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Full Length Research Paper

Quality assurance of herbal drug valerian by chemotaxonomic markers

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The quality assurance of valerian (Balchur), a traditional herbal drug of global importance mainly used for nervous disorders, was studied. At global, regional, national and local levels the end users of this drug face the problems of adulteration. Two different botanical sources are commercially marketed in the Indo-Pak subcontinent under the same trade name of Balchur belonging to two different families. One is *Valeriana wallichii* DC. belonging to the family Valeriniaceae and another source is *Acorus calamus* L. belonging to Araceae. In this study, a commercially available drug sample of valerian is authenticated by using basic and advanced chemotaxonomic techniques. Authentication, quality and standardization of this drug related to its originality was achieved using physicochemical markers, TLC fingerprinting, UV and IR analyses, SEM of natural fingerprints (pollen) and anatomical parameters. This study will contribute towards the global recognition and international acceptance of valerian-like herbal drug.

Key words: Chemotaxonomic markers, quality assurance, herbal drug, valerian.

INTRODUCTION

Indo-Pak valerian (Mushk-e-Bala) is a popular herb of the genus *Valeriana* that is used to treat nervous diseases like insomnia, hysteria, depression and epilepsy. The roots and rhizomes of the several species of this genus are used as a sleep aid, mild sedative and gastro-intestinal agents (Bos et al., 1996). The most common commercially traded species in traditional medicine are *Valeriana wallichii* (Indian or Pakistani valerian), *Valeriana. officinale*, (European valerian) and *Valeriana. procera* (Mexican valerian) (Dweck, 1997). These species vary significantly with regard to their chemical constituents and taxonomic features. In the Indo-Pak subcontinent, *V. wallichii* (Mushk-e-Bala) is adulterated by the rhizomes of *Acorus calamus* (sweet flag). However this plant has been banned internationally due to the presence of B-asarene, which has shown carcinogenic properties. This type of adulteration is very common in the Indo-Pak subcontinent.

Authentication and standardization are prerequisite steps while considering source materials for herbal formulation in any system of medicine. The detailed chemotaxonomic identification with internationally accepted nomenclature in Latin is an integral part of authentication. It is necessary to update nomenclature from time to time in order to avoid confusion regarding synonyms; for example Guggul, one of the Unani materials. The correct Latin name for Guggul is *Commiphor wightii* (Arn.) Bhandari, which in literature is also known by synonyms such as *Balsamodendron wightii* (Arn.); *Balsamodendron roxburghii* (Stocks.); *Balsamodendron mukul* (Hook. ex Stocks.) and *Commi-phora mukul* (Hook. ex Stocks.), (Engl et al., 2000).

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 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 medi-

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cal systems (Fransworth and Soejarto, 1991). Quality assurance of herbal medicine seems to be little explored and that is why standardization and authentication have taken a serious turn. It therefore seems obligatory to procure these 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), quality control and good manufacturing practices (GMP).

In the light of previous literature, it was found that the published work on authentication and standardization of valerian aimed at ascertaining its identity and genuine source with reference to traditional medicine are scanty (Ahmad et al., 2008). Employing various basic and advanced taxonomic and chemical techniques, attempts have been made to evaluate this problematic herbal drug as regards its identification by commercial dealers, chemists, herbalists and pharmaceutical industries.

In the present study, chemo-taxonomic markers including TLC, UV and IR analyses, SEM of natural finger prints (pollen), organoleptographic tests and anatomical parameters were used to authenticate the quality of the herbal drug valerian. Such kind of quality based standardization studies are expected as useful in promoting the usage of genuine drugs contributing to the health care of human society.

MATERIALS AND METHODS

Taxonomic markers

Plant samples were collected during field trips from moist temperate forests in the Himalayan range of the Indo-Pak subcontinent. Detailed morphological (macro and microscopic) examination was carried out by using binocular stereo zoom light microscope (Model Kyowa SZF (0.75x - 3.4x). The plant description was also compared by using different floras (Nasir and Ali, 1974, 1975; Hokker, 1875; Tutin and Heywood, 1972; Hooker and K.C.S.I., 1885a, b, 1894; Saldanha and Nicolson, 1976).

