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Chemotaxonomic Authentication of Herbal Drug Chamomile

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The present study is based on the emerging concepts of authentication of problematic medicinal plants by using chemotaxonomic techniques. The major aim and objective of the study is to ascertain and solve the problems of adulteration and confusion of problematic herbal drugs used in traditional medicines. Unani herbal drug chamomile (*Matricaria chamomilla*) was authenticated by chemotaxonomic techniques from its adulterants *i.e.*, *Anthemis nobilis*, *Matricaria aurea* and *Inula vestita*. Thin layer chromatography (TLC), scanning electron microscopy (SEM), light microscopy (LM), organoleptography, UV and IR analysis revealed the authentic source of chamomile is *Matricaria chamomilla*. It is concluded that when classical techniques combined with analytical techniques like SEM, TLC, UV and IR, then these may bring more appropriate results aimed as characterization and may assist the standardization of traditional herbal drugs available in market and utilized in industry.

Key Words: Chemotaxonomy, Authentication, Chamomile, Herbal drug.

INTRODUCTION

Traditional medicine based on using medicinal plants are moving from fringe to mainstream with greater number of people seeking remedies and health approaches from side effects caused by synthetic chemicals. Recently considerable attention has been paid to utilized eco and bio-friendly traditional plant based medicines throughout the world for prevention and cure of human diseases. Considering the adverse effects of synthetic drugs, the western population, European, African and Asian countries are now looking to natural remedies which are safe, effective and inexpensive[1]. It is documented that 80 % of the world population has faith in traditional medicine, particularly plant based drugs for their primary healthcare[2].

Accurate plant identification is the foundation of the safe use of plant based natural health products. Without proper identification as a starting point, the safe use of quality products cannot be guaranteed[3]. There is a recognition within industry and government that there is a need to protect access and choice by consumers

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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.

Authentication and standardization are prerequisite steps, while considering source material for herbal formulation. Frequently, a particular part of the plant is specified *viz.*, root/rhizome, bark/stem, flower, fruit, seed, *etc*. The detailed taxonomical identification with internationally accepted nomenclature in Latin is an integral part of authentication. It is necessary to update nomenclature time to time in order to avoid confusion regarding synonyms *e.g.* for Guggul, one of the Unani materials, the correct Latin name is *Commiphora wightii* (Arn.) Bhandari, in literature it is also known by synonyms such as *Balsamodendron wightii* Arn.; *Balsamodendron roxburghii* Stocks.: *Balsamodendron mukul* Hook ex Stocks and *Commiphora mukul* (Hook. ex Stocks) Engl[4]. Global interest in the application of useful elements of traditional medicine to attain health for all by the year 2000, as expressed by WHO is real. Since the use of plants as therapeutic agents is paramount in virtually all of the systems of traditional medicine and especially in Unani Medicine, it is necessary to develop a mechanism for quality assurance of plants used as drugs in these medical systems[5]. A comprehensive program for the identification, collection, evaluation, preparation, utilization, storage and conservation is to be implemented by following exhaustive research and development. As such, quality assurance of herbal medicine seems to be little explored and that`s why standardization and authentication have taken a serious turn. It therefore seems obligatory to procure the indigenous drugs and certify their identity by taxonomic and chemical methods. While considering the quality of herbal formulation in any system of medicine emphasis should be given for good harvesting practices (GHP), good laboratory practices (GLP) for quality control and good manufacturing practices (GMP).

Enigmatic situation of problematical medicinal plants is chaotic for herbal industry. Numerous diverse problems are confronted to taxonomists in the identification of such medicinal plants. Problems in authentication of genuine drugs arise due to several reasons such as folk classification of medication which grew up in communities without the influence of science and proper documentation. The existence of several common names for the same plant species in different areas. Another problem is the superficial resemblance of plant species with in the same tribe or family such as family Asteraceae[6]. It was stated that the species of *Anthemus*, *Inula* and *Cotula* were utilized instead of *Matricaria chamomella*. Problem of adulteration of medicinal plant arose due to the potential use of different species for similar ailment such as *Boerhaavia procumbens* (Ponernava) and *Trianthema portulcastrum*, both are used for jaundice. The vernacular name "itset" is used for both species in various areas in Pakistan[7]. Adulteration in market samples is one of the greatest drawbacks in promotion of herbal products[8,9]. Many researchers have contributed in checking adulterations and authenticating them[3,7,10-14].

The present research project based on chemotaxonomic authentication of problematical medicinal plant chamomile used in Unani and traditional medicine, commercially marketed in Indo-Pak subcontinent and abroad. In order to promote herbal drugs in the region and developed world, it felt worthy to authenticate and evaluate the confusion and adulteration problems of this drug by following WHO methodologies and guidelines.

