

*Full Length Research Paper*

## Chemotaxonomic clarification of pharmaceutically important species of *Cyperus* L.

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The evaluation of the crude herbal drug which eventually enters the pharmaceutical market is obviously of considerable importance. This operation involves the identification of the material and determination of its quality, purity and of adulterated nature of the adulterants. The present paper is based on the above objectives which confined to chemotaxonomic authentication of *Cyperus rotundus* L. (Nagar mootha) and its other similar species. Chemotaxonomic techniques including morphology, organoleptography, palynology, anatomy and phyto-chemical analysis were carried out in order to clarify the pharmaceutically important species of *Cyperus* that is *Cyperus rotundus*, *Cyperus alopecuroides*, *Cyperus difformis* and *Cyperus niveus*. It is concluded from this study that the genuine source of herbal drug Nagar Mootha is *C. rotundus* instead of other species. Such type of studies is the need of herbal industry to ensure validation process which applied in the manufacturing of herbal medicines and phyto- pharmaceuticals. This will provide the credibility in the regulation and growth of pharmaceutically important herbal medicines for the foreseeable.

**Key words:** *Cyperus* L., chemotaxonomic, pharmaceutical.

### INTRODUCTION

In several industrialized societies, plant-derived prescription drugs constitute an element in the maintenance of health. Medicinal plants are an integral component of research developments in the pharmaceutical industry. Such research focuses on the isolation and direct use of active medicinal constituents, or on the development of semi synthetic drugs, or still again on the active screening of natural products to yield synthetic pharmacologically-active compounds (Hoareau and Dasilva, 1999).

In Germany, for example, over 1500 plant species encountered in some 200 families and 800 genera have

been processed into medicinal products. In South Africa, likewise, some 500 species are commercialized trade products. Today, Bulgaria, Germany and Poland are recognized as major exporters of plant-based medicinal products. The development and commercialization of medicinal plant-based bio-industries in the developing countries is dependent upon the availability of facilities and information concerning upstream and downstream bioprocess, extraction, purification, and marketing of the industrial potential of medicinal plants.

Absence of such infrastructure compounded by lack of governmental interest and financial support restricts the evolution of traditional herbal extracts into authenticated market products. Furthermore the absence of modernized socio-economic and public healthcare systems reinforces reliance of rural and lower-income urban populations on the use of traditional medicinal

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herbs and plants as complementary aids to routine pharmaceutical market products. The prophylactic and therapeutic effects of plant foods and extracts in reducing cardiovascular disease has been reviewed (Walker, 1996).

At the same time, accurate plant identification is the foundation of the safe use of plant based natural health products in pharmaceutical sciences. Without proper identification as a starting point, the safe use of quality products cannot be guaranteed. There is recognition within pharmaceutical industry and government that there is a need to protect access and choice by consumers when it comes to natural health products. At the same time, consumers have a right to expect that these products can be used with confidence regarding their safety and quality (Ahmad et al., 2009; Zafar et al., 2010). Authentication of pharmaceutical products originated from traditional herbal medicines would facilitate accurate documentation of taxa traded, medicinal usage and assists in identifying material implicated in poisoning cases. Dried products sold in the medicinal plant trade are generally difficult to identify, as many useful diagnostic characters are lost through desiccation. Many herbs are sold through brokers where the material can change hands several times. The originality of herbal drugs in terms of raw material extraction, preparation and marketability requires proper guide lines according to WHO standards (WHO, 2003).

Lack of authentic sources of herbal drug is one of the common example and important issue in pharmaceutical industry. It is well known that in course of time, drug materials get changed to or substituted with other plant species. Nagar mootha is recent day examples in this regard. On discussion with herbal drug suppliers, herbalists and local medicinal plant collectors, it came to be known that in the past, roots of this herbal drug was obtained from different species of *Cyperus*. But now a day due to lesser availability in field these drugs are sold in market with quite similar roots from different species. Due to lack of proper documentation of genuine drug and research in this field the situation to know authentic drug is complex.

The overall objectives of the present project is to use classical and modern techniques of chemotaxonomy to authenticate the original raw material of correct species marketed at herbal shops. This quality assessment mode is effective for their standardization, modernization and acceptance by the world market.

## MATERIALS AND METHODS

### Morphological characters

Four medicinal plant species that is, *Cyperus rotundus*, *Cyperus alopecuroides*, *Cyperus difformis* and *Cyperus niveus* were collected during field visits. Crude raw material of herbal drugs was procured from herbal markets of Akbari Mandi Lahore and Peshawar herbal shops. The morphological and organoleptography

of plant species and herbal parts were carried out by using light microscope (Koywa SZF 0.75 x -3.4x). The morphological characters were reconfirmed by using various Floras (Hooker, 1875; Tutin and Heywood, 1972; Hooker and K.C.S.I., 1885 ab, 1894; Nasir and Ali, 1974; 1975).

