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Pharmacognostic studies of aerial parts of *Colebrookea oppositifolia* Sm.

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Abstract

Colebrookea oppositifolia Sm., a well-known traditional medicinal plant, belongs to the family, Lamiaceae. The research work is about the pharmacognostic characterization of *C. oppositifolia* which includes; macro and microscopic evaluation, phytochemical and physicochemical properties of leaf, stem and inflorescence. Transverse section (T.S.) of leaf and stem showed the arrangement of the different cells, *i.e.* vascular bundle, collenchyma, parenchyma, cortex, cambium, pith, *etc.* respectively. Histochemistry of T.S. of leaf and stem gave positive results with conc. HCl, phloroglucinol, ferric chloride and Sudan III which indicated the presence of Ca^{+2} oxalate crystals, lignin, tannins and volatile oils, respectively. Powder study along with histochemical analysis of leaf showed the presence of glandular trichomes, lignified fibres, spiral vessels and lignified tracheids while, stem and inflorescence powder showed collenchyma, parenchyma, covering trichomes, papillose cells, uniseriate tapering trichomes, round pollen grains and corolla. In fluorescence analysis, different colors were observed under different lights. Phytochemical analysis of MeOH extract indicated the presence of alkaloids, glycosides, flavonoids, sterols, triterpenoids and tannins. Physicochemical analysis, *i.e.* ash values and extractive values were performed. These results will help in identification and quality control of *C. oppositifolia* medicinal material.

Key words: *Colebrookea oppositifolia*, histochemical analysis, pharmacognostic, phytochemical

1. Introduction

Plants are widely used as raw ingredients for many preparations in conventional medicine system. To confirm the genuineness of the raw ingredients and to detect the presence of adulterant stains. Comprehensive and detailed pharmacognostic evaluations are required for each raw drug. Usually these raw drugs were collected by the traditional workers who were engaged in herbal, ayurvedic or any other system of complementary system of medicine. Their identification is commonly based on macroscopic structural features or other unique visible characteristics. Therefore, in such manual practices there is a chance of accidental collection of improper or wrong plant material. Therefore, an extensive anatomical, physicochemical and phytochemical screening was required that is helpful to avoid any ambiguity (Vaibhav and Kamlesh, 2007). Anatomical findings were supportive in elucidation of a unique drug with a major focus on quantitative microscopy, such as starch grains, Ca^{+2}oxalate crystals, stomatal index and trichomes and qualitative microscopy, such as arrangement of vascular bundles, *i.e.* xylem and phloem tissues and other tissues (Brinda *et al*., 2000).

Colebrookea is a genus of plants in the Lamiaceae, first described in 1806. It contains only one known species, *C. oppositifolia* Sm. The plant possesses antimicrobial, antifungal and antioxidant attributes due to high content of flavonoids and polyphenols. It is used for treating sore eyes, corneal opacity or conjunctivitis due to its anti-inflammatory effects (Torri, 2012; Sandhu *et al*., 2011). The plant material is generally used to cure the diseases like epilepsy, fever, headache, and urinary problems. It possess hepatoprotective, cardioprotective and anti-inflammatory attributes. The essential oil of *Colebrookea* possesses fungitoxic property (Holley and Cherla, 1998; Sharma *et al*., 2013). *Colebrookea* has anthelmintic properties which is used in the management of ringworms and it is also employed in the treatment of dermatitis, nose bleeds, bleeding, bloody coughs and dysentery (Venkateshappa and Sreenath, 2013).

Despite, a lot of therapeutic uses were ascribed to this plant material, there is not any comprehensive pharmacognostical information available on structural morphology and other physicochemical standards, generally needed for the quality control for the plant. Therefore, the present research comprises of anatomical and structural evaluations, assessment of physicochemical parameters and phytochemical nature of the *C. oppositifolia*.

2. Materials and Methods

2.1 Plant material

The plant was collected from Botanical garden of Government College University, Lahore and was authenticated by Miss Uzma Hanif, Lecturer, Department of Botany, Government College University, Lahore Pakistan, based on authenticity established by Gamble, Benthum and Hooker. A specimen of plant was deposited in herbarium of Government College University, Lahore under Voucher Specimen No: GC. Herb. Bot. 2941. Aerial parts of the plants, *i.e.* leaves, stem and inflorescences were separated. All plant parts were dried under shade, were powdered and preserved in brown containers in dry place.

