

PHARMACOGNOSTIC STUDIES ON FLOWERS OF *DREGEA VOLUBILIS*: EVALUATION FOR AUTHENTICATION AND STANDARDIZATIONBHASKAR DAS<sup>1</sup>, ARNAB DE<sup>1</sup>, PIU DAS<sup>1</sup>, AMALESH NANDA<sup>2</sup>, AMALESH SAMANTA<sup>1\*</sup><sup>1</sup>Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India. <sup>2</sup>Department of Biotechnology, National Institute of Technology, Arunachal Pradesh, India. Email: asamanta61@yahoo.co.in

Received: 01 February 2019, Revised and Accepted: 19 March 2019

## ABSTRACT

**Objective:** The various parts of *Dregea volubilis* (Family: Apocynaceae), locally known as Jukti (Bengali), are commonly used in Indian system of medicine to treat various ailments such as inflammation, piles, leukoderma, asthma, and tumors. Literature review suggested that there has been no detailed work on systemic pharmacognostic and phytochemical studies done on the flowers of the plant. The present study is aimed to lay down quality control parameters for *D. volubilis* flowers to confirm its identity, quality, and purity.

**Methods:** The present work was designed to study detailed organoleptic, histological, quantitative standards, physicochemical, spectroscopic, and chromatographic characteristics of the flowers of *D. volubilis*.

**Results:** The total ash, acid insoluble ash, water soluble ash, loss on drying, water, and alcohol soluble extractive values were found to be 11.767±0.130% (w/w), 1.287±0.106% (w/w), 9.140±0.344% (w/w), 14.110±0.061% (w/w), 21.600±0.133% (w/v), and 9.603±0.104% (w/v), respectively. Phytochemical screening of different extracts showed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, flavonoids, tannins, and phenolics. The chromatographic study revealed the presence of rhamnose (103.229±4.994 µg/g), fructose (738.670±25.714 µg/g), glucose (285.532±24.465 µg/g), and maltose (49.082±5.206 µg/g).

**Conclusion:** The characterization parameters of the present study may serve as a reference standard for proper authentication, identification and for distinguishing the plant from its adulterants.

**Keywords:** *Dregea volubilis*, Organoleptic, Phytochemistry, High-performance liquid chromatography, Fourier transform infrared.

© 2019 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2019.v12i5.32257>

## INTRODUCTION

Herbal medicines play an important role in the health-care system to alleviate and treat diseases. There is a great demand for medicinal plants in the herbal industry due to its health beneficiary properties with multi-dimensional chemical structures. Standardization of the medicinal plants is essential to confirm the authenticity and quality to avoid deliberate adulteration and substitution [1].

*Dregea*, a genus of vines, is a rich source of steroidal pregnanes with potential biological activities [2]. *Dregea volubilis* (L.f.) Benth. ex Hook.f. (Synonym: *Wattakaka volubilis* (L.f.) Stapf., *Marsdenia volubilis* (L.f.) Cooke) belongs to the kingdom of Plantae, subfamily of Apocynoideae, family of Apocynaceae, order of Gentianales, series of Bicarpetalatae, subclass of Gamopetalae, class of Dicotyledons and are distributed widely in the tropical zone and South East Asia [3]. *D. volubilis*, a large twining perennial shrub, grows as a woody climber having woody vines and is scattered throughout the India and Car-Nicobar ascending to an altitude of 1500 m [4]. The plant blooms between March and April. The young branches of the plant are green, slender, and smooth; the older branches are gray, very long, and glabrous, often with lenticels or small black dots. Leaves are broadly ovate or somewhat rounded, sub-orbicular, acuminate, 7.5–15 cm long, 5–10 cm wide. Flowers are green or pale green, about 1 cm in a radius, bisexual and sweet-scented in a drooping umbel. Follicles are usually two, slightly tapering to a very blunt point, glabrous, and striated. The seeds are elliptic, concave, flattened, smooth, and shining. Different parts of the plant have been traditionally used in Ayurveda in India for the treatment of various ailments such as asthma, inflammation, tumors, piles, leukoderma, application to boils, rat bite, and urinary discharge [5]. The vernacular names of the medicinally important plant are Jukti (Bengali), Akadbel

(Hindi), Harandodi (Marathi), Velipparuthi (Malayalam), Dudhipaala (Telugu), Koti-p-palai (Tamil), Dugdhive (Kannada), Dudghika (Oriya), Khamal lata (Assamese), Kadvo kharkhodo (Gujarati), and Hemajivanti (Sanskrit). The flowers of *D. volubilis* are eaten as a seasonal vegetable in early summer with bitter esthetic principles. Flowers of the plant are a rich source of biologically active phytochemicals and were reported to contain volubiloside A, volubiloside B, volubiloside C, dregealol, volubilogenone, volubilol, drevogenin D, iso-drevogenin P, 17 $\alpha$ -marsdenin, dregeanin, vicenin-2, vitexin, isovitexin, isoorientin, rutin, quercetin, luteolin, apigenin, gallic acid, ferulic acid, ellagic acid, and cinnamic acid [6–9]. Antioxidant and antidiabetic activities of hydroalcoholic extract of the flowers of the plant were reported earlier by the present authors [9].

In spite of a lot of attention to health beneficiary effects, flowers of *D. volubilis* have been less explored pharmacognostically. A detailed study on the structural morphology and other physicochemical parameters of the flowers of *D. volubilis* are required. The present study is aimed to evaluate the flowers to fix the pharmacognostical parameters for proper identification, authentication, and quality standardization of the plant.

## METHODS

## Plant material

The fresh flowers of *D. volubilis* were collected in the month of April 2017 from Jaynagar Mazilpur, South 24 Parganas, West Bengal, India. It was taxonomically identified and authenticated by Dr. V.P. Prasad, Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah, West Bengal, India. A voucher specimen of the plant was kept at Division of Microbiology and Biotechnology, Department

of Pharmaceutical Technology, Jadavpur University, Kolkata, India. The flowers were dried under shade, powdered using a mechanical grinder and preserved at  $25 \pm 2^\circ\text{C}$  in airtight container at a dry place.

#### Chemicals and instruments

Rhamnose, xylose, fructose, glucose, trehalose, and maltose were purchased from Sigma-Aldrich, St Louis, MO, USA. Ethanol was purchased from EMD Millipore, Bedford, MA, USA. Chloroform, acetone, 2-propanol, ethyl acetate, diethyl ether, petroleum benzene ( $40\text{--}60^\circ\text{C}$ ), methanol for liquid chromatography, acetonitrile for liquid chromatography, sodium carbonate, anthrone, glacial acetic acid, hydrochloric acid, nitric acid, acetic acid, sulfuric acid, phosphoric acid, ammonia solution 25%, Coomassie Brilliant Blue G250, sodium hydroxide, potassium hydroxide, chloral hydrate, and glycerin were procured from Merck Life Sciences Private Limited, Mumbai, India. The water was purified by a Milli-Q water purification system (EMD Millipore, Bedford, MA, USA) and used for all experiments. All other reagents used were of analytical grade.

#### Macroscopic evaluation

The macroscopic study of the crude drug includes evaluation of its morphological characteristics which are examined by the naked eye and magnifying lens. The method is the simplest and quickest mean to check the authenticity of a crude drug [10].

#### Microscopic evaluation

Fresh flowers were collected and washed with water for carrying out the microscopical study. Different parts of the flower were cut into very thin transverse sections (T. S) and boiled in 10% potassium hydroxide solution to remove fatty materials and coloring substances. The sections were stained and observed under Magnus microscope (Olympus [India] Pvt. Ltd., Noida, India). Photomicrographs were captured with Magnus photomicrography units (MIPS USB 2.0 Capture and Display Software) at  $\times 40$  [11].

#### Powder characteristics

The mechanically grinded dried powdered material was sieved through mesh number 80 to get uniform powder. It was cleared with chloral hydrate, stained, and mounted in glycerin to observe under Magnus microscope (Olympus [India] Pvt. Ltd., Noida, India) [12].

#### Quantitative standards

The shade dried powdered material of flowers of *D. volubilis* was evaluated for the determination of ash values, extractive values, and loss on drying (LOD).

#### Ash values

The total ash, water-soluble ash, and acid insoluble ash of the plant material were performed [13].