### Palynological features

The pollen study was carried out under the Meiji light microscope (MX 5200H, Japan). Qualitative characters includes type of pollen, shape of polar view, shape in equatorial view, sculpturing whereas quantitative character including polar diameter, equatorial diameter, P/E ratio, length and width of colpi, exine thickness, fertile and sterile pollen. Each value was taken five times to ensure the accuracy. Scanning Electron Microscopy (SEM) analysis were carried out by the dissection of anther and then placement in the center of clean glass slide with 1 to 2 drops of acetic acid for one minute, crushed to release pollen. Then the pollen were transferred to already marked specimen stub and allow them to air dried and then coated with gold with a SPI-MODEL™ sputter coater. After coating, stubs were placed in Jeol Vacuum evaporator. It takes about 15 min to produce the vacuum and then observations were made by using 30 KV scanning electron microscope (JSM5910, JEOL Japan). Pollen terminology was determined by Ronald (2000).

### Leaf epidermal anatomy

The fresh leaves were taken in a test tube covered with 4 ml of concentrated nitric acid, to which 0.2 g of potassium chloride and 0.1 ml of distilled water was added then mixture was carefully boil and after a few second as soon as the epidermis of leaves were separated in the form of thin pellicle, the contents were emptied into a Petri dish partly filled with water. Macerated leaves were washed with water for 2-3 times then placed in Petri dish containing fresh water. To prepare adaxial surface, the leaf was placed in such a way that it is adaxial side of the leaf with the help of fine forceps. Same procedure was followed for the preparation of adaxial surface of leaf. Leaf epidermal samples were prepared according to the methods of Cotton (1974) and Clark (1960) and modified method of Ahmad et al. (2010).

### Phytochemical study

The plant materials (4 species) were dried under shade and were made into small pieces. Thin layer chromatographic (TLC) studies were performed for flavonoids (Chemotaxonomic markers) finger printing by adopting the method of Ahmad et al. (2010) and Hassan et al. (2007). Commercially available pre-coated polyamide-6 TLC plates (Sor bent Technologies USA) were used. The modified method was applied for flavonoids extraction. TLC plates were observed and photographed under UV lamp (Model UVL-56 Black Ray USA).

## RESULTS AND DISCUSSION

The English names, local names, drug names and families of different species and their distribution, occurrence, morphology, etc were given in Tables 1 to 4, while the species parts and characteristics are given in Figures 1 to 4.