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2.2 Chemicals and instruments

Analytical grade alcohol, methanol, chloroform, *n*-hexane, ethyl acetate, glacial acetic acid, sulphuric acid, hydrochloric acid, ferric chloride, lead acetate, phloroglucinol, safranin, fast green, Sudan III, glycerin were used in the present study. All the chemicals were purchased from Sigma Aldrich, Germany. Distilled water and chemical reagents: (distilled water and all the reagents were prepared in the laboratory during histochemical and phytochemical evaluation): Wagner's reagent, Dragendroff's reagent, Millon's reagent, Molisch's reagent. Microscope (Labomed, U.K.) and Canon Powershot SX 220HS Japan.

2.3 Macroscopic evaluation

All macroscopic evaluations of leaf, stem and inflorescence were carried out on 5 samples of each part. The taxonomical description was made according to the related articles and the data given in different books (Kokate *et al*., 2006).

2.4 Microscopic evaluation

2.4.1 *Transverse section cutting*

Fresh leaf and stem were immediately fixed in formalin: acetic acid:70% alcohol (5:5:90) for 24 h. T.S. of leaf and stem were made by commonly used blade and razor method. Sections were stained with safranin and fast green dye (Sylevester and Ruzin, 1994).

2.4.2 *Histochemistry of* T.S.

The sections of leaf and stem were treated with various chemicals to note the chemical reaction and color change. Inferences were made to evaluate the particular reaction for histochemical analysis in the plant tissues accordingly. Phloroglucinol, Conc. HCl, iodine solution, ferric chloride and Sudan III solution were used to locate the presence of Ca^{+2} oxalate crystals, lignin, starch, tannins and oil globules, respectively. After treatment with particular reagents, the sections were observed under light microscope and their photographs were taken using digital camera. (Christodoulakis *et al.*, 2015).

2.4.3 *Powder study*

Chloral hydrate (75%) solution was used as clearing reagent prior to observation of powder drug using microscope. Slides of powdered leaf, stem and inflorescence were prepared according to the prescribed procedures. Photographs were taken, using microscope by means of digital camera (Upton *et al.,* 2016).

2.4.4 *Histochemistry of powder*

The prepared slides of powdered leaf, stem and inflorescence were treated with chloral hydrate, phloroglucinol, HCl and safranin reagents to view and stain the elements. The slides were seen under microscope and were photographed (Biggs, 1985).

2.5 *Fluorescence analysis*

U.V. fluorescence analysis of powdered leaf, stem and inflorescence was carried out by treating them with different reagents and was observed in ordinary light and U.V. light (short wavelength of 254 nm and long wavelength of 366 nm) (Schoor *et al.,* 2015).

2.6 Phytochemical analysis

The phytochemical analysis was carried out according to the standard procedures (Kumar *et al.,* 2016).

2.7 Physicochemical analysis

Powdered samples were subjected to physicochemical analysis for their extractive values along with total ash, water soluble ash, and acid insoluble ash (Evans, 1989; Harborne, 1998).

2.8 Statistical analysis

The simple statistical analysis was carried out for calculating the mean and the standard error of mean.

3. Results

3.1 Macroscopic characters

Plants is 1-3 m tall, with pale hairy stout square branches (Figure 1). Petiole - (0.8-2.5 cm), leaf blade - (10-20 × 3-7 cm), base - broadly cuneate to rounded, margin - crenulate-serrulate, apex - long acuminate, adaxially rugulose and puberulent, abaxially densely-tomentose to lanate-tomentose. Numerous tiny white flowers occur in panicles of upright spikes - (10-15 cm long) branches - (4-7 cm); verticillasters - 10-18 flowered, globose; bracteoles - (1 mm), densely tomentose outside, glabrous inside. Flowers - (2 mm), pistillate: calyx campanulate - (1.5 mm-6 mm) in fruit, tube very short, visibly ribbed; teeth subulate, later spinescent, ± purple. Corolla tube puberulent, lower lip slightly longer than upper lip, with middle lobe ovate. Stamens inserted on apical part of tube, included. Style 2 × as long as corolla. In bisexual flowers: calyx minute - (0.6 mm), corolla (3 mm); upper lip ovate-oblong - (0.5 mm), straight, emarginate; lower lip elongated, spreading - (1.5 mm), middle lobe ovate-oblong, 2 × as long as ovate lateral lobes. Style erect, slightly longer than corolla. Nutlets are obovoid - (1 mm), yellow-brown in color, with a small basal white scar (Table 1). Flowering period ranges from January-March, through March-April (Madhavan *et at.,* 2011).

