Pak. J. Bot., 45(SI): 165-176, January, 2013.

AUTHENTICATION OF HERBAL MEDICINE HENNA (*LAWSONIA INNERMIS* L.) BY USING TAXONOMIC AND PHARMACOGNOSTIC TECHNIQUES

SHAZIA SULTANA[1*] AND MUHAMMAD ZAFAR[2]

[1]*School of Chemical Engineering, Unversiti Sains Malaysia, Engineering Campus, 14300, Nibong, Tebal, Penang - Malaysia*
[2]*Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan*
[*]*Corresponding author's e-mail: shaziaflora@hotmail.com*

Abstract

This study confined to authentication of herbal medicine namely Henna (*Lawsonia inermis*) the leaves and powdered leaves of which are used for jaundice, hair and skin problems. The study aimed to investigate indigenous medicinal uses, marketing status, macro and microscopical characters (LM & SEM) of pollen, foliar epidermal anatomy, behavior of powdered drug on treatment with different chemical reagents, fluorescence analysis (under visible & UV light) and preliminary phytochmeical tests to differentiate the genuine source from its adulterant. Such investigation may provide basis for authentication, standardization and characterization of genuine drug. The study concludes with authentication of *Lawsonia inermis* from its adulterant *Mirabilis jalapa* based on taxonomic and pharmacognostic characterization. These studies are useful especially for traded herbal drugs for their originality which leads to safe and quality assured herbal formulations for global acceptance.

Introduction

Currently the World Health Organization (WHO) is taking much interest in medicinal plants and alternative systems of traditional medicines and coming forward to exploit the scientific validity of the herbal medicines used since traditions. This revival of great interest in medicinal plants based drug has been initiated throughout the world in current scenario (Shinwari, 2010), concerns are also raised as to conservation issues of medicinal plants (Shinwari & Qaisar, 2011). There are various alternative systems of herbal drugs practiced throughout the world since the dawn of human civilization (Kuroyanagi *et al*., 2012). Some common alternative systems includes; traditional Chinese medicines system, Indian systems of medicines (Ayurveda, Siddah), Unani System of medicines, homeopathic system of medicine, aromatherapy, Bach flower remedy and Tabetan system of medicine (Shah & Seth, 2010). WHO encourages to use indigenous herbal medicines in national health care programs because these traditional drugs are safe, indigenous, easily available and the communities have faith in them. At the same time WHO emphasized much more the need to ensure its quality, purity and authentication by using modern techniques, applying suitable instruments and standards for correct botanical identification (Anon., 2002).

Glamorization and trying to look attractive and adorable are a part of the human nature. Among women, the tradition of coloring their hands, feet, eyes, lips, cheeks and hair has persisted in every age and era. Among the natural aids to beautification, "Henna" has tenaciously maintained its popularity from ancient times beyond memory (Jan *et al*., 2011). This tropical shrub *Lawsonia inermis* L. has been popularly used for coloring the hands, feet and hair since time immemorial. In Henna, nature has generally packed both medicinal and commercial advantages, its popularity is not confined to any country or religion but has traveled for Arabia, Africa, China, Indo-Pak subcontinent and rest of the world. The Jews anoint the dead with Henna in ancient Egypt, shrouds were dyed with henna and henna leaves accompanied the dead to the burial place. Among Muslims, henna perfume is applied to dead to repel insects (Levng, 1980).

While medicinally the leaves and aerial parts of *Lawsonia inermis* L. are frequently used as a herbal remedy for an array of human disorders including wounds, ulcers, cough, bronchitis and jaundice (Shiharta *et al*., 1978), especially by female along with other herbal medicines (Sarwat *et al*., 2012, Gul *et al*., 2012, Marwat *et al*., 2011). Henna is an important but a controversial drug in market in Indo-Pak subcontinent. Due to the adulteration and use of other species as source of henna powder in trade, the drug has become adulterated. In view of the extent of adulteration attached to this drug, it was deemed necessary to study the market samples to ascertain their botanical identity. Detailed taxonomic and pharmacognositc screening have been carried out on *Lawsonia inermis* and its adulterant *Mirabilis jalapa*.

In this study, the herbal drug Henna (*Lawsonia Innermis*) was selected as a case study for methods to authenticate and distinguish it from its adulterant *Mirabilis jalapa*, based on taxonomic and pharmacognostic analyses. The aim of the study was to characterize and accurately identify Henna, a commonly used and traded medicinal plant throughout the world.

Materials and Methods

Collection of plant material and morphological investigations: Morphological observations were made from living plants collected during four field trips and procured from 10 herbal shops. Morphological studies of the plant was also based on examinations of herbarium specimens available at ISL-QAU Herbarium, Islamabad. Further information from taxonomic and floristic sources provided confirmation of morphological characteristics (Hooker, 1875; Tutin & Heywood, 1972; Nasir & Ali, 1974; 1975). Morphological examinations conducted using a binocular stereo zoom light microscope (Model SZF Kyowa, Japan, with eye piece WF 10 x 10/20). Assessment of floral morphology was aided by reconstitution of dried flowers in hot water with detergent. All the field images presented were taken by the author using a Sony Digital Camera (DSC-W50).

Palynological analysis (LM & SEM): A palynological study of *Lawsonia innermis* and its adulterant *Mirabilis jalapa* was conducted using light microscopy (LM) and scanning electron microscopy (SEM). Pollens were removed from floral parts and prepared by the standard procedure of acetolysis (Erdtman, 1960), after which they were mounted in glycerin jelly and sealed with paraffin wax for light microscopy. The glycerin jelly was prepared according to modified method of Zafar *et al*., (2011) ; Ahmad *et al*., (2011). Measurements and morphological observations of pollen grains were performed using a minimum of 15 grains for each species. For Scanning Electron Microscopy (SEM) flowers were opened under a binocular dissecting microscope (Meiji MX5200H) using a dissecting needle. Pollen grains were fixed to aluminum stubs with double sided cellophane tape, air dried at room temperature and coated with a very thin layer of gold (JFC-1100). The specimens were examined using a scanning electron microscope (JEOL-JSM 5910), at 2000x, 5000x and 10000x magnification. The terminology used for sculpturing is based on the work by Erdtman (1960), Barthlott (1984) & Ronald (2000).

