Full Length Research Paper

Use of chemotaxonomic markers for misidentified medicinal plants used in traditional medicines

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Onosma hispida Wall. and G. Don. (Boraginaceae) is an important medicinal plant used in Unani system of medicine as cardiac drug. In Unani, Ayurvedic and traditional system of medicines, the drug is marketed under the trade name rattan jot. In herbal market, this drug is misidentified, confused and adulterer with local available drug obtained from another species, Geranium wallichianum D. Don. ex Sweet. In order to ensure the use of only genuine and uniform material of such herbal drug, work on chemotaxonomic authentication assumes vital significance. Chemotaxonomic study has been carried out covering detailed morpho-anatomical, palynological (SEM), features of crude drug (roots) include organoleptic tests, response to UV and IR light exposure and results of TLC fingerprinting (flvonoids) evaluated in this work would serve as standard reference for correct identification in commercialization at global trade.

Key words: Chemotaxonomic markers, misidentified medicinal plant, Rattan jot, traditional medicine.

INTRODUCTION

Plants are the fundamental resource for providing a viable and renewable base for life on this planet. They are the main source of food, feed, fodder, energy and raw material for human beings, animals and plant based industries. History of using herbal medicines is woven into the histories of people and the civilizations. Early human recognized their dependence on nature in both health and illness. Led by instinct, taste, and experience, primitive man treated illness by using plants, animal parts and minerals that were not part of his usual diet. Physical evidence of the use of herbal remedies goes back some 60,000 years to a burial site of a Neanderthal man uncovered in 1960 (Solecki, 1975). All cultures have long folk medicinal histories that include the use of plants. Even in ancient cultures, people methodically and scientifically collected information on herbs and developed well-defined herbal pharmacopoeias. Earlier efforts were confined to the collection of the wild plants for medicinal use and there were no well planned and organized efforts for cultivation of herbs. Concept of herbs growing has largely developed in ancient Egypt, Christian and Islamic religious traditions and flourished from 10 - 13 countries with the Islamic civilization. The 17\(^{th}\) century is said to have created great change and expansion in styles and patterns in France and other European countries (Shah et al., 2009).

The demand for high quality, safe, effective and clean natural plant products and their formulations with various substances have been growing significantly in the industrialized world. In the past, herbs and essential oil bearing plants were largely harvested from the wild and brought to the market without many questions asked about their origin, methods of cultivation, taxonomic identity, purity, safety, and efficacy. However, with further improvements in communication and education, there has been a growing consciousness in industrialized countries about personal health, environmental safety, sustainable harvesting, and loss of genetic diversity resulting from extensive wild harvesting of the medicinal species. This increased awareness has lead in the US, Canada, China and Europe, to a wider use of alternative
medicinal practices, which include the use of botanicals as medicinal though purchased as dietary supplements, by the consuming public. This phenomenon has been largely media and market driven rather than a scientific/medically driven movement. Yet, with increased consumer usage, and increased advertisement, the expectations by the public have also increased leading to a more stringent demand for quality, correct identification and traceability (Tadmor et al., 2002).

The major problems in the manufacture of herbal drugs and their wider acceptance in developing countries like Pakistan are the adulteration and misidentification (Figure 1). The situation is further complicated in the absence of any quality control system in place in our industry. Some of the manufacturers may have their own in-house standards and specifications of the quality of drugs they produce while the others mostly still practice Organoleptic “testing” like sight, smell, taste and touch to identify a plant that is considered unscientific.

In Pakistan the Greco-Arab System, commonly known as Unani system of medicine or Tibb are considered as the oldest systems of medicine. Plants are generally referred to and published in folk literature by their vernacular or common names rather than botanical names. Due to this confusion, genuine plant drugs are adulterated with closely related species. The main difficulty in fixing the botanical identity of drug plant in ancient Unani literature and traditional systems arises due to local name(s) of drug plants, nomenclatural controversy attributed to more than one plant species. The botanical sources of large number of folk medicine found therapeutically effective in indigenous system are still unknown or doubtful. Many workers (Girach et al., 1998; Afaq, 1998; Ahmad and Khan, 1998; Ahmad et al., 2008, 2009) have stressed the need for authentic botanical identification of herbal drugs used in the Unani system of medicine, in order to maintain their efficacy. The present paper confined to herbal drug commonly known as rattan jot in Unani system of medicine, which is marketed with adulteration throughout the country. This herbal drug imported from neighboring countries; India, Afghanistan and Iran. The drug is adulterer in market with another species which is also locally known as rattan jot. In this study the chemotaxonomic markers including classical taxonomic and modern techniques have been used to detect adulterants from genuine source of the drug and solve the problems of confusion faced by herbalists, pharmacists, taxonomists and medicinal herb traders.

