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Full Length Research paper

Taxonomic and pharmacognostic authentication of the herbal medicine Senna *(Cassia angustifolia Vahl.).*

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The present study deals with the taxonomic and pharmacognostic authentication of the herbal medicine Senna (*Cassia angustifolia* Vahl.). This plant of considerable pharmaceutical importance, has been recommended for constipation, piles, epilepsy, respiratory diseases, skin infections, migraine and heart diseases. No studies are available on the micro, Scanning Electron Microscopy (SEM), pharmacognostic and taxonomic analysis of traded parts of *C. angustifolia* and its substitute *Cassia obtusifolia*. Hence, the present study was undertaken to authenticate genuine source of herbal drug Senna based on microscopic palynomorph, anatomical features, behavior of powdered drug with different chemical reagents and fluorescence analysis. The *C. angustifolia* is a perennial herb up to 80 cm tall with tricolporate pollen, foveolate sculpturing, paracytic stomata and single celled non-glandular trichome. It is differentiated from its substitute, *C. obtusifolia*, which is a perennial shrub up to 3.5 m tall having pollen with widely minute holes on surface, anomocytic stomata and elongated trichomes with pointed tips. The powdered drug of *C. angustifolia* is pale greenish in color, while its adulterant *C. obtusifolia* is dark green. It was concluded from this study that the knowledge of morpho-palynological, anatomical and pharmacognostical analysis may lead to authentication of herbal drugs like Senna for the purpose of employment in quality assurance of pharmaceutical products globally.

Key words: Senna, authentication, herbal drug, pharmacognosy, scanning electron microscopy (SEM).

INTRODUCTON

It is evident from the literature that 80% of the world's population is dependent on herbal drugs. Meanwhile, the limitation encountered in the use and research of herbal medicine is the lack of authentication, standardization, quality and purity of raw material. The need for in-depth and systematic investigations into indigenous drugs use cannot be overemphasized. Authenticity, purity and assay are important aspects of standardization and quality control. Adulteration of botanical medicine is an important hindrance to quality control and standardization (Shinde et al., 2009). Due to over exploitation of certain plants, habitat loss and collection for medicinal purposes, plants species are endangered or rare. These and many other factors like cost of the raw material, may cause the problem of availability of genuine drug, which encourages the adulteration of plant by substitution with inferior commercial varieties; cheaper plant are closely related vegetative parts (Sultana et al., 2011). In spite of botanical authentication, there is confusion with respect to some traditional herbal drugs like Senna, which is intentionally adulterated by its closely related species, *Cassia obtusifolia.*

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Senna is widely used for its numerous benefits. Scientifically, it is known as *Cassia angustifolia,* and belongs to family Leguminosae. There are a number of species of Senna used throughout the world for medicinal purposes. Linnaeus classified these under single species of *Cassia senna* L. Since then, there have been a number of studies which indicate that there are different variants of senna. However, the two most widely used species for medicinal purposes are *Cassia acutifolia* Delile., also known as *Alexandrian senna* and *C. angustifolia* Vabl., also known as Tinnevelly senna or Arabian senna (Khan et al., 2011). Herbal practitioners in Indo-Pak subcontinent, Arabian countries and oriental physicians prefer the use of *C. angustifolia* as herbal drug Senna, while at herbal shops in Arabian countries, India, Pakistan and other South Asian countries, there is an adulteration of broken aerial parts of closely related species *C. obtusifolia.*

C. angustifolia was first discovered growing wild in and around the ancient and blessed city of Makkah, in the heart of the old province Hijaz. The plant grows in abundance and was first used as herbal medicine by the Holy Prophet Muhammad (Peace Be Upon Him) (Ahmad et al, 2010). At herbal shops in India, Pakistan and Arabian countries, it is traded under the name of Senna or sana makkahi, and is considered to be a remedy as a cleanser of the digestive system and tonic for the entire body. The Holy Prophet Muhammad (Peace Be Upon Him) said; “If there is any remedy against death, it is Sana, the gladdened, the graceful one” (Al-Suyui, 1985). Nowadays, Senna (*C. angustifolia*) is distributed worldwide, especially in Pakistan, India, Arabian countries, Sudan, China, Kenya, Europe, Britain, etc. Senna is widely used in traditional medicine of China, Indo-Pakistan, Africa, and is also employed in Western Allopathic System of medicine (Dymock, 1972). *C. angustifolia* is commonly used for digestive disorders, constipation, tonic, depression, asthma, eczema and other skin diseases. However, due to its extensive use and trade, this herbal drug is intensively adulterated by *C. obtusifolia* and other allied species.

The ultimate objective of the present study was to use taxonomic and pharmacognostic techniques for the authentication of genuine crude drug Senna from its adulterant. Hence, the present attempt has been undertaken to authenticate genuine source of herbal drug Senna (*C. angustifolia*) based on microscopic palynomorph, anatomical features, behavior of powdered drug with different chemical reagents and fluorescence analysis.