Anatomical investigations (LM & SEM): For epidermal preparations, leaf samples of 1 to 3 cm were cut from the mid portion of mature foliage leaves. Shultze's method, with modifications, was used (Subrah manyam, 1996; Zafar *et al*., 2011; Sultana *et al*., 2011). The peelings of leaves were washed with distilled water for 2-3 minutes. The leaf blades were placed with the adaxial side upward and then scraped gently with a sharp razor. The same procedure was followed to prepare the abaxial side but the leaf was placed with the abaxial surface upward. The abaxial and adaxial epidermal peelings were kept in lactic acid for few minutes in order to remove mesophyllous tissues and extra chlorophyll. Then the peelings were placed on clean glass slides with 1-2 drops of 88% lactic acid, covered with cover slips and fixed with paraffin wax. Prepared slides were observed under a Meiji light microscope. The microphotographs of adaxial and abaxial surface were taken with a Leica light microscope fitted with a CCD camera (DM-1000). The same procedure was adopted to peal of the leaves from adaxial and abaxial surfaces for SEM study. The peelings were dried at room temperature and then affixed to stubs with double sided tape and coated with the same manner as the pollen. Descriptions of foliar epidermal features follow the terminology of Prat (1932) & Metcalfe (1960).

Pharmacognostic studies: Different pharmacognostic tests, i.e., fluorescence and solubility test (cold and hot) (Tables 1-5), were carried out for crude herbal parts of *Lawsonia innermis* and *Mirabilis jalapa* in order to distinguish which of the 2 plants is found in the herbal drug. For cold method 1 gm of powdered drug was mixed in 5ml of solvent at room temperature (10-15°C), while for hot method the same solution was slightly heat on burner in a test tube. The methods of Harborne (1973), Trease & Evans (1989) & Sofowara (1993) were followed. All the chemicals and solvents used for the different studies were of HPLC grade. For solubility and florescence analysis standard procedures were adopted (Afaq *et al*., 1998; Abid *et al*., 2005). The fresh collected herbal drug (leaf material) of the medicinal plants was dried in outdoor shade for about 10-15 days and made into a powder by using an electric grinder. Crude herbal drug samples (leaf material) procured from markets were also made into powder. This coarse powder was sieved into a fine powder by using a No. 10 sieve. The fine powder was used for the extraction and determination of various physico-chemical properties. One gram of the powdered drug was mixed separately with 5ml of 19 different solvents (Table 2); samples prepared by the hot method were boiled in test tubes. Crude herbal parts, powdered drugs and the extracts were studied under visible light, UV (long & short wavelength) and IR lights following the procedure of Ahmad *et al*., (2010). For color analysis, a paint chip card from Indigo Company (Pakistan) was used for comparison.

Results and Discussion

Morphological authenticity: *Lawsonia inermis* L. (Lytheraceae) is a perennial shrub commonly called Henna (English), Mehndi (Urdu & hindi), native to North Africa and East Asia (Kirtikar & Basu, 1956; Malek zadek, 1968). It is 2-2.5 m tall shrub, its foliage resembles to that of Pomegranate tree (Hannan, 1997). The leaves are 42-84 mm long and 2-24 mm wide and flowers bears in cluster, flower is 4-25cm long petal 4-5mm long, flower whitish, creamy and on wilting become greenish and dark brown (Fig. 1: A1). While *Lawsonia inermis* is quite different morphologically from its adulterant in physical appearance as *Mirabilis jalapa* is herbaceous plant 35-80 cm tall with large perennial tuberous roots, leaves cordate, flowers usually pink, yellow or purplish, perienth sometime variegated, fruit is nut ellipsoid rugose and 1-seeded (Fig. 2: A1). Many other workers also give the detailed morphological description of *Lawsonia inermis* and *Mirabilis jalapa* separately distinguished both species from each other (Nadkarni, 2004 ; Bhattacharjee, 2003; Kakate, 2001; Ansari & Ali, 2000).

Microscopic authentication: Palynologically *Lawsonia inermis* is characterized by the presence of tricolporate pollen which are circular to subprolate in shape (Fig. 1: C1, D1). The polar diameter is 11.87µm and equatorial is 11.25µm and with psilate pollen sculpturing (Fig. 1: D1) While from palynological point of view the *Mirabilis jalapa* can be distinguished from *Lawsonia inermis* by the presence of periporate and subangular to spheoridal pollen, the polar and equatorial diameter 35µm and 31.66µm respectively and pollen sculpturing is scabrate and periporate rather than psilate (Fig. 2: C2, D2). In this way the 2 species *Lawsonia inermis* and *Mirabilis jalapa* can be differentiated on the basis of pollen shape, polar and equatorial outline and sculpturing pattern. Similar type of study was presented by Gearaerts *et al.,* (2009) who discussed the systematic significance of palynology in Ebenaceae with focus on Ebenoideae and found that substantial amount of variation in pollen size, equatorial outline and sculpturing pattern were most discriminating pollen features for subfamily Ebenoideae. Similarly foliar epidermal anatomy of *Lawsonia inermis* revealed the presence of polygonal epidermal cells having length 52.5µm and width 26.25µm, stomata diacytic and Unicellular trichomes (Fig. 1: E1, F1, G1). While its adulterant *Mirabilis jalapa* can be distinguished from *Lawsonia inermis* by the presence of irregular shaped epidermal cells which are 80µm in length and 29.8µm in width, stomata anomocytic and tetracellular trichomes (Fig. 2: E2, F2, G2).

Table 1. Comparative characterization for differentiation of *Lawsonia inermis* and *mirabilis jalapa*.