MATERIALS AND METHODS

Taxonomic authentication

Plant specimens were collected during field trips from Baluchistan, Northern Areas (Skardu, Gilgit and Baltistan), Ayubia National Park, Siran Valley and Hazara Division. Herbal drug samples were procured from Herbal Markets of Lahore, Karachi, Rawalpindi, Hazro and Abbotabad. Morphological characters were studied under binocular stereo zoom light microscope (Model Kyowa SZF 0.75x - 3.4x). The plant description was reconfirmed by using various Floras (Nasir and Ali, 1974, 1975; Hooker, 1875; Tutin and Heywood, 1972; Hooker and K.C.S.I., 1885ab; 1894 and Saldanha and Nicolson, 1976).

Palynological Investigation

Pollen study of fresh poleniferous material was carried out under scanning electron microscopy (SEM). The pollen grains were acetolized according to the modified method (Erdtman, 1952;
Figure 2. Acid hydrolysis of flavonoid glycosides.

Leaf epidermal anatomy

Leaf samples for anatomical studies were prepared according to the modified method of Cotton (1974), who followed Clark’s (1960) technique. The leaves were placed in a tube filled with 88% Lactic acid kept hot in boiling water bath (Model, Memmert-91126-FRG, Germany) for 15 - 30 min. Lactic acid softens the tissues of leaf due to which it is possible to scrape the leaf surface with sharp scalpel. Slides of both abaxial and adaxial surface of leaf were prepared and mounted in clean 88% lactic acid. Features i.e. shape, size of epidermal cells, wall thickness, smooth or undulating wall, trichome (shape and structure), arrangement of stomata in epidermis, the presence or absence of compounds such as mucilage, starch or lignin, or the presence of tissues with characteristic cells were studied which are used in the microscopic authentication of herbal drugs. Microhistological photographs of both surfaces were taken by Leica light microscope (DM 1000) fitted with CCD digital camera (Ahmad et al., 2009).

Thin layer chromatography

For the extraction of flavonoid aglycones a small amount of dried plant material is treated with 2 normal (2N) hydrochloric acid (HCl) and heated for one hour in a water bath at about 100°C. By this treatment normally all flavonoids-O-glycosides are converted to flavonoids aglycones, anthocyanins to anthocyanidins where as the C-glycosides remain unaffected (Figure 2). After cooling, the flavonoid aglycones are extracted with diethyl ether (Et₂O) from the aqueous phase. A second series of extraction by n-butanol quantitatively removes the anthocyanidins (Ahmad et al., 2008).

The technique of TLC finger printing consists of applying the flavonoid sample on commercially available pre-coated polyamide F₂₅₄ plates (Merck- Germany). For analytical work pre-coated aluminum or plastic backed TLC plates which are transparent to ultraviolet light (UV) were used. These plates after being well dried are loaded with the herbal extract to be separated. The plates are then developed in TLC tank (large size 20 x 20 cm Camag, Switzerland. The solvent system used in both the directions is Toluene: Methanol: Methyl Ethyl Ketone 4:3:3 (Hasan, 1976). After drying, the fully developed TLC plates are
### Table 1. *Onosma hispida* Wall and G. Don.