#### Total ash

About 1 g of the material was taken in a previously ignited and tarred silica crucible. The material was spread in even layer and ignited at  $450^\circ\text{C}$  by gradually increasing the temperature until it was white indicating the absence of carbon. It was then allowed to cool in a desiccator. The total ash content (% w/w) of the material was calculated according to the following equation:

$$\text{Total ash (\% w/w)} = (\text{weight of ash/weight of sample}) \times 100$$

#### Acid insoluble ash

The acid insoluble ash was determined by boiling the total ash with 25 ml of 2 (N) hydrochloric acid (HCl) into a China dish. It was covered with a watch glass and gently boiled for 5 min. The watch glass was rinsed with 5 ml of boiled water and the rinsed contents were transferred to the contents of China dish. The insoluble matter of the contents of the china dish was collected on tarred gooch crucible, washed with boiled acidulated water, ignited, cooled in a desiccator

and weighed. Acid-insoluble ash content (% w/w) of the material was calculated with reference to the crude drug according to the following equation:

$$\text{Acid-insoluble ash (\% w/w)} = (\text{weight of ash/weight of sample}) \times 100$$

#### Water-soluble ash

About 25 ml of water was added to the total ash in a China dish and was gently boiled for 5 min. The water-insoluble ash was collected on tarred gooch crucible, washed with boiled acidulated water, ignited, cooled in a desiccator, and weighed. The water-soluble ash was calculated by subtracting the weight of insoluble matter from the weight of total ash. The water-soluble ash content (% w/w) was determined with respect to the air-dried material using the following equation:

$$\text{Water-soluble ash (\% w/w)} = (\text{weight of water-soluble ash/weight of sample}) \times 100$$

#### Extractive values

The extractive values are indicative weights of the extractable chemical constituents of crude drugs in different solvents. The extractive values of crude drugs were determined in water and alcohol [14]. 5 g each of the crude drugs was taken in a 250 ml stoppered conical flask. 100 ml of the respective solvent was added to the 250 ml stoppered conical flask and was allowed to macerate for 24 h with the aid of mechanical shaker for 6 h. It was then filtered and 25 ml of the filtrate was taken in a tarred Petri dish. It was evaporated to dryness in an oven at  $105^\circ\text{C}$  and weighed it again. The extractive value (% w/w) was calculated with respect to the air dried material using the following equation:

$$\text{Extractive value (\% w/w)} = (\text{weight of extracted residue/weight of sample}) \times 100$$

#### LOD

The LOD was performed [15] by taking 1 g of the crude drug in previously weighed LOD weighing bottle. It was dried in an oven for 1 h, cooled in a desiccator and weighed. The LOD (% w/w) was calculated with respect to the crude drug using the following equation:

$$\text{LOD (\% w/w)} = (\text{weight loss/weight of sample}) \times 100$$

#### Fluorescence analysis

The fluorescence analysis was performed by treating the dried powdered material with different chemicals and was observed in daylight and ultraviolet (UV) light [16]. Some of the phytochemicals present in plant material show fluorescence in the visible range in daylight. The UV ray produces fluorescence in many crude drugs which do not fluoresce in daylight. A more powerful source of ultraviolet ray is often needed to produce fluorescence in crude drugs. Different types of reagents are often applied to the crude drugs which do not fluoresce to convert them into fluorescent derivatives. The fluorescence analysis is an important parameter for pharmacognostic evaluation for assessing crude drugs qualitatively. The behavior of powdered drugs after treatment with different chemical reagents and their fluorescent characteristics were observed under UV (254 and 366 nm) and visible light using CAMAG UV CABINET 4.

#### Preliminary phytochemical studies

The shade dried powdered material of flowers of *D. volubilis* weighing about 200 g was soaked with sufficient amount of light petroleum benzene ( $40\text{--}60^\circ\text{C}$ ) in a glass beaker for 24 h and then the flowers were extracted with petroleum benzene ( $40\text{--}60^\circ\text{C}$ ), chloroform, methanol successively using Soxhlet apparatus and the exhausted material was boiled with water. The petroleum benzene ( $40\text{--}60^\circ\text{C}$ ) fraction obtained after extraction using Soxhlet apparatus was combined with the initial fraction of petroleum benzene ( $40\text{--}60^\circ\text{C}$ ) obtained after soaking. The extracts of organic solvents were concentrated using rotary evaporator under reduced pressure and evaporated to dryness. The aqueous extract was concentrated using a water bath

and lyophilized. The extracts were preserved in well-closed container and kept in the dark at a temperature of 10°C for future use. The different extracts obtained were tested individually for the presence of different phytoconstituents [17]. Thin-layer chromatography (TLC) fingerprinting of the extracts was performed using Silica Gel G as an adsorbent. TLC plates (Millipore Corporation, USA) were activated in a hot air oven at 110°C for 30 min. The plates were kept in a desiccator for future use. Different combinations of solvents were used for the preparation of the mobile phases.

#### High-performance liquid chromatography (HPLC) fingerprinting

The chromatographic fingerprinting was performed by ultra HPLC (UHPLC) using a UHPLC+ focused system consisting of a Dionex Ultimate 3000 Pump, a Dionex Ultimate 3000 autosampler column compartment, and a Dionex Ultimate 3000 variable wavelength detector [18]. 1 g of dried and finely powdered (mesh size 85) sample was taken in 10 ml volumetric flask and sufficient Milli-Q water was added and heated in boiling water bath for 20 min and cooled and made up to the volume with Milli-Q water. The solution was filtered through a 0.45 µm syringe filter (Millex, Merck, Germany) which was injected as a test solution and Milli-Q water was injected as blank. Chromatographic separations of phytochemicals of the extract were performed using a C18 column (250 mm×4.6 mm i.d.) with a particle size of 5 µm, Hypersil GOLD (Thermo Fisher Scientific, U.S.A.) and column oven temperature were maintained at 25°C. The chromatographic separation was performed using gradient elution (Table 1) with a flow rate of using 0.2 (% v/v) phosphoric acid as mobile phase A and HPLC grade methanol as mobile phase B, respectively. The UV detector was set at 280 nm and the injection volume was 20 µl. The chromatograms were processed with Chromeleon 7, version 7.2.0.3765 software (Thermo Scientific, U.S.A.).

#### Fourier transform infrared (FTIR)

FTIR spectra of the different extracts of *D. volubilis* were performed in attenuated total reflection (ATR) mode using Nicolet iS10 FT-IR Spectrometer (Thermo Fisher Scientific, USA) with a total of 30 scans at a resolution of 4 cm<sup>-1</sup> in the wave number range between 4000 cm<sup>-1</sup> and 525 cm<sup>-1</sup>. Background spectrum of a clean ATR crystal was collected immediately before collecting the spectrum of the plant extracts. The extracts were then placed in the ATR accessory and pressed for acquiring the FTIR spectra of the samples. The spectral acquisitions were processed with OMNIC software supplied by the manufacturer. FTIR study provided qualitative information on the types of functional groups and chemical bonds present in the phytochemicals of the extracts by analyzing the peak values (cm<sup>-1</sup>) of the spectra [9].

#### Determination of UV-VIS spectra

The different extracts of *D. volubilis* were diluted with respective solvents at a concentration of 0.05% (w/v) in respective solvents and scanned between 200 nm and 800 nm using a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific, USA), and the spectra were recorded [19].

#### Determination of pH

The shade dried powdered material of flowers of *D. volubilis* was mixed with water at a concentration of 1% (w/v), 2% (w/v), and 10% (w/v) and kept in a water bath for 20 min. It was then filtered through Whatman filter paper No. 1 and the pH of the filtrate was measured using a pH meter (Model: 3200P, Agilent Technologies, USA) at 25°C [20].

#### Determination of protein content

The protein content of the sample was determined according to Bradford method with some modifications [21]. 0.5 g powder of *D. volubilis* flower was mixed with 10 ml of water and the mixture was shaken for 10 min followed by filtration using Whatman filter paper No. 1. 0.2 ml of the sample solution was mixed with 5 ml of Bradford's reagent (0.1 g of Coomassie Brilliant Blue G250 was dissolved in 50 ml of ethanol followed by addition of 100 ml 85% (v/v) phosphoric acid and volume was made up to 1 L). The reaction mixture was kept for

10 min for the development of color completely. The absorbance of the reaction mixtures was measured at 595 nm against a blank using a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific, USA). The protein present in the sample was quantified from calibration curves of absorbance versus concentration in µg/ml of bovine serum albumin which was used as a standard.