S. No.	Characters	*Lawsonia inermis* L.	*Mirabilis jalapa* L.
01	Nomenclature	**English name:** Henna Local Names: Mehndi, Barg-e-Mehndi, Henna Trade Names: Mehndi, Henna	**English names:** Four-o-clock, marvel of perll Local Names: Gul-e-Abbasi, Gul-e-Abbas Trade Name: None
02	Geographic distribution	In Pakistan; Hyderabad, Sukhar, Sargodha, Lahore. In World; Henna is native to tropical and subtropical region, South Africa, Southern Asia, Northern Australia, Egypt, India, Kurdistan, Iraq, Pakistan, Turkey, Persia and Syria	In Pakistan; Mianwali, Chakwal, Attock, Rawalpindi, Islamabad, Lahore, Abbotabad, Muzaffarabad. In World; China, Japan, Malaya, Burma, India, North Africa, South and Central Europe Pakistan, Nepal, South America
03	Occurrence & habitat	It is cultivated in Sargodha, Lahore, Hyderabad and Shukkar	It is commonly found wild and cultivated in garden and house lawn
04	Morphology	Tall shrub, heavy, sweet-smelling flowers borne on branches 1-3 m high. Fragrant shrub, mutibranched, glabrous, leaves elliptic, ovate or obovate, actue, obtuse, 6-50 mm long, 2-25 mm broad. Panicles 3-22 cm long, pedicles 2-4 mm long. Sepals ovate, petals 3-4 mm long, 4-5 mm broad, filaments 5 mm long, capsules 5-10 mm in diameter, wall veined, flowers numerous, small, white or rose colored, fragrant, ovary 4 celled, 30-50 seeds per fruit. Flowering July-September (Fig. 26: A1)	Small perennial, glabrous herb, height 0.5-1.2 m, succulent stem & branches, leaves opposite, simple, ovate acute, leaf with long pedicle, pointed leaves, 6-12 cm long, flowers bright red, purple, yellow arises in leaf axils, hermaphrodite, trumpt shaped, 5 petals pink, yellow, varigated, bicolors, flowers have slight vanilla scent, fruit small with black seed coat, flowering April to summer to Fall (Fig. 27: A2)
05	Palynology	Pollen monad, tricolporate, shape in polar view circular, polar diameter 11.87 µm (11.25-12.5 µm), polar length 15 µm (12.5-17.5 µm), pollen in equatorial view subprolate, equatorial diameter 11.25 µm (10-12.5 µm), equatorial length 15 µm (12.5-17.5 µm). P/E ratio 1.05, exine thickness 1.87 µm (1.25-2.5 µm), length of colpi 6.25 µm (5-7.5 µm), width 2.97 µm (2.5-3.45 µm), sculpturing psilate with completely smooth surface (Fig. 26: C1 & D1)	Pollen monad, tricolporate, shape of pollen in polar view subangular, polar diameter 35 µm (32-37.5 µm), polar length is 38.75 µm (35-42.5 µm), shape in equatorial view spheroidal and subprolate, equatorial diameter 31.66 µm (27.5-37.5 µm), equatorial length 37.5 µm (32.5-45 µm), P/E ratio 1.1, exine thickness 4.1 µm (2.5-7 µm), length of colpi 7.5 µm (5-10 µm), width 9 µm (7.5-10 µm), pollen grain periporate with 28 pores, sculpturing scabrate, sculpturing elements with variable shape and size less than 1 µm (Fig. 27: C2 & D2)
06	Leaf epidermal anatomy	**Abaxial surface:** Length of ordinary epidermal cell 35.62 µm (32.5-42.5 µm), width cell 20.62 µm (10-30 µm), stomata anomocytic type, length 15 µm (12.5-17.5 µm), width 5.83 µm (5-7.5 µm). Length of guard cell 27.5 µm (25-30 µm), width 6.5 µm (6-7.5 µm). Stomatal complex 27 µm (25-28.5 µm) long, and 20.62 µm (10-30.5 µm) wide. Subsidary cell 30.5 µm (25-35.5 µm) long, and 18 µm (9-25 µm) wide (Fig. 26: E1) **Adaxial surface:** Ordinary epidermal cell flat, hexagonal with thin smooth walls, length of epidermal cell 52.5 µm (47.5-62.5 µm), width 26.25 µm (22.5-30 µm). Numerous stomata are distributed over the surface, stomata anomocytic type, length 15 µm (12.5-17.5 µm), width 7.5 µm (5-10 µm), length of guard cell 27.5 µm (25-30 µm), width 6.5 µm (5.5-7.5 µm), stomatal complex 28 µm (25-29.5 µm) long, 20 µm (17.5-22.5 µm) wide, subsidary cells 31 µm (27-35 µm) long, and 19 µm (10-24 µm) wide, trichomes and glands absent (Fig. 26: F1)	**Abaxial surface:** Ordinary epidermal cells irregular shaped, length 64.75 µm (55.5-69.5 µm), width 21.75 µm (15-28.5 µm). Stomata irregularly oriented, diacytic type, length 25.75 µm (21-30.5 µm), width 17 µm (13.5-20.5 µm). Length of guard cell 22.5 µm (21-24 µm), width 6.5 µm (4-9 µm). Stomatal complex 22 µm (20.5-23 µm) long, and 29 µm (28-30.5 µm) wide. Subsidary cell 47.5 µm (40-55.5 µm) long, and 20 µm (13-26.5 µm) wide. Length of trichome 75.25 (70-80.5 µm), width 17 µm (13.5-20.5 µm). Glands rounded at end, length 69.5 µm (68-71 µm), width 16.5 µm (15-18 µm) (Fig. 27: E2) **Adaxial surface:** Ordinary epidermal cells irregular shaped, with undulating lobed walls, length of epidermal cell 80 µm (67.5-102.5 µm), width 29.8 µm (20-32.5 µm). Stomata irregularly oriented, abundant, diacytic type, length 30 µm (25-35 µm), width 20.8 µm (17.5-22.5 µm). Length of guard cell 27 µm (25-28 µm), width 7.5 µm (5-10 µm). Stomatal complex 26.4 µm (24-27.5 µm) long, and 33.5 µm (32-35.5 µm) wide. Subsidary cell 48 µm (42-56 µm) long, and 18.5 µm (15-20.5 µm) wide. Trichomes are 4 celled, length 74.5 µm (70-87.5 µm), width 16.66 µm (15-17.5 µm). Glands rounded at end, length of glands 72.5 µm (70-75 µm), width 14.5 µm (15-20 µm) (Fig. 27: F2)

Table 1. (Cont'd.).