METHODOLOGY

The traded part of Senna was procured from different herbal shops of Indo-Pak subcontinent. The fresh plant specimens of both *C. angustifolia* and *C. obtusifolia* were collected from different parts of the country and authenticated by a plant taxonomist, Prof. Dr. Mir Ajab Khan, at the herbarium of Pakistan (ISL), Quaid-i-Azam University Islamabad.

Morpho-palynological Study

Morphological investigation was based on macro and microscopic features of plant, habit, root, stem, leaf, flower and seed using binocular light microscope (Model SZF Kyowa, Japan). Morphological description was further reconfirmed through various Floras (Saldanha and Nicolson, 1976; Nasir and Ali, 1974, 1975; Hooker, 1875; Tutin and Heywood, 1972; Hooker, 1885a, 1894). For palynological studies, standard procedure of acetolysis (Erdtman, 1960; Ahmad et al., 2011) was used. For scanning electron micrographs, the methodology of Zafar et al. (2011) was adapted. Palynological description was characterized using the terminology of various authors (Barthlott, 1984; Erdtman, 1960; Ronald, 2000).

Leaf epidermal anatomy (LM and SEM)

For leaf epidermal anatomy, the modified method of Ahmad et al. (2011) was followed. The peelings of leaves were prepared for qualitative and quantitative features of adaxial and abaxial surfaces by using light microscope (Meiji-Japan). The SEM and LM microphotographs were taken using JEOL-JSM (5910) and Leica (DM-1000) light microscopes, respectively. The qualitative characteristics of the leaf epidermal anatomy was described according to Prat (1932) and Metcalfe (1960) terminology.

Pharmacognostic studies

Different pharmacognostic tests such as the fluorescence and solubility test (cold and hot) were carried out for crude herbal parts of *C. angustifolia* and its adulterant *C. obtusifolia.* For the cold method, 2 g of powdered drug was mixed in 10 ml of solvent at room temperature (25 - 30°C), while for hot method the same solution was slightly heated on a burner in a test tube. The methods of Harborne (1973), Trease and Evans (1989) and Sofowora (1993) were followed. All the reagents were of analytical grade and of Merck (Germany). For solubility and fluorescence analysis, standard procedures were adopted (Afaq et al., 1998; Abid et al., 2005). Crude herbal parts, powdered drugs and the extracts were studied under visible light ultraviolet (UV; long and short wavelength) 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

C. angustifolia sold at herbal shops under the trade name of senna or sana makki is a branched erect perennial shrub used medicinally throughout the world (Anonymous, 1992). It is a small under shrub up to 1 or 1.5 m height with variable branches and with compound pinnate leaves (Figure 1A) This species can be distinguished morphologically from its adulterant allied species *C. obtusifolia*, which is a tall shrub up to 2 or 2.5 m in height with obtuse or elliptic leaves (Figure 2A). Srivastava et al. (2006) distinguished *C. angustifolia* by