#### Determination of carbohydrate content

The total carbohydrate content of the dried powdered material of flowers of *D. volubilis* was determined by the anthrone method with slight modifications [22]. A standard stock solution (10 mg/ml) containing glucose was prepared in Milli-Q water and different concentrations (20, 40, 60, 80, 90, and 100 µg/ml) of standard solutions were prepared by diluting the stock solution for the calibration curve. 100 mg of dried sample was hydrolyzed by keeping it in a boiling water bath for 3 h with 5 ml of 2.5 (N) HCl and cooled down to room temperature. It was then neutralized with solid sodium carbonate until effervescence ceased and volume of the solution made up to 100 ml with Milli-Q water. It was then centrifuged at 2000 rpm and the supernatant was collected. 1 ml each of standard solutions and sample solution was added to 4 ml of anthrone reagent (0.2% [w/v] anthrone in ice cold concentrated sulfuric acid) and heated for 8 min in boiling water bath and cooled to room temperature. A blank solution was prepared by adding 1 ml Milli-Q water to 4 ml of anthrone reagent. The absorbance of the reaction mixtures was measured at 630 nm against the blank using a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific, USA).

#### Determination of free sugar composition

The free sugars were determined by ultra-fast liquid chromatography (UFLC) using a prominence UFLC system (Shimadzu, Japan) equipped with a LC-20AT Solvent Delivery Unit, SIL-20A UFLC version autosampler, and RID-10A refractive index detector [23]. A mixed standard stock solution (10 mg/ml) containing rhamnose, xylose, fructose, glucose, trehalose, and maltose was prepared in Milli-Q water and different concentrations (0.625, 1.25, 2.5, 5, and 7.5 mg/ml) of standard solutions were prepared by diluting the mixed standard stock solution for calibration curves to quantify the sugars present in the sample. 1 g of dried sample was extracted with 40 ml of 80% (v/v) aqueous ethanol at 80°C for 30 min. It was then centrifuged at 15,000 g for 10 min and the supernatant was concentrated at 60°C under reduced pressure. The concentrated sample was defatted 3 times with 10 ml of diethyl ether. The defatted material was concentrated at 40°C and dried. The dried sample was dissolved in Milli-Q water to a final volume of 5 ml. The solution was filtered through a 0.45 µm syringe filter (Millex, Merck, Germany) which was injected as a test solution and Milli-Q water was injected as blank. Chromatographic separations of the sugars were performed using a NH<sub>2</sub> column (250 mm × 4.6 mm i.d.) with a particle size of 5 µm and pore size of 100 Å, Luna NH<sub>2</sub> (Phenomenex, U.S.A.) and column oven temperature was maintained at 40°C. A solvent mixture consisting of seven volumes of acetonitrile and three volumes of Milli-Q water was used as mobile phase. The chromatographic separation was performed with a flow rate of 1.0 ml/min with a run time of 15 min and the injection volume was 10 µl.

#### Statistical analysis

All experiments were performed in triplicate and the results of the quantitative studies are presented as mean±standard error of mean. The statistical analyses were performed with GraphPad PRISM6 software, USA.

## RESULTS AND DISCUSSION

#### Macroscopic evaluation

The flowers were numerous, green or pale green in color, sweet-scented, and bitter in taste. Inflorescences were in lateral drooping umbellate cymes. 2.5–5 cm long slender peduncles were arising from between the petioles. 1–2.5 cm long slender calyx dividing nearly to



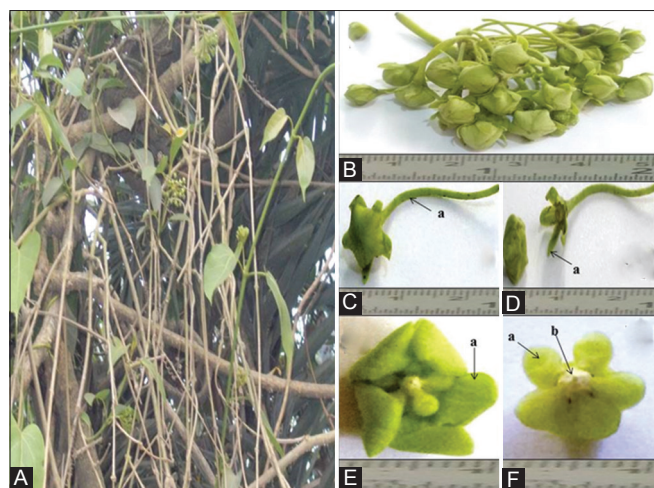
the base was ovate to oblong, obtuse, and ciliate. Corolla was deeply divided and glabrous outside. Lobes were broadly ovate, obtuse and veined overlapping to the right. Stamina column aroused from the base of the corolla and anther tips were membranous, broadly ovate, oblong, and obtuse. Pollen masses were attached to the pollen carriers by very short caudicles (Fig. 1). The macroscopic evaluation of a crude drug is used for its authentication by comparing the diagnostic characters with the prescribed standards of the standard drug. The macroscopic feature helps to evaluate the basic differentiating characteristics between the various species within a single genus [10]. The parameters of the macroscopic evaluation are mostly subjective and there is a chance of existence of adulterants which are closely resembles the genuine drug. The microscopic and physicochemical analyses are more authentic studies to check whether the parameters of the crude drugs conform to the standard or not. The parameters of the macroscopic evaluation for the flowers of *D. volubilis* can be served as reference diagnostic characters for the authentication of the drug.

### Microscopic evaluation

The T. S of the different floral parts of flower (Fig. 2) showed the following observations under the microscope.

### Thalamus

The T. S of thalamus showed that epidermal layer was composed of a single layer of compactly arranged tabular cells with cuticularized outer walls. The cortex was composed of 2–3 layers of collenchyma, known



**Fig. 1:** (A) *Dregaea volubilis* in its natural habitat. (B) Pale green flower in dense drooping umbels. (C) Individual flower of *D. volubilis* showing pedicel (a). (D) *D. volubilis* flower part showing sepal (calyx) (a). (E) *D. volubilis* flower part showing petal (corolla) (a). (F) *D. volubilis* flower part showing androecium (a) and gynoecium (b)

**Table 1:** UHPLC gradient program for the HPLC fingerprinting study

Time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0	1	100	0
10	1	100	0
20	1	90	10
30	1	70	30
40	1	50	50
50	1	30	70
60	1	10	90
70	1	0	100

UHPLC: Ultra-high-performance liquid chromatography,  
HPLC: High-performance liquid chromatography

as hypodermis. Several layers of thin-walled parenchyma cells, known as parenchymatous zone, were present next to the hypodermis in the cortex. The vascular bundles were collateral and open and each bundle was composed of external phloem and internal xylem. Pith was made of parenchyma cells. Trichomes were uniseriate, multicellular with blunt tip. Cluster crystals of calcium oxalate were present.

### Stalk

The T. S of stalk showed that the epidermis was single-layered outermost zone consisting of compactly set tabular living cells, outer walls of which were cuticularized. Cortex was distinctive consisting of hypodermis which was composed of 2–3 layers of collenchyma cells. General cortex was present next to the hypodermis which was composed of several layers of thin-walled parenchyma cells. Vascular bundles were collateral and open consisting of external phloem and internal xylem. Pith was very distinct and large, situated in the center consisting of thin-walled, oval, or polygonal parenchyma cells with abundant intercellular spaces between them. Cluster crystals of calcium oxalate were found to be present. Trichomes were uniseriate, multicellular with blunt tip.

### Calyx

The T. S of sepal showed that there were two epidermal layers, for example, upper and lower epidermis. Both the epidermal layers were uniseriate and composed of compactly arranged tabular cells, the outer walls of which were cuticled. The mesophyll was made of parenchyma cells lying between two epidermal layers. The mesophyll was differentiated into (a) upper closely packed, tubular chloroplast containing cells, known as palisade parenchyma, and (b) lower loosely arranged, more or less rounded cells, called spongy parenchyma. Oil globules and cluster crystals of calcium oxalate were also present. Trichomes were uniseriate, multicellular with blunt tip. Stomata were anomocytic.

### Corolla

The T. S of petal showed that the epidermal layer was uniseriate and composed of compactly arranged tabular cells, the outer walls of which were cuticled. Trichomes were uniseriate, multicellular with blunt tip. Anomocytic stomata and oil globules were found to be present.