S. No.	Characters	*Lawsonia inermis* L.	*Mirabilis jalapa* L.
07	Trade Part & Status	Leaves and leaf powder	Leaves, Stem bark, fruit. It is not traded in Pakistan; it is mixed as adulterant in Henna powder commonly.
08	Organoleptography	Branches, leaves and flowers in dried aerial parts are mixed. Aerial parts brown in color. Branches whitish brown, size from 1.5-11 cm. Branches are hard and irregular ridges are present. Leaves brown in color, leaves have bitter taste and pleasant odor, alternate and lanceolate, size 0.5-1.5 cm. Flowers pinkish brown in color, flowers are white in color and present in cluster form. Flowers size ranges from 0.2-0.3 cm (Fig. 26: B1)	Aerial parts contain flowers, leaves and branches. The branches light green in color and have ridges and furrows on surface. They are cylindrical and smooth. The nodes and internodes are prominent and they are swollen. The size of branches is from 9-24 cm. Branch width varies from 0.5-1.5cm. Leaves are hairy, lenceolate, dark green in colour with wavy margins. Veins on leaves are prominent. Length of leaf ranges from 14-16.5 cm. Taste is pungent and have irritating odor. Flowers red or yellow in color and umbrella shaped. The size of flowers is from 0.4-0.9cm (Fig. 27: B2)
09	Part Use	Leaves & Aerial parts	Aerial parts (mostly leaves)
	Medicinal Uses	Hair tonic, jaundice, skin diseases	Piles, dye, rheumatism, wounds
10	Indigenous herbal recipes	Henna leaf powdered paste is used in marriage, festivals and other traditional ceremonies in Pakistan for decorating hands, nails, feet and head. Among elder ladies it is commonly used as hair dye. According to local people in Punjab, Khyber Pakhtoon Khawa and Sind provinces, the people decorate their horses, cows and donkeys with henna to protect them from evil and accidents. According to an old lady 50 g of leaf powder mixed in 150 ml of boiling water to prepare paste. After cooling, this paste is applied on hair and leave it for 20-30 minutes. This recipe is recommended for cooling effects, hair dye for healthy hair. Similarly elder men used this recipe to make their hair and beard orange According to the young ladies, the dye prepared from henna is useful for strong hair. According to her heat 300 g coconut oil. Add handful of henna leaf powder and heat up to boiling point. After cooling, stored in air tight container. This mixture is recommended to apply on head for 2-3 weeks to make hair strong, healthy and lengthy	Fresh leaves are applied on wound. Leaves are dried in shade, ground to obtain powder. ½ teaspoon with water is taken twice a day for 8-10 days to treat piles, inflammation and rheumatism. A decoction prepared from fresh leaves in boiling water to treat abcesses. Leaf juice is also recommended to treat wound
11	Toxicity	None	None

Table 2. Fluorescence analysis and solubility tests (Cold method) of powdered drug of *Lawsonia inermis* in various solvents.

S. No.	Treatments	Under visible light	Under short wavelength (UV) 254 nm	Under long wave length (UV) 365 nm	On filter paper (under short wavelength UV)	On filter paper (under long wavelength UV)	Solubility Analysis
1.	Dried Plant Powderd	Muddy brown	Brown	Chocolate brown	-	-	-
2.	Powdered drug+50% KOH	Conker	Dark brown	Algal green	Pink	Pink	Soluble
3.	Powderd drug+10% aq. $Fecl_3$	Conker	Black	Brownish black	Dark brown	Chocolate brown	Soluble
4.	Powderd drug+Distl. H_2O	Dark reddish brown	Brown	Chocolate brown	Pink	Glowing pink	Soluble
5.	Powderd drug+HCL Conc.	Greenish black	Black	Black	Pink	Pinkish brown	Partially Soluble
6.	Powderd drug+HCL 50%	Greenish brown	Black	Blackish brown	Yellow	Yellow	Soluble
7.	Powderd drug+H_2SO_4 Conc.	Golden glimmer	Dark green	Greenish black	Dark brown	Brown	Partially soluble
8.	Powderd drug+H_2SO_4 50%	Golden green	Dark brown	Brown	Yellow	Yellowish brown	Partially soluble
9.	Powderd drug+HNO_3 Conc.	Golden brown	Brown	Dark mustard	Pink	Pink	Soluble
10.	Powderd drug+HNO_3 50%	Dark golden brown	Brown	Brown	Yellow	Yellowish brown	Partially Soluble
11.	Powderd drug+Conc. CH_3OH	Brown	Dark reddish brown	Reddish brown	Mustard	Mustard pink	Soluble
12.	Powderd drug+CH_3OH 50%	Mustard brown	Dark brown	Chocolate brown	Pink	Pink	Partially Soluble
13.	Powderd drug+Conc. $CHCl_3$	Brown	Reddish brown	Chocolate brown	Pink	Pinkish brown	Soluble
14.	Powderd drug+$CHCl_3$ 50%	Pale brown	Brown	Brown	Yellow	Golden yellow	Soluble
15.	Powderd drug+Conc. C_2H_5OH	Reddish brown	Dark brown	Greenish brown	Grey	Greyish brown	Soluble
16.	Powderd drug+C_2H_5OH 50%	Reddish brown	Chocolate brown	Chocolate brown	Pink	Pinkish brown	Soluble
17.	Powderd drug+Conc. CH_3COOH	Greenish brown	Chocolate brown	Reddish brown	Pink	Pink	Soluble
18.	Powderd drug+CH_3COOH 50%	Greenish brown	Black	Blackish brown	Yellow	Yellowish brown	Soluble
19.	Powderd drug+Conc. C_6H_6	Golden mustard	Dark red	Reddish black	Offwhite	Offwhite	Soluble
20.	Powderd drug+C_6H_6 50%	Reddish brown	Dark red	Dark red	Yellow	Yellowish brown	Soluble

Table 3. Fluorescence analysis and solubility tests (Hot method) of powdered drug of *Lawsonia inermis* in various solvents.