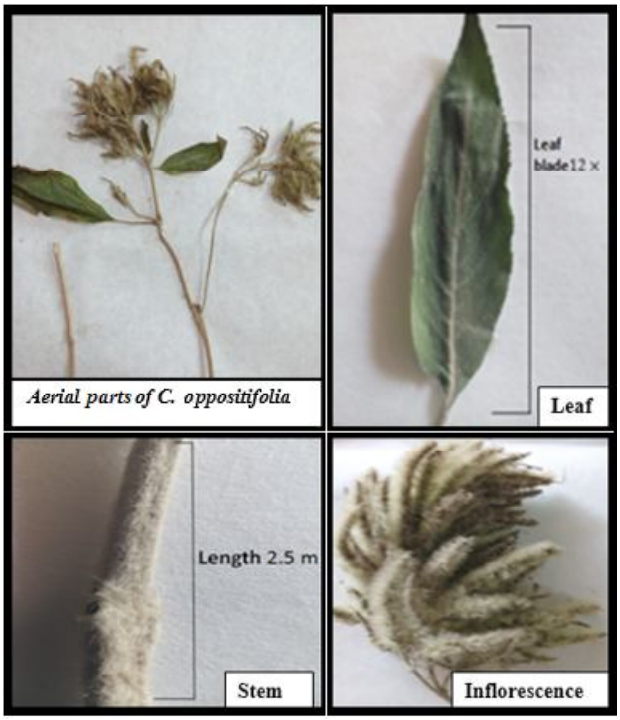

Figure 1: Macroscopic characters of *C. oppositifolia*

Table 1: Macroscopic characters of *C. oppositifolia*

Plant Part	Features	Observations
Leaf	Leaf blade	(10-20 × 3-7 cm)
	Base	Broadly cuneate to rounded
	Margin	Crenulate-serrulate
	Apex	Long acuminate, adaxially rugulose and puberulent, abaxially densely-tomentose to lanate-tomentose
	Color	Dirty dark green
	Texture	Hairy
	Fracture	Crisp
Stem	Height	1-3 m
	Color	Dust brown
	Texture	Glabrous
	Fracture	Woody and fibrous
Inflorescence	Appearance	Panicles of upright spikes
	Color	White
	Length of flowering branch	4-7 cm
	Size	Tiny (2 mm)
	Bracteoles	Present (1 mm)
	Pistillate	Yes
	Calyx	Campanulate
	Corolla	Puberulent
	Stamens	Inserted on apical part
	Style	Erect and long
	Flowering period	January-March, through March-April.

3.2 Microscopic characters

3.2.1 *Transverse section*

T.S. of leaf showing; vascular bundles with xylem and phloem in a lunar shape, ground tissue and collenchymas, trichomes (uniseriate, multicellular), epidermis, cuticle (Figure 2).

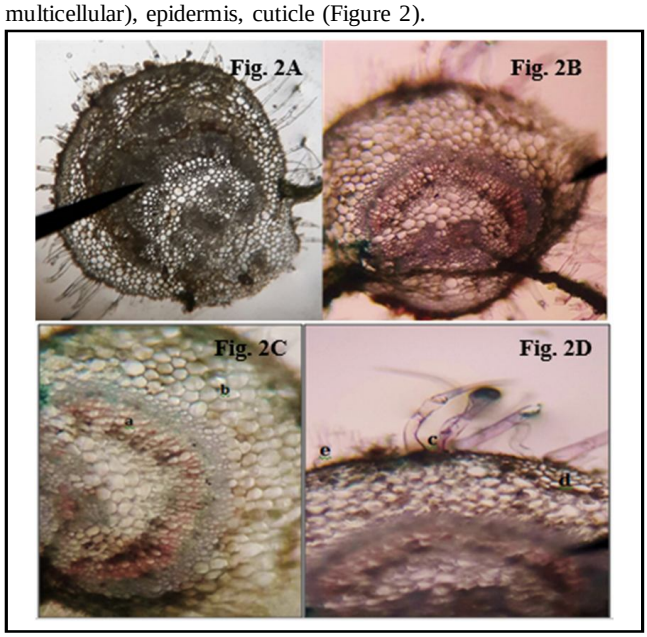

Figure 2 (A,B,C,D): Microscopy of the leaf of *C. oppositifolia*. (A) T.S. of leaf before staining, (B) T.S. of leaf after staining, (C) T.S. of leaf showing: a) vascular bundles with xylem and phloem in a lunar shape, b) ground tissue and collenchyma, (D) c) Trichomes (uniseriate, multicellular), d) epidermis, e) cuticle.