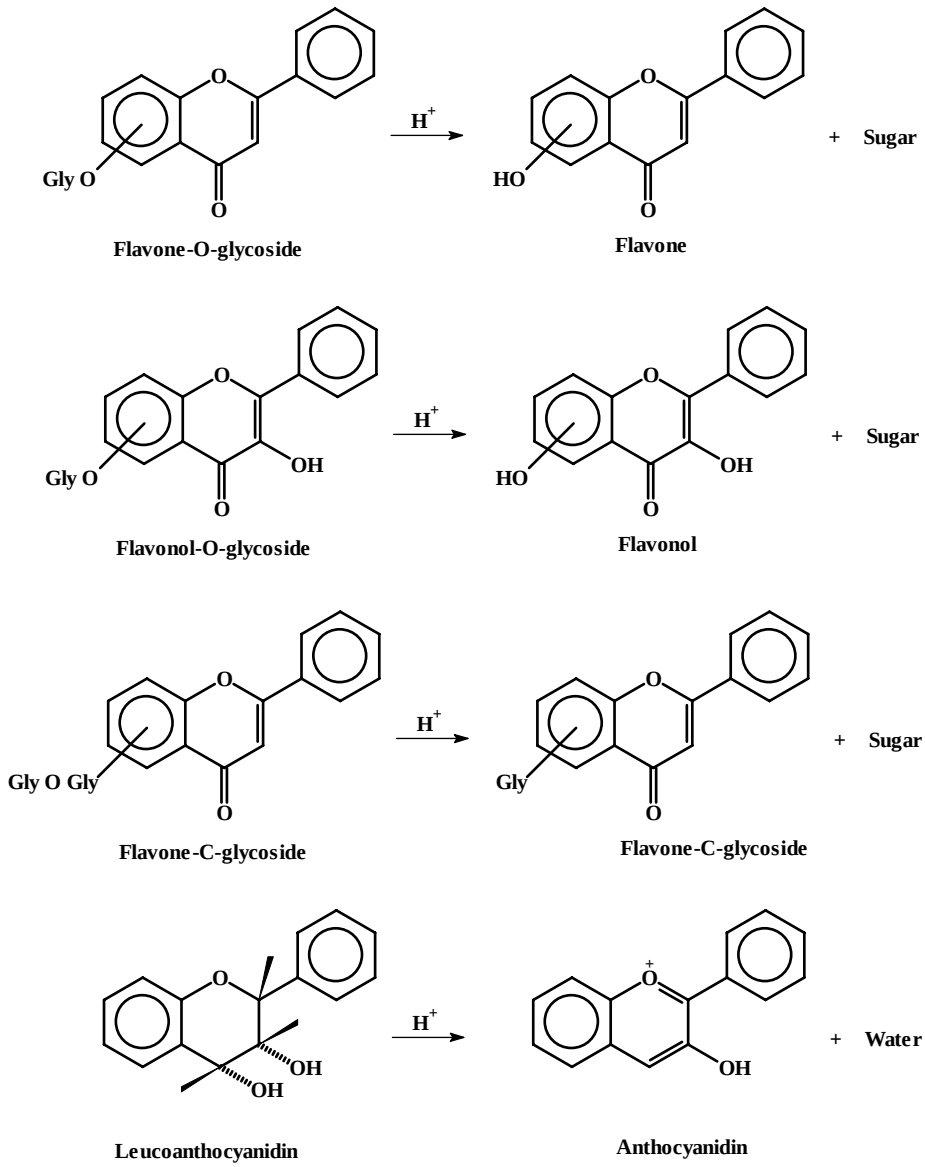
EXPERIMENTAL

Morphological examination: Morphological examination was carried out by binocular dissecting microscope, (Model Kyowa SZF 0.75x-3.4x) using eye piece, WF 10 × 10/20. The description of plant species were also compared by using different Floras[15-20]. Organoleptography confined to the inspection of organoleptic markers such as shape, color, texture and odour of the herbal drug.

Palynological examination: Microscopic examination focused on scanning electron microscopy (SEM) and light microscopy (LM) of natural fingerprints of plants *i.e.* pollen. Pollen samples of fresh collection was used for SEM and LM. The pollen grains were prepared for scanning microscopy (SEM) and light microscopy (LM) according to the method of Erdtman[21]. For light microscopy the pollen grains were mounted in stained glycerine jelly[22] and observations were made with a Meiji Light Microscope (Model SWH 10X/22 Japan). For SEM studies, pollen grains suspended in a drop of 45 % acetic acid were directly transferred with a fine pipette to aluminum stub using double adhesive cello tape and coated with gold in a sputtering chamber (SPI-Module Sputter Coater). The SEM examination was carried out on a Jeol-JSM 5910, scanning electron microscope. The measurements of pollen are based on 25-30 readings from each specimen. Shape of pollen in polar and equatorial view, polar and equatorial diameter, P/E ratio, spines, number of spines between colpi, length of spine, length and width of colpi and exine thickness were studied. The terminology used is in accordance with Walker and Doyle[23] and Ronald[24].

Chemo-taxonomy: A very important class of secondary metabolites flavonoids is considered one of the most reliable chemotaxonomic markers[25]. For the extraction of flavonoid aglycones, 5 g dried powder material (aerial parts) is treated with 200 mL HCl (2 N) and heated for 1 h in a water bath (Model, Memmert-91126-FRG, Germany) at *ca.* 100 ºC. By this treatment normally all flavonoids-O-glycosides are converted to flavonoids aglycones, leucoanthocyanidin to anthocyanidins whereas the C-glycosides remain unaffected (**Scheme-I**). After cooling, the flavonoid aglycones are extracted with diethyl ether (Et_2O) from the aqueous phase. A second series of extraction by *n*-butanol quantitatively removes the anthocyanidins.

Thin layer chromatography: The technique of TLC finger printing consists of applying the flavonoid sample on commercially available pre-coated polyamide F_{254} plates (Merck-Germany). For analytical work pre-coated aluminum backed



Scheme-I: Acid hydrolysis of flavonoid glycosides

TLC plates which are transparent to UV lights were used. These plates are loaded with the herbal extract for its analysis. The plates are then developed in TLC tank (Size 20 cm × 20 cm Camag Switzerland) with solvent system. The solvent system used in both the directions is toluene:methanol:methyl ethyl ketone 4:3:3[26]. After drying the fully developed TLC plates are viewed under 366 nm UV light. This is a reliable and reproducible method of authentication of a particular herbal drug.

Photography: Digital camera (Sony, DSC-W50) was used in photography of herbal part of the drugs under short wavelength UV light (UVGL-58 Lamp, 254/365 nm), IR lights and visible lights. This high resolution photography in UV, IR and visible lights provide authentic approach toward the authentication of doubtful and problematic plant species and herbal products.

RESULTS AND DISCUSSION

Gul-e-Baboona

***Matricaria chamomilla* L.**

English name (s): German chamomile/Scented Mayweed; **Local name:** Baboona; **Tib name:** Gul-e-Baboona; **Family:** Asteraceae; **Distribution in Pakistan:** Balouchistan, Mastnag, Nushki in Balouchistan; **Distribution in world:** Wide spread through out Europe and most of temperate Asia. **Occurrence and conservation status:** Very rare, only found in Mastung and Nushki only.

Description: Erect or ascending, profusely branched above, up to 50 cm tall, glabrous herb with sulcate or obtusely angled internodes. Leaves on short, basally dilated and sheathing petioles, oblong, (1.5-)2.5–6(-7) cm long, up to 2 cm wide, finely bipinnatisect into narrowly linear to filiform, 3-4 mm long, c. 1 mm wide, ± acute to shortly mucronate ultimate segments. Capitula solitary terminal, on up to 8 (-10) cm long, filiform peduncles, 1-1.5 (-2) cm across, sweet-smelling. Receptacle sharply conical, 5.6 mm long, foveolate. Involucre hemispherical, phyllaries 2-3-seriate, imbricate, broadly lanceolate to oblong, 1-2.5 (-3) × 1-1.25 mm, greenish with pale membranous margins, obtuse. Rayflorets female, with white, oblong, 5-8 × 3-3.5 mm, shortly 3-fid, deflexed ligule from c. 1 mm long tube. Disc-florets yellow, 5-lobed, with the externally sparsely glandulose corolla tube becoming campanulate above the constriction; style branches truncate-penicillate. Cypselas oblong-cylindrical, ± curved, c. 1 mm long, greyish-brown, sparsely glandular, with 5 ribs on ventral surface, ray-cypselas with an irregularly toothed, c. 1 mm long, membranous auricle, disc-cypselas slightly coronate or epappose (Plate 1A).

