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Comparative physicochemical and histo-anatomical study of vague traditional and magical herb *Lavandula stoechas* L. with *Lavandula officinalis* Chaix.

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Lavandula stoechas L. (Lamiaceae) is a medicinal plant of traditional Unani, Iranian and Tunisian systems of medicine. It has a unique attribute to expel out and remove all impurities and obstructions and reinvigorate the brain power, honored as broom of brain in classical Unani literatures. To establish clear identification between *L. stoechas* (LS) and *L. officinalis* (LO) standard operating parameters, TLC fingerprinting and physicochemical and histo-anatomical studies were done. LS exhibited significantly higher physical values for their standardization and quality parameters with respect to LO. Histo-anatomical disquisition and chemo-microscopic studies of the drug revealed that presence of substantial and marked diagnostic feature to LS having large bract, brown colored flexible peduncle, presence of exorbitant number of uniseriate and multi seriate covering and uniseriate glandular trichomes leading to soft stem and peduncle. It has distinguished and well-developed phloem, centrally filled with parenchymatous cells, with camphoric stench along with bitter in taste while LO has lesser hairs, hard stem and peduncle, greenish color, presence of centrally hollow pith, indiscriminate bract, and flowers are without stalks with pleasant aroma. Seeds of LS are yellowish brown, frivolous weight and very small in size while LO have blackish seeds and are comparatively larger in size and weight.

Keywords: *L. stoechas, L. officinalis*, Magical herb, Traditional, Ustukhuddoos

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Lavandula stoechas L. (Lamiaceae), is an indigenous shrubby plant and widely distributed taxon from the Arabian, Mediterranean to Asia Minor region. It is well sounded with other vernacular names like French or Arabian Lavender in English, *Ustukhuddoos* in Arabic, *Dharu* in Hindi, *Lavendara na phula* in Gujarati[1], *Tantna* in Bengali and *Jarub Dimagh* in Farsi[2]. This worthy plant is extensively cultivated all over the world, particularly in France, Bulgaria, Russia, Italy, Spain, England, United States and Australia with peerless and characteristic sessile, oblong-linear leaves, small dark, purple, dense, short peduncled spiked flowers[3], tapered and is situated in the axils of downy, heart shaped bracts. The upper bracts of the flower are abortive, form a purple tuft at the top of the spike[4]. This reputed medicinal plant has various fateful applications in the Unani system by ancient practioner and recommended this drug in specially cerebral disorders[5]. In folk medicine it is used as antispasmodic, analgesic, tranquillizer and antiseptic[6,7] and for epilepsy and migraine[1,8]. It is a good stimulant, general carminative, diaphoretic, expectorant, antiphologistic and emmenagogue. Essential oil from the flowers is used in colic and chest affections and to relieve biliousness, headache, rheumatism and neuralgic pains[1]. In Tunisian traditional system it is used to protect headache, depression, diabetes[9-11], rheumatism, chill and digestive disorders[12], urinal infections, cardiac diseases, hypotensive and eczema[13,14]. It also has a specific function to expel the black bile and relieved to head, heart and exhilarate it through its action of cleansing[15].

Lavandula officinalis Chaix. also has the same taxonomical crede with straight and woody branches. *L. angustifolia* Mill has a long-standing history as a medical remedy traditionally. It has been prescribed to treat several complications like infertility, infection, anxiety, fever, depression, spasm, flatulence, emesis and diuretics. In recent years, its essential oil has gained a

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strong reputation in aromatherapy and as a holistic relaxant to treat stress, fatigue or insomnia[16]. It is similar to Ustukhuddoos in most applications for various therapeutic purposes in Unani system[17]. It has good antioxidant[18], sedative[19], antitumor, anti inflammatory, antihistaminic, antidiabetic, antimicrobial activity along with central nervous system modulator[20-25]. It is also useful for nervous breakdown, nervous tension[26-28], alopecia[29], enhancing cognitive performance and mood behaviour[30]. It has analgesic, anticonvulsive[9] and local anesthetic effect[31]. On the basis of above descriptions, it is vindicated that this traditional plant is also have very worthy and almost similar medicinal applications, but as per Unani physicians, ambiguous descriptions mentioned in classical Unani literature provide indistinct idea of identification to this plant. Therefore, in this study, we aimed to discriminate them on the basis of chemo-microscopy and physicochemical studies.

