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Quality assessment and phytochemical analysis of *Clerodendrum serratum* roots

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Abstract
Objectives: *Clerodendrum serratum* (L.) Moon. (Verbenaceae) commonly known as *bharangi* is an important medicinal plant growing in India having traditional value to treat pain, inflammation, rheumatism, respiratory disorders and fever. The aim of present study is to develop pharmacognostical, physicochemical and phytochemical parameters for quality assessment of *Clerodendrum serratum* roots (CSR).
Methods: The roots of *C. serratum* were evaluated for pharmacognostical, physicochemical parameters such as ash values (total ash, acid insoluble ash and water soluble ash), extractive values (alcohol and water soluble), moisture content, crude fiber content and foaming index according to WHO guidelines on quality control methods for medicinal plant materials. The phytochemical analysis was performed using chemical tests, ultraviolet spectroscopy, thin layer chromatography and quantitative estimation of secondary phytoconstituents.
Results: The pharmacognostical and physicochemical parameters evaluated for the roots were found in compliance with the Pharmacopoeial standards of crude drug. The qualitative and quantitative phytochemical analysis of methanolic extract of CSR showed presence of phenolics, flavonoids, saponins and carbohydrates.
Conclusion: The present investigation will be helpful for the identification and quality control of CSR.
Keywords: *Clerodendrum serratum*; quality issues; phytochemical analysis; phenolics; flavonoids; saponins

1.Introduction

Clerodendrum serratum (Linn.) Moon (family: Verbenaceae), commonly known as bharangi is a perennial woody shrub up to 3-8 ft in height with blunt quadrangular stems. The roots of *C. serratum* have been indicated in traditional systems of medicine like Ayurveda and Unani for the treatment of *swasa* and *kapha* (respiratory ailments) includes asthma, inflammatory and infectious disorders. Many Ayurvedic and herbal preparations containing the crude form of *C. serratum* roots such as solid (*Bharngyadi churna*), semisolid (*Kantakaryavaleha* and *Bharangi* guda) and liquid (*Kanakasava* and *Dasamularista*, *Mahapancagavya* ghrita and *Mahavishgarba* taila) are used for the treatment of various disorders especially asthma. Research reports available on chemical constituents of *C. serratum* roots showed presence of saponins (triterpenoids and sterols), phenolics, flavonoids and carbohydrates [1]. Many *in-vivo* and/or *in-vitro* experiments have explained a wide spectrum of pharmacological properties of

crude extract of roots including anti-cancer [2], hepatoprotective [3], anti-bacterial [4], anti-inflammatory [5] and anti-oxidant [6].

Roots of *C. serratum* are often found adulterated in market with roots of many other plants from verbenaceae (*Clerodendrum indicum* (L.) Kuntze, *Gmelina arborea* Roxb. *Premna obtusifolia* R. Br. and *P. herbacea* Roxb.), rubiaceae (*Gardenia latifolia* Aiton, *G. resinifera* Roth, and *G. turgida* Roxb.) and simaroubaceae (*Picrasma quassioides* (D. Don) Benn.) families [1]. A plant species can be easily identified by taxonomists in flowering stage [7] but the identification of leaves and roots seems much more challenging, especially in the processed plant material (e.g., powdered). However, detailed phytochemical analysis serves a major tool for ease of its identification. Since the plant, *C. serratum* is useful in traditional medicine for the treatment of various ailments; it is important to develop standardization methods and quality control parameters. In this regard, the main aim of the present work is to develop pharmacognostical, physicochemical and phytochemical profile of *C. serratum* roots. The present study will be helpful in differentiating other plants used intentionally or unintentionally for adulteration and serve as an important tool for the quality assessment of the roots of *C. serratum*.

2. Materials and methods

2.1 Chemicals and reagents

Oleanolic acid, gallic acid, quercetin and diosgenin were purchased from Sigma Aldrich Chemical Co. (India). Folin-ciocalteau reagent was procured from Sisco Research Lab (India). Glucose was purchased from local supplier. TLC Aluminum sheets precoated with silica gel 60F254, thickness 0.2mm, (20 x 20cm) were purchased from Merck (Germany). All the chemicals and solvents used were of standard analytical grades.