Flowering period: April-August; **Voucher No.:** ISL-MZ-26; **Palynology:** The pollen is monad, tricolporate and echinate. The shape of pollen in polar and equatorial view is circular. Polar diameter with spines is 19.94 µm (17-23 µm) and equatorial diameter is 16.5 µm (15-175 µm). P/E ratio is 1.20 µm. Spines are apiculate broad based and tapering at the end. Number of spines between colpi are 4-6 and length of colpi is 4.9µm (4-4.5 µm) and width of colpi is 6.6 (5-6.5 µm). Exine thickness is 1.3 µm (1-1.5 µm) (Plate 1E, F).;**Part used:** Aerial parts; **Folk medicinal uses:** Insomnia, tension, allergy, hysteria, dyspepsia, sexual debility, rheumatism & colic; **Preparation and dosage:** Aerial parts are dried under the shade and grounded into powder ¼ teaspoon is recommended at night for a month for insomnia per day tension sexual debility, hysteria and dyspepsia. ½ per day teaspoon of the powder drug is used for rheumatism and colic; **Toxicity:** Non-toxic but excessive

use may cause drowsiness; **Marketing status:** Marketed under the name of Gul-e-Baboona but genuine drug not available throughout the country. **Organoleptography (Aerial parts):** Herbal drug consist of dried aerial parts. Mostly branches and flowers are mixed. Branches are 10-35 cm in length, cylindrical containing white hairs. Flowers heads are yellowish in color white ray florets. Ray florets are 3-7 mm × 2-3 mm, cypselas oblong. Aerial parts are with characteristic herbal smell and tasteless (Plate 1B); **Finger printing:** TLC shows the presence of four major phenolic acids, four flavones and one aurone when viewed under UV light (Plate 1D).