Organoleptography

Materials for organoleptic analysis was procured from herbal markets of Lahore, Swat, Karachi, Rawalpindi, Abbotabad and Quetta (Pakistan). All parts of the herbal drugs including bark, roots and rhizomes of the original valerian and its adulterants were studied in detail.

Scanning electron microscopy (SEM) of pollen

Scanning electron microscopy (SEM) of pollen (natural finger prints) from fresh specimens and market samples was carried out. The pollen grains were acetolized according to the modified method (Erdtman, 1952, 1960; Ronald, 2000; Ahmad et al., 2008). For SEM pollen, they were directly transferred with a fine pipette to an aluminum stub using double adhesive cello-tape 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. Sculpturing, shape of pollen in polar and equatorial views, spine and colpi morphologies, and exine surface were studied. The terminology used is in accordance with Walker and Doyle (1976), Punt et al. (1994) and Ronald (2000).

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 a boiling water bath (Model, Memmert-91126-FRG, Germany) for 15 to 30 min. Lactic acid softens the tissues of leaves which make it possible to scrape the leaf surface with a sharp scalpel. Slides of both abaxial and adaxial surfaces of leaves were prepared and mounted in clean 88% lactic acid. Features, that is 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. Micro-histological photographs of both surfaces were taken by Leica light microscope (DM 1000) fitted with CCD digital camera.

Chemical markers

TLC fingerprinting of flavonoids, UR and IR analyses of herbal parts were used as chemical markers.

Acid hydrolysis

For the extraction of flavonoid aglycones, a small amount of dried plant material is treated with 2 normal (2 N) hydrocholoric acid (HCl) and heated for 1 h in a water bath at about 100°C. By this treatment normally, all flavonoids-O-glycosides are converted to flavonoids aglycones and anthocyanins to anthocyanidins where as the C-glycosides remain unaffected (Figure 1). 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.

TLC finger printing

TLC is a simple, quick and inexpensive procedure for authentication and standardization of herbal drugs and problematic medicinal plants used in traditional medicines. 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 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 a TLC tank (large size 20 x 20 cm Camag, Switzerland). The solvent system used in both directions is toluene : methanol : methyl ethyl ketone (4:3:3; Hasan, 1976). After drying, the fully developed TLC plates are viewed under 366 nm UV light. This is a very reliable and reproducible method of authentication of a particular herbal drug and differentiation of problematic medicinal plants on the basis of TLC fingerprints.

Digital photography

A digital camera (Sony, DSC-W50) was used in the photography of the herbal drugs under a short wavelength of UV (UVGL-58 Lamp,