T.S. of stem shows; raphides, druses and Ca^{+2}oxalate crystals along with cellular content and oil globules in collenchyma of hypodermis, sclerenchyma. Vascular bundles were visible showing xylem and phloem tissue. Cambium and sclerenchyma was also observed, pith, ground tissue and parenchymatous tissue with medullary rays of primary parenchymatous tissue were also seen (Figure 3).

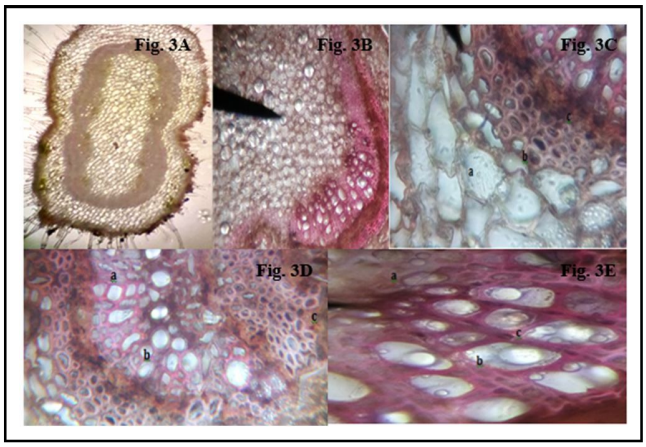

Figure 3 (A, B, C, D, E): Microscopy of the stem of *C. oppositifolia*. (A) T.S. of stem before staining, (B) T.S. of stem after staining, (C) T.S. of stem where: a) raphides and druses and Ca^{+2}oxalate crystals along with cellular content and oil globules in collenchyma of hypodermis, b) sclerenchyma, c) vascular bundles showing xylem and phloem, (D) T.S. of stem where: a) cambium and sclerenchyma between cortex and vascular bundles, b) vascular bundles showing primary and secondary xylem, c) pith and parenchymatous tissue, (E) T.S. of stem where: a) pith and ground tissue, b) oil globules in collenchyma: c) vascular bundles and medullary rays of primary parenchymatous tissue.

3.2.2 *Histochemical analysis of* T.S.

Histochemistry of leaf shows the presence of Ca^{+2} oxalate crystals, tannins, volatile oils and lignins (Figure 4).

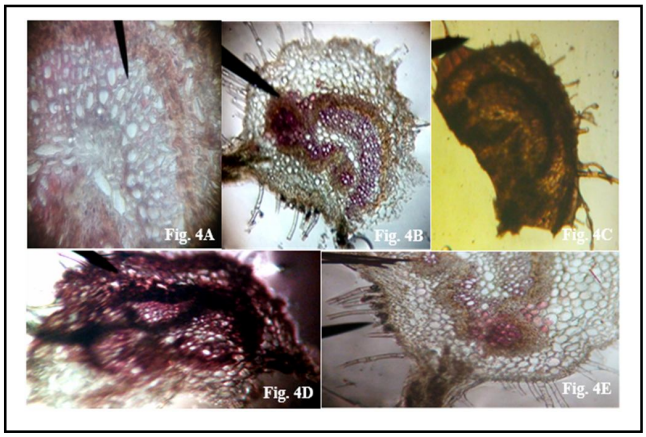

Figure 4 (A, B, C, D, E) : Histochemical analysis of powder of leaf of *C. oppositifolia*. (A) Conc. HCl gave effervescence with Ca^{+2}-oxalate crystals, (B) A magenta coloration in the vascular bundle indicated positive result, (C) Iodine solution did not give blue color to the starch granules, (D) Ferric chloride turned the tannins black giving a +ve result, (E) Sudan III gave +ve result and turned the oil globules red.

Histochemistry of stem shows the presence of Ca^{+2} oxalate crystals, tannins, volatile oils and lignins (Figure 5).

