Pharmacognostical and Phytochemical Evaluation of Rare and Endangered Habenaria spp. (Riddhi and Vriddhi)

Rath Chinmay#, Suman Kumari*, Dhar Bishnupriya#, Mohanty RC##, Singh Arjun#, Padhi MM#, Lavekar GS#

#Central Council For Research in Ayurveda & Siddha, Dept. of Ayush, Ministry of Health & F.W., Janakpuri, New Delhi-58
## Dept. of Botany, Utkal University, Vani Bihar, Bhubaneswar, Orissa

INTRODUCTION

Despite impressive accomplishment of modern medicines or products (or many) disease remains uncured or leads to iatrogenic disorders as a result people are diverging towards the alternative system of medicine. The increasing demand and poor cultivation leads to destructive harvesting system to fulfill the market demand that reduces the affluence and prosperousness of plant species in nature. The continuous exploitation of several medicinal plant species from wild and considerable loss of their habitats during past few years have resulted in population decline of many high yield value of medicinal plant species. In India out of 44% of medicinal plants 22% are found in himalayan region. Himalayan region have been found rich source of varied species of medicinal plants. Habenaria is one of the important genus of family Orchidaceae and found in the habitat of Himalaya.

Habenaria intermedia D. Don. and Habenaria edgeworthii Hook. f. are two plant known as Riddhi and Vriddhi in Ayurveda. Acharya Charaka and Sushruta mentioned these drugs under Astavarga group. These are one of the endangered species which are going to be extinct due to heavy exploitation for medicinal purpose, poor regeneration, low seed germination and seedling establishment, habitat loss, grazing, forest fire, competition with other dominant species of community. So due to scarcity of these plant drugs in market some drugs are sold to be adulterated or substituted which leads to deteriorate the quality and efficacy of drug. Some times shows toxic effect on body. Quality control of herbs and formulations is a major problem which effects the efficacy and reproducibility of the results in formulation.

The present study will be helpful to the ayurvedic practitioners, researchers and industries to identify and standardize the drug.

MATERIAL AND METHODS

The adventitious roots and tubers of authenticated plants of H. intermedia (R5) and H. edgeworthii (V5) were collected from Medicinal Plants Garden of Regional Research Institute (Ayu.), Tarikhet and Indian Medicines Pharmaceutical Corporation Limited, Mohan (Almora).
Some market samples were collected in the name of Riddhi viz. R1, R2, R3, R4 and vridhhi viz. V1, V2, V3, V4 from Delhi, Lucknow, Mandi and Jaipur respectively. Raw ingredients were washed, cut into pieces and preserved in Formalo-Acetyl-Alcohol (FAA) for pharmacognostical study and some were shade dried and coarse (20 - 30 #) powdered for qualitative tests and standardization as per IP / API / WHO Guidelines.

The thin layer chromatography of 90 % ethanolic extract of all samples were performed on pre-coated silica gel 60 F\textsubscript{254} aluminum plates and the plates were developed using suitable solvent system. The developed plates were observed under UV 254 nm and 366 nm and after derivatization under white light.

RESULTS

Pharmacognosy of Habenaria intermedia D. Don.

Macroscopic Characters

The fresh tubers are 15-35 mm long and 10-25 mm thick, oval, obovate, or oblong in shape, buff to yellowish brown in colour, sometimes with shrunken surface, covered with numerous fine white hairs; internally they are white to creamish in colour and mucilaginous to touch. The dried tubers of the market are hard, difficult to break, rough with fine reticulate surface and creamish brown to light brown in colour; the brown to light brown in colour; the broken surface is uneven exhibiting creamy interior. Both fresh and dry samples show scars of the aerial portion at the apex and beaked or sometimes round base. The tubers are odourless and taste is palatable and mucilaginous.

Microscopic Characters

The T.S. of adventitious roots (Figure 1a-1b) shows a single layered epidermis, some of the cells of which elongate and form unicellular hairs (380-890-1250-1720) \textmu m. The hairs are either straight with tapering to blunt ends, or broad, conical at the base and abruptly tapering to tail like ends or tortuous showing helical bendings (Figure 1e). The epidermis is followed by 8-12 layers of parenchymatous cortex. Some of the outer cortical cells of varying size (30-90-150-240 X 60-105-150-180) \textmu m exhibit presence of bundles of raphides of calcium oxalate (36-45-90 X 75-90-140) \textmu m. Below the endodermis is a layer of thin walled pericycle which encloses a polyarch stele.