*Cyperus* L. (Sedge) belongs to the family Cyperaceae

**Table 1.** *Cyperus rotundus* L.

English name (s)	Brown nut sedge and nut grass
Local name	Dheela
Drug name	Nagar Mootha
Family	Cyperaceae
Distribution in Pakistan and world	Sindh, Balouchistan, Loralai, Peshawar, Tank, D.I. Khan, Lahore, Sialkot, Attock, Burbhan, Hazara, Swat, Chitral, Gilgit and Kashmir.
Occurrence	Commonly found in arid areas of Pakistan.
Morphology	Perennial stoloniferous herb, bearing ovoid tubers at the ends of stolons. A single plant can produce 686 new tubers within a period of one year in the tropical areas. Aerial stems 12 to 42 cm long, glabrous, triquetrous, erect, dilated at the base. Leaves many, 7.6-41 x 0.2-0.5 cm, linear, flat, scabrous towards the apex, sheaths truncate at the mouth. Inflorescence compound umbel, involucral bracts 3 to 4 in number, 3.5-10 x 0.2-0.3 cm. longer than the inflorescence, leaf-like, linear, margins minutely scabrous. Spikelets (3-5)-18-36 x 1-1.5 mm, linear-oblong, distichous, flat, 6-34- flowered, rachilla winged, glumes 3-3.5 x 1.5-2.5 mm, ovate, obtuse or slightly speculate, 5-7 nerved, margins hyaline. Stamen 3, style 3-branched, braches longer than the style, glabrous. Nut 1.2-1.5 x 0.5-0.8 mm, trigonous, ovoid oblong; black (Figure 1A).
Flowering period	April to October
Voucher No.	261
Pollen description	Pollen monad, monoporate, radially symmetrical and isopolar. In equatorial view, shape of the pollen apple shaped to rectangular and in polar view the pollen is circular to intersemiangular. Size of pollen in polar axis is 28.33 $\mu\text{m}$ (25-30 $\mu\text{m}$ ) and in equatorial diameter 35 $\mu\text{m}$ (32.50-40 $\mu\text{m}$ ). The exine thickness 2.41 $\mu\text{m}$ (2.25-25 $\mu\text{m}$ ). The P/E ratio 0.81 $\mu\text{m}$ . Sculpturing foveolate. The depressions are minute and uniformly distributed over the tectum (Figures 1D and E).
Leaf epidermal anatomy	Abaxial and adaxial surfaces with costal and intercostals zonation. Long-cells in costal and intercostals zones rectangular, with markedly sinuous walls. Their length in intercostals zone, abaxial one 71.90 $\mu\text{m}$ (37.50-90 $\mu\text{m}$ ) x 18.13 $\mu\text{m}$ (15-20 $\mu\text{m}$ ). Adaxial one, 112 $\mu\text{m}$ (87.5-125 $\mu\text{m}$ ) x 13.75 $\mu\text{m}$ (12.5-15 $\mu\text{m}$ ), while in costal zone, abaxial one, 70.5 $\mu\text{m}$ (52.5-100 $\mu\text{m}$ ) x 19.40 $\mu\text{m}$ (17.5-22.50 $\mu\text{m}$ ) and adaxial one, 98.13 $\mu\text{m}$ (92.50-105 $\mu\text{m}$ ) x 31.90 $\mu\text{m}$ (27.5-37.5 $\mu\text{m}$ ). Microhairs panicoid type on both the sides, stomata of common type, restricted to abaxial surface, their length, 24.5 $\mu\text{m}$ (22.50-27.5 $\mu\text{m}$ ) and interstomatal cells cross ended. Subsidiary cells low dommed. Short cells present at abaxial side of single nature. Silica bodies only on adaxial surface of granular, butterfly and rounded shapes. Prickles restricted to adaxial surface (Figures 1G and H).
Flowering period	May to November
Part used	Roots
Indigenous Recipes	Paste is used to apply in skin related ailments; it helps in relieving from itching. It is used in increasing the size of breast. It also improves eyesight and is used in eye related ailments. Powder is used in mental diseases and diseases like psychosis and epilepsy. It improves digestive system. It also helps in maintaining normal body temperature. Whole plant including roots (rhizomes) is dried in shade and crushed to obtain powder. Equal quality of water is mixed in powder to make paste. The paste is externally applied on infected skin to cure eczema, scabies and chronic skin sculpturing. The paste is also applied on skin for softness and cooling effects.
Toxicity	It has no toxic effect when consumed in normal dosage.
Organoleptography (Roots)	Root sample contain irregular pieces of length 0.5 to 10 cm and diameter of 0.3 cm to 2.8 cm. root surface has woody dark brown color with vertical and horizontal ridges on surface. Root surface also have tiny hairy projections. Root is branched with some noded regions. Root is slightly soft than above three root samples. Internally root is light brown (skin) in color. Root is bitter in taste (Figure 1B).
TLC Fingerprinting	TLC under UV shows the presence to three minor flavanoles (Figure 1F).