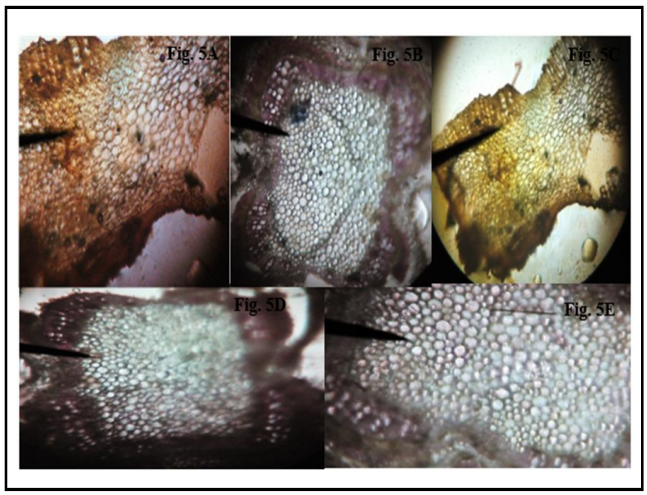

Figure 5 (A, B, C, D, E): Histochemical analysis of powder of stem of *C. oppositifolia*. (A) Conc. HCl gave effervescence with Ca^{+2}-oxalate crystals, (B) A magenta coloration in the vascular bundle indicated positive result for lignins, (C) Iodine solution did not give positive result to the starch granules, (D) Ferric chloride turned the tannins black giving a +ve result, (E) Sudan III gave +ve result and turned the oil globules red.

3.2.3 *Powder microscopy*

The powder microscopy of leaf shows; glandular trichomes with spiral vessels. The lignified spongy mesophyll tissue and along with tracheid vessels and parenchyma tissues of upper epidermis was also observed (Figure 6).

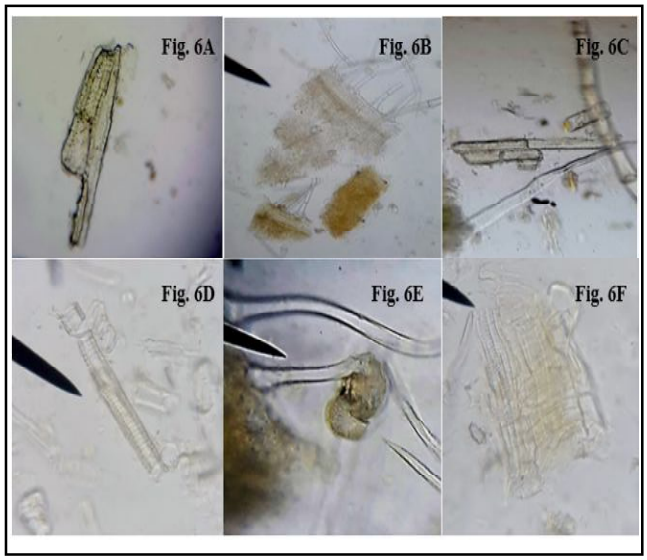

Figure 6 (A, B, C, D, E, F): Powder microscopy of the leaf of *C. oppositifolia*. (A) Lignified fibres with moderately thickened and pitted walls, (B) Capitate glandular trichomes in surface view and part of underlying palisade, (C) Lignified fibro-vascular structures in spongy mesophyll, (D) Fragment of lignified vessel with spiral thickening, (E) Glandular trichome with unicellular stalk and radiate bladder-like head with common cuticle, (F) Tracheid vessel and parecnchyma of upper epidermis.

The powder microscopy of stem shows; trichomes, lignified vessels, paranchyma and collenchyma tissues (Figure 7).

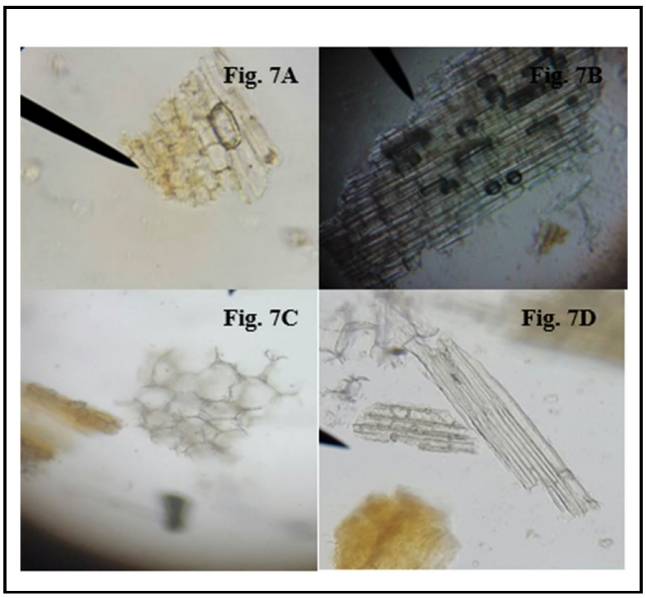

Figure 7 (A, B, C, D): Powder microscopy of the stem of *C. oppositifolia*. (A) Large cells with slightly thickened walls of parenchyma from pith, (B) Thin-walled, longitudinally elongated cells with occasional and scattered covering trichome base in epidermis (surface view), **(**C) Surface view of collenchyma in hypodermis, (D) Group of lignified vessels along with underlying parenchyma.