### Androecium

The T. S showed that the epidermal layer was uniseriate and composed of compactly arranged tabular cells, the outer walls of which were cuticled. The cluster crystals of calcium oxalate were found to be present.

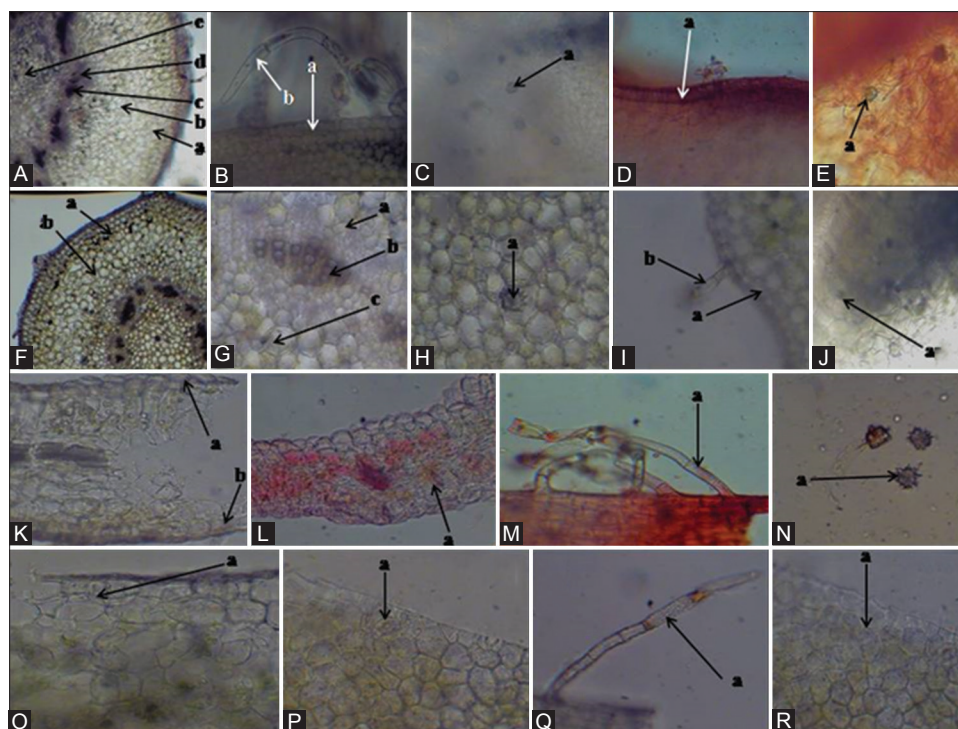
### Gynoecium

The T. S showed the presence of cluster crystals of calcium oxalate.

The detailed microscopic examination of the flowers of *D. volubilis* can be used as a reference to identify the crude drug by comparing the known histological characters. The microscopic examination is an easiest and finest way to set standard parameters depending on the internal anatomy of the plant [15]. The microscopic study alone cannot provide complete evaluation profile for the herbal drugs. The histological characters of the drug along with other analytical parameters can be utilized to set the standardization specifications for the evaluation of the herbal drug.

### Powder characteristics

The powder microscopy showed the presence of fibers, cluster crystals of calcium oxalate, and epidermal trichomes. Some fragments consisted of groups and parts of parenchyma cells and epidermal cells. The observations of the study on the powder of *D. volubilis* flowers can serve as useful parameters for the proper identification of the drug. The dried sample in the powdered form gives characteristic features of the drug under a microscope after proper treatments. The microscopic



**Fig. 2:** Transverse section (T. S) of different parts of *Dregea volubilis* flower. (A). T. S of thalamus showing hypodermis (a), parenchymatous zone (b), xylem (c), phloem (d), and pith (e). (B) Epidermis (a) and trichome (b) in thalamus. (C) Cluster crystals of calcium oxalate (a) in thalamus. (D) T. S of androecium showing epidermis (a). (E) Cluster crystals of calcium oxalate (a) in androecium. (F) T. S of stalk showing hypodermis (a) and general cortex (b). (G) Phloem (a), xylem (b), and pith (c) in stalk. (H) Cluster crystals of calcium oxalate (a) in stalk. (I) Epidermis (a) and trichome (b) in stalk. (J) T. S of gynoecium showing cluster crystals of calcium oxalate. (K) T. S of sepal showing upper epidermis (a), lower epidermis (b). (L) Stomata in sepal (a). (M) Trichome in sepal (a). (N) Cluster crystals of calcium oxalate (a) in sepal. (O) T. S of petal showing epidermis (a). (P) Stomata in petal (a). (Q) Trichome in petal (a). (R) Oil globules in petal (a)

examination of epidermal trichomes and calcium oxalate crystals is extremely valuable for authentication of crude drugs [17].

#### Quantitative standards

The total ash, acid-insoluble ash, and water-soluble ash were found to be  $11.767 \pm 0.130\%$  (w/w),  $1.287 \pm 0.106\%$  (w/w), and  $9.140 \pm 0.344\%$  (w/w), respectively. The water and alcohol soluble extractive values were found to be  $21.600 \pm 0.133\%$  (w/v) and  $9.603 \pm 0.104\%$  (w/v), respectively. LOD was  $14.110 \pm 0.061\%$  (w/w). Therapeutic efficacy of herbal drugs can be ensured by determining the quantitative standards. The ash of crude drugs is consisted of nonvolatile inorganic materials which can be used to set quality control parameter to check the contamination of crude drugs. A high content of ash value beyond the standard limit gives an indication of contamination, substitution, or adulteration [24]. The content of active constituents in a given amount of crude drug is estimated by extractive value in a particular solvent. The extractive values give valuable information regarding the quality of the crude drug whether it is exhausted or not. The high extractive value is an indicative parameter of better extraction of phytoconstituents from crude drugs and it is also helpful for proper selection of solvent that will provide maximum yield [25]. The physical and physicochemical state of the interior of the cell depends on the loss of water. The enzymes present in the cell are responsible for different chemical reactions such as oxidation, hydrolysis, and polymerization of the phytoconstituents present in the plant material when the enzymes come in contact with the active substances during the process of drying. Most of the enzymes present in plant material need sufficient water to act leading to decomposition reactions of the crude drugs. Moisture present in the crude drugs helps in microbial growth leading to degradation of it. It is desirable to keep the water content of the crude drugs at low level to deactivate the enzyme activity as well

as to retard microbial degradation to such an extent that the storage stability of the crude drugs is guaranteed [26]. These standardization parameters are essential to ensure the quality of herbal drugs. Quantitative standards can be applied for the evaluation of crude drugs. These parameters can be utilized for maintaining the identity, purity, and quality of crude drugs. Purity depends on the absence of foreign matter in crude drugs. Quality depends on the concentration of the active constituents present in the crude drugs that exert health beneficiary properties.

#### Fluorescence analysis

The fluorescence analysis of the powdered drug showed various colors after treatment with different chemical reagents and observed visually under daylight, short wavelength ultraviolet light (254 nm), and long wavelength ultraviolet light (366 nm). The results are shown in Table 2.

The fluorescence analysis of the *D. volubilis* flower showed various colors under ordinary light, short wavelength UV light (254 nm), and long wavelength UV light (366 nm) indicating the presence of fluorescent compounds. The analysis is a very important and useful tool for the identification of various phytoconstituents present in the crude drugs which give fluorescence either itself or after derivatization with proper chemical treatment under UV light [16]. The method is very easy and direct method for the identification of fluorescent compounds present in the test sample and the observations can be used as a quality control parameter for the identification of the crude drug.