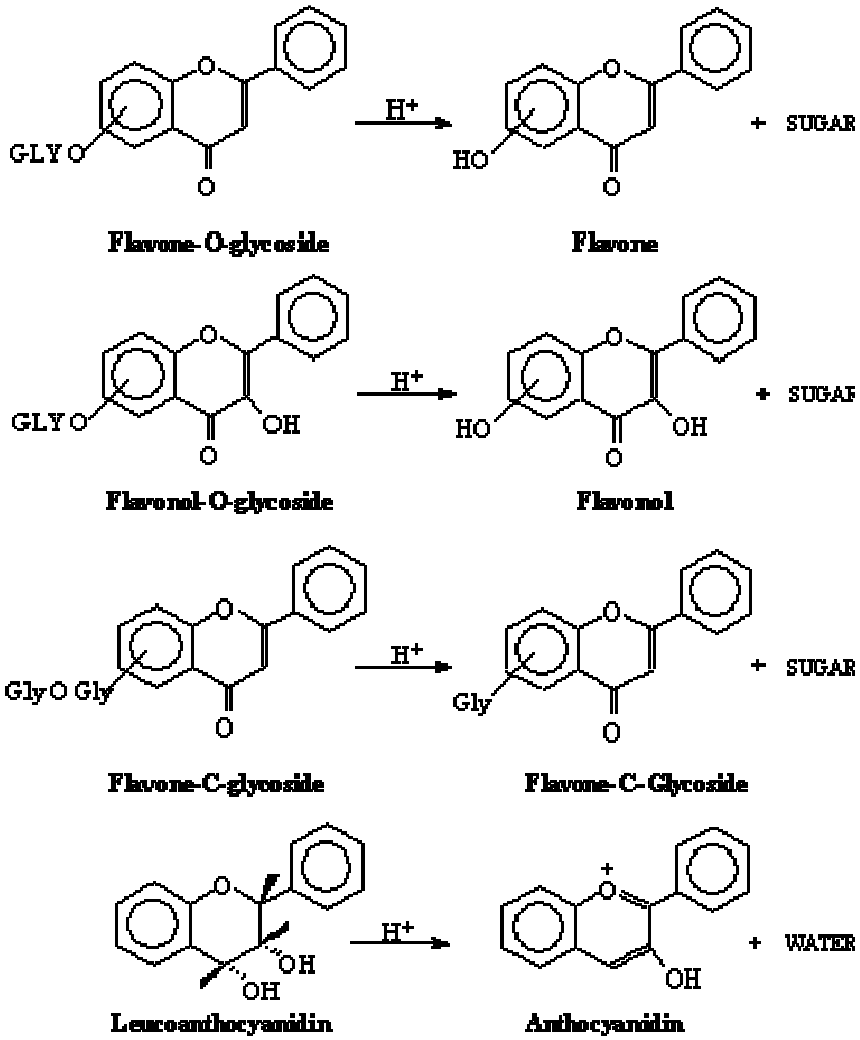

Figure 1. Acid hydrolysis of flavonoid glycosides.

254/365 nm), IR and visible lights. This high resolution photography provides an authentic approach toward the identification of problematic plant species.

RESULTS AND DISCUSSION

V. wallichii commonly known as Indo-Pak valerian and locally known as Mushk-e-Bala or Balchur is one of the important plant species of commerce which belong to the family Valerianaceae (Table 1). It is a commercially important species, sometimes used as substitute for *V. officinale* L., an important sedative in herbal medicine. In Unani, Ayurvedic, folk and homeopathic systems of medicine, it is used as a cardiotonic, sedative, stimulant, cure for epilepsy, hysteria, convulsive affections, heart palpitation and in colic. It has been used in China for over 1000 years for similar purposes (Arora and Arora, 1963).

Valerian is an English name of the drug. In the Indo-Pak subcontinent, this drug is well known as “Balchur or Billilotun”. Actually the drug is obtained from the under ground part (roots) of *V. wallichii*. Aerial part of a one year old plant is 16- 60 cm in height with broad and long leaves. The roots of the Pakistani valerian are 6 - 17 cm long and 1.5 - 12.5 cm in diameter, thick, brownish black in color (Figure 2A). Roots of the European valerian species that is, *V. officinale* are very thin and hairy while roots of the Mexican species that is, *V. edulis* are more or less similar in shape in comparison to the Pakistani valerian but they are thicker (Joshi and Khann, 2005). These species show large differences with regard to chemical constituents as well as their morphology (Jackson and Snowdon, 1990). Organoleptic analysis used in bination with advanced microscopic equipments provides more accuracy for botanical authentication of the valerian drug.

Consequently, the phytomedicines prepared from these species are characterized by different chemical compositions (Dweck, 1997). In the Unani traditional system of

Table 1. Characteristics of *V. wallichii* DC. Syn: *V. jatamansi* Jones.