Materials and Methods

Collection and authentication of samples

Sample of *L. officinalis* Chaix was provided by Central Council for Research in Unani Medicine, Industrial area Janakpuri, New Delhi and two samples of *Lavandula stoechas* Linn (Ustukhuddoos) were procured from local market and Dawakhana Tibbiya College, Aligarh Muslim University (AMU). These samples were authenticated for their botanical identity in the Pharmacognosy research unit of the institute as well as by consulting taxonomist Prof. Wajahat Husain, Dept. of Botany, AMU Aligarh and on the basis of available literatures for *L. officinalis*[32,33] and *L. stoechas*[34,35].

Chemical and reagents

All the chemicals used for the development of standard operating parameters and quality studies of the drugs are of Merck Darmstadt (Germany), double distilled water from own laboratory, instruments like Muffle furnace enabled with pyrometer (Narang scientific works Pvt. Ltd. India), Universal Oven (Narang scientific works Pvt. Ltd. India), water bath rectangular (Narang scientific works Pvt. Ltd. India), pH meter (Biogen scientific India). Microscopic work was performed with Olympus microscope (Japan) and camera lucida. TLC was run on silica gel G 60 F_{254} precoated TLC plates (Merck, Mumbai, India). Spots were visualised by exposing to iodine vapours, UV radiation (366 nm) and spraying with 2% sulphuric acid in methanol.

Methods for standardization and quality parameters

Morphological study

Both the drugs were analysed with external characters to establish possible differences.

Histological study

Slides of floral stem, peduncles, and other aerial parts of LS and LO were analysed with camera lucida and microscope associated with desktop to ascertain their key characteristic features.

Organoleptic evaluation

The organoleptic evaluation refers to evaluation of the drug by colour, odour, taste, appearance, particle size and texture[36].

Loss on drying (L O D)

As per standard procedure designed to measure the amount of water and volatile matters in a sample when it dried under specified conditions[37].

Ash values

As per standard procedure for determination of total ash, acid insoluble ash and water soluble ash is done for testing of drugs to determine authenticity and purity of sample and also these values are important qualitative standards[36,37].

Determination of pH

As per protocol determination of pH of 1% and 10% aqueous solution of drug was prepared (*w/v*) by using digital pH meter[38].

Extractive values

Extracts obtained by exhausting crude drugs are indicative of the nature of phytoconstituents present.

Water soluble extractives

As per standard method, estimation of water-soluble extractives was performed to ensure the nature of hydrophilic and polar phytoconstituents[37].

Alcohol soluble extractives

As per standard method, estimation of water-soluble extractives was performed to ensure the nature of alcohol-soluble phytoconstituents[37].

Qualitative TLC fingerprinting

Thin layer chromatography (TLC) is a powerful analytical technique with separation power. HPTLC is still a better means to separate the various components of a mixture

Preparation of extracts

Alcoholic extracts of the selected plant drug material were prepared and filtered. Alcoholic extracts were facilitated to standardize their quality[39].

Development of solvent system

Solvent systems were developed for establishing the TLC patterns for the different extracts. Various visualization techniques were used to come up with the best TLC fingerprint, like UV at various wave length, iodinisation and spray reagents[39-42].

Results

Flower morphology of *L. stoechas* L.