The powder microscopy of inflorescence revealed the presence of tapering and glandular trichomes and tracheids. There was sinous walled cells in outter epidermis along with papillose straight walled cells (Figure 8).

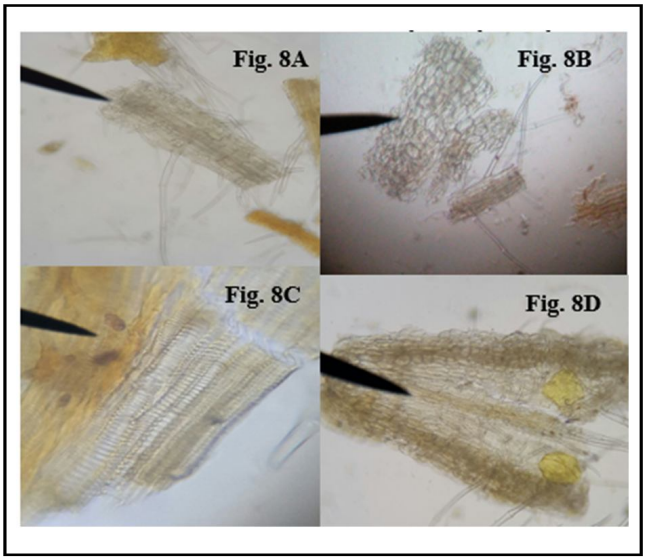

Figure 8 (A, B, C, D): Powder microscopy of the inflorescence of *C. oppositifolia*. (A) Uniseriate tapering trichomes of calyx, (B) Papilllose cells of inner epidermis (corolla) with pinkish purple pigment and wavy walls, (C) Tracheids and vessels with spiral thickening and small celled parenchyma, (D) Corolla outer epidermis with sinous walled cells and inner epidermis with papillose straight-walled cells, and glandular trichomes.

3.2.4 *Histochemical analysis of powder*

Histochemistry of powdered leaf shows venation in palisade mesophylls in hypodermis, lignified fibres, parenchyma and collenchyma tissues (Figure 9).

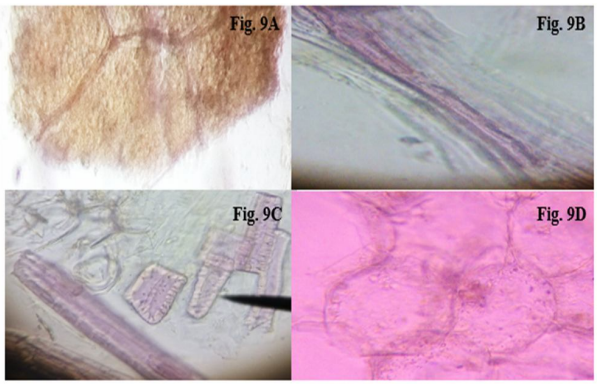

Figure 9 (A, B, C, D): Histochemical analysis of powder of leaf of *C.oppositifolia*. **(**A) Vein terminations in palisade with underlying hypodermis, (B) Large-celled, lignified and pitted parenchyma, (C) Group of lignified fibres with pitted walls, (D) Collenchyma in upper epidermis with underlying hypodermis showing lignification.

The histochemistry of powdered stem shows lignified vessels, glandular and conical covering trichomes and tracheids (Figure 10).

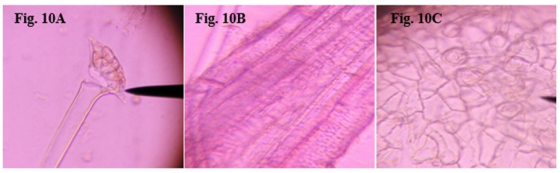

Figure 10 (A, B, C): Histochemical analysis of powder of stem of *C.oppositifolia*. (A) Glandular trichome with multiple thin-walled cells radiating with a common cuticle to form spherical head, (B) Lignified vessels and tracheids form pith, (C) Short conical covering trichomes with swollen base.

The histochemistry of inflorescence shows papillose cells and glandular trichomes (Figure 11).

