

Pharmacognostical Characterization, Phytochemical Screening and Finger Print Profile of the Plant *Desmodium gangeticum* DC

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ABSTRACT

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of any plant seeds, berries, roots, leaves, barks or flowers for the medicinal purposes. From the ancient days the conventional medicines and herbalism is becoming more mainstream for the treatment of various chronic diseases. Herbalism is a traditional medicinal or folk medicine practices based on the uses of plants and plant extracts. Herbalism is also known as botanical medicine, medical herbalism, herbal medicine, herbology, and phytotherapy. Sometimes the scope of herbal medicine is extended to include fungi and bee products, as well as minerals, shells and certain animal parts. The Pharmacognostical studies of the plant *Desmodium gangeticum* DC, Family-Fabaceae were scientifically validated in this present study. The various physicochemical properties were evaluated like ash values, extractive values etc. The preliminary phytochemical analysis revealed that the dried roots of *Desmodium gangeticum* DC were extracted with the solvents of chloroform, ethanol and hydro- alcohol, showed that the presence of alkaloids, carbohydrates, glycosides, phytosterol and flavonoids. The chromatographic analysis was studied with the help of Thin layer chromatography.

Keywords *Desmodium gangeticum* DC, Fabaceae, TLC, Finger print, Extractive values

INTRODUCTION

Traditional and folklore medicines play an important role in health services around the globe. About three quarters of the world population relies on plants and plant products for health care. The plant *Desmodium gangeticum* DC, Family-Fabaceae has been used in folklore medicine in the treatment of various ailments. Many of the Ayurvedic formulations contain this medicinal plant and considered as a Master of Medicinal Plant in Ayurveda due to its wide uses in formulations. Being universal, role of plants has always stood a golden mark to exemplify the outstanding phenomenon of symbiosis. The existence of life in the universe is not endurable without the plants. The plants being in tremendous amount are associated with certain unique and a few common active principles known as secondary plant metabolites. These metabolites are referred to as organic compounds that are not directly involved in the normal growth, development or reproduction of organism. Unlike primary metabolites, absence of secondary metabolites does not cause immediate death, but is involved with the long-term impairment of the organism's survivability or aesthetics and sometimes represents no significant change at all. According to the World Health Organization (WHO), the herbal medicines have been defined as the finished, labeled medicinal products that contain active ingredients, aerial or underground parts of the plant or other plant material or

combinations. For the assessment of safety, efficacy and quality of herbal medicines, specific set of guidelines has been set by WHO. As per the estimation of WHO, around 80% of the world's population presently use herbal medicine for primary health care (WHO technical report series 1996)¹. Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs². *Desmodium gangeticum* DC, a traditional medicinal plant, identifies a well-established role in some of the Ayurvedic preparations that justifies its substantial potential in the modulation of several physiological anomalies. DG hails from the Leguminosae family and is synonymous with *Hedysarum gangeticum*. The roots of DG constitute as one of the components of the Ayurvedic medicine, Dashmoola, also called Dasamularishtam, a dietary supplement, which has marked its use in treating diseases like rheumatism, jaundice, paralysis, puerperal fever, filaria, edema and post-natal care^{3,4}. Popularly known as Prisniparni in Sanskrit, *Desmodium gangeticum* DC, an Indian medicinal plant has been widely used by many Ayurvedic and Unani physicians for curing fever, cataract, typhoid, piles, bronchitis, dysentery, asthma and various other

Table 1: The extractive values after successive extraction of roots of *Desmodium gangeticum* DC.

S. No.	Name of solvent	Total yield (gm)	Percentage
1	Petroleum ether	1.60	0.533
2	Chloroform	9.36	3.12
3	Ethyl acetate	1.20	0.4
4	Ethanol	8.50	2.833
5	Aqueous	7.2	2.4
6	Hydro alcoholic	11.48	3.8

inflammatory conditions arising from 'vata' disorder^{5,6}. Known as Salvan in Satpura hills, India; Powdered root with honey – treatment of mouth ulcer. Known as Shalparni in U.P., India; Leaf paste of *Desmodium gangeticum* DC, applied externally to prevent hair fall. Known as Biyanisaawata in Assam, India; Leaf paste applied topically on infection to cure eczema. Known as Salparni in Odhisha, India; Root decoction- treatment of dysentery. Known as Da ye shanludou in China, Root consumed orally: treatment of diarrhea, root paste applied topically: treatment of toothache; leaf paste applied topically: treatment of headache. Known as Kaganilaakatono, Uganda, Africa; Root chewed to cure premature ejaculation⁷⁻¹².