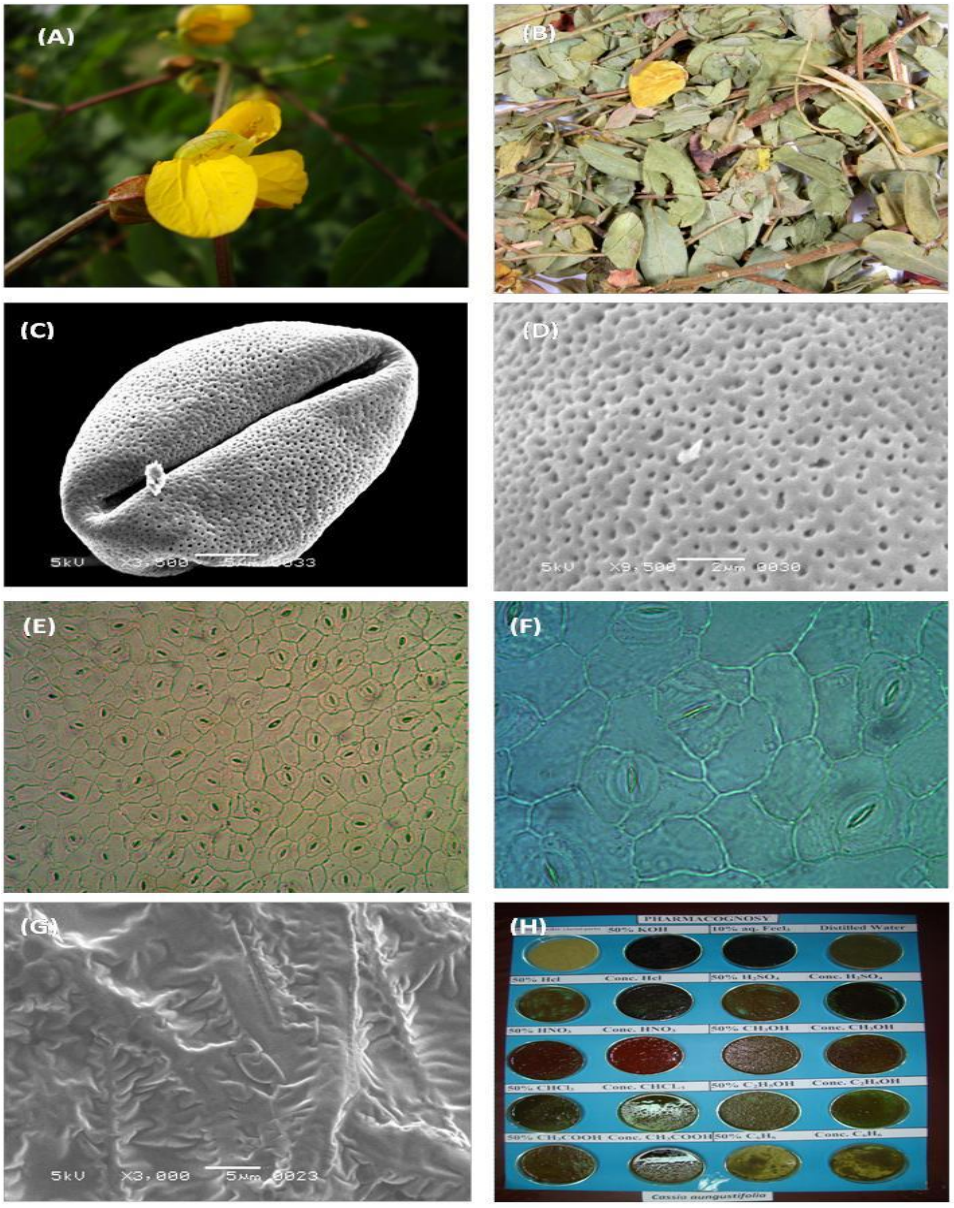

Figure 1. A *Cassia angustifolia;* (B) dried aerial parts; (C) equatorial view of pollen (SEM); (D) pollen sculpturing (SEM); (E) stomata and epidermal cells (abaxial : LM 40X); (F) stomata and epidermal cells (adaxial : LM-40X); (G) trichome and epidermal cells (SEM); (H) pharmacognostic flow chart.

the presence of greenish brown or dark brown pod of 3 – 9 cm in size, while its adulterant *C. obtusifolia* have greenish pod, which is 10 - 15 cm long. From palynological point, pollen of the *C. angustifolia* are tricolporate, which are spherical to subprolate in shape and having polar diameter 30 µM and equatorial 27.5 µM with faveolate sculpturing (Figure 1C and D) and can be distinguished from its adulterant *C. obtusifolia* by the presence of minute holes on pollen. While in *C. obtusifolia*, the pollen are subangular to spheroidal shaped, having polar and equatorial diameter of 29.5 and 15.25 µM, respectively, with widely distributed minute holes on pollen surface (Figure 2C and D).

Similarly based on foliar epidermal anatomy at microscopic level, the *C. angustifolia* can be distinguished from *C. obtusifolia* by the presence of smooth walled polyhedral epidermal cells with paracytic stomata and conical unicellular trichomes (Figure 1E to G), while *C. obtusifolia* have irregular epidermal cells with diacytic stomata and flat trichome (Figure 2E to G). The