2.2 Plant material

The roots of *C. serratum* Linn were collected from Government Ayurvedic Udhyan, Gandhinagar, Gujarat (India) in the month of August, 2011. Herbarium specimen (10EXTPHDP49CS11) was authenticated and deposited in the department of pharmacognosy, institute of pharmacy, Nirma University, Ahmedabad, Gujarat (India). The roots were washed and dried under sun light for 15 days. The dried material was then subjected to pulverization and the powder sample was passed through 60# sieve. The powdered sample was used for further studies.

2.3 Pharmacognostical studies

The morphological and microscopical characters of the roots were studied using standard methods [8]. Various diagnostic characters of transverse section and powder (#60) of roots were studied microscopically with or without staining. Photomicrographs of the different cellular structures and inclusions were taken using trinocular microscope (Jyoti Scientific, Gwalior) with digital Olympus camera [9].

2.4 Physicochemical studies

Physicochemical parameters such as ash values (total ash, acid insoluble ash and water soluble ash), extractive values (alcohol and water soluble), moisture content [8], crude fiber content and foaming index [10] were carried out according to reference methods.

2.5 Preparation of methanolic extract of *C. serratum* roots

The root powder (500 g) was extracted with methanol (1000 mL) by soxhlet extraction process at 60-70 ºC temperature for 13-15 hrs. The extract was filtered and the marc was re-extracted by the same process till exhaustion. The combined filtrate was evaporated near to dryness under reduced pressure to yield dark brown dry extract (yield: 11.67 %w/w) and stored at 4 °C for further use.

2.6 Phytochemical analysis

2.6.1 Preliminary phytochemical screening

Preliminary phytochemical screening for detection of various secondary metabolites was carried out using standard procedures [9].

2.6.2 Ultraviolet spectroscopy of the extract

Ultraviolet (UV) spectroscopy of the methanolic extract of CSR (MCSR; 1mg/mL) was performed within 200 to 400 nm using a UV-VIS spectrophotometer (UV 1800, Shimadzu, Japan) and the characteristic peaks were detected [11].

2.6.3 Chromatographic fingerprinting studies

The extract was analyzed by performing thin layer chromatography (TLC) to develop the finger printing profile of methanolic extract of *C. serratum* roots [12]. The MCSR and oleanolic acid (in methanol) was applied in

the form of bands on precoated aluminum silica gel GF-254 plates to develop Co- TLC. Numbers of solvents were tried for separation of different components of extract, but the satisfactory resolution was obtained in the solvent system; chloroform: glacial acetic acid: methanol (64:32:12 v/v). Prepared plate was developed up to a distance of 85mm in previously saturated twin trough chamber in linear ascending direction. The developed plate was air dried to evaporate solvents from the plate and then derivatized by spraying anisaldehyde-sulphuric acid reagent followed by heating at 110ºC in hot air oven for 5-10 mins. The R_f values and colors of bands resolved were recorded.

2.6.4 Quantitative estimation of phytoconstituents

The total phenolics [13], flavonoids [14], saponins [15] and carbohydrate [16] content of MCSR were quantitatively estimated according to the reference methods.

3. Results and discussion

3.1 Pharmacognostic studies

Macroscopically, roots of *C. serratum* were earthy brown and showed ridges, longitudinal striations, wrinkles, circular warts, rootlets and rootlet scars externally (fig. 1). Root pieces were thin and varying in size. Fracture was found to be hard and fractured surface was pale yellowish brown. Roots possessed characteristic odor and a slight bitter taste. In transverse section (fig. 2a), the root of *C. serratum* showed stratified cork with one to two celled wide phellogen and 3 to 5 celled wide parenchymatous phelloderm layers. It showed narrow cortex containing reddish brown content in the initial layers and plenty of simple starch grains throughout along with groups of lignified stone cells (isolated or in groups of 3 to 4) and mesocortical fibres. Stone cells were found to be oval and thick walled with striations. Fibers were in a group of 3 to 5, lignified and striated. Phloem was parenchymatous and filled with starch grains and observed with few scattered stone cells in this region. Xylem vessels were scattered, lignified and pitted. Medullary rays were 1-4 cell wide and lignified. Abundant starch grains were present in xylem parenchyma and medullary ray cells. Root powder (fig. 2b) showed plenty of simple starch grains, stone cells fragments and cork cells in surface view. Fragments of bordered pitted xylem vessels, pitted xylem fibres and tracheids were also found present in root powder.