Parameter	Character
English name (s)	Indian valerian, Musk root
Local name (s)	Mushk-e-Bala, Bala Mushk, Bililoton
Tib name (s)	Sumbal-ul-Tayyab, Balchhur
Family	Valerianaceae
Distribution in Pakistan	Kurram, Chitral, Swat, Hazra, Murree Hills, Kashmir and Ayubia National Park.
Distribution in the world	East Asia, Afghanistan to South West China, India, Nepal, Japan and Belgium.
Occurrence and conservation status	Common in forests, shrubberies and on open slopes (1500 - 3600 m).
Description	Perennial 16-60 cm tall, tomentose to pilose. Rhizome elongate, with fibrous roots. Stems 3 to 6 in number. Radical leaves cordate or ovate, 2 to 10 cm x 1.5 to 8 cm, sinuate or crenulate. Cauline leaves sessile, smaller, uppermost often 3-fid or -sect. Flowers in lax corymbose cymes or dense corymbs. Upper bracts linear-lanceolate, c. 3 mm long. Corolla and style sometimes pilose. Stigma 3-fid. Achene tomentose, shorter than the upper bracts (Figure 2A).
Flowering period	March-May
Voucher no.	ISL-MZ-33
Palynology	Pollens are tricolpate, semiangular to spheroidal. Polar diameter 37 µm (36 - 39 µm), equatorial diameter 36 µm (35 - 37 µm). P/E ratio is 1.02. Sculpturing echinate with minute / small scattered spines. Visible columellae holding up the tectum of ektexien. Bears veructe elements in the surface. Colpi length is 12 µm and width is 10.5 µm. Exine thickness is 5 µm (4 - 6 µm) (Figure 2 E,F).
Leaf epidermal anatomy	The non glandular and glandular hairs are characteristic features of *V. wallichii* species. The non glandular hairs are frequent on adaxial surface. The non glandular hairs are large size and bicelled or tricelled. Non glandular hairs with thick walls, slightly conical with broad base and tapering towards apex. Stomata are rare and hemiparacytic on adaxial surface. Anomocytic and polycyitic types of stomata are abundant on abaxial surface. The adaxial epidermal cells are 40 µm surface. The adaxial epidermal cells are 40 µm (39.5 - 41.5 µm) in length, 27.5 µm (26 - 28.5 µm) in breadth whereas the abaxial epidermal cells are 34.1µm (33 - 35 µm) in length and 20.8 µm (19 - 22 µm) in breadth. The stomata at adaxial surface is 32.5 µm (31 - 34 µm) in length, 17.5 µm (16 - 19 µm) in breadth where at abaxial side, 22.5 µm (20 - 33 µm) in length and 20.5 µm (19 - 22 µm) in breadth. The subsidiary cells at adaxial side are 26.5 µm (25 - 28 µm) in length, 11 µm (10 - 12 µm) in length and 17 µm (16 - 18 µm) in breadth. The glandular trichome (hair) at abaxial side is 64.5 µm (62.5 - 66 µm) in length and 50.25 µm (49 - 51.5 µm) in breadth where as at abaxial side it is 61.75 µm (60 - 63.5 µm) in length and 27.5 µm (26.5 - 28.5 µm) in breadth. The non glandular trichome on adaxial surface is 344.25 µm (342.5 - 346 µm) in length and 43.25 µm (42.5 - 44 µm) in breadth. Where as on abaxial side it is 350.5 µm (349 - 352 µm) in length and 55.5 µm (54 - 57 µm) in breadth. The glandular hairs are characteristic features of this species. The glandular hairs have a unicellurlar head which is divided into 4 cells (Figure 2G, H).
Part used	Roots
Folk medicinal uses	Insomnia, nerve tonic, cough, dysentery, hysteria, plague, bronchitis, constipation, high blood pressure, restlessness, cholera, heart beat (palpitation), epilepsy and hair diseases.
Preparation and dosage	Roots are dried undershade and ground to obtain powder. 2-3 gm of this powder is taken twice a day for 20 days to relive palpitation, epilepsy, insomnia and constipation. It is also useful as nerve tonic and for baldness. Decoction of root is used in cholera, dysentery and hysteria. 100 gm dried roots are boiled in 1 L of water with 25 gm of liquorice. Half cup of this decoction is recommended for cough, bronchitis and plague. The infusion of the roots is inhaled to cure headache.
Toxicity	Excessive use may dull the mind and may cause mental depression. Do not use during pregnancy or breast feeding.
Marketing status	Commonly marketed under the herbal name Mushk-e-Bala.
Organoleptography (roots)	Roots consist of short, irregular pieces of about 6 - 17 cm in length, 1.5 - 12.7 cm in diameter. Roots are externally marked with transverse ridges and bearing numerous prominent, circular tubercles, to some of which on the under surface, thick rootlets are attached. Rootlets are 2 - 4 cm. The upper surface bears the remains of leaves. The roots are hard and tough internally, greenish-brown in color. Ridges of roots contain powdery material. Roots are scented with strong odor (Figure 2 B).
TLC finger printing	TLC of root extract reveals the presence of four phenolic acids of significant amount and two flavonols when viewed under 366 nm UV light (Figure 2D).