The T.S. of a mature tuber, about 22 mm thick (Fig. 1c-1d) shows 2-3 layered epidermis bound proximally by a distinct exodermis. A number of outer epidermal cells (15-21-45-60 X 60-90-150-210) \textmu m elongate to form unicellular hairs (540-930-1840-2100 \textmu m) similar to as described earlier. The exodermis is followed by 15-20 layers of cortical parenchyma. The cells of which is proximity of the cortex. Below the cortex a typical polystelic condition is found and it consists of 14-16 steles arranged in a single and 7-10 steles distributed within the parenchyma in the central region (Figure 1c). Each stele of the tuber itself represents a clear diarch condition (Figure 1c, 1d).

A few parenchymatous cells of outer cortex contain bundles of raphides of calcium oxalate (45-60-90 X 60-120-150)\textmu m (Figure 1d). The schizontogenous mucilage canals (180-240-360 \textmu m) lined by an epithelium of usually 7-9 cells are found distributed throughout the parenchymatous tissue. The starch grains, mostly of simple type, consisting of both small (3-6-12\textmu m) and (30-45-60-90\textmu m) large grains are found distributed in abundance throughout the parenchyma as well as in the epithelial cells of mucilage canals. The small starch grains are mostly round with helium as point or cleft and larger ones are round to oval in shape with helium in form of a point or cleft or central triangular or 2-3 stellate clefts (Figure 1f).

Pharmacognosy of Habenaria edgeworthii Hook. f.

Macroscopic Characters

The fresh tubers are 12-28 mm. long and 9-12 mm. thick, oval, obovate or oblong in shape, fleshy, buff in colour with shrunken surface and covered with a large number of fine white hairs; internally the tubers are white and slimy to touch. The completely dried tubers of the market are hard, difficult to break, rough with fine reticulate markings, grayish brown to brown in colour; broken uneven surface shows creamy-yellow central area. Both fresh and dry samples show scar or remains of the aerial stem at the apex and finely beaked or sometimes round base. The tubers are odourless and the taste is bland and mucilaginous.

Microscopic Characters

Adventitious root in T.S. shows a single layered epidermis, some of the cells of which elongate to form unicellular hairs (482-958-1375-1665 \textmu m). It is followed by 3-15 layers of parenchymatous cortex. A few cortical cells contain bundles of raphides of calcium oxalate (45-60-96 X 66-81-124)\textmu m. The endodermis is distinct. Below the endodermis is a layer of thin walled pericycle which encloses a polyarch stele (Figure 2a-2b).

The T.S. of tuber, about 10 mm thick (Figure 2c-2d) shows 2 layered epidermis bounded proximally by a distinct exodermis. A number of cells of outer layer of epidermis elongate to form unicellular hairs (380-860-1230-1540)\textmu m similar in shape to those described for
Pharmacognostical and Phytochemical Evaluation of Rare and Endangered *Habenaria* spp.

**Figure 1.**

**Legend:**

- **Figure 1a:** T.S. of a portion of adventitious root of *Habenaria intermedia* D.Don. showing poly-arch condition (diagrammatic)
- **Figure 1b:** T.S. of a portion of adventitious root of *Habenaria intermedia* D.Don. showing cellular details
- **Figure 1c:** T.S. of a portion of tuber of *Habenaria intermedia* D.Don. showing polyystelic condition
- **Figure 1d:** T.S. of a portion of tuber of *Habenaria intermedia* D.Don. showing cellular details
- **Figure 1e:** Fragments of tracheids
- **Figure 1f:** Starch grains

Figure 1.
Pharmacognostical and Phytochemical Evaluation of Rare and Endangered Habenaria spp.

Legend:

Figure 2a: T.S. of a portion of adventitious root of Habenaria edgeworthii Hook.f. showing poly-arch stele (diagrammatic)

Figure 2b: T.S. of a portion of adventitious root of Habenaria edgeworthii Hook.f. showing cellular details

Figure 2c: T.S. of a portion of tuber of Habenaria edgeworthii Hook.f. showing poly-stelic condition (diagrammatic)

Figure 2d: T.S. of a portion of tuber of Habenaria edgeworthii Hook.f. showing cellular details.

A : Epidermis to outer cortex region
B-C: Cortical region
D : A stele showing tetra-arch condition
E : Central region

Figure 2e: Starch grains

Figure 2.
The exodermis is followed by 10-12 layers of cortical parenchymatous cells of sizes (60-120-165 X 45-90-160)µm, the cells of which in proximity of exodermis are smaller as compared to the remaining cells of cortex. Below the cortex a typical polystellic condition is observed and this consists of 8-19 steles arranged in a ring and 1-8 steles distributed within the parenchyma in the central region. Each stеле in itself represents a clear mono to pentarch condition (Figure 2c-2d.D).