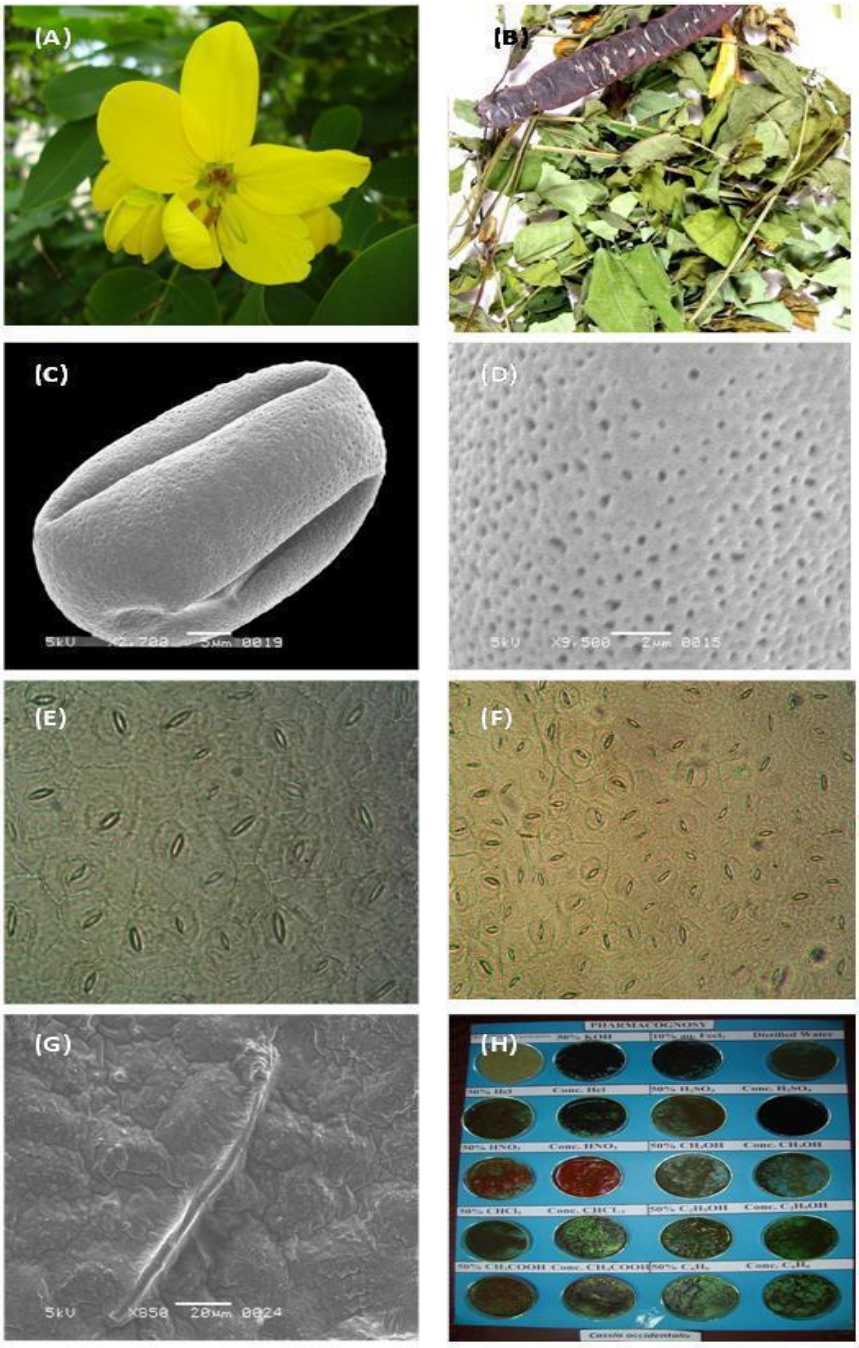

Figure 2. (A) *Cassia obtusifolia*; (B) dried aerial parts; (C) equatorial view of pollen (SEM); (D) pollen sculpturing (SEM); (E) stomata and epidermal cells (abaxial: LM 40X); (F) stomata and epidermal cells (adaxial: LM-40X); (G) trichome (SEM); (H) pharmacognostic flow chart.

results of preliminary phytochemical tests for the presence or absence of active constituents is reported herein. The behavior of powdered drug on treatment with different chemical reagents and the fluorescence analysis under ultraviolet and visible day light are shown in Tables 1 to 5. Powder drug analysis with various chemical reagents also showed positive results for secondary metabolites. Drug powder of *C. angustifolia* is pale

Table 1. Comparative characterization for differentiation of *Cassia angustifolia* and *Cassia obtusifolia*.