Flowers are (5-9 mm) in size, short stalk and bluish, purple and dark brown in colour and are present in whorls on the spike inflorescence. The whorls are closely arranged towards the top with a terminal whorl. Peduncle is soft and hairy. Rhomboid bracts purple to light purple in colour 4-6 mm in length and longitudinally marked with reticulated vein. Calyx is brown, 3-5 mm in length, tubular with longitudinal marked with 10-12 ridges and persistent. Corolla is light purple in colour, fused at the lower end and expanded at the upper end. Four stamens, in 2 sets (didynamous condition). Seeds are small and light, 0.9-1.2 mm in length, smooth, shiny, oval shape and yellowish brown in colour. Each flower has two seeds. Flowers have camphoric odour and bitter in taste (Fig. 1, 2 and 3).

Flower microscopy of *L. stoechas* L.

Under microscope observation it showed numerous trichomes in form of tuft as well as singly on the bracts and calyx. Glandular trichomes appeared as multicellular stalk and unicellular heads present on calyx and corolla and long glandular trichomes are also present in the inner surface of the corolla. Non glandular trichomes are unicellular and multicellular, uniseriate and branched located on the surface of bracts, calyx and corolla. Anther is brown in colour, two celled, filled with smooth and rounded pollen grains. While the cross-sections of flower stalk (Fig. 1 and 3) showed quadrangle shaped with four prominent ridges with abundant trichomes. Non

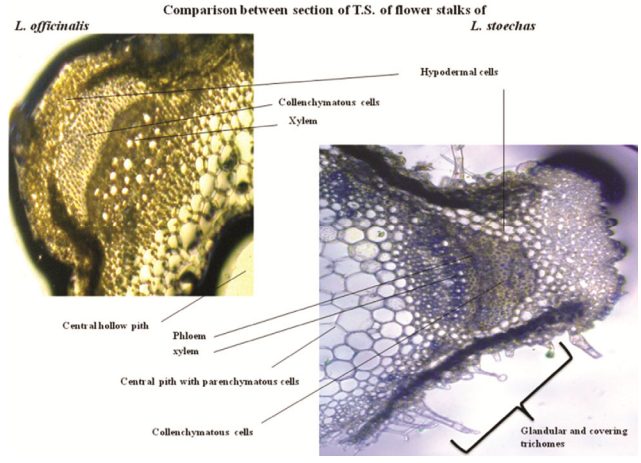

Fig. 1 — Study of T.S. of flower stalk of both plant species under microscope associated with camera at 40 X.

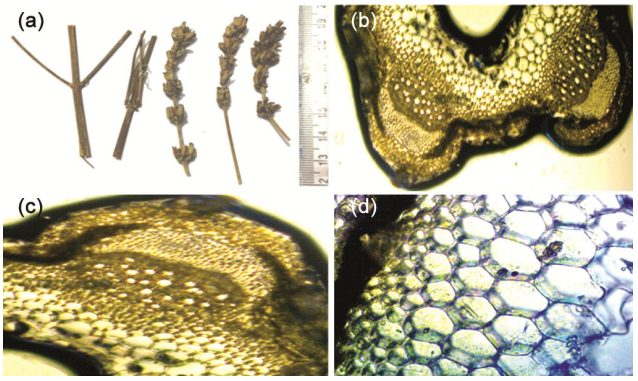

Fig. 2 — Microscopical study of sample of *L. officinalis*: A-Flowers with flower stalk, B- Layout of T.S. of flower stalk, C- vascular bundle present in flower stalk, D- Hypodermal cells in flower stalk (picture B- at 4X, C at 10X and D at 40X)