PLANT PROFILE¹³⁻¹⁵

Biological source

Shalparni consists of dried roots of *Desmodium gangeticum* DC (Fabaceae), *Desmodium gangeticum* DC is found in the outskirts of Himalayas, up to 5,000 ft and also available throughout India, usually growing in the dry hills areas. Mostly they are used in the medicine as an anti-asthmatic, bronchitis, aphrodisiac, anthelmintic, astringent to the bowels, cures typhoid fevers due to mental disorder, inflammations and piles. Outer Himalayas, up to 5,000 ft. and throughout India to Ceylon and Burma, Malay Peninsula-malay islands, China, Philippines and tropical Africa. The plant is widely distributed in tropics and sub-tropics. It is cultivated also in the plains.

Botanical Name	<i>Desmodium gangeticum</i> DC
Family	Fabaceae
Common name	Salpan
Synonym	
Sanskrit	Sthira, Anshumati
Kannada	Murelehona
Malayalam	Moovila, Orilla
Telugu	Kolakuponna
Tamil	Pulladi
Locality & Altitude	
Locality	Lucknow, UP, Kollam, Maruthamonapilly
Altitude	Up to 1500 MSL

Description^{16,17}

Desmodium gangeticum DC have conserving of systemic tap root, poorly developed but lateral roots are 15-30 cm long, and 0.1-0.8 cm in thickness, uniformly cylindrical

with a number of branches, light brown lenticels, light yellow fracture fibers, the odor of the plant is not characteristic and taste of the plant is Sweetish and mucilaginous and its contain the Alkaloids, Vitamins, oils and minerals like calcium, phosphorus, magnesium and it is used in the treatment of Antipyretic, digestive, diuretic, aphrodisiac, it is also beneficial in the treatment of typhoid, piles and dysentery.

MATERIALS AND METHOD

Pharmacognostic evaluation

Collection and authentication of plant

The plant was collected from National Botanical Research Institute/Aminabad, Lucknow (U. P) and authenticated from National Botanical Research Institute, Lucknow by Dr. Alok Lahri Ref.No: NBRI/CIF/492/2015, Scientist and Head, Pharmacognosy and Ethanopharmacology Division.

Preparation of Powder

The fresh roots of *Desmodium gangeticum* DC were washed and cut in to small pieces. The same were dried in controlled hot air oven at the temperature of 30-40°C. The dried materials were coarse powdered by means of mechanical grinder and resulting powdered material were used for further studies.

Physicochemical determination

A. Ash values

Determination of Ash Values^{17,18}

Determination of Total Ash

Accurately weighed 2 gm of powdered drug was incinerated in tarred silica dish at a temperature not exceeding 450°C until free from carbon, cooled, weighed, repeated the procedure to get a constant weight of the same sample and calculated for the percentage of ash with reference to that of air dried drug.

Determination of Acid insoluble ash

Boiled the ash obtained from total ash with 25 ml of dilute hydrochloric acid for 5 minutes then collected the insoluble matter on an ashless filter paper, washed with hot water and ignited at a temperature not exceeding 450°C to get constant weight, then calculated the percentage of acid-insoluble ash with reference to the air dried drug.

Determination of Water soluble ash

Boiled the ash with 25 ml of water for 5 minutes then collected the insoluble matter on an ashless filter paper and washed with hot water and ignited at a temperature not exceeding 450°C to get constant weight and subtracted the weight of insoluble matter from the weight of ash; the difference in weight representing the water soluble ash. The percentage of water-soluble ash with reference to the air dried drug was then calculated.

B. Determination of extractive values

Determination of Alcohol soluble extractive

5 gm of air dried coarsely powdered drug was macerated with 100 ml of alcohol in a closed flask for 24 hrs, shaking frequently during 6 hrs and allowed to stand for 18 hrs. It was then filtered rapidly taking precautions against loss of solvent and then evaporated 25 ml of filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105°C to constant weight and weighed.