Table 2. Characteristics of *Acorus calamus* L.

Parameter	Character
English name (s)	Sweet flag, Sweet sedge, Flag root
Local name	Bach
Tib Name	Bach
Family	Araceae
Distribution in Pakistan	Kashmir, Abbotabad, Hazara, Kaghan, Swat.
Distribution in the world	North and Central America, Europe, Asia, India, Northern Latitude countries around the world.
Occurrence and conservation status	Sweet flag is found in marshy places and along river banks from (600-) 1000 - 2000 m.
Description	Perennial herb up to 80 cm tall. Rootstock stout, 1 - 1.5 cm broad, creeping, with long fibrous roots from the lower surface. Stem erect, glabrous, grooved at one side, and ribbed at the opposite. Leaves ensiform or linear, 55 - 100 x 8 - 1.5 cm. Spathe leaf-like, up to 46 cm long, not enclosing the spadix. Spadix 5 - 6.5 cm long, cylindrical, obtuse, 1 - 1.4 cm broad. Tepals c. 2 mm long, oblong-obovate, slightly curved, margin membranous, surface with embedded raphides. Filaments 2 mm long, flat, anthers less than 1 mm long, ± orbicular. Ovary 3 mm long, obconical; seeds obconical, 2 mm long (Figure 3A).
Flowering period	May - July.
Voucher no.	ISL-MZ-158
Part used	Root
Folk medicinal uses	Analgesic, toothache, headache, aphrodisiac, cough, colic, indigestion, diabetes, skin disorders, asthma, bronchitis, constipation.
Preparation & dosage	The roots are sun dried and powdered. 2 - 3 gm root powder is given with teaspoon of honey for twice a day to the patients for acidity, constipation, colic and indigestion. Root decoction is drunk as aphrodisiac tonic. 2 - 3 pieces of roots (2 - 3 cm) are eaten fresh. It cures diarrhea, gastritis, toothache and headache.
Toxicity	Fresh roots are poisonous. It should be taken only under the advice of a physician.
Marketing status	Commonly traded under the name of Bulchur/Bach.
Organoleptography (roots)	Root pieces are 4 - 9 cm in length, 1 - 1.7 cm in thickness. 10 pieces of equal size (8 x 1.5 cm) are 54 gm. Surface is dark brownish to light brownish; texture is rough, convoluted which contain nodes and internodes. Nodes bear root hair which is cylindrical and light whitish in color. Size of root hair is 1 - 2 cm. Root surface is porous in nature and it contains buds like human eyes. Internally, root texture is solid and appearance is just like wheat flour. Powder of roots is with characteristic smell like fish meat (Figure 3B).
TLC finger printing	TLC of root extract reveals the presence of one phenolic acid and one flavone of significant amount when viewed under 366 nm UV light (Figure 3D).

medicine, the decoction or syrup of Balchur on drinking produces a tonic effect and increases the sexual potency of a man. Traditional herb healers (Hakims) recommend this drug to their patients in case of nervousness, rheumatism, colic, menstrual troubles, digestive problems, liver disorders and jaundice. When patients purchased this drug (roots) from herbal shops (Pansar store), they sometimes get the roots of entirely different species; *A. calamus* under the name of Balchur. In this way the roots of *V. wallichii* become adulterated by the substitution of *A. calamus* roots. Jackson and Snowdown (1990) provide a detailed account of the microscopy of *V. officinalis*, suggesting that adulteration of the *V. officinalis* with the *Valeriana* species is very common. However, previously available microscopic work on *Valeriana* species does not provide a detailed comparative account among these commercial species; *V. wallichii* and its adulterant species *A. calamus* are confused in the market due to their morphological similarity in roots and nomenclatural confusion (Table 2). The present work assists in the identification of *V. wallichii* (Mushk-e-Bala) in trade using microscopic characters. The pollens (natural finger prints) of *V. wallichii* are tricolpate, semiangular to spheroidal and with echinate sculpturing (Figure 2E, F).