#### Preliminary phytochemical studies

The results of preliminary qualitative phytochemical studies of the different extracts of flowers of *D. volubilis* are presented in Table 3. The TLC studies of the different extracts were performed in

Table 2: Fluorescence analysis of powdered flowers of *D. volubilis*

Sl. No.	Treatment	Daylight	UV light	
			254 nm	366 nm
1	Powder as such	Light brown	Dark green	Reddish brown
2	Powder+Acetic acid	Brown	Dark brown	Brownish black
3	Powder+Ferric chloride (5% FeCl <sub>3</sub> )	Greenish brown	Brownish black	Black
4	Powder+Conc. Hydrochloric acid (HCl, 5N)	Yellowish brown	Brown	Bluish-black
5	Powder+Conc. Nitric acid HNO <sub>3</sub>	Reddish brown	Brownish black	Black
6	Powder+Conc. Sulfuric acid (H <sub>2</sub> SO <sub>4</sub> )	Brownish black	Brown	Brownish black
7	Powder+Iodine solution (1%)	Reddish yellow	Brown	Brownish black
8	Powder+Methanol	Light brown	Dark brown	Brownish black
9	Powder+Picric acid	Yellowish brown	Brownish yellow	Brownish black
10	Powder+NaOH Solution (1 N)	Reddish yellow	Dark brown	Brownish black
11	Powder+Distilled water	Yellowish brown	Dark brown	Brownish black
12	Powder+Liquid ammonia (NH <sub>3</sub> )	Deep brown	Dark brown	Brownish black
13	Powder+Conc. HNO <sub>3</sub> +NH <sub>3</sub>	Reddish brown	Dark brown	Brownish black
14	Powder+Dilute HNO <sub>3</sub>	Yellowish brown	Brown	Brownish black
15	Powder+10% potassium dichromate solution	Deep yellow	Dark brown	Black
16	Powder+Acetone	Brown	Yellowish brown	Bluish black

*D. volubilis*: Dreyea volubilis, UV: Ultraviolet

Table 3: Preliminary phytochemical analysis of *D. volubilis* flower extracts

Phytochemicals	Petroleum benzene extract	Chloroform extract	Methanol extract	Aqueous extract
Carbohydrates	-	-	+	+
Proteins	-	-	+	+
Amino acids	-	-	+	+
Steroids	+	+	-	-
Glycosides	-	+	+	+
Alkaloids	-	-	+	+
Tannins and Phenolics	-	-	+	+
Flavonoids	-	-	+	+

*D. volubilis*: Dreyea volubilis

different solvent systems on trial and error method. The retention factor ( $R_f$ ) values of the different extracts are presented in Table 4. The phytochemical studies of the different extracts of *D. volubilis* flower showed the presence of different types of plant metabolites which are responsible for the medicinal values of the plant. The extraction with different solvents gives rise to the separation of medicinally active portions of the plant according to the polarity of the solvents. The purpose of the standardized extracts is to obtain the therapeutically active compounds and to eliminate unwanted materials by treatment with a selective solvent known as menstruum. The extracts of the crude drugs can be considered a good source of useful drugs [27]. The various types of phytochemicals present contribute medicinal as well as physiological properties to the plants. The TLC analysis of the different extracts was carried out for the development of characteristic fingerprint profile which may be used as a reference for the quality evaluation and standardization of the drug. The bands of the different extracts in the TLC plates were obtained at different  $R_f$  values which can be used as identifying markers [11]. The extracts can be utilized as medicinal agents after standardization in different dosage forms of pharmaceutical interest. The preliminary phytochemical studies are of great importance in the field of standardization of crude drugs.

#### HPLC fingerprinting

The different phytoconstituents present in the sample were separated on C18 column using UHPLC (Fig. 3). The different peaks along with their retention times (min), area (mAU\*min), height (mAU), relative area (%), and relative height (%) are presented in Table 5. HPLC plays an important role as an important analytical tool for the quality control of drugs [28]. Natural products have a unique chemical diversity which results in diversity in their biological activity leading to the development of lead compounds which will play an important role

in the discovery of drugs for treating various ailments. The modern analytical technique (HPLC) with high power of separation and reproducibility can be used to separate multidimensional chemical structures present in the plant materials. The peak number 7 and 26 with a retention time of 4.912 min and 34.855 min, respectively, are the two more intense peaks among the others in the chromatogram generated after HPLC study. The peak number 7 accounts for 27.54% and the peak number 26 accounts for 23.92% relative area. The chromatograms generated after HPLC study can be used to establish reference HPLC fingerprints of the flower of *D. volubilis* against which raw materials can be evaluated and finished products containing the plant material can be analyzed.

#### FTIR

The FTIR spectra of the different extracts of *D. volubilis* flowers are presented in Fig. 4. The petroleum benzene and chloroform extracts exhibited characteristic bands for the asymmetrical stretching vibrations of the C-H bonds in CH<sub>2</sub> and CH<sub>3</sub> groups between 2980 and 2810 cm<sup>-1</sup>, C-H bending vibrations between 1480 and 1400 cm<sup>-1</sup>, C=O stretching vibrations between 1870 and 1540 cm<sup>-1</sup>, interactions of O-H bending and C-O stretching in the C-O-H group between 1390 and 1350 cm<sup>-1</sup>, and the secondary C-O vibrations in the C-O-H group between 1060 and 1025 cm<sup>-1</sup> which are characteristics of phytosterols [29]. The aqueous, chloroform, and methanol extracts showed characteristic bands for the O-H stretching vibrations between 3550 and 3200 cm<sup>-1</sup>, C-O stretching vibration band between 1060 and 1000 cm<sup>-1</sup>, and O-H bending vibration band between 1420 and 1330 cm<sup>-1</sup> which are due to the presence of phenolic compounds. The extracts also exhibited characteristic O-H stretching vibration band near 3000 cm<sup>-1</sup> and C=O stretching vibration band near 1700 cm<sup>-1</sup>, C-O stretching vibration band between 1320 and 1210 cm<sup>-1</sup>, and O-H bending vibration band between 1440 and 1395 cm<sup>-1</sup> which



Table 4: Thin-layer chromatography of *D. volubilis* flower extracts

Sl. No.	Solvent system	Extracts	No. of spots (254 nm)	R <sub>f</sub> values (254 nm)	No. of spots (366 nm)	R <sub>f</sub> values (366 nm)
1	Chloroform: ethyl acetate (6:4)	Aqueous	1	0.48	-	-
		Methanol	4	0.04, 0.09, 0.13, 0.47	1	0.25
		Chloroform	16	0.04, 0.06, 0.10, 0.12, 0.19, 0.30, 0.35, 0.41, 0.48, 0.56, 0.61, 0.72, 0.79, 0.95, 0.96, 0.98	9	0.08, 0.16, 0.30, 0.35, 0.41, 0.93, 0.95, 0.97, 0.98
		Petroleum benzine	7	0.07, 0.23, 0.48, 0.56, 0.72, 0.91, 0.96	3	0.84, 0.92, 0.96
2	Chloroform: ethyl acetate (8:2)	Aqueous	2	0.04, 0.15	-	-
		Methanol	4	0.02, 0.06, 0.10, 0.16	3	0.01, 0.05, 0.14
		Chloroform	9	0.02, 0.05, 0.08, 0.10, 0.15, 0.16, 0.88, 0.91, 0.96	4	0.06, 0.09, 0.90, 0.96
		Petroleum benzine	12	0.10, 0.20, 0.24, 0.29, 0.52, 0.64, 0.67, 0.79, 0.86, 0.89, 0.91, 0.97	3	0.66, 0.89, 0.96
3	Chloroform: methanol: glacial acetic acid (4:5:1)	Aqueous	1	0.69	2	0.10, 0.73
		Methanol	7	0.13, 0.17, 0.24, 0.53, 0.66, 0.76, 0.90	5	0.24, 0.69, 0.78, 0.90, 0.97
		Chloroform	2	0.84, 0.93	2	0.74, 0.87
		Petroleum benzine	3	0.46, 0.48, 0.72	4	0.46, 0.72, 0.78, 0.90
4	Chloroform: ethylacetate: glacial acetic acid (4:5:1)	Aqueous	3	0.06, 0.17, 0.88	1	0.88
		Methanol	8	0.03, 0.04, 0.07, 0.12, 0.19, 0.33, 0.71, 0.87	5	0.04, 0.13, 0.16, 0.70, 0.96
		Chloroform	8	0.02, 0.04, 0.42, 0.55, 0.86, 0.90, 0.94, 0.95	4	0.04, 0.85, 0.94, 0.96
		Petroleum benzine	1	0.94	1	0.94
5	Chloroform: 2propanol: glacial acetic acid (5:4:1)	Aqueous	1	0.05	1	0.05
		Methanol	9	0.03, 0.12, 0.19, 0.48, 0.57, 0.62, 0.72, 0.92, 0.96	7	0.05, 0.13, 0.21, 0.48, 0.62, 0.69, 0.92
		Chloroform	4	0.12, 0.72, 0.88, 0.94	1	0.97
		Petroleum benzine	1	0.94	1	0.97
6	Chloroform: ethanol: triethylamine (6:3:1)	Aqueous	3	0.06, 0.08, 0.11	-	-
		Methanol	12	0.05, 0.13, 0.16, 0.19, 0.31, 0.37, 0.41, 0.47, 0.61, 0.65, 0.90, 0.96	5	0.06, 0.89, 0.92, 0.95, 0.97
		Chloroform	3	0.44, 0.84, 0.95	2	0.91, 0.96
		Petroleum benzine	2	0.81, 0.90	3	0.82, 0.89, 0.96
7	Chloroform: methanol: glacial acetic acid (5:4:1)	Aqueous	6	0.08, 0.12, 0.38, 0.50, 0.69, 0.88	2	0.52, 0.69
		Methanol	11	0.04, 0.09, 0.16, 0.34, 0.44, 0.62, 0.68, 0.78, 0.87, 0.95, 0.98	7	0.16, 0.40, 0.65, 0.79, 0.83, 0.95, 0.98
		Chloroform	2	0.80, 0.92	3	0.79, 0.87, 0.93
		Petroleum benzine	2	0.80, 0.96	2	0.87, 0.96

