatum verticillatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Ash (% w/w)</td>
<td>3.32</td>
<td>5.32</td>
<td>4.89</td>
<td>4.02</td>
</tr>
<tr>
<td>Acid insoluble ash (% w/w)</td>
<td>0.83</td>
<td>1.54</td>
<td>0.73</td>
<td>0.90</td>
</tr>
<tr>
<td>Water soluble ash (% w/w)</td>
<td>1.23</td>
<td>1.82</td>
<td>1.83</td>
<td>1.43</td>
</tr>
<tr>
<td>Water soluble extractive (% w/w)</td>
<td>49.51</td>
<td>43.44</td>
<td>38.08</td>
<td>48.25</td>
</tr>
<tr>
<td>Alcohol soluble extractive (% w/w)</td>
<td>33.13</td>
<td>29.54</td>
<td>25.05</td>
<td>35.54</td>
</tr>
<tr>
<td>TLC Under (Fig. 3)</td>
<td>0.51, 0.59, 0.93</td>
<td>0.51, 0.93</td>
<td>0.51, 0.59, 0.93</td>
<td>0.51, 0.59, 0.66, 0.84, 0.93</td>
</tr>
<tr>
<td>UV 254 nm (R Values) Under</td>
<td>0.52, 0.82, 0.93</td>
<td>0.52, 0.80</td>
<td>0.40, 0.51, 0.82, 0.92</td>
<td>0.40, 0.51, 0.82, 0.92</td>
</tr>
<tr>
<td>UV 366 nm (R Values)</td>
<td>0.18, 0.51, 0.73, 0.91, 0.94</td>
<td>0.18, 0.51, 0.72</td>
<td>0.18, 0.51, 0.72</td>
<td>0.12, 0.18, 0.51, 0.59, 0.72, 0.83, 0.91, 0.94</td>
</tr>
</tbody>
</table>

The T.S. of rhizome (Figure 2b) showed 5-10 layers of cork cells (15-21-45 X 45-90-120 µm) with lenticels at places. Cork followed by wide zone of parenchymatous ground tissue, the outer region has large mucilage canals (124-165-180-210 µm) surrounded by 7-8 epithelial cells and bundles of raphides of calcium oxalate (27-50-90 X 42-70-115 µm). The middle and central region of ground tissue, however, exhibits a large number of scattered vascular bundles. The vascular bundles mostly of amphivasal type but few have conjoint, collateral arrangement as in *P. verticillatum* and both types being enclosed by a bundle sheath of thin walled cells. Mucilage canals also scattered in this region. The phloem consists of normal elements. The xylem shows conspicuous absence of xylem fibres. The vessels and tracheids have mostly reticulate thickening on their walls and are without any peg like outgrowth or irregular lateral projections as in *P. verticillatum*. However, an abrupt narrowing in the middle region observed.

**Comparative Studies of Market samples**
The detail macro and microscopical characters of all the market samples PV1 – PV4 & PC1-PC4 were studied and compared with both *Polygonatum* species. It was observed that samples PV2 and PV4 shows more or less similar macro and microscopical details with that of *P. verticillatum* however some part of the stem pieces are found along with root and rhizomes of *P. verticillatum* in the market sample PV3, which are remain absent in other samples. Therefore, the sample PV3 is found to be adulterated one. The Physicochemical analysis (Table 1) and TLC of 90 % ethanolic extract (Figure 3) of powders of all the samples were carried out and the observations compared.

The pharmacognostic characters of PC1 and PC2 are identified same as *P. cirrifolium*. Sample PC4 resembles the pharmacognostic characters of *Asparagus racemosus* (Figure 5a-e).
Table 2: Observations Physicochemical parameters of powdered samples of Maha-Meda