S. No.	Treatments	Under visible light	Under short wavelength (UV) 254 nm	Under long wave length (UV) 365 nm	Solubility Analysis
1.	Powdered drug+50% KOH	Blackish brown	Dark green	Greenish brown	Soluble
2.	Powderd drug+10% aq. $Fecl_3$	Dark brown	Black	Black	Soluble
3.	Powderd drug+Distl. H_2O	Reddish oxide	Black	Brownish black	Soluble
4.	Powderd drug+HCL Conc.	Black	Black	Black	Insoluble
5.	Powderd drug+HCL 50%	Golden brown	Black	Blackish brown	Insoluble
6.	Powderd drug+H_2SO_4 Conc.	Black	Black	Black	Insoluble
7.	Powderd drug+H_2SO_4 50%	Black	Black	Black	Insoluble
8.	Powderd drug+HNO_3 Conc.	Yellowish cream	Dark mustard	Mustard	Soluble
9.	Powderd drug+HNO_3 50%	Fresh orange	Orange brown	Orange brown	Insoluble
10.	Powderd drug+Conc. CH_3OH	Dark brown	Dark reddish brown	Reddish brown	Partially soluble
11.	Powderd drug+CH_3OH 50%	Chocolate brown	Dark brown	Dark brown	Partially soluble
12.	Powderd drug+Conc. $CHCl_3$	Chocolate brown	Reddish brown	Conker	Soluble
13.	Powderd drug+$CHCl_3$ 50%	Golden brown	Dark brown	Red oxide	Soluble
14.	Powderd drug+Conc. C_2H_5OH	Red oxide	Brown	Chocolate brown	Soluble
15.	Powderd drug+C_2H_5OH 50%	Reddish brown	Chocolate brown	Blackish brown	Soluble
16.	Powderd drug+Conc. CH_3COOH	Greenish brown	Brown	Chocolate brown	Soluble
17.	Powderd drug+CH_3COOH 50%	Brown	Dark brown	Chocolate brown	Soluble
18.	Powderd drug+Conc. C_6H_6	Brown	Dark red	Reddish brown	Partially soluble
19.	Powderd drug+C_6H_6 50%	Brown	Dark red	Reddish maroon	Soluble

Table 4. Fluorescence analysis and solubility tests (Cold method) of powdered drug of *Mirabilis jalapa* in various solvents.

S. No.	Treatments	Under visible light	Under short wavelength (UV) 254 nm	Under long wave length (UV) 365 nm	On filter paper (under short wavelength UV)	On filter paper (under long wavelength UV)	Solubility Analysis
1.	Dried Plant Powderd	Fresh green	Green	Dark green	-	-	-
2.	Powdered drug+50% KOH	Golden glimmer	Dark green	Algal green	Pink	Pink	Partially soluble
3.	Powderd drug+10% aq. $Fecl_3$	Dark brown	Brown	Chocolate brown	brown	Chocolate yellow	Partially soluble
4.	Powderd drug+Distl. H_2O	Yellowish green	Grayish yellow	Grayish green	Pink	Pink	Partially soluble
5.	Powderd drug+HCL Conc.	Dark leaf green	Brown	Greenish brown	Mustard	Mustard brown	Partially soluble
6.	Powderd drug+HCL 50%	Greenish yellow	Dark green	Blackish green	Pink	Pink	Partially soluble
7.	Powderd drug+H_2SO_4 Conc.	Greenish yellow	Golden glimmer	Light leaf green	Brown	Chocolate brown	Partially soluble
8.	Powderd drug+H_2SO_4 50%	Spring green	Blackish green	Blackish green	Light brown	Yellowish brown	Partially soluble
9.	Powderd drug+HNO_3 Conc.	Light yellow brown	Brown	Dark brown	Light brown	Light muddy brown	Partially soluble
10.	Powderd drug+HNO_3 50%	Mustard	Dark brown	Greenish brown	Brown	Light brown	Partially soluble
11.	Powderd drug+Conc. CH_3OH	Spring green	Buckingham green	Reddish green	Yellow	Yellow	Soluble
12.	Powderd drug+CH_3OH 50%	Golden glimmer	Pale green	Forest green	pink	Light pink	Partially soluble
13.	Powderd drug+Conc. $CHCl_3$	Spring green	Dark green	Reddish green	Yellow	Light mustard	Soluble
14.	Powderd drug+$CHCl_3$ 50%	Leaf green	Algal green	Pine forest	Pink	Pink	Partially soluble
15.	Powderd drug+Conc. C_2H_5OH	Spring green	Bottle green	Reddish green	Yellow	Yellow	Soluble
16.	Powderd drug+C_2H_5OH 50%	Leaf green	Pale green	Dark green	Pinkish white	Glowing white	Partially soluble
17.	Powderd drug+Conc. CH_3COOH	Leaf green	Pine forest	Reddish brown	Yellow	Pale yellow	Partially soluble
18.	Powderd drug+CH_3COOH 50%	Leaf green	Pine forest	Redo green	Yellow	Pale yellow	Partially soluble
19.	Powderd drug+Conc. C_6H_6	Golden glimmer	Shocking green	Reddish green	Pink	Pink	Soluble
20.	Powderd drug+C_6H_6 50%	Leaf green	Pine forest	Reddish green	Yellow	Yellowish mustard	Soluble

Table 5. Fluorescence analysis and solubility tests (Hot method) of powdered drug of *Mirabilis jalapa* in various solvents.