*D. volubilis*: *Dregea volubilis*, R<sub>f</sub>: Retention factor

are due to presence of phenolic acids. All the extracts showed characteristic absorption bands for the out-of-plane bending of ring C-H bonds between 900 and 675 cm<sup>-1</sup>, in-plane bending bands between 1300 and 1000 cm<sup>-1</sup>, and C-C stretching band within the ring between 1600 and 1585 cm<sup>-1</sup> and 1500 and 1400 cm<sup>-1</sup> which are due to the presence of mononuclear and polynuclear aromatic hydrocarbons [30]. The characteristic absorption bands of the different extracts of the plant material confirm the presence of different types of phytoconstituents in *D. volubilis* flowers. FTIR is a useful analytical tool in the field of standardization of drugs. FTIR spectrum is useful in the identification of drugs by comparing the spectrum of the test material with that of the reference material. The FTIR spectra of the different extracts can be served as reference

FTIR fingerprints of *D. volubilis* flowers for the quality control of raw materials and finished products containing it [9]. The study also provides qualitative information on the types of chemicals present in the different extracts of the flowers of *D. volubilis*.

#### Determination of UV-VIS spectra

The UV-VIS spectrum of different extracts of *D. volubilis* flowers is presented in Fig. 5. The aqueous extract showed the peaks at 250 nm, 290 nm ( $\lambda_{max}$ ); the methanol extract showed the peaks at 230 nm and 300 nm ( $\lambda_{max}$ ); the chloroform extract showed the peaks at 250 nm, 300 nm ( $\lambda_{max}$ ), 410 nm, 510 nm, 540 nm, and 670 nm; and the petroleum benzine extract showed the peaks at 300 nm ( $\lambda_{max}$ ), 400 nm, 500 nm, 530 nm, and 670 nm. UV-VIS spectrum

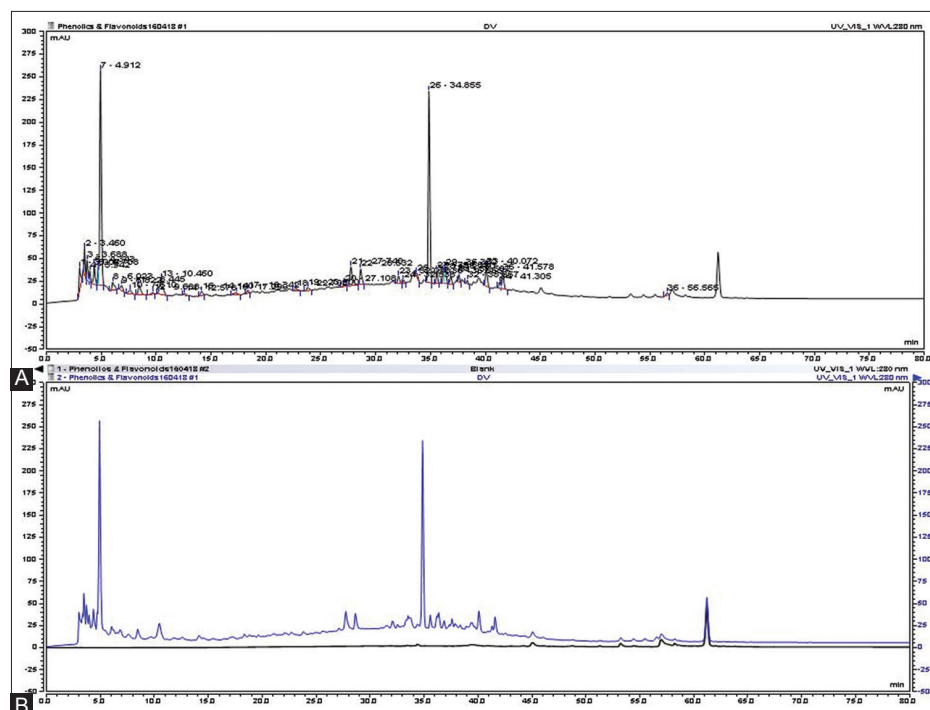


Fig. 3: Ultra-high-performance liquid chromatograms of a sample solution of the flower of *Dregea volubilis* (A) and an overlapped chromatogram (black colored chromatogram represents blank solution and blue colored chromatogram represents sample solution) (B) as detected at 280 nm.

Table 5: HPLC peaks present in the sample solution of the flower of *D. volubilis* as detected at 280 nm

Sl. No. of HPLC Peaks	Retention time (min)	Are(mAU*min)	Height (mAU)	Relative area (%)	Relative height (%)
1	3.007	3.639	26.758	2.52	3.24
2	3.460	2.992	29.535	2.07	3.58
3	3.688	2.887	22.366	2.00	2.71
4	3.942	1.443	13.231	1.00	1.60
5	4.343	3.521	22.164	2.44	2.68
6	4.708	1.774	19.838	1.23	2.40
7	4.912	39.758	236.210	27.54	28.60
8	6.023	2.299	9.147	1.59	1.11
9	6.822	1.329	5.662	0.92	0.69
10	7.610	1.339	4.054	0.93	0.49
11	8.445	2.820	10.939	1.95	1.32
12	9.688	0.501	1.924	0.35	0.23
13	10.460	5.894	17.178	4.08	2.08
14	12.578	0.595	2.365	0.41	0.29
15	14.140	1.131	4.393	0.78	0.53
16	17.290	1.123	2.568	0.78	0.31
17	18.342	0.735	3.604	0.51	0.44
18	22.708	0.772	3.080	0.53	0.37
19	23.800	0.936	3.735	0.65	0.45
20	27.108	0.644	3.177	0.45	0.38
21	27.740	6.010	21.011	4.16	2.54
22	28.632	3.555	17.369	2.46	2.10
23	32.073	1.885	8.404	1.31	1.02
24	32.538	0.623	4.004	0.43	0.48
25	33.720	0.911	4.209	0.63	0.51
26	34.855	34.533	210.94	23.92	25.54
27	35.565	2.167	14.434	1.50	1.75
28	36.180	2.312	14.081	1.60	1.71
29	36.352	3.113	17.537	2.16	2.12
30	36.855	1.667	8.962	1.15	1.09
31	37.582	1.252	8.645	0.87	1.05
32	38.357	0.592	4.046	0.41	0.49
33	40.072	4.243	21.997	2.94	2.66
34	41.305	1.173	6.969	0.81	0.84
35	41.578	3.454	18.030	2.39	2.18
36	56.565	0.749	3.239	0.52	0.39
Total		144.370	825.81	100	100

HPLC: High-performance liquid chromatography, *D. volubilis*: *Dregea volubilis*



plays an important role in the identification and quantification of many drugs. This analytical tool is very useful for the quality control of drugs [31]. The UV-VIS spectrum of the different extracts of *D. volubilis* flowers can be served as a reference spectrum for quality control of drugs.

#### Determination of pH

The pH of the 1% (w/v), 2% (w/v), and 10% (w/v) aqueous solutions of the dried powdered material was found to be  $5.02 \pm 0.02$ ,  $4.89 \pm 0.02$ , and  $4.74 \pm 0.02$ , respectively. pH can also serve as a quality control tool for the identification of the drugs. A change in the value of pH from the standard value indicates the deterioration of the quality of the product. The aqueous solution of *D. volubilis* flower was found to be acidic in nature. The pH value is of great importance in product development to estimate stability and dissolution of the product. The pH value is also

helpful in the development of suitable extraction procedure for the phytoconstituents from the plant [15].