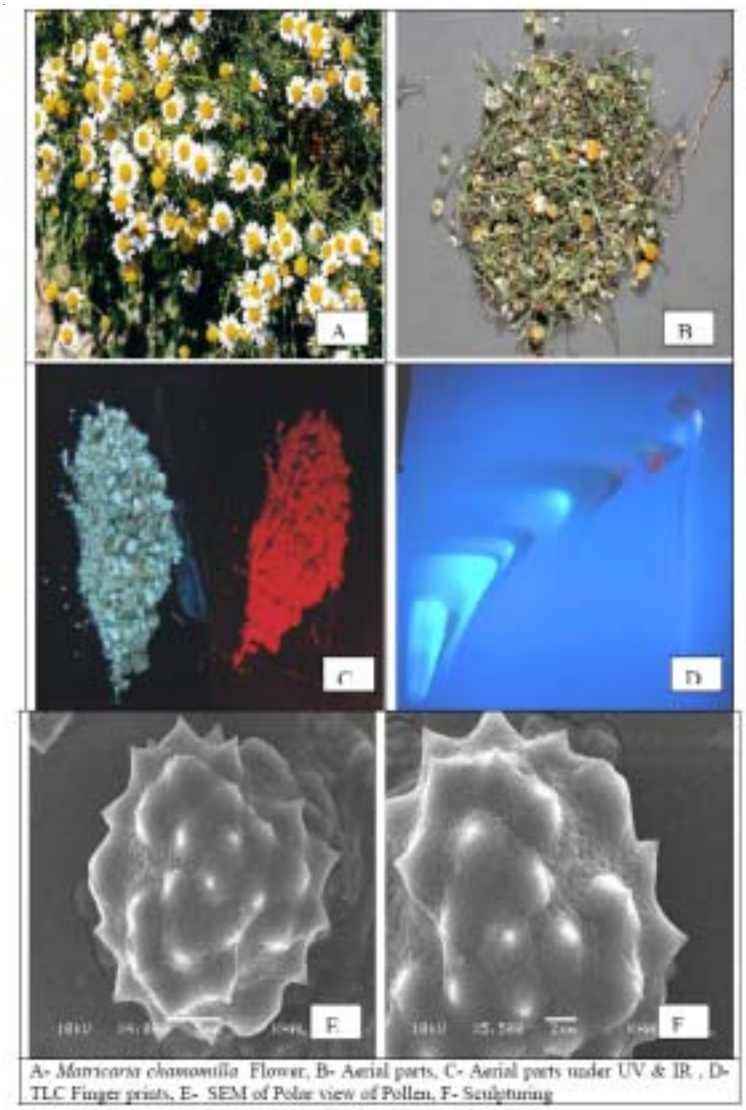


Plate 1. Chemotaxonomic authentication profile of *Matricaria chamomilla*

***Matricaria aurea* (Loefl.) Schultz: Syn: *Cotulea aurea* Loefl.**

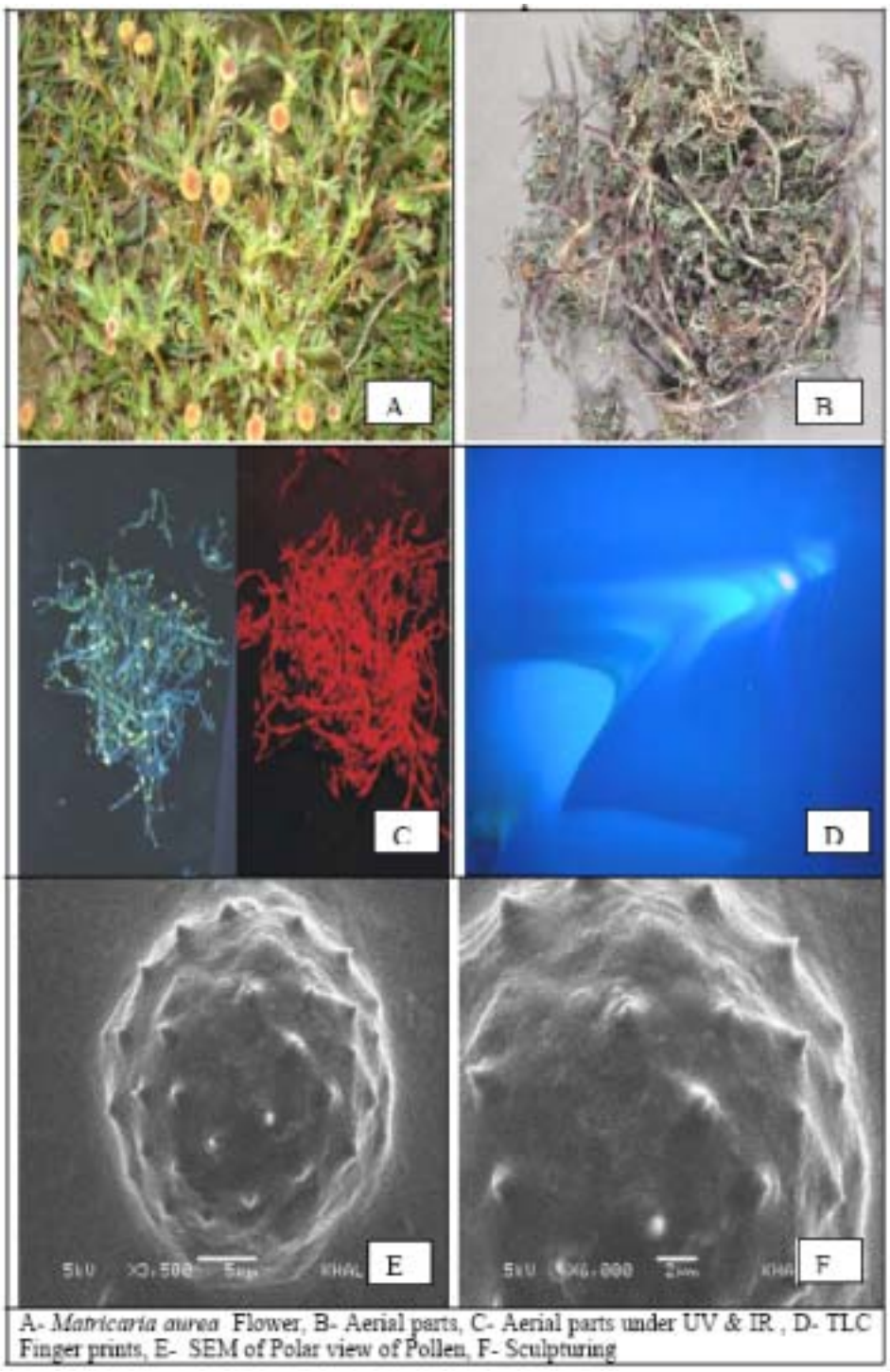
English name: Rounded chamomile; **Family:** Asteraceae; **Distribution in Pakistan:** Peshawar, Dir, Lower Hazara, Attock, Hazro, POK; **Distribution in world:** South Europe, North Africa, Middle East, South West Asia to Central Asia. **Occurrence and conservation status:** In silt soils and silt-floored basins and as weed in gardens or near gardens with loamy-silt.

Description: Annual, prostrate to erect, branched, up to 20 cm tall, glabrous or with short sparse whitish hairs. Leaves shortly petiolate to ± sessile, with rhachis and petiole slightly dilated and semiamplexicaule towards the base, linear-lanceolate to oblong, (0.7) 1.5-3 (-5) cm long, 2-3-pinnatisect into linear-filiform, 0.2 mm wide, apically shortly cartilaginous mucronulate ultimate segments. Capitula discoid, heterogamous, globose, 5-6 mm across, pedunculate, solitary or 2-3 in a ± corymb. Involucre broadly napiform, phyllaries oblong, 2-2.5 mm long, glabrous, margins and obtuse apices broadly brown scarious or sometimes whitish membranous. Receptacle sharply conical. Florets yellow, all tubular; marginal female and fewer than the disc-florets, with 1 mm long, irregularly 2-toothed with long exserted styles; disc-florets slightly constricted in the middle, dilated upwards into a 4-lobed limb. Cypselas oblong to ovoid, 0.5-0.7 mm long, brownish, mildly 3-ribbed. Pappus absent (Plate 2A).

Flowering period: May-July; **Voucher No.:** ISL-MZ-19; **Palynology:** Pollen is monad, echinate and tricolporate. Shape in polar view is circular and in equatorial view is perprolate. Polar diameter is 10.7 µm (10-12 µm) and equatorial diameter is 9.93 µm (9.75-10 µm) P/E 0.302 µm. Length of colpi is 0.87 µm (0.5-1 µm) and width of colpi is 2.3 µm (2-2.5 µm). Length of spine is 1.1 µm (1-1.25 µm) and No. of spines between colpi is 8-9. Exine thickness is 2.3 µm (2-2.5µm) (Plate 2E, F); **Marketing status:** Commonly available in herbal market under the name of Baboona; **Organoleptography (Aerial parts):** Herbal drug consist of parts which are dried broken branches, leaves and flowers. General appearance is greenish in color. Branch contain whitish long hairs. Leaves linear to lanceolate 1-3 cm long. Leaves are divided into pinnatisect leaflets. Branch are cylindrical bearing sabtary flowers. Branch are 8-16 cm in length. Flowers heads are orange in colour, some time yellowish 4-6 mm in diameter. Aerial parts are odourless and tasteless (Plate 2B); **Finger printing:** TLC of aerial parts extract reveals the presence of five major amount of phenolic acids when viewed under 366 nm UV light (Plate 2D).

***Anthemis nobilis* L. : Syn: *Chamaemelum nobile* (L.) All.**

English name: Roman chamomile; **Local name:** Baboona; **Tib name:** Gul-e-Baboona; **Family:** Asteraceae; **Distribution in Pakistan:** Cultivated in POK, wild in Siran Valley Manshera, Abbotabad, Kaghan, Naran, Chitral and Swat. **Distribution in world:** Europe, North Africa, West Asia, North America; **Occurrence and conservation status:** Cultivated commonly in hilly and plain areas. Wildly distributed in moist temperate zone of Pakistan.



A- *Matricaria aurea* Flower, B- Aerial parts, C- Aerial parts under UV & IR., D- TLC Finger prints, E- SEM of Polar view of Pollen, F- Sculpturing