Aerial parts of *Desmodium gangeticum* DC



Root of *Desmodium gangeticum* DC



Legume of *Desmodium gangeticum* DC

Figure 1a: Different images of *Desmodium gangeticum* DC

The percentage of alcohol-soluble extractive with reference to the air dried drug was then calculated.

Determination of water-soluble extractive

5 gm of air dried coarsely powdered drug was macerated with 100 ml of chloroform water in a closed flask for 24 hrs, shaking frequently during 6hrs and allowed to stand for 18 hrs. The extract was then filtered and evaporated 25 ml of filtrate to dryness in a tarred flat bottomed shallow dish and dried at 105°C to constant weight and weighed. The percentage of water-soluble extractive with reference to the air dried drug was then calculated. Results are present in the Table No.5 & 6.

Phytochemical Analysis

Extraction with different solvent²⁰

Shade dried roots of *Desmodium gangeticum* DC were extracted with different solvents like Petroleum Ether, Chloroform, Ethyl Acetate, Ethanol and Aqueous by using hot extraction method.

Phytochemical screening^{19,21}

The extracts and fractions were subjected to chemical tests and chromatography to determine chemical nature of the constituents present.

Chemical Test

Test for alkaloids

Extracts or fractions were treated with Mayer's reagent (Potassium-mercuric Iodide), appearance of cream coloured ppt. confirmed alkaloids.

Extracts or fractions were treated with Wagner's reagent (Iodine in potassium iodide solution), when appearance of reddish brown ppt. confirmed alkaloids.

Extracts or fractions were treated with Dragendorff's reagent (solution of Potassium Bismuth Iodide), when appearance of reddish brown ppt. confirmed alkaloids.

Test for Carbohydrates

Molisch's test

Extracts or fractions were treated with α -naphthol followed by addition of Conc. Sulphuric acid, when appearance of purple colour confirmed the presence of carbohydrates.

Benedict's test

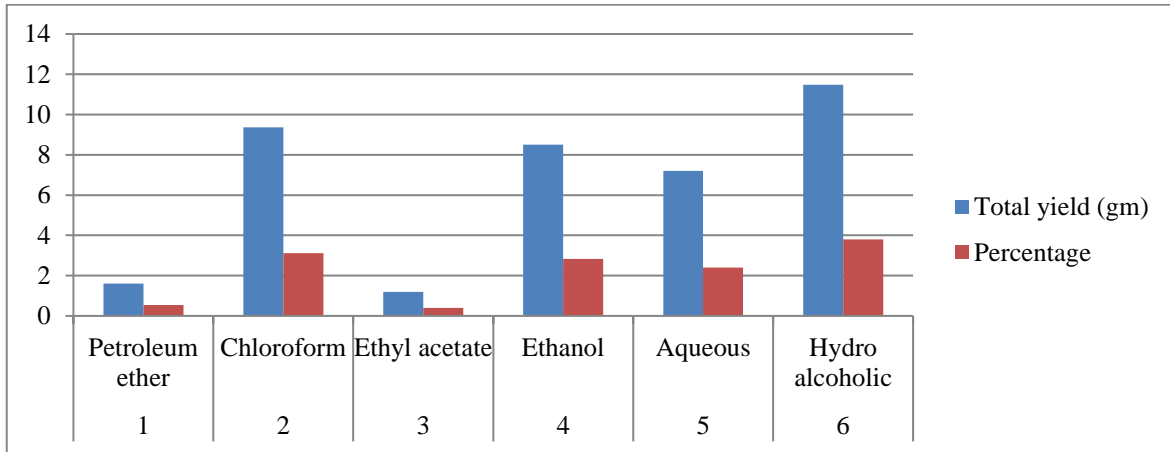


Figure 1: Showing the extractive yield of the dried roots extract of Desmodium gangeticum DC