#### Determination of protein content

Proteins are important biomolecules with multiple functions within organ and the molecules are differing from one another primarily in their sequence of amino acids. The protein content of the sample was found to be  $2.112 \pm 0.058$  mg/g of the sample. The protein content of the powder of *D. volubilis* flower can be served as a quantitative parameter for standardization of the plant material. Any deviation from the standard value of protein content reflects the changes in the quality of the crude drug. Estimation of protein of herbal drugs plays a crucial role in assessing the nutritional significance and health effects [32]. The parameter can be used as a reference for the quality control of crude drugs.

#### Determination of carbohydrate content

The carbohydrate content of the dried powdered material of *D. volubilis* flowers was found to be  $124.243 \pm 3.573$  mg/g of the sample. Carbohydrate is one of the most widely used substances in nature and is the main ingredient of food. The quantitative analysis for the estimation of the carbohydrate content of crude drugs can be considered as a quality control parameter for assessing the crude drugs [33].

#### Determination of free sugar composition

The UFLC analysis (Fig. 6) was performed to identify and quantify the free sugars present in the *D. volubilis* flowers. The retention times of rhamnose, xylose, fructose, glucose, trehalose, and maltose were found to be 4.982, 5.456, 5.734, 6.198, 7.351, and 8.032 min, respectively. The sugars present in the sample were identified by comparing the retention times of the standards with that of the sample. Xylose and trehalose were not detected in the crude drug. The study showed that the flower contained rhamnose ( $103.229 \pm 4.994$  µg/g), fructose ( $738.670 \pm 25.714$  µg/g), glucose ( $285.532 \pm 24.465$  µg/g), and maltose ( $49.082 \pm 5.206$  µg/g). Characterization of sugars present in crude drugs is very important for their quality control [33]. The sugars present in the flower can be considered as markers for the standardization of the crude drug.

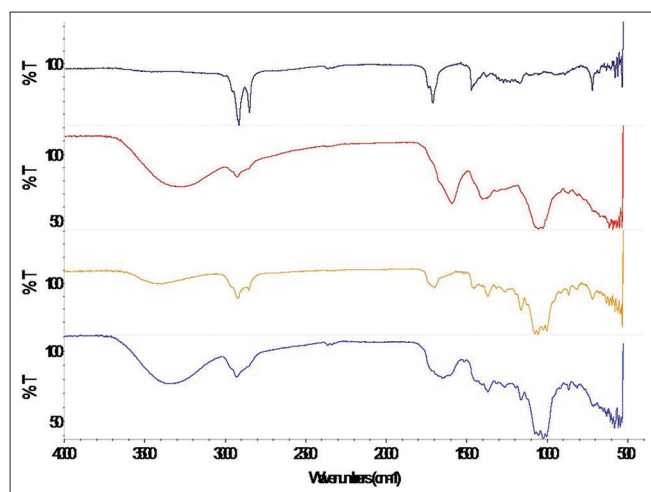


Fig. 4: Fourier transform infrared spectra of the petroleum benzene extract (A), aqueous extract (B), chloroform extract (C), and methanol extract (D) of the flower of *Dregea volubilis*

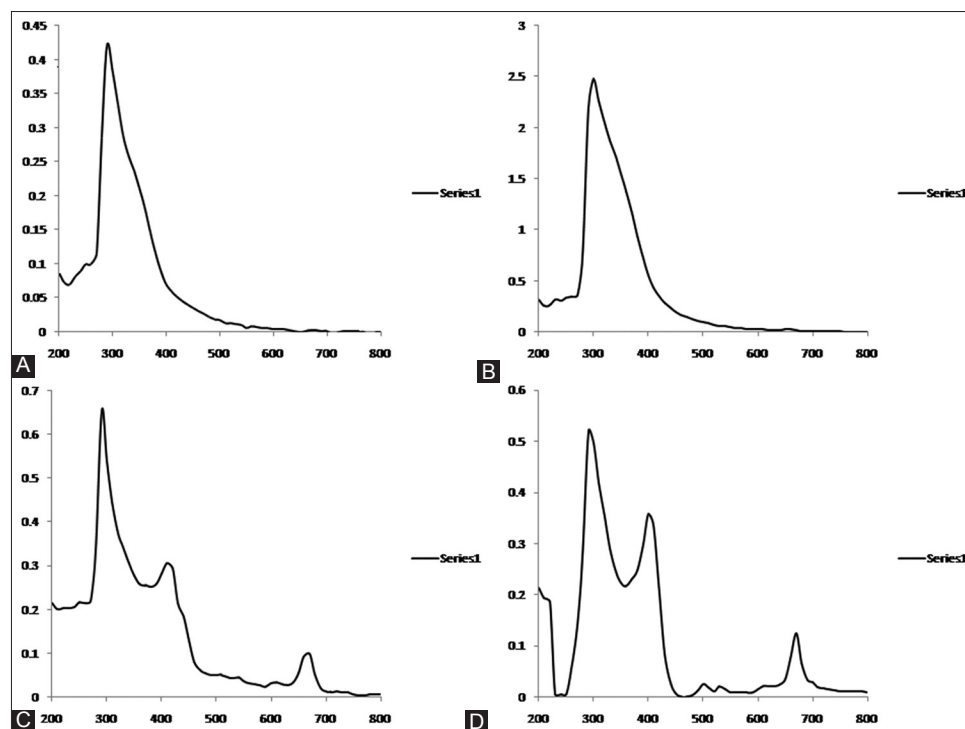


Fig. 5: Ultraviolet-visible spectra of the aqueous extract (A), methanol extract (B), chloroform extract (C), and petroleum benzene extract (D) of the flower of *Dregea volubilis*

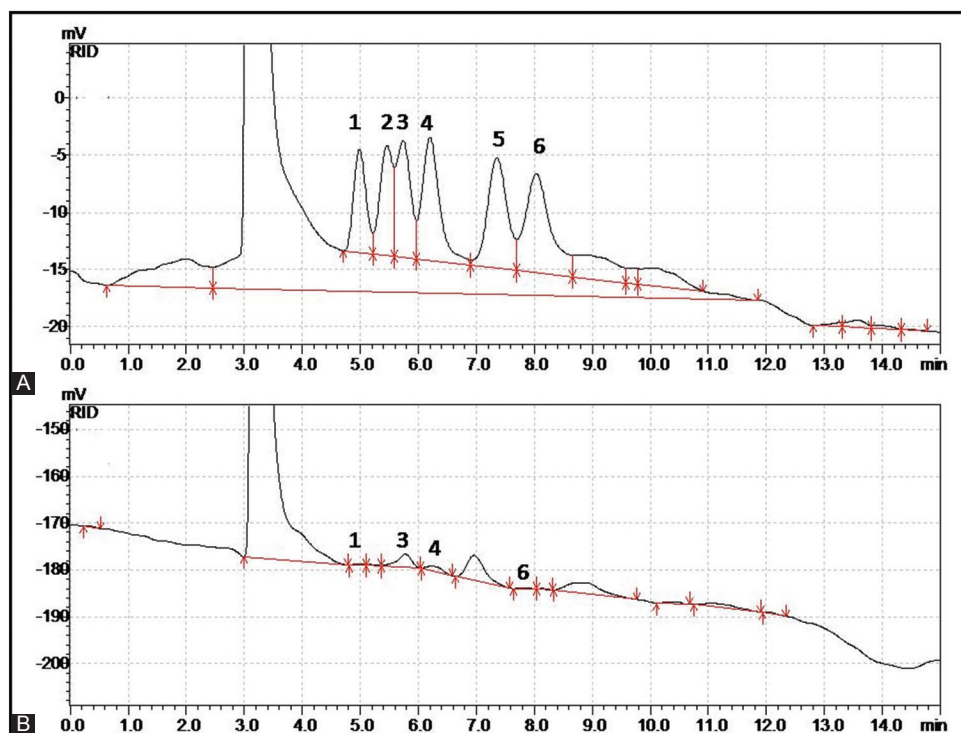


Fig. 6: High-performance liquid chromatography chromatograms of the mixed standard of sugars (A) and the sample solution of the flower of *Dregea volubilis* (B) as detected with refractive index detector (1: Rhamnose, 2: Xylose, 3: Fructose, 4: Glucose, 5: Trehalose, 6: Maltose)

## CONCLUSION

Herbal drugs are subjected to variability in quality as they derived from heterogeneous sources. The main concerned area is that the activity of the plant material may vary and even inferior quality material may be produced which may impart a quality impact on the products of the pharmaceutical industry. Standardization of herbal drugs is an important topic of great concern. The present study of pharmacognostical evaluation on the flowers of *D. volubilis* has laid down standard parameters for proper identification, authentication, and for distinguishing the material from its adulterants and substitutes. The detailed study also set the parameters which can be utilized as a pharmacopeial reference for recognition of its distinctiveness, genuineness, and quality. The study also contributes to the documentation of the nutritional composition on the flowers of *D. volubilis* which are consumed as a vegetable.