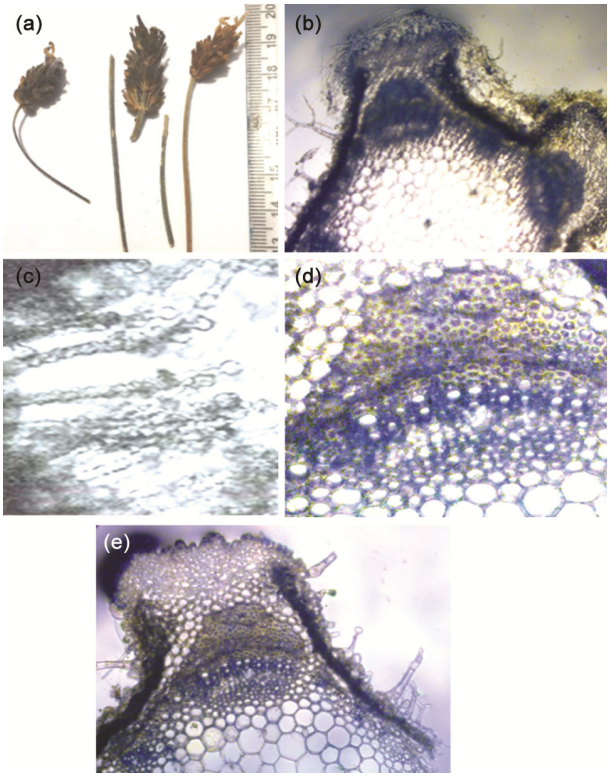

Fig. 3 — Microscopical study of sample of *L. stoechas*: E Flower with flower stalk, F- Layout of T.S. of flower stalk bearing plenty of trichomes, G- Uniseriate glandular trichomes in inner surface of corolla, H-Vascular bundle surrounded by hypodermal cells, F-Glandular and covering trichomes on the surface of flower stalk. (Picture F is at 4X, H and I at 10X, while G at 40 X).

glandular trichomes are unicellular and multicellular, uniseriate and branched located on the surface. The four ridges have 3-4 cell lined hypodermis and a well-developed angular collenchyma upto 4-5 layers and large vascular bundles with distinct phloem 4-6 layers and sclerenchyma upon the phloem. Between the vascular bundles there are large parenchymatous cells and central pith (Fig. 4).

Thin layer chromatography of *L. stoechas* L.

TLC of the petroleum ether extract of the drug (Fig. 3) was developed on Aluminum TLC plate precoated with silica gel 60 F_{254} using toluene: ethyl acetate (4:1, *v/v*) as mobile phase. It showed five spots at R_f 0.28 (yellowish), 0.63 (yellowish) 0.68 (light green), 0.75 (green) and 0.80 (green) under solar light and five spots under UV 365 nm at R_f 0.17 (reddish), 0.25 (reddish), 0.31 (red), 0.45 (reddish) and 0.48 (brownish). On exposure to iodine vapors five spots appeared at R_f 0.16, 0.24, 0.30 and 0.49, all are yellow in colour. Eight spots were observed at R_f 0.15 (brown), 0.35 (brown), 0.53 (purple), 0.63 (blue), 0.67 (brown), 0.75 (green), 0.83 (light brown), 0.92 (brown) colors when treated with 5% sulphuric acid reagent in methanol followed by heating at 105ºC for 5-10 min (Fig. 5).