<table>
<thead>
<tr>
<th>Parameters %</th>
<th>Polygonatum cirrifolium</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Ash (% w/w)</td>
<td>2.9</td>
<td>6.54</td>
<td>3.5</td>
<td>6.52</td>
<td>5.0</td>
</tr>
<tr>
<td>Acid insoluble ash (% w/w)</td>
<td>0.78</td>
<td>2.78</td>
<td>1.52</td>
<td>1.34</td>
<td>0.5</td>
</tr>
<tr>
<td>Water soluble ash (% w/w)</td>
<td>1.12</td>
<td>2.12</td>
<td>1.90</td>
<td>2.83</td>
<td>2.60</td>
</tr>
<tr>
<td>Water soluble extractive (% w/w)</td>
<td>73.52</td>
<td>65.08</td>
<td>70.22</td>
<td>48.60</td>
<td>37.4</td>
</tr>
<tr>
<td>Alcohol soluble extractive (% w/w)</td>
<td>13.82</td>
<td>10.02</td>
<td>11.30</td>
<td>12.36</td>
<td>20.7</td>
</tr>
<tr>
<td>TLC (Fig.4) Under UV 254 nm (Rf Values)</td>
<td></td>
<td>0.20, 0.55, 0.70</td>
<td>0.20, 0.55</td>
<td>0.20, 0.55</td>
<td>-</td>
</tr>
<tr>
<td>Under UV 366 nm (Rf Values)</td>
<td></td>
<td>0.11, 0.20, 0.35, 0.55</td>
<td>0.20, 0.35, 0.55</td>
<td>0.11, 0.20</td>
<td>0.35, 0.55</td>
</tr>
<tr>
<td>In Iodine vapors under white light (Rf Values)</td>
<td></td>
<td>0.20, 0.55, 0.70, 0.80</td>
<td>0.55, 0.80</td>
<td>0.55, 0.80</td>
<td>0.55</td>
</tr>
</tbody>
</table>
**Mobile Phase:** Toluene: Ethyl acetate: Glacial acetic acid (5.6: 4.1 : 0.3)

*Figure 3:* TLC fingerprint of 98% ethanolic extract of samples

**Mobile Phase:** Toluene: Ethyl acetate: Glacial acetic acid (5.3: 4.2 : 0.5)

*Figure 4:* TLC fingerprint of 98% ethanolic extract of samples

**LEGENDS:**
Samples of *Polygonatum verticillatum* from:
PV2: Jaipur (Rajasthan), PV3: Manli (Himachal Pradesh), PV4: New Delhi, PV5: Tarakeshwar (Uttar Pradesh)

Samples of *Polygonatum aristolochium* from:
PC1: Ludhiana (Uttar Pradesh), PC2: Jaipur (Rajasthan), PC3: Manali (Himachal Pradesh), PC4: New Delhi, PC5: Tarakeshwar (Uttar Pradesh)
Figure 2a: T.S. of adventitious root of *P. cirrifolium* (diagramatic)

Figure 2b: T.S. of rhizome of *P. cirrifolium* (cellular details)

- Cuticle
- Epidermis
- Intercellular space
- Idioblast
- Starch grains
- Macerage cavity
- Cortex
- Xylem
- Phloem

Epidermis with root hairs

Stomata

Trachied

Vessel
Legends:
Figure 1a: T.S. of adventitious root of Polygonatum verticillatum Alt. (diagrammatic)
Figure 1b: T.S. of adventitious root of Polygonatum verticillatum Alt. showing cellular details
Figure 1c: T.S. of rhizome of Polygonatum verticillatum Alt. (diagrammatic)
Figure 1d: T.S. of rhizome of Polygonatum verticillatum Alt. cellular details
Market sample PC3 is identified as rhizomes of *Asparagus racemosus* admix with pieces of roots of *Eulophia campestris* Wall. The T.S. of adventitious roots (Figure 6a-b) of *E. campestris* show a single layered epidermis, some of the cells of which elongate to form unicellular hairs (380-450 - 570 µm).

DISCUSSION AND CONCLUSION

From ongoing studies it has been revealed that certain characters are common in both the species like in roots presence of wide cortex, endodermal cells showing thickening on radial and inner tangential walls except in thin walled passage cells and a polyarch stele which is only present in *P. verticillatum* similarly rhizomes show wide ground tissue in which a large number of mucilage canals and vascular bundles scattered in both the species. However, both the species can also be distinguished from each other for example in *P. verticillatum* 6-9 layers of cork cells, wide parenchymatous ground tissue, the outer region of which shows only large mucilaginous canals, large number of vascular bundles mostly being enclosed by bundle sheath, vessels and tracheids greatly vary in shape and sizes with either peg like outgrowth or irregular lateral projections and scalariform thickening present in rhizome. However number of cork cells 5-10 layers, large mucilage canals surrounded by 7-8 epithelial cells and bundles of raphides of calcium oxalate, number of scattered vascular bundles and absence of fibers in xylem observed in *P. cirrifolium*.

Like the anatomical structure of roots of *Asparagus racemosus* is quite characteristic and can be differentiated by having the cells of innermost layer are thick walled, lignified with simple pits, the bundles of raphides of calcium oxalate are present in cortex and the primary stele is polyarch from both the Polygonatum species.

The observation of physico-chemical evaluation indicates that most of the drugs available in market are not genuine or some what adulterated which leads to deteriorate the quality and efficacy of drug.

REFERENCES