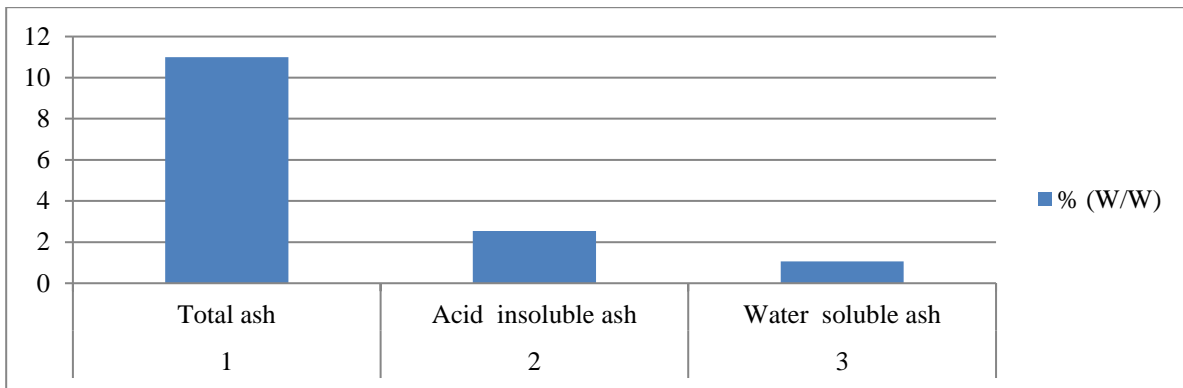


Figure 2: Showing the Ash values of the dried root extracts of Desmodium gangeticum DC

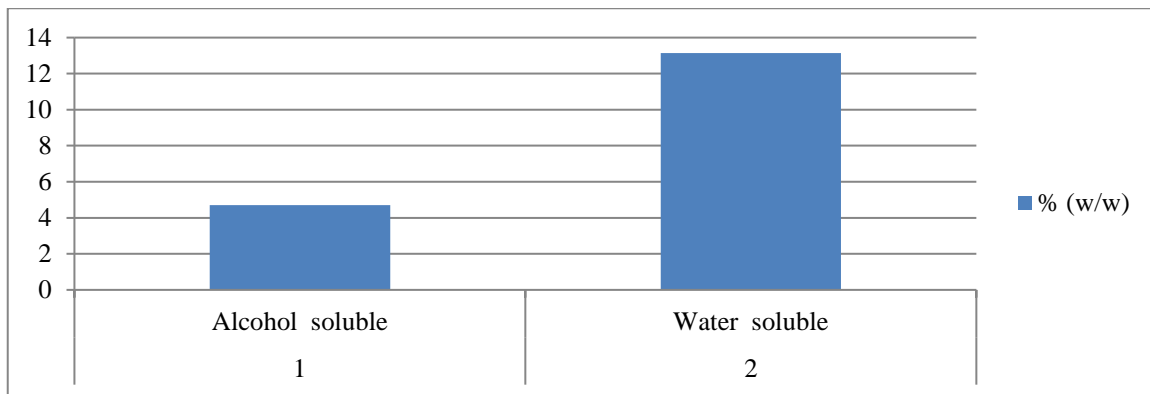


Figure 3: Showing the extractive values of the dried root extracts of Desmodium gangeticum DC

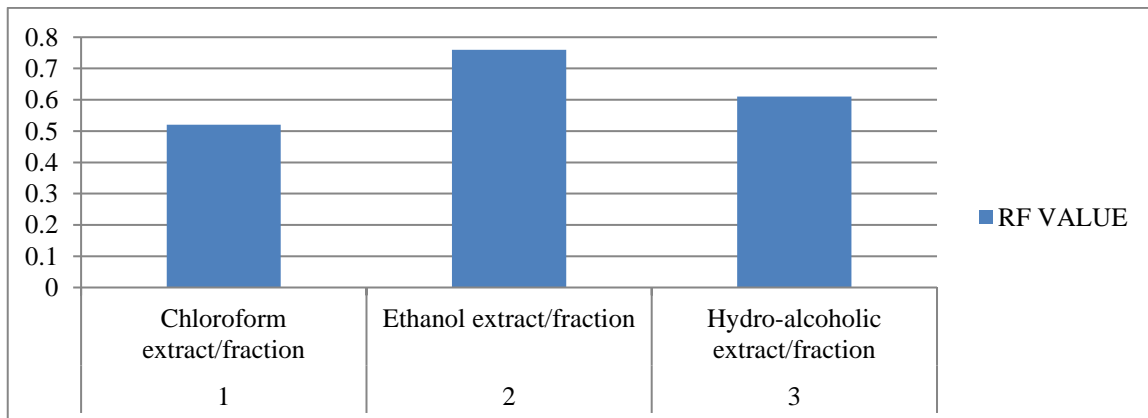


Figure 4: Showing the R_f Values of the dried root extracts of Desmodium gangeticum DC