**Table 2.** *C. alopecuroides* Rottb. Syn: *Juncellus alopecuroides* (Rottb.) C. B. Clarke.

English name (s)	Foxtail flatsedge
Local name	Mootha
Drug name	Nagar Mootha
Family	Cyperaceae
Distribution in Pakistan and world Occurrence	In Pakistan; Mianwali, D. I. Khan, Peshawar, Karachi, Thatta, Tharparkar. In World; Madagascar, North Tropical Africa, Macaronesia, West Indies, Guadelou; Pakistan, India, Malayasia, North East Australia. Rarely found in moist places
Morphology	Perennial, tufted with short rhizome. Aerial stem up to 140 cm long and 3 to 7 mm diameter, trigonous, smooth. Leaves up to as long as stem; sheaths up to 35 cm, leaf blade up to 60 cm long and up to 10 mm broad, keeled, apex trigonous, scabrous. Inflorescence a compound umbel; involucre bracts up to 5-7, leaf like, up to as long and as wide as leaf, primary branches 8-12; secondary branches up to 5-7, leaf like, up to as long and as wide as leaf, primary branches 8-12; secondary branches up to more than 10, with several foliose bracts; some primary and most secondary branches ending with cluster of spikes, sometimes with small teritary anthelodia; cluster of spikes 2-6 x 1-2 cm, each cluster with 70 spirally arranged spikes; spikes 5-15 x 1.5 mm, compressed; glumes 2 mm, keeled, midnerve strong, greenish, side yellowish or grey, with reddish brown stripes, margins narrowly scarious, stamens 2, stigmas 2 or 3. Nut elliptic to obovate-oval, biconvex, slightly flattened (or obcompressed-trigonous in tricapellary pistil), yellowish brown, finely reticulate or almost smooth (Figure 2A).
Flowering period	April to September
Voucher No.	299
Pollen description	Pollen monad, monoporate, radially symmetrical and isopolar. In equatorial view, shape of pollen prolate and in polar view the pollen circular to angular and intersemiangular. Polar diameter 19.16 $\mu\text{m}$ (17.5-22.5 $\mu\text{m}$ ) and in equatorial diameter 21.66 $\mu\text{m}$ (20-22.5 $\mu\text{m}$ ), the exine thickness 1 $\mu\text{m}$ (0.75-1.25 $\mu\text{m}$ ). The P/E ratio 0.90 $\mu\text{m}$ . Exine with scabrate type of sculpturing. The sculpturing elements less than 1 $\mu\text{m}$ . The granules uniformly distributed over the tectum (Figure 2D and E).
Leaf epidermal anatomy	Abaxial and adaxial surface with costal and intercostals zonation. Long cells in costal zone narrower and smooth, rectangular, shiel with markedly sinuous walls in intercostals zone, at adaxial surface the long cells rectangular to hexagonal. Their length in intercostals zone 86.90 $\mu\text{m}$ (72.5-100 $\mu\text{m}$ ) x 23.13 $\mu\text{m}$ (20-25 $\mu\text{m}$ ), adaxial one 109 $\mu\text{m}$ (87.5-130 $\mu\text{m}$ ) x 41 $\mu\text{m}$ (35-47.5 $\mu\text{m}$ ), while in costal zone, abaxial one is 108.13 $\mu\text{m}$ (100-117.5 $\mu\text{m}$ ) x 20 $\mu\text{m}$ (17.5-22.5 $\mu\text{m}$ ) and adaxial one 78 $\mu\text{m}$ (75-90 $\mu\text{m}$ ) x 30 $\mu\text{m}$ (20-35 $\mu\text{m}$ ). Stomata paracytic type and only at abaxial surface with in abundance, their length 46.25 $\mu\text{m}$ (42.50 to 50 $\mu\text{m}$ ) x 25.5 $\mu\text{m}$ (22.5-27.5 $\mu\text{m}$ ). Interstomatal cells cross ended. Subsidiary cells low dommed. Short cells absent at both sides Silica bodies on Both surfaces in abundance with different types. Prickles restricted to abaxial side. (Figures 2G and H).
Part used	Roots
Indigenous recipes	Roots are collected, washed and dried in shade. Roots are cut into small pieces and ground to obtain powder. 5-10 gm of powder is taken with amla juice ( <i>Phyllanthus emblica</i> ) in order to cure menstrual cycle, digestive problems and pain in body. $\frac{1}{2}$ teaspoon of root powder is taken twice a day for 3-4 days to cure diarrhea.
Toxicity	Excessive use may cause toxicity
Organoleptography (roots)	Root length varies from 5 cm to 14 cm and irregular pieces of 0.5 to 3 cm diameter. Externally root is hard and dark brown in color with irregular vertical ridges. Root is branched with swollen nodes. Internally root is comparatively light brown as compared to outer surface. Root is slightly bitter. Root have woody smell (Figure 2B).
TLC fingerprinting	TLC under UV shows the presence of three minor flavonls (Figure 2F).