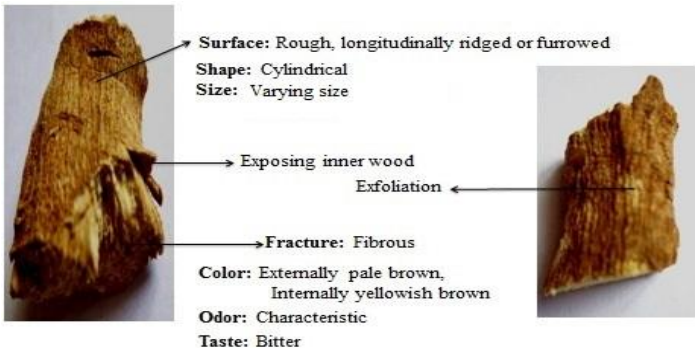
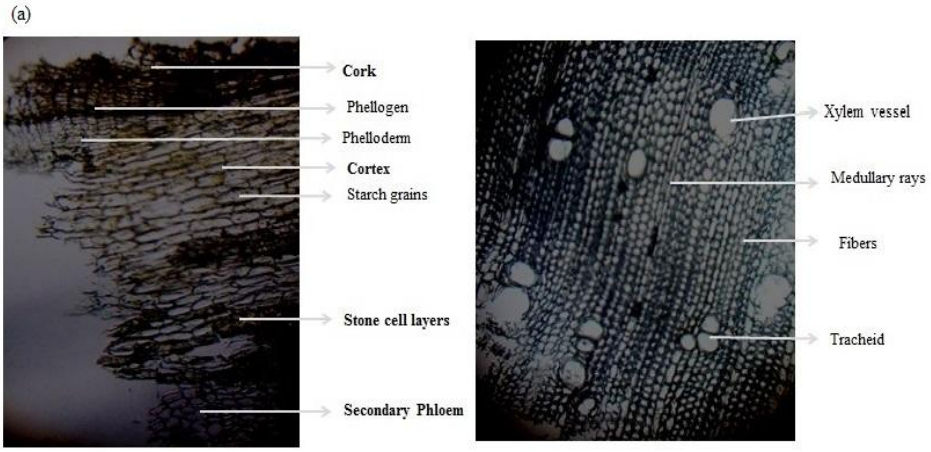


Figure 1: Macroscopic studies of *C. serratum* roots



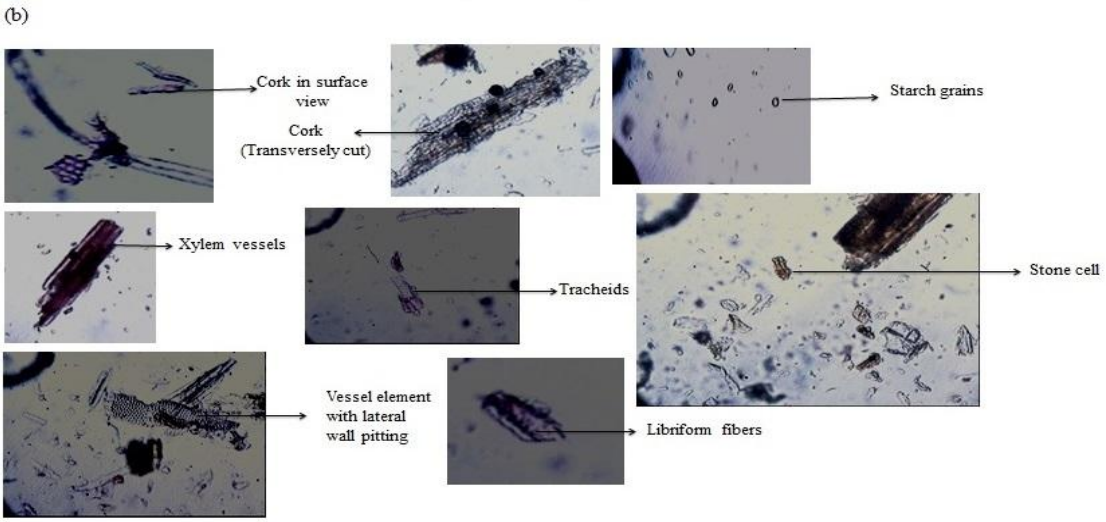


Figure 2: Microscopic studies of *C. serratum* roots ((a) Transverse section; (b) powder study)