<table>
<thead>
<tr>
<th><strong>English name</strong></th>
<th><strong>Golden drops</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Local name (s)</strong></td>
<td>Rattan Jot, Gao Zaban.</td>
</tr>
<tr>
<td><strong>Tib name</strong></td>
<td>Rattan Jot, Gul-e-Ljari.</td>
</tr>
<tr>
<td><strong>Family</strong></td>
<td>Boraginaceae.</td>
</tr>
<tr>
<td><strong>Distribution in Pakistan</strong></td>
<td>Baluchistan, Karrum, Landikotal, Chitral, Swat, Kaghan, Kashmir, Skardu.</td>
</tr>
<tr>
<td><strong>Distribution in world</strong></td>
<td>Afghanistan, Russia, South West Asia, Syria, Turkey, Mongolia, South Europe to North Africa, India, Iran</td>
</tr>
<tr>
<td><strong>Occurrence and conservation status</strong></td>
<td>Very rare. Limestone slopes and rocks to 1600 m. Crevices in rocks and cliffs.</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>Herb annual. Stem usually branched from base, 5.0-25.0 cm tall; branches ascending to decumbent, hispid, short strigose, with glandular hairs. Leaves sessile, hispid, sparsely hirsute. Basal and lower stem leaves linear-oblanceolate, 3.0-6.0 cm × 4.0-10.0 mm; upper stem leaves linear-lanceolate, smaller. Inflorescences to 15.0 cm after anthesis, short strigose and hirsute. Pedicel 3 mm; bracts linear-lanceolate, 1.5-5.0 cm. Calyx 5.0-8.0 mm, 5-parted to middle; lobes lanceolate-triangular. Corolla purple-red, 0.8-1.2 cm; appendages below throat 2.0-lobed; limb to 1/3 as long as tube; lobes ovate to suborbicular, margin entire or subdentate. Stamens inserted slightly above middle in corolla tube, included; anthers 1.4 mm. Style 4.0 mm; stigma subglobose, slightly 2.0-cleft. Gynobase slightly convex. Mature nutlets black-brown, reniform, 4.0 mm, glabrous or sparsely pubescent before maturity, horizontally wrinkled, apex keeled; attachment scar cupular, margin finely dentate. Seeds gray-brown, reniform; radicle superior; cotyledons obovate-oblong (Plate 1A).</td>
</tr>
<tr>
<td><strong>Flowering period</strong></td>
<td>June-August.</td>
</tr>
<tr>
<td><strong>Voucher No.</strong></td>
<td>ISL-MZ-107.</td>
</tr>
<tr>
<td><strong>Palynology</strong></td>
<td>Pollen grains are prolate to spheroidal with rudimentary spines. Reticulate sculpturing. Pollen are trizonoporate, with polar diameter 18.5 µm (17-20 µm), equatorial diameter is 17.5 µm (16.5-18.5 µm), P/E ratio is 1.057 µm and exine thickness is 2.75 µm (Plate 1E,F).</td>
</tr>
<tr>
<td><strong>Leaf epidermal anatomy</strong></td>
<td>In abaxial surface, ordinary epidermal cells are trigonal, pentagonal, rhomboidal, slightly undulate and smooth doubled walled. Length of ordinary epidermal cells is 61 µm (30-80 µm). Breadth of ordinary cells is 25 µm (15-30 µμ). Stomata are numerous and irregularly oriented. Stomata sometimes in groups of 3-4. They are paracytic, anomocytic, desmocytic, axillocytic, and copolocytic. Length of stomata is 15 µm (10-20µm). Width of stoma is 11.6 µm (10-15 µm). Lengt of stomatal opening is 11.6 µm (10-15 µm). Width of stomatal opening is 2.8 µm (1-5 µm). Subsidiary cells are tubular. Length of subsidiary cells is 28.3 µm (25-30-µm). Breadth of subsidiary cells is 16.6 µm (10-15 µm). Non-glandular macro hairs with thick and smooth wall are numerous. They have prominent lumen and circular base. Tip of macro hair rounded. Length of macro hair is 636.6 µm (250-1250 µm). In adaxial surface, ordinary epidermal cells are irregular, tubular, tetragonal, and pentagonal. Length of ordinary cells is 55 µm (50-65 µm). Breadth of ordinary cells is 20 µm (15-25 µm). Stomata are rarely present. They are paracytic, anisocytic. Length of stomata is 23.3 µm (20-25). Width of stomata is 20 µm (15-25 µm). Length of stomatal opening is 10 µm (5-10 µm). Width of stomatal opening 5.8 µm (2.5-10µm). Non-glandular macrohair with thick and smooth walls numerous. They have prominent lumen and circular base. Length of macrohair is 636.6 µm (250-1250 µm) (Plate 1G, H).</td>
</tr>
<tr>
<td><strong>Part used</strong></td>
<td>Roots.</td>
</tr>
<tr>
<td><strong>Folk medicinal uses</strong></td>
<td>Tonic, cardiac diseases, eye diseases.</td>
</tr>
</tbody>
</table>
Preparation and dosage | Roots are crushed to obtain powder. Half tea spoon of the powder thrice a day after meal is recommended for cardiac diseases and skin infection. It is also use as tonic and to cure eye diseases.