S. No.	Treatments	Under visible light	Under short wavelength (UV) 254 nm	Under long wave length (UV) 365 nm	Solubility Analysis
1.	Powdered drug+50% KOH	Yellowish green	Reddish green	Reddish green	Partially soluble
2.	Powderd drug+10% aq. $Fecl_3$	Yellowish brown	Brown	Brown	Partially soluble
3.	Powderd drug+Distl. H_2O	Yellowish green	Yellowish brown	Light brown	Partially soluble
4.	Powderd drug+HCL Conc.	Blackish green	Black	Black	Soluble
5.	Powderd drug+HCL 50%	Golden glimmer	Dark green	Blackish green	Partially soluble
6.	Powderd drug+H_2SO_4 Conc.	Orange red	Dark brown	Brown	Insoluble
7.	Powderd drug+H_2SO_4 50%	Reddish brown	Dark brown	Chocolate brown	Insoluble
8.	Powderd drug+HNO_3 Conc.	Orange	Orange	Light brown	Soluble
9.	Powderd drug+HNO_3 50%	Pale brown	Woody brown	Fresh green	Insoluble
10.	Powderd drug+Conc. CH_3OH	Spring green	Fresh green	Reddish green	Soluble
11.	Powderd drug+CH_3OH 50%	Gold dust	Pine forest	Leaf green	Partially soluble
12.	Powderd drug+Conc. $CHCl_3$	Signal green	Buckingham green	Red	Soluble
13.	Powderd drug+$CHCl_3$ 50%	Leaf green	Pine forest	Reddish brown	Partially soluble
14.	Powderd drug+Conc. C_2H_5OH	Buckingham green	Spring green	Red	Soluble
15.	Powderd drug+C_2H_5OH 50%	Pale green	Leaf green	Spinach green	Partially soluble
16.	Powderd drug+Conc. CH_3COOH	Pine forest	Dark green	Redo green	Soluble
17.	Powderd drug+CH_3COOH 50%	Golden brown	Reddish brown	Dark brown	Soluble
18.	Powderd drug+Conc. C_6H_6	Leaf green	Algal green	Dark green	Soluble
19.	Powderd drug+C_6H_6 50%	Fresh green	Spring green	Reddish green	Soluble

Fig. 1a. Lawsonia inermis.

Fig. 1b. Dried Aerial parts.

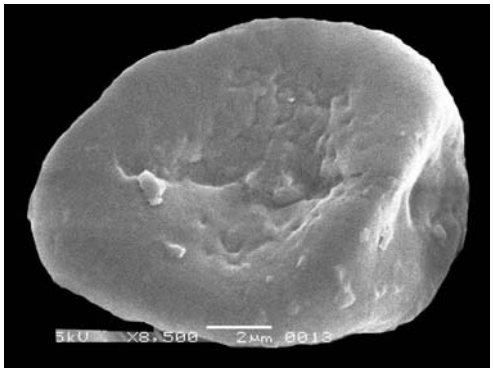

Fig. 1c. Polar View of Pollen (SEM).

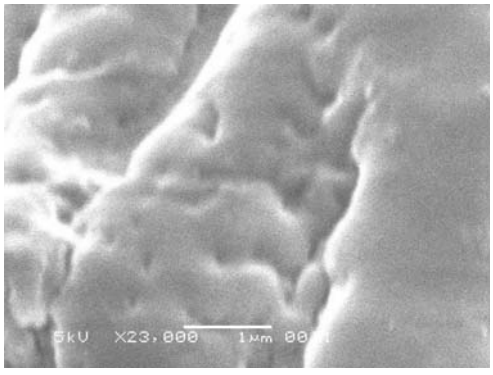

Fig. 1d. Pollen Sculpturing (SEM).

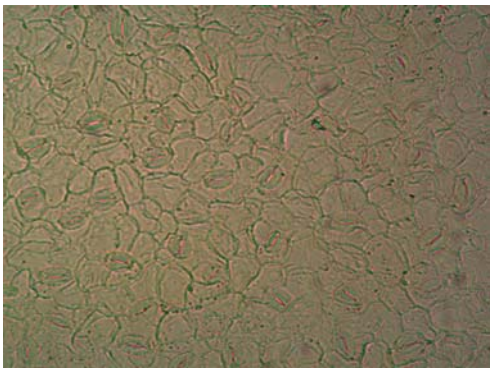

Fig. 1e. Stomata & Epidermal Cells (Abaxial : LM 40x).

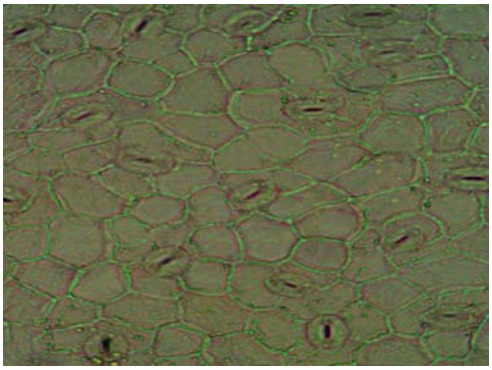

Fig. 1f. Epidermal cells (Adaxial : LM-40x).

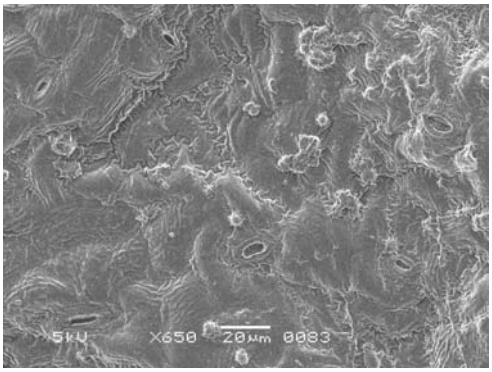

Fig. 1g. Stomata (SEM).

Fig. 1h. Pharmacognostic Flow Chart.

Fig. 2a. Mirabilis jalapa

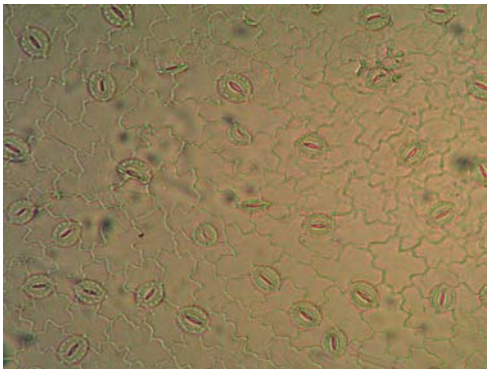

Fig. 2e. Stomata & Epidermal Cells (Abaxial : LM 40x)