No.	Characters	*Cassia angustifolia* **Vahl**.	*Cassia obtusifolia* **L.**
01	Nomenclature	English Names : Tinne velly Senna, Indian Senna Local Names : Senna, Sana, Sanna Makki Trade Names: Senna, Sanna Makki	English Name : Coffee Weed, Sicklepod Local Name : Chota Amaltas Trade Name : None
02	Geographic distribution	In Pakistan; Punjab and Sindh province. In World; Pakistan (Punjab, Sind); India (Bombay, Madras); Algeria; Libya; Egypt, Sudan, Saudi Arabia. It has also been found in Kenya and Mozambique.	In Pakistan; Chakwal, Gujrat, Salt Range. In World; Central and South America.
03	Occurrence and habitat	In Pakistan, it is cultivated but very rare.	It is commonly cultivated along roads, garden and house lawns.
04	Morphology	Perennial, 60-80 cm tall, branches glabrous to subglabrous, stipules lateral, 1.6 mm long, leaf paripinnate, 5.5-10.5 cm long, Leaflets 5-9 pairs, lamina 1.2-4.5 cm long, 3.5-10 mm wide, glabrous hairy on both sides, lanceolate to ovate, tip acute. Inflorescence terminal or axillary raceme, up to 15 cm long. Young flowers with 8-9 mm long cup-shaped bracts. Pedicel 3-4 cm long. Sepals 5, subequal, 10-13 mm long, 6-9 mm broad, spoon shaped or cup shaped, light yellow in color. Petals 5, subequal, 14-17 mm long, 7-10 mm wide, obviate, stamens 10, rest perfect, 2 lower largest, ovary hairy, stipitate. Fruit 5-6 cm long, 17-23 mm broad, sparsely hairy, turning black at maturity, generally 5-10 seeded, flowering April – June (Fig. 20: A1).	An erect shrub, 0.5-3.5 m tall. Leaf stipulate, stipule 3-.8 mm long leaflets 3 pairs, 1.7-7 cm long, obovate, glabrous, rounded at the top, apex minutely acute. Racemes short, peduncle 2 mm long, bearing 1-2 flowers. Bracts linear-acute, glabrous except the edges, 3-9 mm long, pedicel 1.2-3.5 cm long, hairy. Sepals membranous, ovate, 6-7 mm long. Petals unequal in size, more or less ovate, obtuse or rounded, 2-15 mm long; Stamens 10, 3 lower longest, 4 lateral smaller, 3 upper stamens staminoidal. Ovary glabrous, stigma truncate. Pods, glabrous to subglabrous, indehiscent, 10-25 cm long, 4-8 mm wide, septate, 20-50 seeded, 5-10 mm long, albumen copious. Flowering March - September (Fig. 21: A2).
05	Palynology	Pollen monad, tricolporate, shape in polar circular view, polar diameter 30 µM (27.5-32.5 µM), polar length 35 µM (32.5-37.5 µM), shape in equatorial view subprolate, equatorial diameter 27.5 µM (25-30 µM), equatorial length 38.75 µM (37.5-40 µM), P/E ratio 1.09, exine thickness 2.13 µM (1.25-5 µM), length of colpi 4.25 µM (2.5-6.25 µM) and width 8.75 µM (7.5-10 µM), sculpturing faveolate with holes or depressions which are evenly distributed over the surface. These holes are minute and variable in size (Fig. 20: C1 & D1).	Pollen monad, tricolporate, shape in polar view subangular, polar diameter 30 µM (27.5-32.5 µM), polar length 33.75 µM (32.5-35 µM), shape in equatorial view spheroidal, diameter 15.25 µM (10-30 µM), equatorial length 32.5 µM (30-35 µM), P/E ratio 1.9, exine thickness 3.12 µM (2.5-3.75 µM), length colpi 5.62 µM (5-6.25 µM) and width of colpi 6.87 µM (6.25-7.5 µM) (Fig. 21: C2 & D2).
06	Leaf epidermal	**Abaxial surface:** Ordinary epidermal cells of various shapes, length of ordinary epidermal cell 30 µM (22.5-37.5 µM), width 17.5 µM (15-20 µM), stomata irregularly oriented, paracytic, length 16.87 µM (16.25-17.5 µM), width 8.75 µM (7.5-10 µM), length of guard cell 21.25 µM (20-22.5 µM), width 15 µM (12.5-17.5 µM), stomatal complex: 21.5 µM (19.5-23.5 µM) long, 22.75 µM (20-23.5 µM) wide. Subsidary cell 28.5 µM (22-35 µM) long, and 19.5 µM (15-24 µM) wide. Trichomes unicellular and pointed at the tip, length 121.25 µM (87.5-155 µM), width 17.5µM (15-20µM) (Fig. 20: E1).	**Abaxial surface:** Epidermal cells variously shaped, length 29µM (22.5-35.5 µM), width 18.5 µM (16-21 µM), stomata length 18.25 µM (17-19.5 µM), width 14 µM (12-16 µM), length of guard cells 17 µM (16-18 µM), width of guard cells 3.25 µM (3-5.5 µM), stomatal complex: 20.8 µM (20-21.5 µM) long, and 23.8 µM (23-25 µM) wide, subsidary cells: 20.5 µM (16-25.5 µM) long, 15.5 µM (12-18 µM) wide (Fig. 21: E2). **Adaxial surface:** Epidermis cells narrowly elongated and tetragonal or variously shaped. Ordinary epidermal cell, length 33.33 µM (27.5-42.5

Table 1. Contd.