Toxicity | Non toxic.

Marketing status | Commonly traded under the name of Rattan jot throughout the country.

Organoleptography (Roots) | Roots pieces are 5-10 cm in length, 6-12 cm in diameter. Roots are dark reddish and yield dye. Root surface is glaucous and consist of layers just like colorful papers (Plate 1B).

Finger printing | TLC of root extract reveals the presence of one major amount of flavonol, eight flavones and two aurones when viewed under 366 nm UV light (Plate 1D).

viewed under 366 nm UV light. This is a very reliable and reproducible method of authentication of a particular herbal drug, and differentiates misidentified medicinal plants on the basis of TLC fingerprints.

**RESULTS**

The result of this study is given in Tables 1 and 2 and in Plates 1 and 2.

**DISCUSSION**

Rattan jot (*Onosma hispida*) is an example of unknown authentic plant. It is an important Unani, Tib and Ayurvedic traditional medicine. It is sold commonly in Indo-Pak subcontinent under the name of Rattan Jot. On discussion with suppliers and herb traders, it is imported from Iran and India. But the source of this drug is very confusing, according to some herbalists, it is the roots of *Arnebia euchroma* and suppliers and traders said that in past the roots of *Ventilago madraspatana* was the only source of Rattan jot (Mitra and Kanan, 2007). According to the magazine published monthly by Qarshi herbal industries private (Ltd.), Well naturally products (Ltd.) and Mahima herbal products which are the exporters, importers, suppliers, raw materials, herbs, species and crude drugs in India (Fazal and Razzak, 1978).

It is interesting to know that an endangered species *O. hispida* is sold in plenty as Rattan jot. Sometime the adulterated materials originated from the roots of *G. wallichianum* which is locally known as Rattan jot in Murree, Ayubia, Kashmir, hazaara and in Abbotabad. Tough both the plants are available in plenty throughout the herbal shops of Pakistan. *G. wallichianum* only known as Rattan jot in Murree, Abotabad and Hazara because of its common distribution (Shah et al., 2009). This species is unknown by major herbal markets in Pakistan such as Akbari and Papri Mandi (Lahore), Baluchistan, Sind and Central Punjab. In these markets the *O. hispida* is the only authentic source of Unani drug “Rattan jot”.

**Medicinal importance**

*O. hispida* (Boraginaceae) known as Rattan jot, laljari is an annual herb. The roots are bruised and used as an external application to skin eruptions. A red dye obtained from the root is used as an Alkana substitute (Omkar et al., 2006). In indigenous system of medicine, the roots (Rattanjot) of *O. hispida* are used for cardiac and eye diseases. Some herbalists recommend the roots as tonic, whereas the toots of *G. wallichianum* is traditionally used to prepare sweet dish which is commonly used by local communities for sexual debility and backache (Shah et al., 2009). Root powder is also available.
### Table 2. *Geranium wallichianum* D. Don ex sweet.

<table>
<thead>
<tr>
<th>Local name</th>
<th>Buxton’s blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibet name</td>
<td>Rus Jot</td>
</tr>
<tr>
<td>Family</td>
<td>Geraniaceae</td>
</tr>
<tr>
<td>Distribution in Pakistan</td>
<td>Swat, Baluchistan, Murree hills, Hazara, Chitral, Dir, Gilgit, Shogran valley and Lowari top.</td>
</tr>
<tr>
<td>Distribution in world</td>
<td>East Asia - Himalayas from Afghanistan to Bhutan, Nepal</td>
</tr>
<tr>
<td>Occurrence and conservation status</td>
<td>Commonly found in Forests, shrubberies and open slopes, 2400 - 3600 m.</td>
</tr>
</tbody>
</table>

#### Description
Perennial 40-60 cm tall, eglandular, pubescent to pilose, rhizome stout, vertical. Stem ascending, diffuse. Leaves 5-angled, palmate-partite or sect, 3-11 cm broad, pilose-pubescent; segments broadly ovate- rhombic, pinnatifid or deeply so into acute lobes. Peduncles up to 13 cm long, 2 flowered, pubescent-retrosely hairy. Flowers 3-4 cm road. Pedicles 4-65 cm long, retrosely hairy, deflexed in fruit. Bracts 10-15 mm long, broad lanceolate, acuminate. Mericarps patent hairy. Seed 5 mm long, oblong, minutely reticulate. Flower is pink to purplish (Plate 2A).