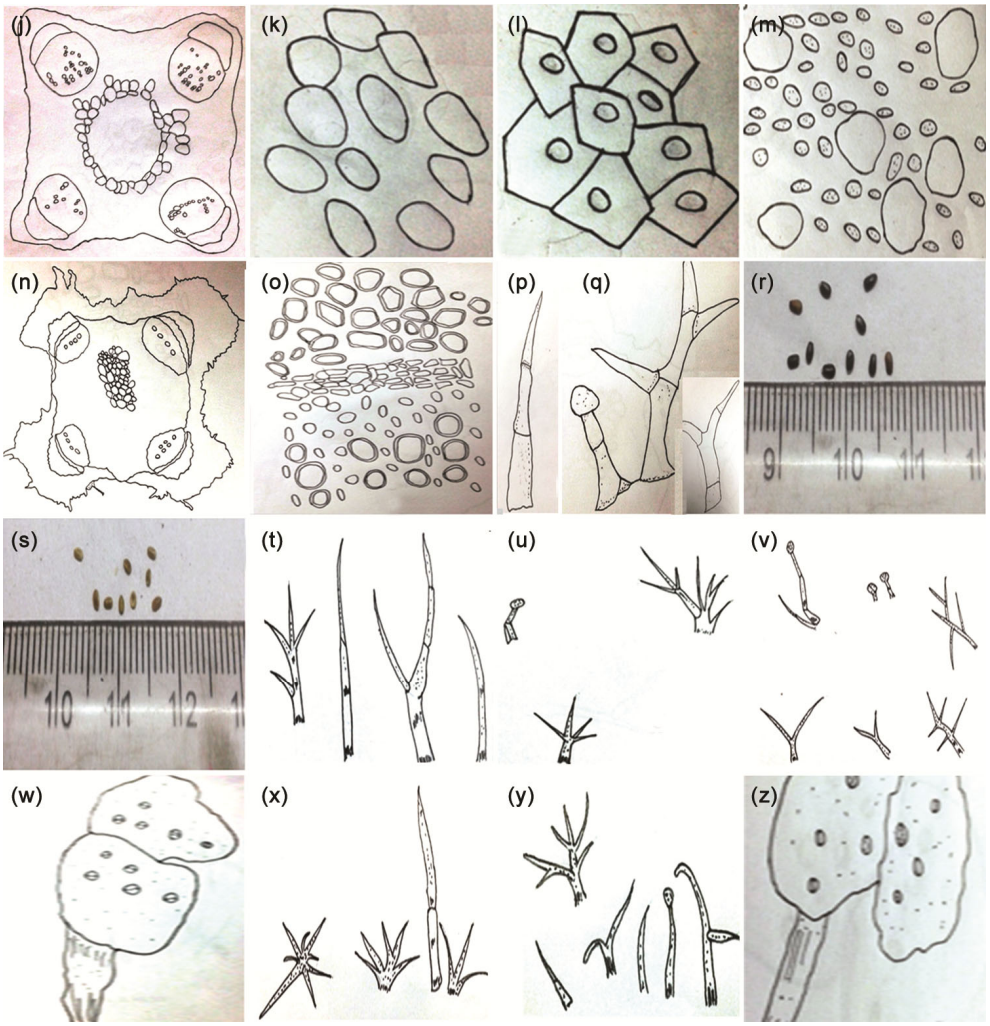

Fig. 4 — (J-Q, with application of Camera Lucida), picture J and N are at 4X and remaining K-Q is at 40X while figures T-Z are at 10X. Magnification: 4X=9.2 cm, 10X= 21.5 cm, and 40X= 90 cm; *L. officinalis* flower stalk: J- Layout of T.S., K- Hypodermal cells below epidermal layer, L- collenchymatous cells, M- vascular tissue (xylem), Q-Trichomes on the surface of peduncle (flower stalk), X-Covering trichomes on the surface of calyx, Y- Covering and glandular trichomes on the surface of corolla, Z- Anther with of filament and R- Seeds of *L. officinalis*; *L. stoechas* flower stalk: N- Layout of T.S., O- Vascular bundles, phloem between upper collenchymas and lower xylem, Q- Glandular and covering trichomes on the surface of peduncle and S- Seeds of Ustukhuddoos, T- Covering trichomes on the surface of bracts, U- Covering and glandular trichomes on the surface of calyx, V-Covering and glandular trichomes on the surface of corolla, W-Anther with of filament.