A few parenchymatous cells of outer cortex contain bundle of raphides of calcium oxalate. The schizogenous mucilage canals (180-240-330) µm lined by an epithelium of usually 6-9 cells are found distributed throughout the parenchymatous tissue (Figure 2c, 2d, C, E). Starch grains, mostly of simple type are found in abundance and are distributed throughout the parenchyma including the epithelial cells of mucilage canals. The smaller starch grains (6-9-16-24) µm are mostly round with hilum as point or cleft and larger ones (30-65-135-150) µm are round to oval in shape with hilum in form of a point or cleft or central triangular or 2-7 stellate cleft (Figure 2e).

**Comparative pharmacognostic characters of market samples**

The detail macro and microscopical characters of all the market samples V1-V4 & R1-R4 were studied and compared with both Habenaria species and it was observed that only sample V1 tubers are found very hard to break which shows more or less similar macro and microscopical details with that of H. edgeworthii however the characters of market samples V2 and V3 were 1-6 × 0.5-1 cm pseudo-bulb, conical, translucent, reddish brown in colour, covered with light brown sheathing leaves, membranous with parallel venation, rough surface and slightly mucilaginous in taste. The transverse section passing through bulb shows a single layer of cuticle and a layer of thick walled sclerified epidermal cells followed by 1 or 2 layers of large sclerified epidermal cells and these extend unevenly into irregular ground parenchymatous tissue, with large air spaces at some places, prominent vascular bundles scattered throughout the ground tissue surrounded by thick walled sclerenchymatous cells which resembles to the characters of Microstylis wallichii Lindl. (Figure 6a-6c)(Family Orchidaceae).

The physicochemical analysis (Table 2) and TLC of 90 % ethanolic extract (Figure 4) of powders of all the samples were carried out using solvent system Toluene: Chloroform: Methanol (2.7 : 6.0 : 1.3 ) and the observations were compared.

The pharmacognostic characters of R1 and R3 were identified same as H. intermedia while characters of sample R4 was found similar to the Dioscorea bulbifera L. (Figure 5a-5f) (Family Orchidaceae) showing 9-40 mm

<table>
<thead>
<tr>
<th>S. No.</th>
<th>parameters %</th>
<th>Habenaria edgeworthii</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
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<tbody>
<tr>
<td>1.</td>
<td>Total ash (% w/w)</td>
<td>3.1</td>
<td>4.2</td>
<td>3.5</td>
<td>2.9</td>
<td>4.2</td>
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<tr>
<td>2.</td>
<td>Acid insoluble ash (% w/w)</td>
<td>0.6</td>
<td>0.98</td>
<td>1.2</td>
<td>1.0</td>
<td>1.5</td>
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<tr>
<td>3.</td>
<td>Water soluble extractive (% w/w)</td>
<td>29.9</td>
<td>20.3</td>
<td>15.3</td>
<td>16.2</td>
<td>12.7</td>
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<tr>
<td>3.</td>
<td>Alcohol soluble extractive (% w/w)</td>
<td>21.2</td>
<td>17.4</td>
<td>6.2</td>
<td>6.8</td>
<td>10.2</td>
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<tr>
<td>5.</td>
<td>TLC(figure 4) Under UV 254 nm (Rf Values)</td>
<td>0.18, 0.25, 0.32, 0.41, 0.45, 0.89, 0.96</td>
<td>0.07, 0.11, 0.15, 0.25, 0.29, 0.38, 0.43, 0.47, 0.85, 0.89</td>
<td>0.04, 0.07, 0.10, 0.22, 0.30, 0.30, 0.37</td>
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<td></td>
<td>Under UV 366 nm (Rf Values)</td>
<td>0.10, 0.19, 0.30, 0.32, 0.48, 0.67, 0.81, 0.89, 0.95</td>
<td>0.03, 0.06, 0.27, 0.36, 0.38, 0.43, 0.47, 0.57, 0.80, 0.85</td>
<td>0.06, 0.13, 0.21, 0.30, 0.31, 0.80, 0.83, 0.95</td>
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<tr>
<td></td>
<td>In Iodine vapors under white light (Rf Values)</td>
<td>0.17, 0.24, 0.43, 0.95</td>
<td>0.07, 0.17, 0.23, 0.30, 0.38, 0.44, 0.88</td>
<td>0.05, 0.26, 0.31, 0.31, 0.43</td>
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</table>
Pharmacognostical and Phytochemical Evaluation of Rare and Endangered Habenaria spp.