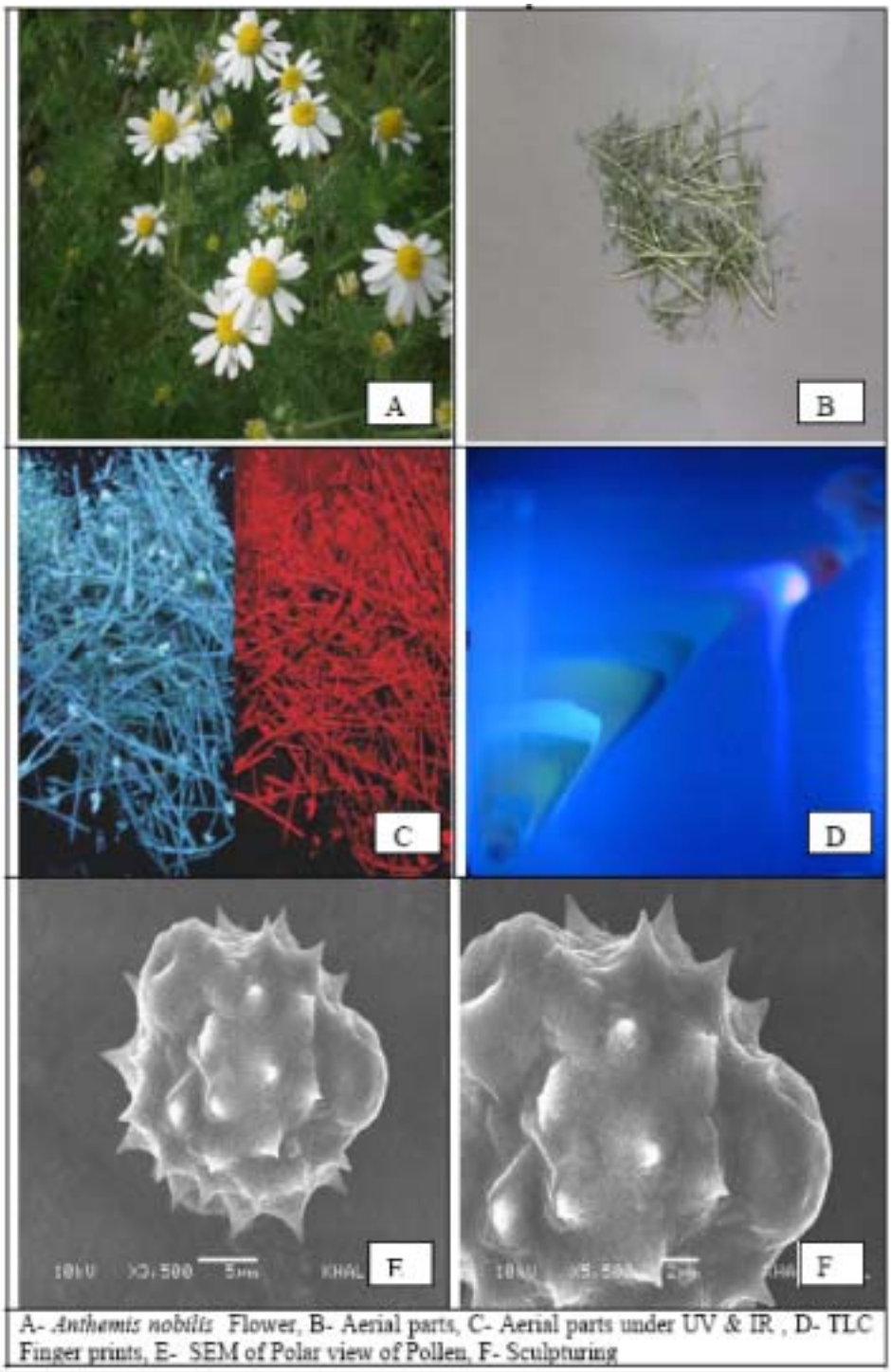
Plate 2. Chemotaxonomic authentication profile of *Matricaria aurea*

Description: A perennial, pubescent, 10.30 cm tall, pleasantly aromatic herb with decumbent or ascending shoots from the base. Leaves sessile, oblong, 1.5.5 cm long, 2-3-pinnatisect into linearsubulate or filiform, ± hairy, mucronate ultimate segments. Peduncles 2-4 cm long, unthickened in fruit. Capitula radiate, 1.8-2.5 cm across. Phyllaries oblong, 3.5 mm long, 1.5-2 mm wide, obtuse with broad scarious margins, sparsely hairy on midrib. Receptacle conical, chaffy all over, paleae oblong, keeled, obtuse. Ray-florets female, fertile, ligules 7.10 mm long, white, occasionally absent. Disc-florets yellow, as long as paleae, corolla tube basally swollen, hairy. Cypselas obovoid, 1.1-5 mm long, smooth, with 3 faint striae on inner side, bald (Plate 3A).

Flowering period: March to May; **Voucher No.:** ISL-MZ-15; **Palynology:** Pollen is monad and echinate. Shape in polar view is intersub angular and in equatorial view is perprolate. Polar diameter is 21.8 µm (21.5-22 µm), equatorial diameter is 22.4 µm (22-22.7 µm), P/E is 1.37 µm, colpi length 5 µm and width 6.5 µm. Length of spines is 1.18 µm (1-1.25 µm). No. of spines is 8-9. Exine thickness is 5 µm (4.25-5.5 µm) (Plate 3E,F); **Part used:** Aerial parts; **Folk medicinal uses:** Indigestion, Insomnia, nausea, sleeping sickness, menstrual and stomach disorder; **Preparation and Dosage:** One kg aerial parts are collected, dried under shade and crushed to obtain powder. Herbal tea is prepared by boiling 10 g of this powder in about 0.5 L of water. A cup of this tea trice a day is used against gastrointestinal complaints such as gas, motion, sleeping sickness and insomnia. Decoction of 8 g of dried flowers in 250 mL water is prepared. Half cup of this decoction is taken daily at night for insomnia, menstrual pain and stomach disorder. Crushed aerial parts of the plant are applied externally on head for headache. Decoction of the plant is used to wash the eyes. 2 g dried powder of the plant is taken twice a day for kidney stone. Decoction is also used for deafness; **Toxicity:** It should be avoided by allergic people and during early pregnancy; **Marketing status:** Abundantly available under the name of Baboona at herbal shops; **Organoleptography (Aerial parts):** Dried aerial parts are greenish in colour, market samples are light yellowish in colour which consist of mostly flowers, broken petals and leaves. Dried parts have pleasant aromatic smell. Leaves are filiform 1-5 cm long and hairy. Flowers are yellowish, petals whitish with scarious margins (Plate 3B); **Finger printing:** TLC of aerial parts extract reveals the presence of five major amount of phenolic acids and two flavonols when viewed under 366 nm UV light (Plate 3D).

***Inula vestita* Wall ex. DC.**

English name: Golden samphire; **Family:** Asteraceae; **Distribution in Pakistan:** Sind, Hyderabad, NWFP, Peshawar, Noshewara, Swat, Lahore, Hasan Abdal, Tilla, Jhelum, Attock; **Distribution in world:** East Asia, Himalayas from Pakistan to Kashmir, India, China; **Occurrence and conservation status:** Abundant in waste places, dried and arid areas.