Table 2: Qualitative phytochemical results

Qualitative Phytochemical screening	Pet. Ether	Chloroform	Ethyl Acetate	Ethanol	Hydro-alcoholic	Aqueous
ALKALOIDS						
Mayer's reagent	-	+	-	-	+	+
Wagner's reagent	-	+	+	+	+	+
Hager's reagent	-	+	+	+	+	-
Dragendroff reagent	-	+	-	+	+	+
CARBOHYDRATES AND GLYCOSIDES						
Molish's test	-	-	-	-	+	-
Fehling's test	+	-	+	-	-	-
Benedict's test	+	-	+	-	-	-
Legal test	-	-	-	-	-	-
Borntrager test	-	-	-	-	-	-
Kellarkillani test	-	-	-	-	-	-
Baljet's test	-	-	-	-	-	-
SAPONINS						
Foam test	-	-	-	-	+	+

Extracts or fractions were treated with Benedict Reagent (173gm sod. Citrate + 100gm sod. Carbonate + 17.3gm copper sulphate) followed by boiling for 2 min then allowed to cool, when appearance of red, yellow or green ppt. depending on amount of sugar confirmed the presence of carbohydrates.

Tests for Flavonoids and Coumarins

Mineral acid test

Extracts or fractions were treated with Conc. Sulphuric acid, when appearance of yellow orange colour confirmed the presence of Flavonoids.

Sodium Hydroxide test

Extracts or fractions were treated with 10% sod. Hydroxide solution, when appearance of yellow orange colour confirmed the presence of Flavonoids.

Shinoda's test (Mg-HCl reduction test)

Extracts or fractions in alcohol were added few fragment of magnesium ribbon followed by addition of conc. Hydrochloric acid drop by drop, appearance of pink to crimson red color after few minutes confirmed the presence of Flavonoids.

Test for steroids

Libermann-Burchard Sterol reaction

To the extracts or fractions glacial acetic acid was added followed by addition of conc. Sulphuric acid, when a colour change from rose, through red, violet and blue to green confirmed the presence of steroids.

Salkowski reaction

To the extracts or fractions chloroform was added followed by addition of conc. Sulphuric acid, when appearance of red colour in chloroform layer confirmed the presence of steroids.

Test for triterpenoids

Hirchorn test

To the extracts or fractions Trichloroacetic acid was added followed by warming, when appearance of yellow to red colour confirmed the presence of triterpenoids.

Salkowski reaction

To the extract or fractions chloroform was added followed by addition of conc. Sulphuric acid, when appearance of

red colour in chloroform layer confirmed the presence of triterpenoids.

Test for tannins

Ferric chloride test

To the extracts or fractions freshly prepared Ferric chloride solution was added, when appearance of blue-black or brownish colour confirmed the presence of tannins. Results are tabulated in the Table No 2 & 3.

TLC OF ALKALOIDS²²

Presence of alkaloids in various extracts and fractions was investigated by using the following mobile phases. Benzene: Methanol (95: 5), Methanol: Chloroform 40:60 and stationary phase was used silica gel G and the visualizing agents were used the development was done by using Anisaldehyde: H₂SO₄: GAA (0.5: 5: 10) as the developing agent and Iodine chamber was used as detector and observation of spots either in normal or UV light and the R_f value was calculated by Distance moved by the substance front / Distance moved by the solvent front Results are tabulated in Table No 4.

RESULT AND DISCUSSION

The plant *Desmodium gangeticum* DC belongs to family Fabaceae. The roots of *Desmodium gangeticum* DC was universally used as a medicine. The roots were included in Ayurvedic pharmacopoeia of India, Vol-III. So we felt in worthwhile to validate it scientifically the folk claims for its therapeutic activity. The plant had been investigated in systemic way covering its pharmacognostical, phytochemical and TLC profile aspect.