Table 1: Observations of physicochemical parameters of powdered samples of Riddhi

<table>
<thead>
<tr>
<th>S. No.</th>
<th>parameters %</th>
<th>Habenaria intermedia</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
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<tr>
<td></td>
<td>Total ash (% w/w)</td>
<td>4.5</td>
<td>6.4</td>
<td>3.2</td>
<td>5.2</td>
<td>6.96</td>
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<tr>
<td></td>
<td>Acid insoluble ash (% w/w)</td>
<td>0.81</td>
<td>1.2</td>
<td>0.9</td>
<td>0.98</td>
<td>2.11</td>
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<tr>
<td></td>
<td>Water soluble extractive (% w/w)</td>
<td>32.2</td>
<td>23.7</td>
<td>22.3</td>
<td>28.3</td>
<td>18.34</td>
</tr>
<tr>
<td></td>
<td>Alcohol soluble extractive (% w/w)</td>
<td>15.2</td>
<td>9.5</td>
<td>18.9</td>
<td>9.8</td>
<td>5.18</td>
</tr>
<tr>
<td></td>
<td>TLC(Under UV 254 nm (Rf Values))</td>
<td>0.06, 0.11, 0.16, 0.30, 0.49, 0.63</td>
<td>0.90</td>
<td>0.05, 0.33, 0.50, 0.61, 0.92</td>
<td>0.14, 0.30, 0.35, 0.39, 0.49, 0.55, 0.59</td>
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<tr>
<td></td>
<td>Under UV 366 nm (Rf Values)</td>
<td>0.05, 0.11, 0.24, 0.06, 0.12, 0.26, 0.90</td>
<td>0.05, 0.08, 0.13, 0.61, 0.92</td>
<td>0.08, 0.11, 0.18, 0.39, 0.49, 0.55, 0.59</td>
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<td></td>
<td>Under UV 366 nm (Rf Values)</td>
<td>0.39, 0.42, 0.46, 0.39, 0.48, 0.89, 0.46, 0.52, 0.55, 0.87</td>
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<td></td>
<td>Under UV 366 nm (Rf Values)</td>
<td>0.50, 0.56, 0.62, 0.94</td>
<td>0.49, 0.53, 0.61, 0.66, 0.91</td>
<td>0.46, 0.52, 0.55, 0.87</td>
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<tr>
<td></td>
<td>Under UV 366 nm (Rf Values)</td>
<td>0.73, 0.89</td>
<td>0.66, 0.91</td>
<td>0.46, 0.52, 0.55, 0.87</td>
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<tr>
<td></td>
<td>Under UV 366 nm (Rf Values)</td>
<td>0.31, 0.35, 0.44, 0.03, 0.30, 0.42, 0.58</td>
<td>0.06, 0.29, 0.33, 0.08, 0.30, 0.35, 0.39, 0.48, 0.55, 0.87</td>
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<tr>
<td></td>
<td>Under UV 366 nm (Rf Values)</td>
<td>0.53, 0.59, 0.65, 0.49, 0.57, 0.80</td>
<td>0.47, 0.56, 0.62, 0.39, 0.48, 0.55, 0.87</td>
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</tr>
<tr>
<td></td>
<td>Under UV 366 nm (Rf Values)</td>
<td>0.73, 0.91</td>
<td>0.93</td>
<td>0.60</td>
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<td></td>
</tr>
</tbody>
</table>

Legend:
Samples of Habenaria intermedia from:
R1: New Delhi, R2: Lucknow (Uttar Pradesh), R3: Mumbai (Maharashtra), R4: Jaipur (Rajasthan), R5: Tiruchirapalli (Tamil Nadu)

Samples of Habenaria edgeworthiana from:
V1: New Delhi, V2: Lucknow (Uttar Pradesh), V3: Mumbai (Maharashtra), V4: Jaipur (Rajasthan), V5: Tiruchirapalli (Tamil Nadu)

Figure 3 and Figure 4.
Pharmacognostical and Phytochemical Evaluation of Rare and Endangered *Habenaria* spp.