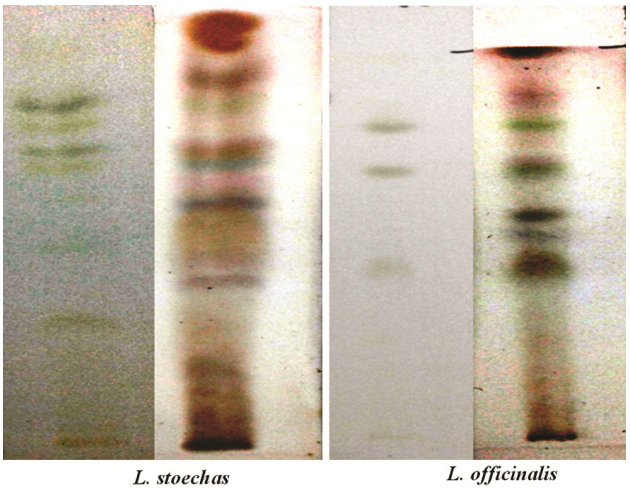

Fig. 5 — Fingerprinting pattern of *L. stoechas* and *L. officinalis* by thin layer chromatography.

Flower morphology of *L. officinalis* Chaix

Flowers of *L. officinalis* are 6-8 mm in length and are present in whorls of 8-10 at intervals on the spike inflorescence. The whorls are closely arranged towards the top with a terminal whorl. Bract is absent. Calyx is green, tubular with longitudinal markings and persistent. Corolla is light purple colour, fused at lower end and expanded at upper end. Calyx and corolla both have trichomes. Four stamens, in 2 sets (didynamous condition). It has a pleasant smell and a slight bitter taste. Seeds of *L. officinalis* are blackish in colour; oval shaped 1.5-2.0 mm long and 0.9-1.1 mm in width, slightly compressed, smooth, and shiny and each flower has 2 seeds. The narrow leaves are opposite, regular margin, lanceolate and simple, with no stipules arranged on quadrangle stem. Leaves are hairy and green in colour. Flower stalk is green, quadrangle shaped with numerous hairs which are non glandular and glandular (Fig. 2).

Flower microscopy of *L. officinalis* Chaix

Non glandular trichomes are multicellular, uniseriate and branched located on the surface of calyx and corolla. Glandular trichomes appeared as multicellular stalks and unicellular heads present on corolla. Anthers are light brown in colour, two celled, filled with smooth rounded brown pollen grains which are smaller in size as compared to Ustukhuddoos. The cross-sections of *L. officinalis* flower stalk (Fig. 1 and 2) showed quadrangle shaped with four prominent ridges with plenty of trichomes but lesser in number as compared to Ustukhuddoos. The four ridges have 3-4 cell lined hypodermis and a well-developed angular collenchyma upto 4-5 layers and large vascular bundles with distinguishing xylem while phloem is not well distinguished. Between the vascular bundles, there are large parenchymatous cells with hollow pith in the central space.

Thin layer chromatography of *L. officinalis* Chaix

TLC of the Pet ether extract of the drug was developed on Aluminum TLC plate precoated with silica gel 60 F_{254} using toluene: ethyl acetate (4:1, *v/v*) as mobile phase. It showed four spots at R_f 0.40 (yellowish), 0.59 (green), 0.60 (green) and 0.71 (yellowish) under solar light and 5 spots under UV 365 nm at R_f 0.22 (reddish), 0.25 (reddish), 0.34 (brown), 0.49 (blue), and 0.48 (brown). On exposure to iodine vapors five spots appeared at R_f 0.24, 0.40, 0.75, 0.85 and 0.92, all are yellow in colour. Eight spots were observed at R_f 0.40 (brown), 0.49 (green), 0.55 (light grey), 0.66 (green), 0.71 (brown), 0.79 (green), 0.88 (brown) and 0.98 (brown) colour when treated with 5% sulphuric acid in methanol followed by heating at 105ºC for 10 min (Fig. 5).