	anatomy	**Adaxial surface:** Ordinary epidermal cells pentagonal, hexagonal or various shaped, mostly narrow elongated cells with very thin smooth walls. Length of ordinary epidermal cells 41.25 µM (32.5-50 µM), width 30 µM (25-35 µM), stomata irregularly oriented and much abundant, paracytic, length 16.25 µM (15-17.5 µM), width 7.5 µM (6.25-8.75 µM), length of guard cell 21.25 µM (20-22.5 µM), width 14.37 µM (12.5-17.5 µM), stomatal complex: 22.33 µM (21.5-23.5 µM) long, 24.3 µM (23.5-25 µM) wide, subsidary cell 32.75 µM (25-40.5 µM) long, 24.5 µM (19.5-29 µM) wide, glands surrounded by six cells and six spikes are protruding out. Trichome single celled, non glandular, hair like structures attached at base, tapering at the tip, by 6-7 cells, length 343.5 µM (325-362 µM), width of larger trichomes 23.75 µM (20-27.5 µM) (Fig. 20: F1).	µM), width 22.5 µM (20-25 µM), anomocytic type of stomata, uniformly distributed over the surface, length 20.9 µM (20-22.5 µM), width 17.5 µM (15-20 µM), length 20 µM (19-21 µM), width 5.83 µM (5-7.5 µM), stomatal complex: 20.8 µM (20-21.5 µM) long, and 23.8 µM (23-25 µM) wide, subsidary cell 23.5 µM (18-28 µM) long, 18 µM (14-20.5 µM) wide. Long trichomes pointed at tip. Length of trichome 122.6 µM (95.5-141.8 µM) and width 15.6 µM (8.9-20.5 µM) (Fig. 21: F2).
and 07	Trade part Status	Leaves and aerial parts are traded commonly at herbal shops throughout the country, under the name of Sana makki.	Leaves and aerial parts are generally mixed in genuine drug Sana Makki.
08	Organoleptgraphy	In dried herbal drug leaves, branches and flowers are mixed. Aerial parts odorless and good in taste. Branches brown in color, size 0.6-1.5 cm, branches hard, narrow, pointed and have rough surface. Leaves green in color, irregular margins and have rough texture. The size of leaves ranges from 0.8-2.5 cm. Flowers yellowish brown in color. Flowers have delicate surface and 0.5-0.7cm in length (Fig. 20: B1).	Flowers, branches and leaves mixed in dried herbal drug. The aerial parts odorless and has herbal taste. Branches cylindrical and greenish brown in color. The length of branches 12.2 cm-22 cm. Branches hard and narrow, diameter of branches is 0.8-1.9 cm. Leaves are green in color and broad. The size of leaves 1-1.5 cm in length. Pods also mixed with aerial parts. Pods black brown in color, segmented 7cm-11cm in size(length). Flowers yellow in color (Fig. 21: B2).
9	Part use	Leaves and aerial parts	Leaves, pods, flowers, aerial parts
10	Medicinal uses	Constipation, digestive disorder, piles, migraine, epilepsy, heart diseases, respiratory diseases, skin diseases.	Constipation, digestive disorder
11	Indigenous herbal recipes	Dried leaves are ground to obtain powder. 2 g of powdered leaves are mixed with equal amount of quality powdered rose petals. 5-10 g of this mixed powder is given with water to patients for 5 days regularly at night to cure constipation, digestive disorders and respiratory diseases. In 2-3 g powdered leaves, mix with equal amount of *Plantago ovata* husk. Take 5-8 g of this powder with glass of milk at night for 10-20 days to cure piles. In 2-5 g powdered leaves of Senna, mix equal amount of pure honey to make tablets. Take one tablet with glass of fresh water at night for a month to treat migraine, heart diseases and epilepsy.	Dried leaves and flowers are used medicinally for constipation and digestive disorder. According to women at Khyber Pukhton Khawa and Punjab Provinces. Dried leaves and pods are ground to obtain powder. 1-2 g of this powder is given with water to patients suffering from constipation and stomach disorders.
12	Toxicity	Non toxic	Non toxic

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

S/N	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 Powdered	Dull muddy green	Green	Green	-	-	-
2	Powdered drug+50% KOH	Pine forest	Black	Brownish black	Yellow	Yellow	Partially soluble
3	Powdered drug+10% aq. $Fecl_3$	Pine forest	Dark green	Dark green	Dark brown	Chocolate brown	Partially soluble
4	Powdered drug + Distilled H_2O	Leaf green	Greenish brown	Greenish brown	White	Whitish pink	Partially soluble
5	Powdered drug + HCL Conc.	Golden glimmer	Black	Greenish black	Pink	Pinkish brown	Partially soluble
6	Powdered drug + HCL 50%	Golden glimmer	Reddish brown	Red oxide	Yellow	Pinkish yellow	Partially soluble
7	Powdered drug+H_2SO_4 Conc.	Dark red	Reddish brown	Brown	Brownish white	Whitish brown	Partially soluble
8	Powdered drug + H_2SO_4 50%	Red oxide	Black	Reddish black	Brown	Brown	Partially soluble
9	Powdered drug+HNO_3 Conc.	Red oxide	Black	Reddish black	Brown	Chocolate brown	Soluble
10	Powdered drug+HNO_3 50%	Golden brown	Reddish brown	Reddish brown	Yellow	Yellowish brown	Partially soluble
11	Powdered drug + Conc. CH_3OH	Leaf green	Dark green	Reddish brown	Yellow	Yellow	Soluble
12	Powdered drug + CH_3OH 50%	Golden glimmer	Pine forest	Leaf green	Pink	Pink	Partially soluble
13	Powdered drug + Conc. $CHCl_3$	Golden glimmer	Leaf green	Spring green	Pink	Pinkish white	Soluble
14	Powdered drug + $CHCl_3$ 50%	Copper	Dark brown	Reddish brown	Pinkish yellow	Pinkish yellow	Soluble
15	Powdered drug + Conc. C_2H_5OH	Golden glimmer	Fresh green	Spring green	Glowing white	Shiny white	Soluble
16	Powdered drug + C_2H_5OH 50%	Dull green	Leaf green	Spring green	Yellow	Yellow	Partially soluble
17	Powdered drug + Conc. CH_3COOH	Golden glimmer	Dark green	Leaf green	Pink	Pink	Partially soluble
18	Powdered drug+CH_3COOH 50%	Golden glimmer	Green	Dark green	Yellow	Yellow	Partially soluble
19	Powdered drug + Conc. C_6H_6	Golden glimmer	Leaf green	Reddish green	Pink	Pink	Soluble
20	Powdered drug+C_6H_6 50%	Golden glimmer	Leaf green	Black	White	Whitish yellow	Partially soluble