**Table 3.** *C. difformis* L.

English name (s)	Variable flat sedge
Local name	Bari Ghuien
Drug name	Nagar Mootha
Family	Cyperaceae
Distribution in Pakistan and world Occurrence	In Pakistan, Peshawar, Darya Khan, K. I. Khan, Mianwali, Nowshera, Kohat, Attock, Rawalpindi, Sargodha, Lahore, Dadu, Karachi, Thar Parkar and Kashmir. In world; Tropical and sub-tropical regions of the world. Very common in moist and shady places
Morphology	Annual herb with fibrous roots. Aerial stems 48-64 cm tall, sharply triquetrous, soft, glabrous. Leaves sessile, sheathed, 3-10 x 0.1-0.15 cm, leaf blade linear, glabrous, sheaths closed, surrounding the stems bases. Inflorescence umbellate; rays 5-8 in number, 0.5-3 cm long, each bearing a congested head of small sessile spikelets; involucre bracts 3-4 in number, 6.25-10 x 0.15-0.4 cm, longer than the inflorescence, linear oblong, mid vein and margins scabrous. Spikelets 3-4 x 1 mm, suborbicular, margins hyaline. Stamen 1; style 3-branched. Nut 0.5-0.6 x 0.3-0.4 mm, obvoid-ellipsoid, trigonous, yellowish-brown (Figure 3A).
Flowering period	July to December
Voucher No.	296
Pollen description	Pollen monad, monoporate, radially symmetrical and isopolar. In equatorial view, shape of the pollen apple shape to rhomboidal and in polar view the pollen circular to rhomboidal. Polar diameter 30.25 $\mu$ m (28-32 $\mu$ m). Equatorial diameter 28.75 $\mu$ m (27.50-32.5 $\mu$ m). Exine thickness 2.15 $\mu$ m (1.75-2.5 $\mu$ m). P/E ratio 1.05 $\mu$ m. Exine with striate elements. The sculpturing elements less than 1 $\mu$ m. The granules are uniformly distributed with narrow spaces over the tectum (Figures 3D and E).
Leaf epidermal anatomy	Abaxial and adaxial surfaces are with costal and intercostal zonation. Long cells in costal zone narrower and smooth, rectangular, while with markedly sinuous walls in intercostals zone, and similar at abaxial surface. Their length in intercostals zone, abaxial one is 80 $\mu$ m (57-95 $\mu$ m) x 13.75 $\mu$ m (12.5-15 $\mu$ m) abaxial one, 65.75 $\mu$ m (37.5-100 $\mu$ m), while in costal zone, abaxial one, 31.25 $\mu$ m (27.5-35 $\mu$ m) x 14.375 $\mu$ m (12.5-15 $\mu$ m) and adaxial one, 188 $\mu$ m (175-200 $\mu$ m) x 12.5 $\mu$ m (17.5-20 $\mu$ m). Microhairs panicoid type on both the sides, stomata common, comparatively in smaller number at abaxial surface, their length, abaxial one is 22.50 $\mu$ m (18-25 $\mu$ m) x 18.75 (17.5-20 $\mu$ m) and adaxial one is 25 $\mu$ m (22.5-27.5 $\mu$ m) x 12.5 $\mu$ m (15.625-17.5 $\mu$ m). Interstomatal cells cross ended. Subsidiary cells slightly triangular. Short cells absent at both sides. Silica bodies only on adaxial surface (Figures 3G and H).
Part Used	Roots
Indigenous Recipes	Fresh aerial parts are crushed to obtain paste which externally applies on eyes to reduce redness, pain and for conjunctivitis. The paste is applied for 15-20 days on skin to cure eczema and scabies. The aerial parts are dried in shade and ground to obtain powder. Half teaspoon at morning before breakfast and at night with glass of water is taken for 20-25 days to reduce obesity.
Toxicity	None
Organoleptography (Roots)	Root length is 4-8 cm and 1 to 3.5 cm in diameter. Root texture is hard; color of surface is dark brown. Ridges and furrows on the root surface. Small prickles on root surface. Internally root is light brown in color. Root has smell like wet soil. Taste of root is slightly bitter (Figure 3B).
TLC fingerprinting	TLC under UV shows the presence of two minor flavonls (Figure 3F).

**Table 4.** *C. niveus* Retz.

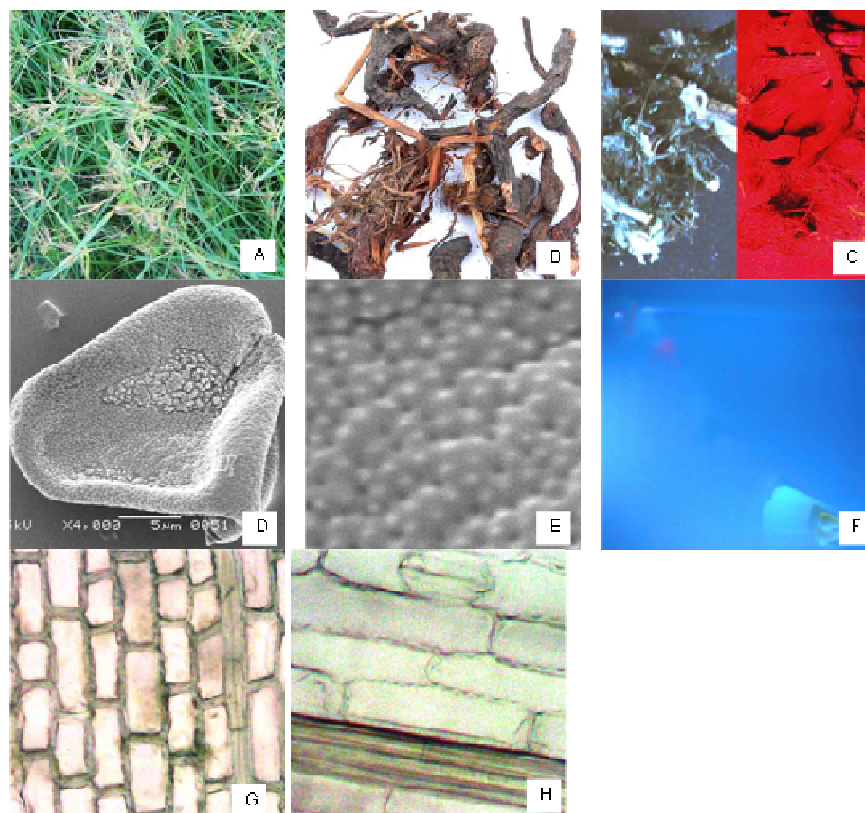
English name	Snow white sedge
Local name	Mootha
Drug name	Nagar Mootha
Family	Cyperaceae

Table 4. Contd.