Discussion

This indigenous knowledge of tribal, rural and ancient practitioners and information from conventional healers attracted new generations for the cure of various ailments along with the base for new research[45]. Detailed disquisition and evaluation of physico-anatomical data including floral stalk, floral parts with microscopic evaluation enabled with camera lucida (Fig. 4) and microscope (Fig. 1, 2 and 3) illuminated *L. stoechas* has similar anatomical credentials of stem, flowers, leaves and seeds as descripted for Ustukhuddoos in classical Unani literatures applied for their traditional therapy by the ancient physicians[3,4,34,35,43-44]. On the basis of chemo-microscopy, pharmacognostical & physico-chemical investigations, it was revealed that these two plants have distinguishing characters and different physical values for their standardization parameters (Table 1) and exhibited characteristic diagnostic differences to each other (Table 2). Distinguished fingerprinting pattern of TLC is also an important step for the establishment of chemical profile of these plants that will further provide a quality control tool to researchers. The pet ether extracts of both plants showed good resolution with their selected mobile systems which have been established for the first time, represented the genetic and chemical level of plants, and are a vital step in the authentication of botanicals and for medicinal purposes. So in this

Table 1 — Comparative physicochemical parameters of *L. stoechas* Linn. and *L. officinalis* Chaix

S. No	Parameters	*L. stoechas* L.			*L. officinalis* Chaix		
		Batch I	Batch II	Batch III	Batch I	Batch II	Batch III
1	Loss on drying 105 ºC (%, *w/w*)	10.20	10.21	10.22	9.00	9.70	10.4
2	Total ash (%, *w/w*)	9.85	10.50	11.00	5.00	5.30	5.50
3	Acid insoluble ash (%, *w/w*)	5.00	5.25	5.50	0.90	1.00	1.10
4	Water-soluble ash (%, *w/w*)	0.50	0.80	1.10	0.50	0.90	1.00
5	Pet ether-soluble extractive (%, *w/w*)	1.90	2.00	2.10	1.40	1.60	1.80
6	Alcohol-soluble extractive (%, *w/w*)	7.00	7.20	7.40	4.50	4.60	4.80
7	Water-soluble extractive (%, *w/w*)	13.60	13.80	14.00	10.20	10.30	10.40
8	pH of 1% aqueous solution	5.70	5.71	5.72	6.09	6.10	6.11
9	pH of 10% aqueous solution	5.34	5.35	5.37	5.72	5.74	5.75

Table 2 — Key differences observed between *L. officinalis* Chaix and *L. stoechas* Linn.

S No.	*L. officinalis* Chaix	*L. stoechas* L.
1	Flower stalk is hard and greenish.	Peduncle is soft and hairy, more number of trichomes are present and brownish in colour.
2	Central hollow pith is present in peduncle.	Centrally parenchymatous cells are present in peduncle.
3	Phloem is not well developed in peduncle.	Phloem is well distinguished in the peduncle.
4	Aroma is pleasant	Camphoric odour and bitter in taste
8	Bract is not distinguished and flowers are without stalk.	Brownish-coloured large bract is present in shortly stalked flowers.
6	Seeds are blackish and larger in size.	Seeds are yellowish brown, light weight and smaller in size.

concern, development of authentic quality control methods for herbal drugs is able to ensure the phytochemical composition and quantitative analyses of bioactive compounds and other major constituents of interest. For any drug, identity is very important in order to differentiate itself from adulterants and sustain the product quality (Fig. 5).

Conclusion

Ustukhuddoos is a medicinal plant of repute since ancient times in the traditional Unani system of medicine with characteristic therapeutic implications. It can be distinguished from its other closely related species *L. officinalis* by having unique distinguishing features. To overcome the impediment of distinguishing between these plants and ambiguous description mentioned in classical Unani literature it is substantially revealed that Ustukhuddoos is *L. stoechas* L. and specific traditional claims are vested with this only. Further it is also needed to validate the therapeutic claims scientifically with above diagnostic featured plant in future. This study will provide very significant information to establish key characters for diagnosis and authentication of the genuine plants from their substitute and adulterants along with multi-component formulations.

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Conflict of interest

All authors declare there is no conflict of interest.

Authors' contribution

J M designed and performed the study and drafted the manuscript, M R had provided all technical support, K MMA provided facility for microscopical analysis, J SS and K AA facilitated the whole experimental procedure.

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