A- *Anthemis nobilis* Flower, B- Aerial parts, C- Aerial parts under UV & IR , D- TLC Finger prints, E- SEM of Polar view of Pollen, F- Sculpturing

Plate 3 Chemotaxonomic authentication profile of *Anthemis nobilis*

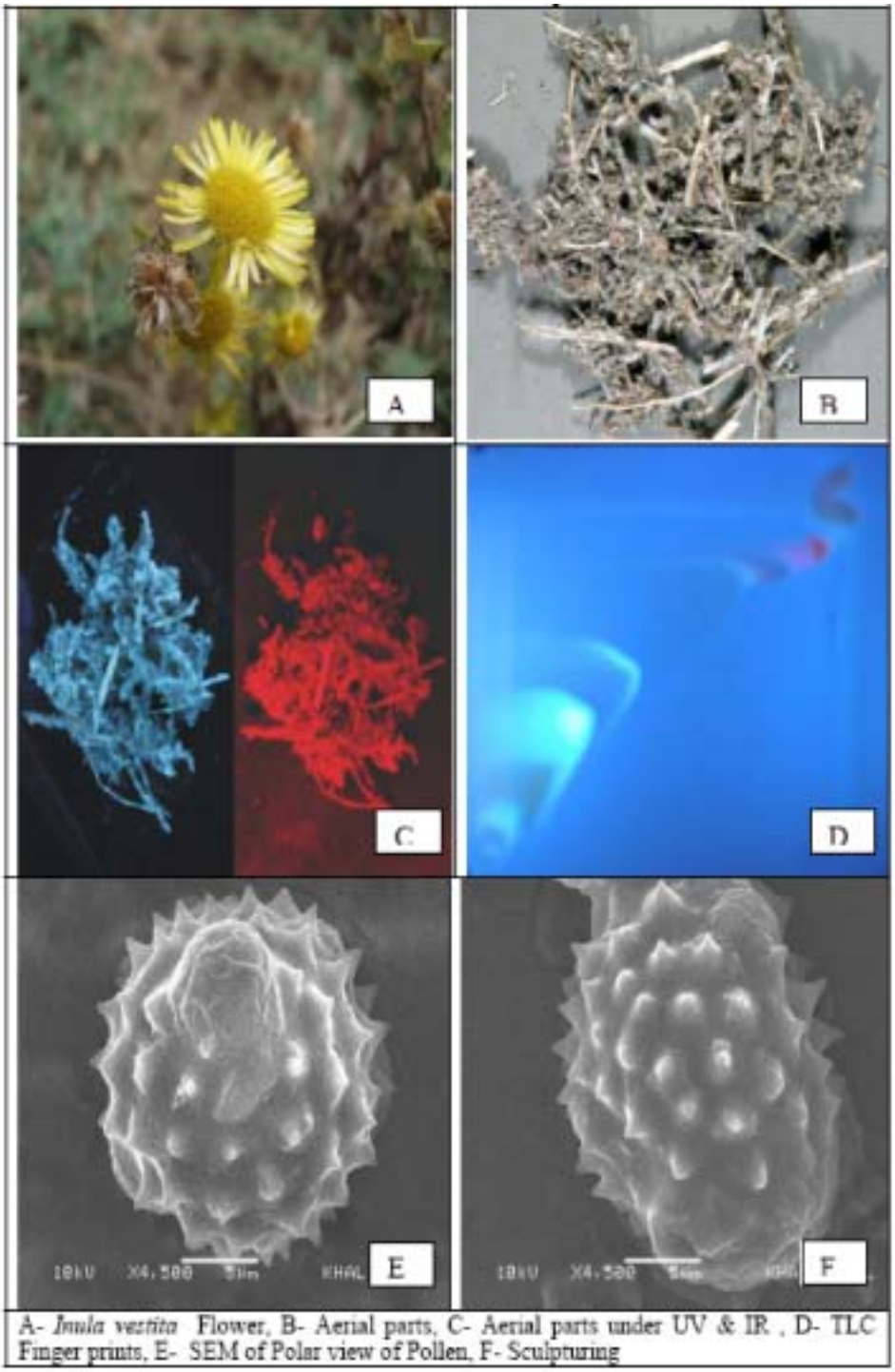
Description: Plants 10-40 (-75) cm. Leaves: basal blades lanceolate, (3-)6-7 cm × 8-20(-30+) mm; cauline blades lance-elliptic to lancelinear, 2-5+ cm × 5-12 (-20+) mm, bases ± cordate, clasping, margins entire or serrulate (abaxial faces usually villous, adaxial sparsely strigillose to glabrate). Involucres 7-9(-15) mm diam. Outer phyllaries lance-linear, 4-6 ± 0.5-0.8 mm (bases sericeous); inner phyllaries similar, more scarious. Ray florets 40-70+; corolla laminae 10-15+ mm. Disc corollas 4-6 mm. Cypselae 1-1.5 mm, puberulent or glabrate; pappi of 15-25 distinct or basally connate bristles 4.6 mm (Plate 4A).

Flowering period: Mid to late summer; **Voucher No.:** ISL-MZ-23; **Palynology:** Pollen are monad, echinate sculpturing and tricholporate. Spheridal in polar view and suboblate in equatorial view. The polar diameter is 19.5 µm (18-21 µm) and equatorial diameter is 20 µm (19-21 µm). P/E ratio is 0.975 µm and exine thickness is 3.5 µm (2.5-4.5 µm). The colpi length is 4.5 µm and width 5.5 µm. No. of spines are 12-14 (Plate 4 E,F); **Marketing status:** Abundantly available at herbal shops under the name of baboona; **Organoleptography (Aerial parts):** Herbal drug consist of broken branches, leaves and flowers. The general appearance is grey in colour and in crumble form. Branches are 8-12 cm in length, cylindrical. Leaves lanceolate 4-12 cm in size. Outer phyllaries are linear . lanceolate. Cypralas 1-1.5 m. Aerial parts are odourless and tasteless (Plate 4B).

Finger printing: TLC of aerial parts extract reveals the presence of three phenolic acid of significant amount, one flavone and one aurone when viewed under 366 nm UV light (Plate 4D).

Authentication of Chamomile: Authentication of botanical material is a critical step in the use of these materials for both research purposes and commercial preparation. Microscopic evaluation and comparison of authenticated and unauthenticated samples of whole, cut, or powdered plant material is a cost effective and accurate means of identifying herbal ingredients. Microscopy can be a useful tool for the detection of botanical and non-botanical adulterants such as pharmaceutical drugs, microbial contaminants, and inorganic materials. Advances in microscopic technology and improvements in light, fluorescence, phase contrast and scanning electron microscopy have improved the accuracy and capabilities for botanical authentication. Organoleptic analysis, used in combination with advanced microscopic equipment provides further accuracy for botanical authentication[27].