#### Flowering period
March to April

#### Voucher No.
ISL-MZ-79

#### Palynology
Pollen grains are tricolporate, monad, psillate. Pollen has granulated sculpturing. Pollen are spheroidal to semiangular. Polar diameter is 61.5 µm (55-68 µm), equatorial diameter is 62.5 µm (60-65 µm), length of colpi 3.5 µm and width is 2.5 µm and exine thickness is 2.75 µm.

#### Leaf epidermal anatomy
Ordinary cells are irregular, elongated in shape. In abaxial surface Length of ordinary epidermal cells is 32.5 (25-37.5). Breadth of ordinary cells is 20 (15-25). Stomatal orientation irregular. They are of animocytic type. Length of stomata is 11.28 (10-12.5). Width of stomata is 5.41 (5-6.25). Subsidiary cells are elongated to flatten. The Glandular and non-glandular hairs have been observed on both abaxial and adaxial surfaces. Non-glandular hair is of two types. Micro and macrohairs. A micro hair is unicellular or bicellular. Macrohairs unicellular with characteristic base. The hairs are surrounded by a series of spheroidal cells. Hair with thick, smooth and prominent walls. The epidermal hairs have thin undulating walls. Length of hairs is 134.16-(115-162). Length of micro hairs is 40(35-45). Glandular trichomes with multicellular and unicellular stalk and base present. Some glandular trichomes with bicelled stalk and unicellular head. Length of glandular trichomes is 18.3 (12.5-20). Length of stalk is 5.4(3.75-7.5). Length of head is 6.6 (5-7.5). Width of head is 6.6 (5-7.5) (Plate 2E,F).

#### Part used
Roots

#### Folk medicinal uses
Backache, sexual debility, joint pain, colic, jaundice, kidney and spleen disorder.

#### Preparation and dosage
Dried roots are ground into powder, 100 gm powders is mixed in 250 gm of wheat flour, 250 gm of loaf sugar and roasted in ghee to prepare a sweet dish. It is taken once a day at night for sexual debility and backache. During this treatment sour and cold things should be avoided.

Roots are collected, dried under shade and ground to obtain powder. Half teaspoon of this powder is taken with water twice a day for jaundice. One teaspoon of the powder is taken once a day with a glass of water for fifteen days in cases of kidney and spleen disorder.

#### Toxicity
Non toxic.

#### Marketing status
Marketed in NWFP, Murree, Islamabad only under the name of Rattan Jot.

#### Organoleptography (Roots)
Herbal drug consist of dark colored dried root pieces. Roots pieces are 3-7 cm in length, 0.5-1.7 cm in diameter. 5-equal size pieces are 27 gm. Internally and externally the root pieces are hared in texture. Root surface contain undulating ridges and whitish root hairs. Roots are with characteristic odor and taste (Plate 2B).

#### Finger printing
TLC of root extracts reveals the presence of one aurone and two phenolic acids, when viewed under 366 nm UV light.
recommended for jaundice, kidney and spleen disorders.

**Morphology and anatomy**

*O. hispida* is an annual herb belonging to family Boraginaceae with stem usually branched from the base. Branches are ascending to decumbent, hispid, short strigose (Plate 1 A). The *G. wallichianum* is a perennial herb which belongs to family Geraniaceae with stem ascending (Plate 2 A). Palynologically the *Onosma hispida* can be distinguished from *G. wallichianum* by the presence of Trizonoporate pollen, prolate to spheroidal in shape, with rudimentary spines and reticulate sculpturing (Plates 1 E and F). Similarly both the species can be distinguished on the basis of leaf epidermal characteristics. The leaf epidermal cells in case of *O. hispida* are elongated, quadrangular or pentagonal with non-glandular hairs but glandular hairs are absent (Plate 1 G, H). There is diversity of stomata such as paracytic, anomocytic, desmocytic, axillocytic...
G. wallichianum shows the presence of one aurone and two phenolic acids (Plate 2 D).

Conclusion

The chemotaxonomic characteristics in the present study have brought out several distinguishing features of the crude herbal drug (roots) Ratan jot on the basis of which identification of the genuine source, Onosma hispida can easily be ascertained. The morpho-anatomical and palynological characters in combination with UV and IR and TLC fingerprinting data will not only provide criteria for the correct taxonomic authentication but would also serve as future standard data for the quality assessment of the pharmaceutical preparation of botanical drugs.

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