Fig. 2b. Dried Aerial parts

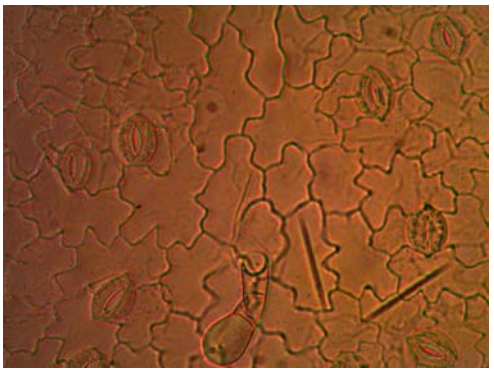

Fig. 2f. Trichomes & Glands (Adaxial : LM-40x)

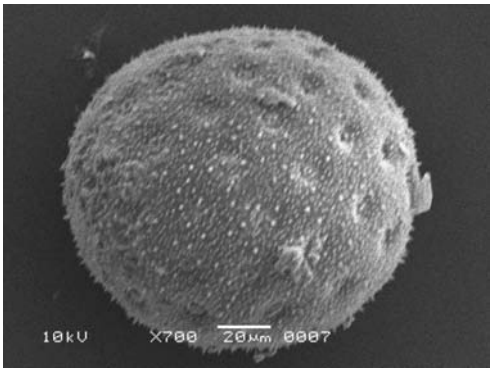

Fig. 2c. Polar View of Pollen (SEM)

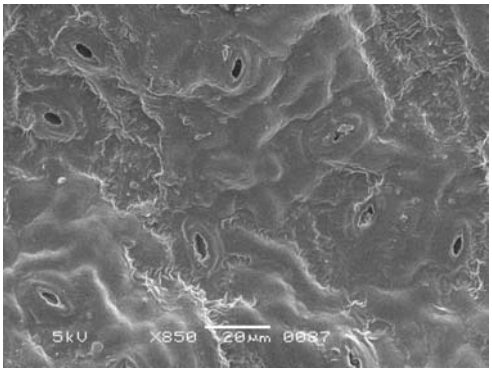

Fig. 2g. Stomata (SEM)

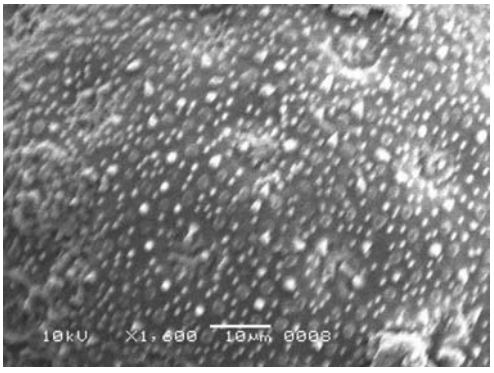

Fig. 2d. Pollen Sculpturing (SEM)

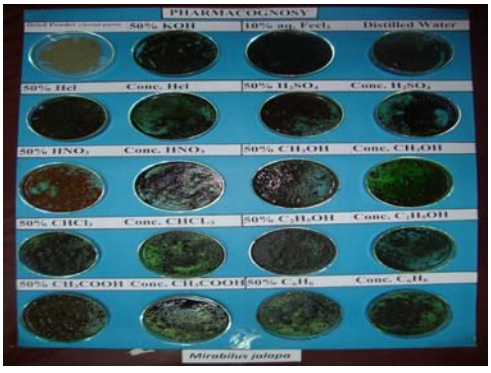

Fig. 2h. Pharmacognostic Flow Chart

Pharmacognostic screening: Details of pharmacognostic tests including solubility tests in hot and cold conditions, fluoresence analysis and preliminary phytochemical screening of both *Lawsonia inermis* and *Mirabilis jalapa* were presented in Tables 1-5. The powdered leaf of *Lawsonia inermis* exhibited different fluorescence under different conditions. In this way the fluorescence method is adequately sensitive and accurate for determination of satisfactory authentication of powder drug Henna without several time consuming dilution steps prior to analysis of pharmaceutical samples (Pimenta *et al*., 2006). The macroscopical, microscopical, pharmacognostic and preliminary phytochemical characters observed in the present study were useful to distinguished the genuine source of herbal drug Henna. A comparison, based on present study and published literature (Jain *et al*., 2010; Ansari & Ali, 2000, Hanna, 1997) revealed that taxonomic and pharmacognostic analysis of market samples for herbal drug Henna are of great extent to be used as salient features to distinguish the genuine source *Lawsonia inermis* from its adulterant *Mirabilis jalapa*.

Acknowledgement

Authors are grateful to HEC-Pakistan and Institute of Post Graduate Studies, School of Chemical Engineering, Universiti Sains Malaysia for financial support of this project.

References

Abid, M., M. Ahmad, A. Jabeen, M. Zafar and S. Nadeem. 2005. Pharmacognostic studies of some indigenous medicinal plants of Pakistan. *J. Ethnobotanical Leaflets*.

Afaq, S.H. 1998. A comparative introduction of the Unani and Tibetan medical traditions, *Ayur Vijnana*, Volume: 6. (http://www.ittm.org/publications/AyurVijnana/Vol_06/AV_V06_5.htm).

Ahmad, M., M.A. Khan, M. Zafar, M. Arshad, S. Sultana, B.H. Abbasi and S.U. Din. 2010. Use of chemotaxonomic markers for misidentified medicinal plants used in traditional medicines. *J. Medicinal Plant Res*., 4(13): 1244-1252.

Ahmad, F., M. A. Khan, M. Ahmad, M. Hameed, R. B. Tareen, M. Zafar and A. Jabeen. 2011. Taxonomic application of foliar anatomy in grasses of tribe Eragrostideae (poaceae) from salt range of Pakistan. *Pak. J. Bot.*, 43(5): 2277-2284.

Anonymous. 2002. WHO Traditional Medicine Strategy 2002-2005. World Health Organization, Geneva.

Ansari, S.H. and M. Ali. 2000. Phytochemical investigation on *Mirabilis jalapa*. *Hamdard Medicus*, 43(3): 65-67.

Barthlott, W. 1984. Microstructural features of seed surfaces. In: *Current Concepts in Plant Taxonomy*. (Eds.): V.H. Heywood and D.M. Moore, Academic Press, London, pp. 95-105.

Bhattacharjee, S.K. and L.C. De. 2003, *Medicinal Herbs and Flowers*, Aavishkar Publishers and Distributors, pp. 366.