Distribution in Pakistan and world	In Pakistan; Sindh, D. I. Khan, Mianwali, Rawalpindi, Islamabad, Baluchistan, Tahl-Kurram, Peshawar, Kohat, Chitral, Swat, Barikot, Mingora, Karakar, Hazara, Siran Valley, Nathia, Sakesar, Kahuta, Saidpur, Murree road, Poonch, Kashmir, Jhelum, Domel, Kahuta, Bimbar. In world; Central, South and Southeast Asia.
Occurrence	Commonly occurs in arid areas
Morphology	Perennial herb perennating by woody rhizome. Aerial stems 16-50 x 0.1-0.15 cm, tufted, erect, triquetrous, glabrous. Leaves 4-25 x 0.1-0.3 cm, basal, linear, shorter than the stems, mid-vein and margins scabrous. Inflorescence capitate of sessile spikelets, involucre bracts 2-3 in number, 2-10 x 0.1 cm, leaf like, linear. Spikelets 1.5-3.0 x 0.4-0.5 cm, narrowly oblong, 8-54 flowered, white, rachilla not winged, glumes 4-4.5 x 2-2.5 mm, ovate lanceolate, boat shaped, mucronulate, 13-14 nerved. Stamens 3, style 3-branched. Nut 1.3-1.5 x 0.9-1 mm, obovoid-ellipsoid, trigonous (Figure 4A).
Flowering period	April to October
Voucher No.	531
Pollen Description	Pollen circular in polar view and spheroidal to oblate spheroidal in equatorial view. Polar diameter 21.89 $\mu\text{m}$ (20-25 $\mu\text{m}$ ) and equatorial diameter 23.18 $\mu\text{m}$ (20-27.5 $\mu\text{m}$ ). P/E ratio 0.94. Pollen monoporate and ectoporate. Pore diameter 1.5 $\mu\text{m}$ (1.0-2.0 $\mu\text{m}$ ) and exine thickness 0.89 $\mu\text{m}$ (0.75-1.0 $\mu\text{m}$ ). Pollen fertility 84.16 %. Sculpturing is foveolate. The sculpturing elements or depressions are minute and laxly thinly distributed over the tectum (Figures 4D and E).
Leaf epidermal anatomy	Abaxial intercostal long cells with thin sinuous walls, 60-65 $\mu\text{m}$ long and 10-12.5 $\mu\text{m}$ wide. Number of rows of long cells between two costal zones, 5-9. Number of stomatal rows between two costal zones, 2-3. Stomatal complex 15.25-20 $\mu\text{m}$ long and 15.25-17.5 $\mu\text{m}$ wide, guard cells dumb bell shaped, subsidiary cells triangular to high dome shaped. In Costal zone: silica bodies saddle shaped, 12.5-20 $\mu\text{m}$ long and 11.25-15 $\mu\text{m}$ wide. Short cells with sinuous walls, 22.5 - 25 $\mu\text{m}$ long and 12.5-13.25 $\mu\text{m}$ wide. Prickles 31.25-41.25 $\mu\text{m}$ long and 12.5 - 18.75 $\mu\text{m}$ wide. Adaxial intercostal zone: Adaxial intercostal long cells with sinuous walls, 32.5 - 55 $\mu\text{m}$ long, 6.25 - 10 $\mu\text{m}$ wide. Silica bodies saddle shaped, 7.5 - 8.0 $\mu\text{m}$ wide horizontally and 11 - 12.25 $\mu\text{m}$ wide vertically. Short cells 12.5 - 16.25 $\mu\text{m}$ long and 8.75 - 10 $\mu\text{m}$ wide. Prickles 25-30 $\mu\text{m}$ long and 8.75 - 10 $\mu\text{m}$ wide (Figures 4G and H).
Part used	Roots
Indigenous recipes	Roots are collected, clean and washed. Roots are dried in shade and ground to obtain powder. 5-8 gm of root powder is taken twice a day for a week to cure infection internally. $\frac{1}{2}$ teaspoon of powder is taken with water at night time for stimulant, sedative and diuretic.
Toxicity	Excessive use may cause toxicity
Organoleptography (roots)	Root length varies from 1 cm - 5 cm and diameter of root is from 2-5.5 cm. root is globular with both ends slightly pointed. Root surface is dark brown in color. Surface of root is rough and contain small hair. Root is hard in texture. Internally root has comparatively light brown color than external surface. Root is odor less and slightly bitter (Figure 4B).
TLC Fingerprinting	TLC under UV shows the presence to two minor flavonols, two minor phenolic acid and one minor aurone (Figure 4F).