Table 3. Fluorescence analysis and solubility tests (Hot method) of powdered drug of Cassia angustifolia in various solvents.

S/N	Treatments	Under visible light	Under short wavelength (UV) 254 nm	Under long wave length (UV) 365 nm	Solubility analysis
1	Powdered drug + 50% KOH	Brownish black	Reddish brown	Reddish brown	Soluble
2	Powdered drug+10% aq. $Fecl_3$	Brownish green	Dark green	Dark green	Partially soluble
3	Powdered drug + Distilled H_2O	Redo green	Dark green	Pine forest	Partially soluble
4	Powdered drug + HCL Conc.	Brownish black	Black	Black	Partially soluble
5	Powdered drug + HCL 50%	Golden glimmer	Dark brown	Chocolate brown	Partially soluble
6	Powdered drug + H_2SO_4 Conc.	Reddish black	Black	Black	Partially soluble
7	Powdered drug + H_2SO_4 50%	Blackish brown	Black	Black	Partially soluble
8	Powdered drug + HNO_3 Conc.	Red orange	Dark red	Dark red	Soluble
9	Powdered drug + HNO_3 50%	Orange	Dark brown	Dark brown	Partially soluble

Table 3. Continued.

10	Powdered drug + Conc. CH_3OH	Pine forest	Dark green	Dark green	Partially soluble
11	Powdered drug + CH_3OH 50%	Mustard	Leaf green	Dark green	Soluble
12	Powdered drug + Conc. $CHCl_3$	Gold dust	Leaf green	Spring green	Soluble
13	Powdered drug + $CHCl_3$ 50%	Red oxide	Dark brown	Reddish brown	Partially soluble
14	Powdered drug + Conc. C_2H_5OH	Golden glimmer	Dark green	Reddish green	Soluble
15	Powdered drug + C_2H_5OH 50%	Lemon color	Leaf green	Light green	Insoluble
16	Powdered drug + Conc. CH_3COOH	Golden glimmer	Reddish green	Reddish green	Partially soluble
17	Powdered drug + CH_3COOH 50%	Fresh orange	Dark green	Dark green	Partially soluble
18	Powdered drug + Conc. C_6H_6	Gold dust	Leaf green	Red	Partially soluble
19	Powdered drug+C_6H_6 50%	Golden glimmer	Leaf green	Dark brown	Soluble

Table 4. Fluorescence analysis and solubility tests (Cold method) of powdered drug of Cassia obtusifolia in various solvents.