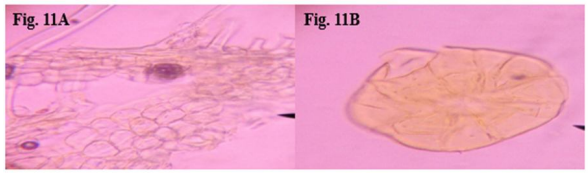

Figure 11 (A, B): Histochemical analysis of powder of inflorescence of *C. oppositifolia*. (A) Inner epidermis of corolla with papillose cells, (B) Multicellular base of glandular trichome.

3.3 Fluorescence analysis

The fluorescence analysis revealed various colors of the extracts under ordinary light, short wavelength (254 nm) U.V. light, and Long wavelength (366 nm) U.V. light (Table 2).

Table 2: Fluorescence analysis of *C. oppositifolia*

S. No.	Reagent	Leaf			Stem			Inflorescence		
		Ordinary light	Short wavelength (λ=254 nm)	Long wavelength (λ=366 nm)	Ordinary light	Short wavelength (λ=254 nm)	Long wavelength (λ=366 nm)	Ordinary light	Short wavelength (λ=254nm)	Long wavelength (λ=366 nm)
1	Powder	Dirty Green	Dark Green	Light Green	Saw dust Brown	Light Brown	Light Green	Brownish Green	Brownish Green	Light Green
2	Water	Dirty Green	Light Brown	Light Green	Light Brown	Light Brown	Light Green	Dirty Brownish Green	Light Brown	Light Green
3	5% NaOH	Yellowish Green	Brown	Orange Brown	Amber Yellow	Orange Brown	Green	Dark Brown	Orange	Green
4	5% $FeCl_3$	Dark Brown	Yellowish Brown	Black	Dark Brown	Brown	Black	Dark Brown	Yellowish Brown	Black
5	50% H_2SO_4	Light Green	Brown	Pistachio Green	Light Brown	Orange	Light Green	Greenish Brown	Orange Brown	Light Green
6	50% HNO_3	Light Brown	Orange	Pistachio Green	Mustard Yellow	Orange	Light Green	Brownish Yellow	Yellowish Orange	Light Green
7	50%HCl	Translucent Green	Dark Brown	Light Green	Dirty Yellow	Orange Brown	Light Green	Greenish Brown	Orange Brown	Light Green
8	$CHCl_3$	Emerald Green	Brown	Neon Red	Brown	Brown	Peach	Greenish Yellow	Orange Brown	Peach
9	Aniline	Blackish Brown	Dark Brown	Orange	Maroon	Orange	Yellowish Brown	Ruby Red	Yellowish Brown	Black
10	Petroleum ether	Amber Yellow	Yellow	Peach	Hazy Brown	Light Yellow	White	Dirty White	Orange	White
11	MeOH	Emerald Green	Brown	Neon Red	Light Green	White	Neon Blue	Light Green	White	Neon Blue
12	Picric acid	Amber Yellow	Orange	Black	Sharp Yellow	Orange	Black	Neon Yellow	Yellowish Orange	Black

3.4 Phytochemical screening

The phytochemical screening of plant material mainly revealed the presence of terpenoids, sterols, glycosides, flavonoids, alkaloids, carbohydrates, tannins, phenols and lignins (Table 3).

Table 3: Phytochemical analysis of *C. oppositifolia*

Group	Name of tests	Leaf	Stem	Inflorescence
Terpenoids	Liebermann's test	+	+	+
Sterols	Salkowaski test	+	+	-
Glycoside	Keller-Killiani test	+	-	+
Flavonoids	NaOH test	+	+	+
Alkaloids	Dragendroff's test	+	+	+
Proteins	Millon's test	-	-	-
Carbohydrates	Molisch's test	+	+	+
Saponin	Foam test	-	-	-
Lipids	Soap formation test	-	-	-
Tannins	Braymer's test	-	+	+
Phenols	Ferric chloride test	+	-	+
Lignins	Lignin test	-	+	-
Fixed oils and fats	Spot test	-	-	-

3.5 Physicochemical constants

The extractive values of leaf in distilled H_2O and MeOH are high while, for inflorescence and stem, ethyl acetate showed more extractive value as compared to the rest of the solvents (Table 4).The ash values of leaf and stem showed high content of water-soluble ash followed by acid insoluble ash but inflorescence showed the highest acid insoluble ash (Table 5).

