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Preliminary Phytochemical Evalution and Antioxidant potential of Aerial parts of *Desmodium gangeticum*

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ABSTRACT

Standardization plays a significant role in the production of phytopharmaceutical of standard quality as the quality standards are based on proper selection of raw materials. *Desmodium gangeticum* (DC) commonly known as salpan, salvan and sarivan belonging to family-Fabaceae. In India, *Desmodium gangeticum* (L.) DC. Has a considerable reputation as a bitter tonic, febrifuge, digestive, anti-emetic, antipyretic and anti-catarrhal. It is also widely used in Ayurveda for the treatment of neurological disorders. Present investigation includes examination of morphological and microscopic characters, ash value, extractive values and Phytochemical evaluations including qualitative chemical examination of active constituents were carried out along with radical scavenging activity.

Keywords: *Desmodium gangeticum* (L.) DC, Phytochemical, Phyto constituent, Pharmacognostic, Antioxidant.



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INTRODUCTION

Standardization plays a momentous role in the production of phytopharmaceutical of standard quality as the quality standards are based on proper selection of raw materials. As very little specific standards are mentioned in the official monographs evaluation of the crude drugs is of great consequence for the pharmaceutical industry. This involves the determination of identity and purity of quality. Many organic & inorganic contaminants which are virtually impossible to avoid while collecting crude drugs affect the purity of any crude drug which needs proper assessment & detection based on different pharmacognostic & phytochemical parameters¹. An herbal drug constitutes a major part in all traditional systems of medicine. There areapproximately 1250 Indian medicinal plants which are used in formulating therapeutic preparations according to Ayurvedic and other traditional systems of medicine². Desmodium gangeticum (DC) commonly known as salpan, salvan and sarivan in Hindi; belonging to family-Fabaceae. Salparni is found throughout tropical India into the lower portions of the Himalayans range, and it related species are also found in regions of China (Desmodium styracifolium, Desmodium pulchellum). The meaning of its Sanskrit name 'Leaves like sala' suggests that its leaf structure is similar to those of the tree Shorea robusta14. Its synonyms are Aakuparnijaa, Amshumati, Atiguha, Atiruha, Deergmoolika, Dhurva, Guha, Mahaakleetaanika, Parninee, Peethanee, saumya, Sthira, Triparni, vidyarigandha³.Desmodium gangeticum DC is common on the lower hills and plains throughout India, on the Himalayans it ascends to 5,000 feet. It is spread east to Pegu and Ceylon and is distributed to China. It is a large genus of woody stem without stocks distributed in the Himalayans zones, about 24 species are found in India with various medicinal and therapeutic values⁴.



Desmodium gangeticum

Morphology

Desmodium gangeticun is a perennial herbs, subshurbs, shrubs, or rarely small trees, pubscent with straight or uncinate hairs, Leaves green with 1 or 3 leaflet, stopellate, stipules scarious, distinct. Flowers papilionacious, white in colour terminal and auxiliary or rarely leaf-opposed, recemose, paniculate or rarely fasciculate inflorescences, principal bracts striate, persistent or

caducous, each subtending 2 to several flowers born at base of calyx; calyx campanulate 2 lipped or 5 lobed, corolla exceeding the calyx. Stem woody stem, prostrate to erect or araching, angular, striate, uncinulate. Pods compressed 1-2 joined to many jointed constricted on lower or both sutures to form one seeded indehiscent articles. Seed compressed, ellipsoid to subqurdrate; often rimarillate. Fruit sessile to shortly stipitate, up to 7-8 articled⁵⁻⁶. In India, *Desmodium gangeticum* (L.) DC. has a considerable reputation as a bitter tonic, febrifuge, digestive, anti-emetic, antipyretic and anti-catarrhal. It is also widely used in Ayurveda for the treatment of neurological disorders⁷.

MATERIALS AND METHOD

Collection and authentication

Aerial parts of *Desmodium gangeticum* were collected from herbal garden of Dehradun (Green Biotech). The plant was identified and authenticated at the Botanical Survey of India (BSI), Northern regional centre, Dehradun with the accession number BSD-112743.