## ACKNOWLEDGMENT

The authors gratefully acknowledge the University Grants Commission, New Delhi, for providing the instrumental facilities under UGC-UPE Phase-II program at the Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

## AUTHOR'S CONTRIBUTIONS

Bhaskar Das was involved in performing all the experiments and preparing the manuscript. Arnab De, Piu Das, and Amalesh Nanda were involved in the plant identification, methodology, and interpretation of data. Dr. Amalesh Samanta had revised and finalized the manuscript.

## CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

## REFERENCES

- Sharma C, Irshad S, Khatoon S, Arya KR. Pharmacognostical evaluation of Indian folk-traditional plants *Coelogyne cristata* and *Pholidota articulata* used for healing fractures. *Indian J Exp Biol* 2017;55:622-7.
- Sanyacharemkul S, Itghiarbha A, Kongtawelert P, Meepowpan P, Nuntasaeen N, Pompimon W. A new polyoxypropygnane glycoside from the roots of *Dregea volubilis* (L.f) Benth. Ex Hook. F and its chondroprotective effect. *Am J Biochem Biotechnol* 2009;5:202-9.
- Karthika KS, Sanjaya KS, Hari KR, Jyothi T. A pharmacognostic evaluation on moorva bheda (*Dregea volubilis* (L.f) Benth. Ex Hook.f). *Int Res J Pharm* 2012;3:127-30.
- Biswas M, Haldar PK, Ghosh AK. Antioxidant and free-radical-scavenging effects of fruits of *Dregea volubilis*. *J Nat Sci Biol Med* 2010;1:29-34.
- Nandi D, Besra SE, Vedasiromoni JR, Giri VS, Rana P, Jaisankar P. Anti-leukemic activity of *Wattakaka volubilis* leaf extract against human myeloid leukemia cell lines. *J Ethnopharmacol* 2012;144:466-73.
- Sahu NP, Panda N, Mandal NB, Banerjee S, Koike K, Nikaido T. Polyoxypropygnane glycosides from the flowers of *Dregea volubilis*. *Phytochemistry* 2002;61:383-8.
- Panda N, Mondal NB, Banerjee S, Sahu NP, Koike K, Nikaido T, et al. Polyhydroxypropygnanes from *Dregea volubilis*. *Tetrahedron* 2003;59:8399-403.
- Panda N, Mandal D, Mandal NB, Sahu NP, Banerjee S. Flavonoid and flavones C-glycosides from *Dregea volubilis*. *Nat Prod Commun* 2006;1:731-3.
- Das B, De A, Das M, Das S, Samanta A. A new exploration of *Dregea volubilis* flowers: Focusing on antioxidant and antidiabetic properties. *S Afr J Bot* 2017;109:16-24.
- Chanda S. Importance of pharmacognostic study of medicinal plants: An overview. *J Pharmacogn Phytochem* 2014;2:69-73.
- Borah S, Kakoti BB, Mahato K, Chakraborty D, Lahkar S, Gogoi B, et al. Pharmacognostic and preliminary phytochemical studies on shoot of *Calamus leptospadix* Griff. An ethnomedicinal plant of Assam. *Indian J Nat Prod Resour* 2014;5:320-5.
- Hemalatha K, Abirami P. Pharmacognostic studies on *Talinum portulacifolium* (Forssk.) Asch. Ex Schweinf. *Asian J Pharm Clin Res* 2018;11:470-3.
- Upreti K, Semwal A, Upadhyaya K, Masiwal M. Pharmacognostical and phytochemical screening of leaf extract of *Zanthoxylum armatum* DC. *Int J Tradit Herb Med* 2013;1:6-11.
- Arambewela LS, Arawawala LD. Standardization of *Alpinia calcarata* roscoe rhizomes. *Pharmacogn Res* 2010;2:285-8.
- Aslam I, Afridi MS. Pharmacognostic characterization of *Beaumontia*

- grandiflora* (Roxb.) Wall. Leaf for taxonomic identification for quality control of a drug. J Appl Res Med Aromat Plants 2018;8:53-9.
16. Mallya R, Bhitre MJ. Pharmacognostic standardisation and chromatographic fingerprinting of leaves and fruits of *Zanthoxylum rhetsa*. Int J Pharm Pharm Sci 2018;10:101-4.
  17. Rabinarayan A, Switu J, Harisha CR, Vinay S. Pharmacognostical genetic barcoding and phytochemical analysis on leaves of *Dalbergia volubilis* Roxb. An extra pharmacopoeial drug of Ayurveda. Int J Pharm Pharm Sci 2018;10:117-24.
  18. Adams SJ, Kuruvilla GR, Krishnamurthy KV, Nagarajan M, Venkatasubramanian P. Pharmacognostic and phytochemical studies on Ayurvedic drugs ativisha and musta. Rev Bras Farmacogn 2013;23:398-409.
  19. Cheng D, Zhang Y, Xin X, Gao D. Comparative pharmacognosy of *Pyrrosia petiolosa* and *Pyrrosia davidii*. Rev Bras Farmacogn 2014;24:368-80.
  20. Wahab S, Hussain A, Ahmad P, Usmani S. Ethanobotanical, pharmacognostical and physicochemical studies of stem bark of *Bombax ceiba* L., commonly growing in eastern Uttar Pradesh region of India. Pharmacogn J 2012;4:55-60.
  21. Sharma M, Sharma V, Majumdar DK. Entrapment of  $\alpha$  amylase in agar beads for biocatalysis of macromolecular substrate. Int Sch Res Not 2014;1:1-8.
  22. Baloch AB, Xia X, Sheikh SA. Proximate and mineral compositions of dried cauliflower (*Brassica Oleracea* L.) grown in Sindh, Pakistan. J Food Nutr Res 2015;3:213-9.
  23. Heleno SA, Barros L, Sousa MJ, Martins A, Ferreira IC. Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high performance liquid chromatography. Microchem J 2009;93:195-9.
  24. Kokate CK, Purohit AP, Gokhale SB. Test Book of Pharmacognosy. 42<sup>nd</sup> ed. Pune: Nirali Prakashan; 2006.
  25. Folashade O, Omoregie H, Ochogu P. Standardization of herbal medicines a review. Int J Biodivers Conserv 2012;4:101-12.
  26. Madhav NV, Upadhyaya K, Bisht A. Phytochemical screening and standardization of polyherbal formulation for dyslipidemia. Int J Pharm Pharm Sci 2011;3:235-8.
  27. Yadav RN, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol 2011;3:10-4.
  28. Farooqui NA, Dey A, Singh GN, Easwari TS, Pandey MK. Analytical techniques in quality evaluation of herbal drugs. Asian J Pharm Res 2014;4:112-7.
  29. Ubeyitogullari A, Ciftci ON. Phytosterol nanoparticles with reduced crystallinity generated using nanoporous starch aerogels. RSC Adv 2016;6:108319-27.
  30. Silverstein RM, Webster FX, Kiemle DJ. Spectrometric Identification of Organic Compounds. 7<sup>th</sup> ed. New Jersey: John Wiley and Sons, Inc.; 2005.
  31. Gad HA, El-Ahmady SH, Abou-Shoer MI, Al-Azizi MM. A modern approach to the authentication and quality assessment of thyme using UV spectroscopy and chemometric analysis. Phytochem Anal 2013;24:520-6.
  32. Hussain J, Khan AL, Rehman NU, Hamayun M, Shinwari ZK, Ullah W, et al. Assessment of herbal products and their composite medicinal plants through proximate and micronutrients analyses. J Med Plants Res 2009;3:1072-7.
  33. Zhao J, Ma S, Li S. Advanced strategies for quality control of Chinese medicines. J Pharm Biomed Anal 2018;147:473-8.