Extractive value

The extractive values of *Desmodium gangeticum* DC showed that more fractions of material were available in hydroalcoholic (3.8%), chloroform (3.12%), ethanol (2.83%) and aqueous (2.4%). This showed that more fractions are available in these fractions compare to that petroleum ether (0.53%) and ethyl acetate (0.4%). These values may be due to presence of alkaloids present in the drug. values are tabulated below in (Table No. 1)

Pharmacognostical and phytochemical studies

Table 3: Qualitative phytochemical results

Qualitative screening	Phytochemical	Pet. Ether	Chloroform	Ethyl Acetate	Ethanol	Hydro-alcoholic	Aqueous
PHYTOSTEROL							
Lieberman burchard test		-	-	+	-	-	-
Salkowski reaction		+	-	+	+	+	+
FIXED OILS AND FATS							
Spot test		+	-	+	-	-	-
Saponification test		+	-	+	-	-	-
PHENOLIC COMPOUNDS AND FLAVONOIDS							
Ferric chloride test		-	+	-	-	-	-
Gelatin test		-	-	-	+	-	-
Lead acetate test		+	-	+	-	-	-
Shinoda test		-	-	-	-	-	-
GUMS AND MUCILAGE							
Alcohol 95% test		-	-	-	-	-	-
Gums		-	-	-	-	-	-
PROTEINS AND AMINO ACIDS							
Million's test		+	-	-	-	-	-
Biuret test		-	-	-	-	-	-
Ninhydrin test		-	-	-	-	-	-
TEST FOR VITAMINS							
Vitamin C		-	-	-	-	-	-
Vit .D		-	-	-	-	-	-
VOLTILE OILS							
SOLUBILITY TEST							
		-	-	-	-	-	-

Table 4: TLC of Chloroform, ethanol and hydro alcoholic extracts of *Desmodium gangeticum* DC.

S. No.	TLC of extract	RF Value
1.	Chloroform extract/fraction	0.52
2.	Ethanol extract/fraction	0.76
3.	Hydro-alcoholic extract/fraction	0.61

Table 5: Ash values of the dried roots of *Desmodium gangeticum* DC

S. No	Type of ash	% (W/W)
1	Total ash	11
2	Acid insoluble ash	2.54
3	Water soluble ash	1.065

Table 6: The alcohol soluble extractive values and the water soluble extractive values with different polarity of the solvent were carried out and result are as follows

S. No	Type of extract	% (w/w)
1	Alcohol soluble	4.696
2	Water soluble	13.136

The taxonomical identification of the plant, its morphological characteristics further provided additional information about this plant drug. Analytical parameter like ash values and extractive values were carried out. The total ash, acid insoluble ash, water soluble ash values were carried out and the result are present in (Table No 5 & 6). Qualitative phytochemical analysis Both successive extracts and hydro-alcohol (Maceration) were subjected to

chemical test for the detection of the phytoconstituents. The result revealed alkaloids, carbohydrates, glycosides, phytosterol and flavonoids are reported. (Table No 2 & 3). *Thin layer chromatography*

The R_f values of chloroform, ethanol and hydro-alcoholic extracts were found to be 0.52, 0.61 and 0.91 respectively. Values are present below:

DISCUSSION

Desmodium gangeticum DC is one of the important ingredients of Dasmulakwatha of Ayurveda. The species *Desmodium gangeticum* DC is a plant that has been confused with other species due to their relative similarities, therefore many a times some other materials are mixed or adulterated during the preparation of medicines. As standardization of a crude drug is an integral part of establishing its correct identity for its inclusion in herbal pharmacopoeia. For which pharmacognostical parameters and standards must be established. The total cash value is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. As there is no pharmacognostical work on record of this much valued traditional drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant.

CONCLUSION

In conclusion, the pharmacognostical features examined in the present study that includes physico-chemical and TLC may serve as a tool for identification/ validation of the raw material and standardization of its formulations in fixing quality control parameters as well as answer to the latest

GMP norms and FDA guidelines on standardization of herbal drugs. The present study revealed different physicochemical parameters which can be used for authentication of the crude samples. Physico-chemical as well as various aspects of the sample were studied and the sample of *Desmodium gangeticum* DC exhibits a set of physicochemical characters, which will help to identify the drug in dried condition. It has been concluded from this study that estimation of pharmacognostical evaluation is highly essential for raw drugs or plant parts used for the preparation of compound formulation drugs. The periodic assessment is essential for quality assurance and safer use of herbal drugs. As per phytochemical investigation, the main constituents present are alkaloids, carbohydrates, glycosides, phytosterol and flavonoids. The therapeutic activity of this plant may be due to the presence alkaloids. Further studies on the isolation of the compound will be carried out in future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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