**Figure 5.**

Legend:

*Figure 5a:* T.S. of an adventitious root showing poly-arch condition (diagrammatic)

*Figure 5b:* T.S. of advanced stage of an adventitious root (diagrammatic)

*Figure 5c:* T.S. of a portion of an adventitious root showing cellular details

A. Epidermis to inner cortical region

B. Cortex to central region showing details of stele

*Figure 5d:* T.S. of a portion of tuber (diagrammatic)

*Figure 5e:* T.S. of a portion tuber showing cellular details

A. Epidermis to outer cortical region

B-C. Cortical region showing vascular bundle and group of vessels

D. Central region

*Figure 5f:* a-i. Isolated elements of the tuber

a. Epidermis

b. Cortex

c. Cortex lignified

d. Phloem

e. Vessel

f. Vascular bundle

i. Vessel
Figure 6c: Powder characteristics of Microstylis wallichii Lindl.

Legend:

Figure 6a: T.S. pseudo bulb of Microstylis wallichii Lindl. (diagrammatic)
Figure 6b: T.S. pseudo bulb of Microstylis wallichii Lindl. (portion enlarged)
Figure 6c: Powder characteristics of Microstylis wallichii Lindl.

Figure 6.
thick, globose to pyriform light brown to brown colored tuber having rough surface usually covered with abundant small adventitious roots. The transverse section showed single layered epidermis followed by a narrow strand of stone cells of varying size (150-270-390-660) µm, 2-3 layers thick. The stone cells were mostly elongated with tapering to blunt ends and their walls were thick, lignified and bear simple pits on them (Figure 5f, a-h). It was followed by 2-4 layers of cork cells (12-21-30 X 18-36-60) µm below which was the ground tissue consisting of thin walled parenchymatous cells (45-75-180 X 54-90-180) µm. However, outer 2-5 layers of cortical cells become thick walled and lignified of sizes (21-45-66 X 27-54-90) µm (Figure 5e.A). Within the ground tissue a large number of vascular bundles consisting of lignified scalariform vessels and soft phloem tissues were distributed. A large number of schizogenous mucilage canals (127-210-320) µm lined by epithelium of 7-9 cells and numerous starch grains of varying sizes (15-27-45-60) µm were also distributed in the ground tissue.

The pharmacognostic characters of sample R2 resembles to H. edgeworthii. The physicochemical analysis (Table 1) and TLC (Figure 3) of 90 % ethanolic extract of powders of all the samples were carried out using solvent system Toluene : Chloroform : Methanol (2.0 : 6.0 : 2.0 ) and the results compared to the authentic sample.

DISCUSSION AND CONCLUSION

From the foregoing observations, it has been seen that both species of Habenaria viz. H. intermedia and H. edgeworthii resemble to a great extent in their morphological and histological characters like in adventitious roots, presence of unicellular hairs with tapering or blunt ends or tortuous showing helical bendings, polychrom primary stele, bundles of raphides of calcium oxalate present in outer cortex and both species are devoid of starch grains.

Similarly in tubers of both species, epidermis are 2-3 layered, single layered exodermis, scalariform thickening on vessels and each stele is diach and the no. of starch grains per mg. powder is 56,210 in case of H. intermedia while 61,319 in case of H. edgeworthii. Like the anatomical structure of tubers of D. bulbifera are quite characteristic and can be differentiated by having single layered epidermis ruptured at places, stone cells either elongated or rectangular with simple pits on their walls forming continuous ring below the epidermis, ground tissue consists of thin walled parenchymatous cells except a few layers of outer cells which are thick walled & lignified, and no. of starch grains per mg powder is 72,516 from both Habenaria species.

Similarly the anatomical structure of M. wallichii showing a single layered, thick walled, sclerified epidermis having acicular crystals of calcium oxalate, followed by mesophyll adjacent to the upper epidermis composed of 2 to 4 layers of elongated cells with lignified reticulate thickening, devoid of chloroplast; vascular bundles prominent, phloem well developed with large sieve plates, surrounded by sclerenchymatous bundle sheath; single layer cuticle and a layer of thick walled sclerified epidermal cells; below this lie 1 or 2 layers of large sclerified cells and these extend unevenly into ground parenchymatous tissue; ground parenchyma irregular, with large air spaces with passage cells in the form of small protuberances at some places; vascular bundles scattered throughout the ground tissue surrounded by thick walled sclerenchymatous cells, which occasionally extend into intercellular spaces.

The Drugs under study are not easily available in market and which are available the observation of Physico-chemical evaluation of the same indicates that the drugs are not genuine. Hence the species are considered to be rare and endangered.

REFERENCES