Table 4: Extractive values of *C. oppositifolia*

Parameters	Percentage yield ± (SEM)		
	Leaf	Stem	Inflorescence
n-Hexane	1.367 ± 0.001	0.359 ± 0.004	1.287 ± 0.006
Chloroform	1.867 ± 0.009	0.532 ± 0.005	1.352 ± 0.005
Methanol	2.629 ± 0.001	1.759 ± 0.020	2.584 ± 0.006
Ethyl acetate	1.296 ± 0.002	4.392 ± 0.015	3.967 ± 0.012
Distilled H_2O	2.416 ± 0.001	3.265 ± 0.008	2.470 ± 0.015

Table 5: Ash values of *C. oppositifolia*

Parameters	Values % (w/w)		
	Leaf	Stem	Inflorescence
Total ash	20.8233 ± 0.0052	5.8473 ± 0.0327	20.933 ± 0.6187
Acid insoluble ash	27.7908 ± 2.1744	7.9894 ± 0.08694	28.0743 ± 4.2496
Water soluble ash	72.0166 ± 1.4668	157.4138 ± 2.6247	16.480 ± 5.5786

4. Discussion

Natural remedies recorded from medicinal plants are favorable choices over synthetically formulated drugs. It is of importance to individualize the consumption of herbal products not merely based on the knowledge of folklore use but through systematic studies (Dewick, 2002). Pharmacognostic, physicochemiscal and phytochemical evaluations are of robust entitlement of entire crude drug profile in context of its pharmaceutical and pharmacological importance (Panda, 2004).

C. oppositifolia is a local plant, commonly used by natives for folklore use. The transverse section of leaf and stem showed the basic profile of botanical anatomy with a little contrast in having lunar shaped vascular bundles in the leaf while a continuous vascular bundle with primary and secondary xylem and phloem with presence of sclerenchyma all around; fibres and trachieds with annular and spiral thickening present in the medullary region and parenchyma along with collenchyma in papillose form with oil globules were indicated in prominence are all represented in Figures 1 and 2.

Histochemical evaluation of transverse sections were performed to get a clear picture on preliminary scale at cellular level using conc. HCl, phloroglucinol which showed presence of lignins in the leaf and stem while iodine solution gave negative result for starch, Ferric chloride solution specified the presence of tannins and Sudan III dye coloured the oil globules in the ground tissue of leaf and pith area of stem (Figures 4 and 5).

The photomicrograghic evaluations revealed microscopic features particular to each part of *C. oppositifolia* used for powder microscopy in general and basic characters; they were prominent especially the covering trichomes shown in Figures 6, 7 and 8 (Beck, 2010). While, the HCl and phloroglucinol treated powder macerates of *C. oppositifolia* leaf, stem, and inflorescence were stained with safranin and observed the results were given in Figures 9, 10 and 11 (Jensen, 1962)

The fluorescence analysis was a valuable and modest method for the identification of fluorescent compounds. Different compounds give fluorescence when exposed to U.V. light. The powder of leaf, stem and inflorescence gave various fluorescence in short and long wavelength of U.V. light given in Table 2 (Joshi, 2012).

The methanolic extracts of *C. oppositifolia* leaf, stem and inflorescence established the presence of various important active constituents like terpenoids, sterols, glycosides, flavonoids, alkaloids, carbohydrates, *etc.* (Table 3), which may be responsible for the folklore uses of this plant.

Quantitative analysis using the physicochemical parameters of extractive values of *C. oppositifolia* leaf, stem and inflorescence were determined for further standardization of the powder and evaluation was performed with *n*-hexane, chloroform, MeOH, ethyl acetate and distilled water; the results were tabulated in Table 4 (Mukherjee, 2002). Ash values for the powdered plant parts was helpful in the determination of extraneous material content adhered to the plant as well as the amount of siliceous matter in the left over residue, respectively; the results were presented in Table 5 (Khandelwal, 2008).

5. Conclusion

The current investigation reveals the pharmacognostic features and physicochemical properties of *Colebrookea oppositifolia*. The present findings are associated with standardization of parameters like macroscopic and microscopic characters, phytochemical screening, fluorescent analysis and physicochemical quantification of *C. oppositifolia*. It was found that H_2O and MeOH extractive

values of leaf and ethyl acetate for stem and inflorescence were significant. Ash values added more strength to crude drug standardization with prominent results indicating the involvement of extraneous matter. Such study on the macro and microscopic anatomy, preliminary phytoconstituent screening and physicochemical parameters are important informations which may be useful in verification and contamination for quality control of this therapeutic plant afterwards.

Acknowledgements

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Conflict of interest

We declare that we have no conflict of interest.

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