which contains medicinally important species used in traditional systems of medicines pharmaceutical industries in Pakistan, India, China and other Asian countries. The herbal drug obtained from *Cyperus* is commonly called as Nagar Mootha in Urdu, Hindi and Tibb while Nagar Musta in Sanskrit (Bhagwat et al., 2009). *Cyperus* is known in

Chinese as "xiangfu or xiang fuzi" means fragrant and usually applied to strong and pleasant fragrances, such as those occurring in culinary species, perfumes and incenses (Yang, 2002). The character "fu" is the same as that used to describe aconite (*Aconitum heterophyllum*). The term was likely to be used because the appearance



**Figure 1.** *C. rotundus*, A: Flower branch, B: Roots, C: Roots under UV and IR, D: Polar view of pollen (SEM), E: Pollen sculpturing (SEM), F: TLC finger prints, G: Leaf epidermal cells and stomata, H: Stomata.

of the *Cyperus* rhizomes, the part used, remind herbalists of the aconite roots (Haung and Yuxia, 1993). In much of the rest of the world, the *Cyperus* is referred as “nut grass” or purple nut sedge (sedge is the term indicating blade like leaves). The nut is the rhizome (or tuber) which forms rounded or elongated roots.

### Phytotherapeutic uses

*Cyperus* is considered by many traditional practitioners to be the best herb used for depression, circulation, skin disorders, digestive complaints etc. (Nadkarni, 1976; Williamson, 2002). *Cyperus* is included in dozens of traditional herb formulas. *C. rotundus* (Nagar mootha) is thought to have originated in Indo-Pak sub continent and these spread the past 2000 years (it first appears in a Chinese medicine book around 500 A.D.). The rhizome is used in Ayurvedic, Unani and Chinese medicines mentioned in the ancient Caraka Samhita (ca. 100 A.D.). Its uses in modern medicines are primarily for treating fevers and digestive system disorders (diarrhea, vomiting and indigestion). It is also known as menagogue to (treat delayed menstruation) and an analgesic use for dysmenorrheal for (painful menstruation) (Nadkarni,

1976).

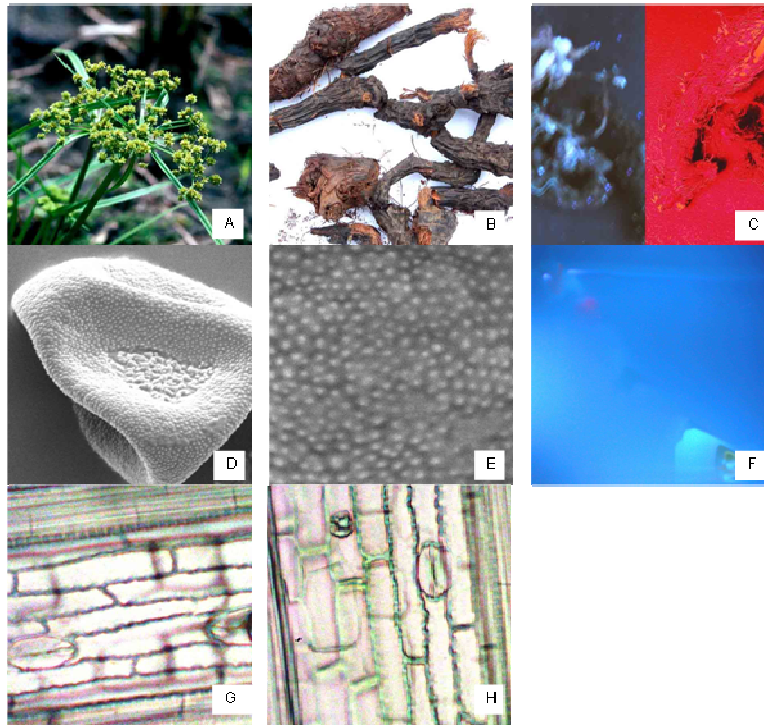
### Problems in authentication

In herbal markets of Indo-Pak sub-continent and other South Asian countries, under the name of herbal drug Nagar Mootha different types and forms of rhizomes are sold which belong to various species of genus *Cyperus*. It was observed during market survey that most of the herbal shops in Attock, Mianwali, Rawalpindi, Lahore (Punjab), Sindh, NWFP and Balouchistan where the adulterant rhizomes are available. In this way some other species of *Cyperus* can be mistaken for genuine Nagar Mootha (Purple nut sedge) including *C. alopecuroides*, *C. difformis* and *C. niveus* which have leaves and rhizome resembling those of *C. rotundus*.