3.2 Physico-chemical studies

The physicochemical studies like ash values, solvent extractive values, moisture content, crude fiber content and foaming index were evaluated to judging the quality and purity of the roots. The results of physicochemical parameters studied are mentioned in table 1. Estimation of ash value is an important in the evaluation of purity of drugs, the presence or absence of foreign inorganic matter such as metallic salts or silica. The ash values usually represent the residue remaining after incineration and indicate the inorganic composition or earthy materials and other impurities present along with roots [9]. The total ash value (3.24±0.06 %w/w) was found to be relatively high which may be due to high content of carbonates, phosphates, silicates and silica. This is also in accordance with low content of acid insoluble ash (0.53±0.09% w/w) and water soluble ash (0.26±0.02 %w/w). Extractive value determinations are primarily useful for the identification of exhausted drugs. The amount of the extract that drug yields in a solvent is often an approximate measure of the amount of certain constituents that the drug contains. The high water soluble extractive value (13.28±0.38 %w/w) in comparison with alcohol (7.5±0.21 %w/w) indicates the possibility of considerable amount of polar compounds such as sugar, saponins and phenolics in the roots [17].

Determination of moisture content of the herbal drugs is an important factor responsible for the deterioration of the drugs and formulation. The higher or lower percentage of moisture content indicates the improper storage conditions. Excessive moisture content may favor the growth of fungal or other micro-organic contamination leads to the deterioration of drug. Low moisture content is always desirable for higher stability of drugs and to prevent bacterial, fungal or yeast growth. Moisture content was found to be 5.16±0.03 %w/w which is not too high, hence could discourage bacterial, fungal or yeast growth. Crude fiber is the fraction of carbohydrate that remains after treatment with acid and alkali which was found to be 30.00±0.28 %w/w. Foaming index of *C. serratum* roots was found to be 9 cm due to presence of high amount of saponins [10].

Table 1: Physico-chemical parameters of *C. serratum* root

Sr. No.	Parameters	Result*	Reference value (Anonymous, 1999)[18]
1.	Ash values		
	Total	3.24±0.06 % w/w	NMT 11% w/w
	Acid insoluble	0.53±0.09 % w/w	NMT 1% w/w
	Water soluble	0.26±0.02 % w/w	-
2.	Extractive values		
	Alcohol soluble	7.5±0.21 % w/w	NLT 6% w/w
	Water soluble	13.28±0.38 % w/w	NLT 12% w/w
3.	Moisture content	5.16±0.03 % w/w	-
4.	Crude fiber content	30.00±0.28 % w/w	-
5.	Foaming index	9 cm	-

*n=3, Values are mean±SEM

3.3 Phytochemical analysis

Preliminary phytochemical investigation of roots showed presence of phenolics, flavonoids, saponins and carbohydrates in varying amount in MCSR (table 2) while alkaloids are found to be absent in roots. Furthermore, the UV spectroscopic analysis of the MCSR showed peaks at 329.80, 308.00, 288.40, and 264.60 nm wavelengths (figure 3) with absorbance 0.440, 0.383, 0.413 and 0.299, respectively. The results of UV spectroscopic analysis indicates possible presence of phenolics and flavonoids as the spectra for phenolic compounds (tannins) and flavonoids typically lie in the range of 230-290 nm [19]. TLC chromatogram of MCSR (table 3) exhibited 14 grey-bluish (visible) bands in the R_f range 0.1-0.9 after derivatization with anisaldehyde sulphuric acid reagent. The characteristic purple violet color on derivatization with anisaldehyde sulphuric acid reagent by saponins (steroidal or terpenoidal) and flavonoids was observed in our TLC which strongly suggests the presence of steroidal/terpenoidal saponins and flavonoids (fig. 4). The results of qualitative phytochemical analysis further facilitated their quantitative estimation of secondary phytoconstituents present in roots. The Subsequent quantification of phytoconstituents (table 4) revealed the higher saponin content followed by phenolic, flavonoid and carbohydrate in the roots. From the standard curve of gallic acid ($y = 0.0025x + 0.0118$, $R^2 = 0.9978$), quercetin ($y = 0.0014x + 0.0128$, $R^2 = 0.9988$), diosgenin ($y = 0.002x + 0.0369$, $R^2 = 0.9941$) and glucose ($y = 0.0085X + 0.05$, $R^2 = 0.991$), total phenolic, flavonoid, saponin and carbohydrate content in the MCSR were determined, respectively. Plant polyphenols are the widest spread secondary metabolite and have been reported for many pharmacological activities including antioxidant, anti-inflammatory, anticancer, antibacterial, anti-asthmatics, etc. These observations support the usefulness of this plant in folklore remedies in the treatment of asthma, allergy, fever, inflammation and liver disorders [1].