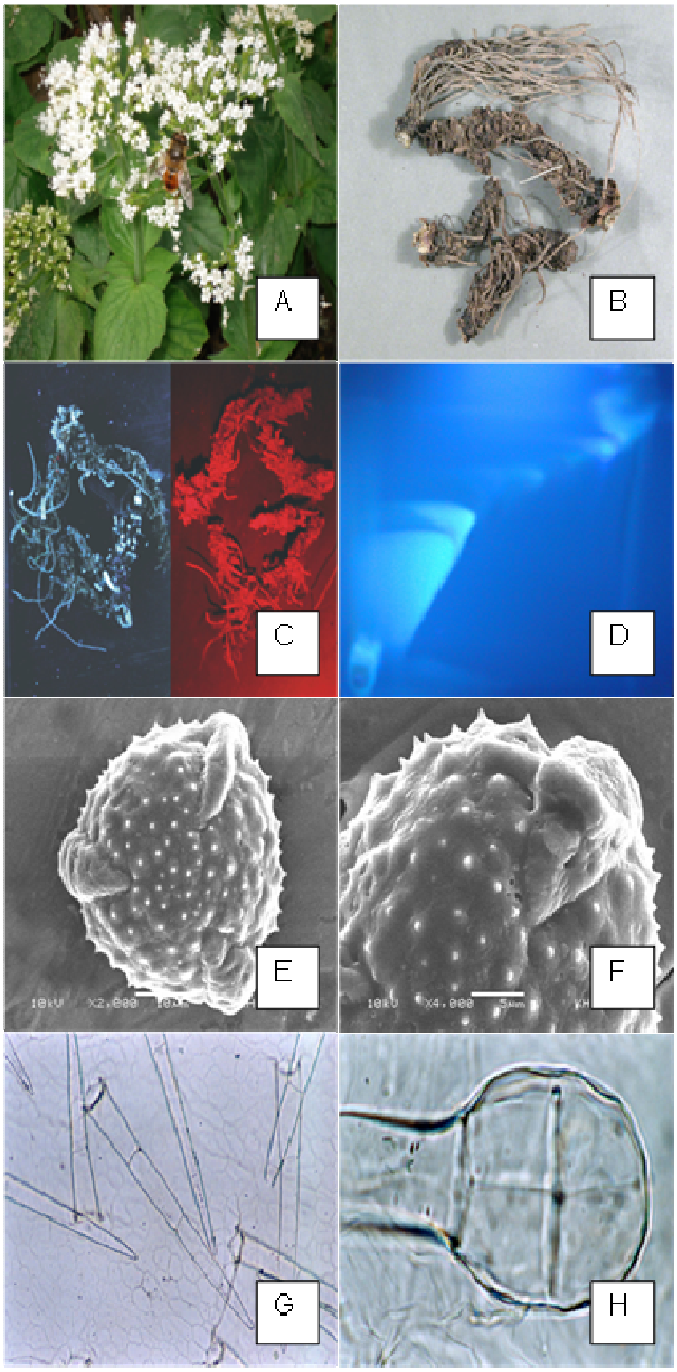

Figure 2. Chemotaxonomic photographic profile of *V. wallichii*; A-Flower, B- Roots, C- Roots under UV and IR, D- TLC fingerprints of root, E- SEM polar view of pollen, F- SEM sculpturing of pollen, G-leaf epidermal trichomes and H- epidermal gland.

The polar and equatorial diameters are 37 and 36 µm, respectively. Similarly the microscopic leaf epidermal features revealed the presence of glandular and non glandular hairs. The stomata are characteristic having hemiparacytic at adaxial and anomocytic to polycytic on abaxial surface (Figure 2G, H). In this account, organoleptographic analysis and TLC fingerprints easily