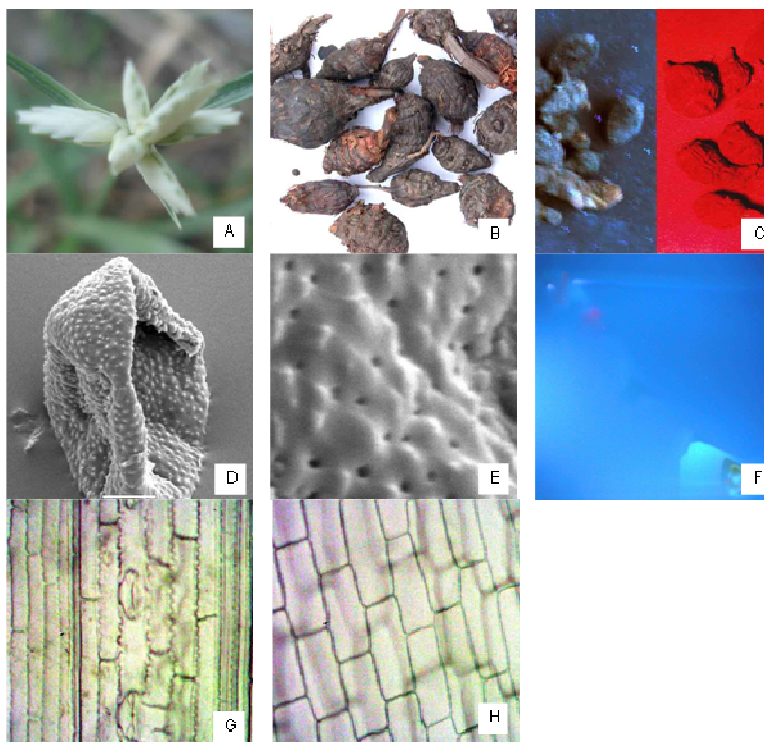
### Organoleptography and chemotaxonomic differentiation

Morphologically the *C. rotundus* has slender leaves that are connected to a network of underground rhizomes, roots and tubers (Nishimoto et al., 1998). *C. rotundus* is





**Figure 3.** *C. difformis*, A: Floral branch, B: Roots, C: Roots under UV and IR, D: Polar view of pollen (SEM), E: Pollen sculpturing (SEM), F: TLC Finger prints, G: Leaf epidermal cells, H: Stomata.



**Figure 4.** *C. niveus*, A: Flower, B: Roots, C: Roots under UV and IR, D: Polar view of Pollen (SEM), E: Pollen sculpturing (SEM), F: TLC finger prints, G: G. Leaf epidermal cells and stomata H: Stomata.



perennial stoloniferous herb, bearing ovoid tubers at the end of stolens (Figure 1A). Rhizomes has dark brown color with vertical and horizontal ridges on surface (Figure 1B). While *C. alopecuroides* is the perennial, tufted with short rhizome. Externally the rhizome is hard, dark brown color with irregular vertical ridges (Figure 2B). Similarly the *C. niveus* is perennial herb with woody rhizomes which are globular and dark brown in color (Figure 4B). Gupta et al. (1998) investigated the anti-inflammatory activities of oil isolated from the rhizomes of *C. scariosus* and other closely resembling species. Palyno-anatomical features of *C. rotundus*, *C. alopecuroides*, *C. difformis* and *C. niveus* have quite resemblance to each other and it is difficult to differentiate these species on the basis of microscopic features like pollen and leaf epidermal characters. While it is found that TLC fingerprinting can differentiate the genuine rhizome from its adulterants. TLC under UV shows the presence of three minor flavanoles in the rhizome of *C. rotundus* (Figure 1F), while in *C. alopecuroides* and *C. difformis* there are two and three minor flavanoles respectively (Figures 2 and 3F). But *C. niveus* is quite different which contain two minor flavanoles, two minor phenolic acids and one minor aurone (Figure 3F) indicating that these species of *Cyperus* can be distinguished due to flavanole pattern. It has been observed during rhizome study that there is a lot of variation in rhizome size which might determine the quality of variant. Further study is required to find the area and soil of the best variant of Nagar Mootha (*C. rotundus*). It is concluded that the subject of herbal drug standardization is massively wide and deep. There is so much to know and so much seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function.

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