Chamomile is one of the most widely used and well documented plant based drug in the world and it is included in the pharmacopeias of 26 countries[28]. However authentic source of chamomile is generally confused with the *Anthemis nobilis*, *Matricaria aurea* and *Inula vestita* in herbal markets of the world in Indo-Pak subcontinent. Problems within identification and adulteration in commercial samples have been found to exist in *Matricaria chamomilla* L. and its allied taxa. The plant species *i.e. Anthemis nobilis, Corchorus depressus* L. and *Matricaria chamomilla* are reported under only one Unani name 'babuna' at different places in literature. This complex of taxa has created a lot of confusion and misuse of real drug,



A- *Inula vestita* Flower, B- Aerial parts, C- Aerial parts under UV & IR , D- TLC Finger prints, E- SEM of Polar view of Pollen, F- Sculpturing

Plate 4 Chemotaxonomic authentication profile of *Inula vestita*

M. chamomilla[29]. Khan *et al.*[6] reported that specimens of *M. chamomilla* (babuna) were obtained from 10 local shops in Rawalpindi and Islamabad, but it was actually *Cotula* spp. In only 4 shops had real *M. chamomilla*.

M. chamomilla is a remarkable medicine used both in Tib and homeopathic systems of medicine. It is considered to be stimulant, carminative, aphrodisiac, antispasmodic, anti convulsion, used in tension, insomnia, anxiety, hysteria, dyspepsia, fevers, rheumatism, flatulence and colic. It is prescribed for all birth related difficulties[30]. It is of special value in uterine reflex disturbances of women[31]. *Chamomilla* was called 'earth apple' by the ancient Greeks because of its smell[32]. In this study samples were procured from herbal shops of Hyderabad, Karachi, Quetta, Peshawar, Abbotabad, Attock, Mianwali, Lahore and Rawalpindi. In all these shops it was found that there were adulterers of original drug. These adulterers are morphological similar to original source *i.e. Matricaria chamomilla* plant.

Morphology: Morphological features of *Matricaria chamomilla, Matricaria aurea, Anthemis nobilis* and *Inula vestita* have been investigated. Photographs of these plants have been presented (Plates 1-4). *M. chamomilla* is a small, much branched, annual or perennial herb. Stem is 8-40 cm in length, leaves 2-3 pinnately dissected into short very narrowly linear segments and of lengths 2-3 cm, the capitulum is radiate. Flowers are inner tubular, yellow, 4-5 toothed, outer ligulate, white rays becoming reflexed and flower heads ovoid to conical. The hollow receptacle is surrounded by flattened imbricated involucre. There is no pappus and the achenes are small.

Matricaria aurea is a smaller herb, as compared to *M. chamomilla*. It is annual, prostrate and nearly glabrous. Stem is 5-22 cm in length and leaves are 2-3 pinnatisect, segments very finely divided and sub-divided, length 1-3 cm. The capitulum is solitary or 2-3 at the apex of each branch, discoid, involucre more or less obtuse. Ray florets are absent and there is no pappus. Achenes are oblong, brown, 1 mm, flattened and striated. *Antehmis nobilis* is much branched. The branches are procumbent; length 12-35 cm. Leaves linear, 1-2 cm. Numerous unicellular hairs on peduncle and the receptacle is solid and conical. Flower heads solitary, radiate with rays white and disc yellow. The scales of receptacle are membranous. The pappus is absent and the achenes are oblong.

Morphologically the *Inula vestita* can be distinguished form rest of the species by the presence of basal blades lanceolate serrulate leaves. Involucres 7-9 mm in diameter, outer phyllaries lanceolate-linear where as inner phyllaries are scarious. *A. nobilis* and *M. chamomilla* can be readily distinguished as the receptacle is not hollow in *A. nobilis*[33]. *M. aurea* differ from both *M. chamomilla* and *A. nobilis* mainly in having discoid capitulum, while in the case of *M. chamomilla* and *A. nobilis* the capitulum is radiate. The *M. aurea* smell differ form *M. chamomilla*. Moreover the decoction of chamomile taste bitter while *M. aurea* does not.

Palynology: Pollen characters such as polar and equatorial diameter, colpi length, spine length, number of spine between colpi and exine ornamentation were

observed to discriminate between three taxa. Clark *et al.*[34] showed the potential value of pollen studies in distinguishing some groups in the Asteraceae. They observed that some genera could be distinguished by pollen size and spine rows between colpi.

Matricaria chamomilla and its related genera *Anthemis nobilis*, *Matricaria aurea* and *Inula vestita* are characterized by tricolporate, caveate, echinate and tectate pollen (Plate 1 E, F). However they can be distinguished on the basis of different palynological characters, for instance, the pollen of *M. chamomilla* are smaller than *A. nobilis* and larger than *M. aurea*, as described to be the values of equatorial and polar diameter.

Anthemis nobilis pollen can be distinguished from the *M. chamomilla*, *M. aurea* and *I. vestita* on the basis of prominent colpi with highest length (Plate 3 E, F). The colpi of *M. aurea* (Plate 2 E, F) are less prominent while the least value of colpi length has been found in *M. chamomilla*. The number of spine rows between colpi is more or less same in the three taxa. However, the spines are of very small length in *M. aurea* than in *M. chamomilla* and *Anthemis nobilis*. The pollen of *Inula vestita* are echinate and spheroidal to suboblate. The polar and equatorial diameter is 19.5 and 20 μm, respectively.

The general appearance of the pollen of *M. chamomilla* has more resemblance with pollen of *Anthemis nobilis* but differ from *M. aurea*. This character is also correlated with their general morphology, *i.e.* overall morphology of *M. chamomilla* is closer to *Anthemis nobilis* than to *M. aurea*. It can be concluded that *M. chamomilla* and *Anthemis nobilis* are phylogenetically closer to each other as compared to *M. aurea*. However, the pollen of *M. chamomilla* are foraminate while those of *M. aurea* and *Anthemis nobilis* are non-foraminate and *Anthemis nobilis* pollen show much prominent colpi than *M. chamomilla*.

Although these genera are very closely related even then with the help of palynology, considering all these characters, then we are able to discriminate between these genera. For commercial purpose, if there is a bulk of plants, we can distinguish between all these species with the help of palynological characters.

TLC Fingerprints: TLC fingerprints can easily distinguished genuine herbal drug *M. Chamomilla* (Gul-e-Baboona) from *A. nobilis*, *M. aurea* and *I. vestita*. TLC fingerprints of *M. chamomilla* showed the presence of four major phenolic acids, 4 flavones and one aurone (Plate 1 D). In case of *M. aurea* TLC fingerprints shows the presence of only five major phenolic acids (Plate 2 D), while in *Inula vestita* there are three phenolic acids, one flavone and one aurone (Plate 4 D). *Anthemis nobilis* is distinguished by the presence of two flavonols and 5 phenolic acids (Plate 3 D).