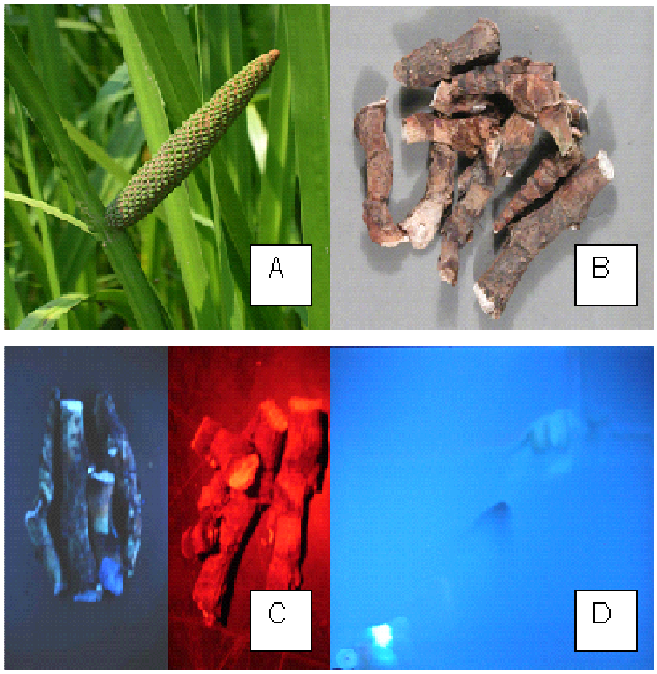

Figure 3. Chemotaxonomic photographic profile of *A. calamus*; A-Flower, B- Roots, C- Roots under UV and IR, and D-TLC fingerprints of root.

distinguished these two species. Roots of *V. wallichii* are externally marked with transverse ridges and bearing numerous prominent, circular tuber cells (Figure 2B) while the roots of *A. calamus* are short (4 - 9 cm) in length and root texture is rough and convoluted containing nodes and internodes (Figure 3B). Roots of *V. wallichii* are scented which contain fragrant essential oil. The smell of this essential oil attracts cats and because of this, it is known as Billilotun. In South Asia it is used in scented candle and other perfumed materials at saints' graves (Mazars) and holy tombs (Maqbaras). Due to the occurrence of volatile oil, it is used in perfumery and medicine. It is also mentioned in the bible as a costly perfume due to its property of scent (Conert and Der, 1980). One of the most common patent preparations of *V. wallichii* in Pakistan is "Jawarish jalinus" which is known as the best herbal medicine for stomach and abdomen diseases. It is available in every herbal shop (Dawakhana). Valerian is a pithy drug which has a long history of efficacy and is still used by others but often ignored because of adulterated with *A. calamus* roots.

TLC is a widely applied technique in herbal authentic-cation and used in majority of pharmacopoeia's monograph for correct identification. TLC separates a mixture of com-pound plate coated with an adsorbent such as silica gel, polyamide and cellulose. By Co-TLC, unknown compounds can be easily identified with an authentic compound. Usually thin layer chromatography (TLC) is widely adopted because it is less time consuming, semi quantitative and a cheap technique.

Furthermore, it also provides finger prints of the material under consideration, if the marker compound is known then it becomes more precise. Thus, TLC is an important tool for monitoring the identity and purity of the plant material under consideration; in addition it will also provide information about substitution and adulteration (Handa, 1993). TLC fingerprints of flavonoids in roots of *V. wallichii* DC. revealed the presence of four phenolic acid and two flavonols (Figure 2D) in *A. calamus*, TLC of roots showed the presence of one phenolic acid and one flavonone of significant amount (Figure 3D).To date no reference is available about the use of IR and UV lights for the purpose of authentication of a plant species. However Davidhazy (2004) has reported the use of infrared light for the blooming of houseplants. It is reported that infrared photography is of interest to amateur and commercial photographers and to scientists and technologists because it produces images that are not possible with conventional photography. In its practice, there is not much difference between infrared and normal photography. The same cameras and light sources can usually be used, together with the same processing solutions. Infrared photography, however, is usually only attempted by skilled photographers, scientists and technicians with a particular purpose in mind. The peculiarities of infrared photography lie in its ability to record what the eye cannot see. In the same article of Davidhazy (2004), infrared photography has been used in forest survey to distinguish between stands of coniferous and deciduous trees. Thus, the use of Infrared photography is a unique and reliable method for authentication of plant species whenever a doubt about the identity of a plant species is in question.

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