S/N	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 Powdered	Fresh green	Green	Buckingham green	-	-	-
2	Powdered drug + 50% KOH	Mustard green	Black	Blackish brown	Pink	Purple blue	Partially soluble
3	Powdered drug + 10% aq. $FeCl_3$	Dark brown	Black	Black	Pale brown	Dark brown	Partially soluble
4	Powdered drug + Distilled H_2O	Light green	Light grey	Grayish white	Light blue	Pink with white outline	Insoluble
5	Powdered drug + HCL Conc.	Leaf green	Dark green	Dark green	Pink	Light purple	Partially soluble
6	Powdered drug + HCL 50%	Golden glimmer	Pine forest	Pine forest	Purple	Light pink	Partially soluble
7	Powdered drug+ H_2SO_4 Conc.	Pink	Dark green	Buckingham green	Black	Blackish brown	Partially soluble
8	Powdered drug + H_2SO_4 50%	Reddish black	Black	Blackish brown	Black	Chocolate brown	Insoluble
9	Powdered drug + HNO_3 Conc.	Reddish brown	Dark brown	Dark brown	Light brown	Brownish purple	Partially soluble
10	Powdered drug+ HNO_3 50%	Golden glimmer	Leaf green	Golden glimmer	Light brown	Woody brown	Partially soluble
11	Powdered drug + Conc. CH_3OH	Spring green	Brown green	Reddish green	Light purple	Fresh pink	Soluble
12	Powdered drug+CH_3OH 50%	Pale green	Dark green	Leaf green	Brownish green	Pinkish green	Partially soluble
13	Powdered drug + Conc. $CHCl_3$	Pine forest	Dark green	Reddish brown	Bluish brown	Brown	Soluble
14	Powdered drug + $CHCl_3$ 50%	Spring green	Algal green	Dark green	Dark green	Green	Soluble
15	Powdered drug + Conc. C_2H_5OH	Spring green	Fresh green	Reddish green	Dull brown	Soil brown	Soluble
16	Powdered drug + C_2H_5OH 50%	Pale green	Leaf green	Lemon green	Pink	Purple	Partially soluble
17	Powdered drug + Conc. CH_3COOH	Leaf green	Reddish brown	Reddish brown	Bluish brown	Pink flash	Partially soluble
18	Powdered drug + CH_3COOH 50%	Leaf green	Leaf green	Redo green	Light blue	Pinkish brown	Partially soluble
19	Powdered drug + Conc. C_6H_6	Pine forest	Leaf green	Reddish green	Pinkish blue	Light pink	Soluble
20	Powdered drug + C_6H_6 50%	Light green	Leaf green	Spring green	Brown with blue shade	Pinkish brown	Partially soluble

Table 5. Fluorescence analysis and solubility tests (Hot method) of powdered drug of Cassia obtusifolia in various solvents.

S/N	Treatments	Under visible light	Under short wavelength (UV) 254 nm	Under long wave length (UV) 365 nm	Solubility analysis
1	Powdered drug + 50% KOH	Greenish black	Black redo green	Fresh green Reddish black	Soluble
2	Powdered drug + 10% aq. $FeCl_3$	Orange brown	Black	Greenish black	Soluble
3	Powdered drug + Distilled H_2O	Light green	Golden glimmer	Golden glimmer	Partially soluble
4	Powdered drug + HCL Conc.	Brownish green	Brown	Blackish brown	Partially soluble
5	Powdered drug + HCL 50%	Brownish green	Black	Dark brown	Partially soluble
6	Powdered drug + H_2SO_4 Conc.	Black	Black	Black	Insoluble
7	Powdered drug + H_2SO_4 50%	Black	Black	Black	Insoluble
8	Powdered drug + HNO_3 Conc.	Orange	Dark brown	Dark brown	Soluble
9	Powdered drug + HNO_3 50%	Light brown	Dark green	Golden glimmer	Soluble
10	Powdered drug + Conc. CH_3OH	Buckingham green	Spinach green	Red	Partially soluble
11	Powdered drug + CH_3OH 50%	Light green	Brownish green	Golden green	Soluble
12	Powdered drug + Conc. $CHCl_3$	Redo green	Fresh green	Red green	Soluble
13	Powdered drug + $CHCl_3$ 50%	Leaf green	Dull green	Brown green	Partially soluble
14	Powdered drug + Conc. C_2H_5OH	Fresh green	Spring green	Reddish green	Soluble
15	Powdered drug + C_2H_5OH 50%	Leaf green	Algal green	Pine forest	Insoluble
16	Powdered drug + Conc. CH_3COOH	Leaf green	Reddish brown	Reddish brown	Partially soluble
17	Powdered drug + CH_3COOH 50%	Redo green	Pine forest	Pine forest	Partially soluble
18	Powdered drug + Conc. C_6H_6	Spring green	Dark green	Red	Partially soluble
19	Powdered drug+C_6H_6 50%	Leaf green	Brown	Brownish green	Soluble

greenish in day light and showed various shades under UV light.

This is the first report of the pharmacognostic standardization of powder drug *C. angustifolia* in comparison with its adulterant *C. obtusifolia.* Similar phytochemical standardization of herbal drug such as *Mimosa pudica* L. and *Biophytum sensitivum* DC. was carried out by Saritha and Brindha (2008). From such type of studies, it is clear that the knowledge of morpho-palynological, anatomical and preliminary phytochemical characters used in pharmacognostic investigation is important in the identification and delimitation of taxa like *C. angustifolia* for the purpose of employment in pharmaceutical preparations.

Conclusion

The present work focuses on the taxonomic and pharmacognostic authentication of the herbal drug Senna marketed in Indo-Pak subcontinent and other parts of the world, with its common adulterant *C. obtusifolia* which is a medico botanically different species. Since there is no such authentication screening on record for this much valued Senna drug, the present work was aimed at establishing standards that could be used in deciding the genuineness of the aforementioned drug from its closely related adulterant. The colored macro and micro photographs of plant, drug part, pollen, foliar epidermis and pharmacognostic tests might facilitate the researchers for correct identification and pharmaceutical industries in providing safe and authentic drug of genuine source.

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