Erdtman, G. 1960. The acetolysis method. A revised description. *Sven. Bot. Tidskr*., 54: 561-564.

Geeraets, A., J.A.M. Raeymaekers, S. Vinckier, A. Pletsers, E. Smets and S. Huysmans. 2009. Systematic palynology in Ebenaceae with focus on Ebenoideae: Morphological diversity and character evolution. *Review of Paleobotany and Palynology*, 153: 336-353.

Gul, F., Z.K. Shinwari and I. Afzal. 2012. Screening of indigenous knowledge of herbal remedies of skin diseases among local communities of north west Punjab. *Pak. J. Bot*., 44(5): 1609-1616.

Hannan, H.A. 1997. Medicinal Properties of *Henna. Hamdard Medicus*. 40(4): 17-21.

Harborne, J.B. 1973. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman & Hall, London. pp. 302.

Hooker, J.D. 1875. *Flora of British India*, Rananculaceae to Sapindaceae. Vol. 1. 1-23.

Jain, V.C., D.P. Shah, N.G. Sonani, S. Dhakara, N.M. Patel and R.P. Ellis. 2010. Pharmacognostical and preliminary Phytochemical investigation of *Lawsonia inermis* L. *Leaf. Rom. J. Biol*., 55(2): 127-133.

Jan, H.U., Z.K. Shinwari and A.A. Khan. 2011. Staining effect of dye extracted from dry leaves of *Lawsonia Inermis* Linn. (Henna) on angiospermic stem tissue. *Pak. J. Bot*., 43(1): 383-389.

Kabata-Pendias, A. and H. Pendias. 2001. *Trace elements in soils and plants*, Washington, D.C., CRC Press.

Kirtika, KR., B.D. Basu. *Indian Medicinal Plants*, Vol. 3. International book distributors, New Delhi, India, 1993.

Kuroyanagi, M., M. Murata, T. Nakane, O. Shirota, S. Sekita, H. Fuchino and Z.K. Shinwari. 2012. Leishmanicidal active withanolides from a Pakistani medicinal plants, *Withania coagulans Chem. Pharm. Bull*., 60(7): 892-897.

Leung, A.Y. 1980. Encyclopeida on common natural ingredients used in food, drugs and cosmetics, John Willey and Sons, New York. 409.

Malekzadek, B.C. 1968. Studies of medicinal plants from Margallah Hills Islamabad. *Hamdard Medicus*., 51(2): 112-125.

Marwat, S.K., F.U. Rehman, M.A. Khan, M.A. Khan, M. Ahmad, M. Zafar and Said Ghulam. 2011. Medicinal folk recipes used as traditional phytotherapies indistrict Dera Ismail Khan, KPK, Pakistan. *Pak. J. Bot*., 43(3): 1453-1462

Metcalfe, C.R. 1960. *Anatomy of the Monocotyledons. I. Gramineae*. Clarendon Press, Oxford.

Nadkarni, K.M. 2004. Elementary *Pharmacology* and Toxicology. A *Textbook of Pharmacognosy*, Srishti Book Distributors, pp. 214.

Nasir, E. and S.I. Ali. 1974. Family Verbenaceae. *Flora of West Pakistan* (*Verbenaceae)*, 77: 4-10.

Nasir, E. and S.I. Ali. 1975. Family Lythraceae. *Flora of West Pakistan. (Lythraceae)*, 78: 6-7.

Prat, H. 1932. L'Épiderme des graminées. Étude anatomique et systématique. *Ann. Sci. Nat. Bot*., 14: 117-324.

Primenta, A.M., M.C. Montenegro, A.N. Ara Ujo and J.C. Mart'inez. 2006. Application of sequential injections analysis to pharmaceutical analysis. *Journal of Pharm. Biomed. Annuls*, 40: 1452-1457

Ronald, O.K. 2000. Pollen and spores. 2nd Ed. *American Association of Stratographic Palynologists*, pp. 13-21.

Sarwat, Z.K. Shinwari and N. Ahmad. 2012. Screening of potential medicinal plants from district Swat specific for controlling women diseases. *Pak. J. Bot*., 44(4): 1193-1198.

Shah, B. and A.K. Seth. 2010. Text book of pharmacognosy and phytochemistry. Elsevier, a division of Read Elsevier India Private Ltd. pp. 10-21.

Shiharta, I.M., A.G. Hussain and G.Y. Mayah. 1978. Pharmacological effects of *Lawsonia inermis* leaves (el-henna). *Egypt J. Vet. Sci*., 15: 31.

Shinwari, Z.K. 2010. Medicinal plants research in Pakistan. *Journ. Med. Pl. Res*., 4(3): 161-176.

Shinwari, Z.K. and M. Qaisar. 2011. Efforts on conservation and sustainable use of medicinal plants of Pakistan. *Pak. J. Bot*., 43(Special Issue): 5-10.

Sofowora, A. 1993. *Medicinal plants and traditional medicines in Africa*. John Wiley & Sons New York. pp. 97-145.

Subrahmanyam, N.S. 1996. *Laboratory manual of plant taxonomy*. Vikas Publishing House (Pvt) Ltd., New Dehli.

Sultana, S., M. A. Khan, M. Ahmad, A. Bano, M. Zafar and Z. K. Shinwari. 2011. Authentication of herbal medicine neem (*Azadirachta indica A*.Juss.) by using taxonomic and pharmacognostic techniques. *Pak. J. Bot*., 43: 141-150,

Trease, G.E. and W.C. Evans. 1989. *Pharmacognosy*, 12th Ed. Lodon Press, London. 1-240.

Tutin, T.G. and V.H. Heywood. 1972. *Flora of Europe.* Vol. 3. Cambridge University Press. London.

Zafar, M., M. Ahmad, M.A. Khan, S. Sultana, G. Jan, F. Ahmad, A. Jabeen, G.M. Shah, S. Shaheen, A. Shah, A. Nazir and S.K. Marwat. 2011. Chemotaxonomic clarification of pharmaceutically important species of *Cyperus* L. *Afr. J. Pharmacy & Pharmacology*, 51(1): 67-75.

(Received for publication 1 September 2012)