Photographic authentication: To date no reference is available about the use of infrared spectra for the purpose of authentication of a plant species. However, Sexton[35] and Welsh *et al.*[36] have reported the use of infrared spectra for the blooming of houseplants. In recent report, Davidhazy[37] has reported that infrared photography

is of interest to the amateur and commercial photographer and to scientists and technologists because it produces images that are not possible with conventional photography. Thus, the use of Infrared photography is a unique and reliable method of authentication of plant species whenever a doubt about the identity of a plant species is in question.

Like infrared, no reference is available about the use of ultraviolet light for the authentication of plant species. However, Rorslett[38] has posted his work on the web about photographing and comparing the appearance of a number of ornamental flowers under visible and ultraviolet light. Therefore, viewing of plant species under UV light can provide a very authentic approach towards authentication of doubtful and problematic plant species.

Conclusion

For the identification and authentication of each genuine drug various techniques such as morphology, anatomy, palynology (light microscopy and scanning election microscopy) and TLC fingerprinting of falconoid were used. Photographs from the field, UV and IR photography of market and field collected materials were utilized. The case was so complex due to their long practices for generations and lack of proper documentation of medicinal plants, that it seemed very difficult to search the genuine drug. However, it was tried to discover the genuine drug for the benefit of the industry, herbalists, students, researchers and for general masses/ public.

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REFERENCES

1. M.A. Rizvi, Medicinal Flowers of Pakistan, Part III, Horticultural Society of Pakistan, Karachi, pp. 48-52 (1998).
2. A.K. Roy, *Ind. Phytop.*, **4**, 1 (2003).
3. A. Hasan, M.A. Khan and M. Ahmad, Authenticity of Folk Medicinal Plants of Pakistan, Vol. 1(1), pp. 1-165 (2007).
4. N.P. Singh and S. Karthikeyan, Flora of Maharashtra State Dicotyledons, Botanical Survey of India, Calcutta, India, Vol. 1, pp. 459-459 (2000).
5. N.R. Farnsworth, O. Akerele, A.S. Bingel, D.D. Soejarta and Z. Eno, *Bull. World Health Organ.*, **63**, 965 (1985).
6. A.A. Khan, R.A. Jaiput and K. Umeed, in eds.: Z.K. Shinwari, B.A. Khan and A.A. Khan, Plants in Coexistence with Man and Wild Life at Deosai, Himalayas, In: Proceedings of First Training Workshop on Ethnobotany and its Application to Conservation, National Herbarium/PARC, Islamabad, pp. 46-42 (1996).
7. M.A. Khan, H.H.A. Niazi and S. Khalid, *Hamdard Medicus*, **43**, 61 (2000).
8. A.K. Gupta, S.K. Vats and B. Lal, *Curr. Sci.*, **74**, 565 (1998).
9. M.A. Khan, H.A. Niazi and M.S. Khan, *Hamdard Medicus*, **43**. 23 (2000).

10. N.N. Tewari, *Sachitra Ayurved.*, **44**, 284 (1991).
11. G. Sunita, Substitute and Adulterant Plants, Periodical Experts Book Agency, New Delhi, India (1992).
12. M.R. Uniyal and G.C. Joshi, Historical View of the Basic Principles of Identification of Controversial Drugs, Problems and Suggestions, Sachitra Ayurveda, CSIR & ICMR, New Delhi, India (1993).
13. A. Saraswathy, *Sachitra Ayurved.*, **54**, 63 (2001).
14. A.K. Gupta, Quality Standards of Indian Medicinal Plants, ICMR, New Delhi, India, Vol. 1 (2003).
15. E. Nasir and S.I. Ali, *Flora of West Pakistan (Verbenaceae)*, **77**, 4 (1974).
16. E. Nasir and S.I. Ali, *Flora of West Pakistan (Lythraceae)*, **78**, 6 (1975).
17. J.D. Hooker, Flora of British India, Rananculaceae to Sapindaceae, Vol. 1 (1875).
18. T.G. Tutin and Heywood, Flora of Europe, Cambridge University Press, Vol. 3 (1972).
19. J.D. Hooker and K.C.S.I. Flora of British India, Asclepideae to Amarantaceae, Vol. 4 (1885a).
20. C.J. Saldanha and D.H. Nicolson, Flora of Hassan District Kanataka, India, Amerind Publishing Co. Pvt. Ltd. New York (1976).
21. G. Erdtman, Pollen and Spore Morphology/Plant Taxonomy (An Introduction to Palynology, II. Gymnospermae, Pteridophyta, Bryophyta) Almqvist and Wicksell, Stockholm (1952).
22. M. Zafar, M. Ahmad and M.A. Khan, *Pak. J. Plant Sci.*, **12**, 21 (2006).
23. J.W. Walker and J.A. Doyle, *Ann. Mo. Bot. Gard.*, **62**, 666 (1976).
24. O.K. Ronald, Pollen and Spores, American Association of Stratographic Palynologists, edn. 2, pp. 13-21 (2000).
25. J.B. Harborne, In Comparative Biochemistry of Flavonoids, Academic Press London and New York (1967).
26. A. Hasan, Ph.D. Thesis, Claude Bernard University, Lyon-France (1976).
27. V.C. Joshi and I.A. Khan, *J. AOAC Int.*, **88**, 707 (2005).
28. I. Salmon, *J. Herbs Spices Med. Plants*, **1**, 37 (1992).
29. R.N. Chopra, S.L. Nayar and L.C. Chopra, Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research, New Delhi, India (1956).
30. M. Castleman, The Healing Herbs, Rodale Press, Emmaus, Pennsylvania (1991).
31. W. Dymock, Pharmacographia Indica, The Institute of Health and Tibbi Research under the auspices of Hamdard Foundation Pakistan (1972).
32. S. Dev, *Environ. Health Perspect.*, **107**, 783 (1999).
33. A.M. Rashid and M. Ahmad, *J. Agric. Found.*, **6**, 12 (1994).
34. W.D. Clark, G.K. Brown and R.A. Mays, *Am. J. Bot.*, **67**, 1391 (1980).
35. R.J. Sexton, www.geocities.com/CapeCanaveral/4742/lighting.html (1998).
36. D.F. Welsh and S.D. Cotner, in "Texas Master Gargen Handbook" retirees.uwaterloo.ca/~jerry/orchids/light.html - 38k (2006).
37. A. Davidhazy, Imaging and Photographic Technology Department School of Photographic Arts and Sciences Rochester Institute of Technology, New York.http://www.rit.edu/~andpph/ipt.html (2004).
38. B. Rorslett, www.naturfotograf.com/UV_flowers (2006).

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