Table 2: Preliminary phytochemical screening of *C. serratum* root

Sr. No.	Chemical tests	MCSR
1.	Carbohydrates	
	a) Molisch test	+++
	b) Fehling's test	+++
	c) Benedict's test	++
	d) Barfoed's test	++
2.	Amino acid	
	a) Ninhydrin test	-
3.	Glycoside	
	a) Deoxy sugars (Keller-killani test)	-
	b) Borntrager's test	-
	c) Baljet test	-
4.	Steroids and terpenoids	
	a) Salkowski test	++
	b) Liebermann Buchard's test	+++
5.	Flavonoids	
	a) Shinoda test	+++
	b) Lead acetate test	+++
	c) Sodium hydroxide test	+++
6.	Saponins	
	Foaming test	+++
7.	Alkaloids	
	a) Dragendorff's test	-
	b) Mayer's test	-
	c) Hager's test	-
	d) Wagner's test	-
8.	Tannins (phenolic compounds)	
	a) 5% ferric chloride test	+++
	b) Lead acetate test	+++
	c) Dilute Iodine test	+++
	d) Dilute nitric acid test	++
	e) Potassium dichromate test	++
	f) Acetic acid test	++

Key: +++ = highly present; ++ = moderately present; + = slightly present; - = absent

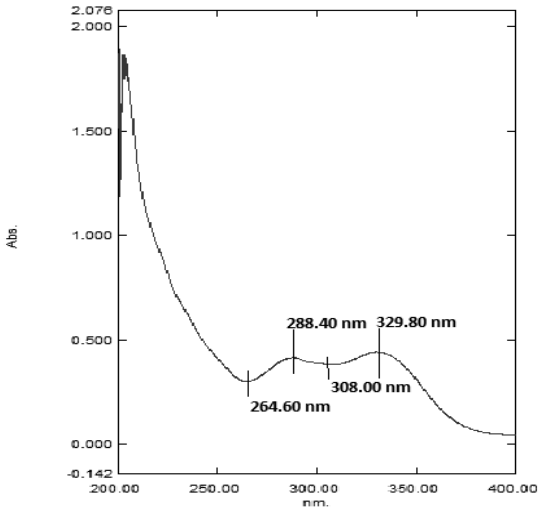


Figure 3: UV spectrogram of methanolic extract of *C. serratum* root

Table 3 TLC screening of methanolic extract of *C. serratum* roots

Preparation	No. of spots	R_f value
Oleanolic acid	1	0.32
MCSR	14	0.15, 0.22, 0.32, 0.40, 0.49, 0.53, 0.55, 0.63, 0.74, 0.78, 0.82, 0.90, 0.95, 0.97

Figure 3: TLC chromatogram of MCSR

(Key: S1= Oleanolic acid, T= MCSR after derivatization with anisaldehyde sulphuric acid reagent followed by heating at 110ºC for 5-10 mins)

Table 4: Quantitative estimation of phytoconstituents of *C. serratum* roots

Type of preparation	Quantitative estimation of phytoconstituents*			
	Total phenolic content	Total flavonoid content	Total saponins content	Total carbohydrate content
MCSR	0.911±0.65	0.568±0.72	1.766±0.50	0.347±0.69

*n=3, Values are mean±SEM and expressed as g per100 g of dry weight extract

4. Conclusion

The present study undertaken with the objective of pharmacognostical and physicochemical studies of *C. serratum* to provide useful information for standardization and quality assessment of the roots. Furthermore, the qualitative and quantitative phytochemical analysis will be beneficial for the correct identification of the *C. serratum* roots available in the market and may be useful to establish monograph details on the roots.

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