Preparation of Plant Extracts

The powder was subjected to successive soxhlet extraction with different solvents in increasing order of polarity at different temperature (i.e. Petroleum Ether <Benzene< Chloroform< Acetone< Ethanol<Chloroform water I.P.

PRELIMINARY PHYTOCHEMICAL SCREENING OF PLANT EXTRACTS:

Determination of solvent extractive values:

Determination of water soluble extractive value:

5 g of the air-dried drug, coarsely powdered were macerated with 100 ml of water in closed flask for 24 hours, shaking frequently during the first 6 hours and allow standing for 18 hours. It was filtered rapidly taking precaution against loss of water, then the filtrate was evaporated 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, and dried at 105^oC then weighed. The percentage of water-soluble extractive with reference to the air dried was calculated.

Determination of alcohol soluble extractive value:

5 gm of the air dried and coarsely powdered drug was macerated with 100 ml of ethanol of the specific strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow standing for 18 hours. There after filter rapidly taking precaution against loss of ethanol. Evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, dry at 105^oc and weigh. The percentage of ethanol soluble extractive with reference to the air dried drug has to be calculated.

Determination of moisture content:

Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Method of determination of moisture content include the loss on drying, the test for loss on drying determines both water and volatile matter in the crude drug. It can be carried out either by heating at 100^oC-105^oC or in a dessicator over phosphorous pentoxide under atmospheric or reduced pressure at room temperature for specific period of time.

Ash value:

Ash value is helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in case where silica may be present or when the calcium oxalate content of the drug is very high.

Total ash value

Weighed accurately about 2 to 3 g of the powdered drug in a tared silica crucible. Incinerated at a temperature not exceeding 450 0 C for 4 hr, until free from carbon, cooled and weighed. The percentage of ash with reference to air-dried was calculated following formula.

% Total ash value = $\frac{\text{Wt. of total ash}}{\text{Wt. of crude drugs}} \times 100$

Water soluble ash value:

Boiled the ash with 25 ml of water. Filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450 °C for 4 hr. Cooled in a desiccator and weighed. Substrate the weight of insoluble matter from the total weight of ash. The difference in weight represented weight of water soluble ash. Calculated the percentage of water soluble ash with reference to the air-dried drug by using the following formula.

Acid insoluble ash value:

Boiled the ash for 5 min with 25 ml of 2 M HCL. Filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450^{0} C for 4 h. cooled in a desiccator and weighed. Calculated the percentage of acid insoluble ash with reference to the air -dried drug was calculated by using following formula,

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% Acid insoluble ash value = <u>Wt. of acid insoluble ash</u> × 100 Wt. of crude drug taken

Preliminary qualitative test

The various extract of *Desmodium gangeticum* was subjected to preliminary qualitative phytochemical investigation. The various tests and reagent used are given below.

Alkaloids

Preparation of test solution: The test solution was prepared by dissolving extracts in the dilute hydrochloric acid.

Mayer' test: The acidic test solution with Mayer's reagent (Potassium Mercuric iodide) gave cream colored precipitate.

Hager's test: The acidic test solution with Hager's reagent (Saturated picric acid solution) gave yellow precipitate.

Dragendorff's test: The acidic solution with Dragendorff's reagent (Potassium bismuth iodide) showed reddish brown precipitate.

Wagner's test: The acidic test solution treated with Wagner's reagent (Iodine in potassium iodide) gave brown precipitate.

Tannic acid test: The acidic test solution treated with Tannic acid gave buff colour precipitate.

Picrolonic acid test: Alkaloids gave yellow colour precipitate with picrolonic acid.

Amino acid:

Millon'test: To the test solution add about 2 ml of millon's reagent white precipitate indicates presence of amino acid.

Ninhydrine test: To the test solution add Ninhydrine solution, boil, violet colour indicates presence of amino acid.

Carbohydrates

Preparation of test solution: The test solution was prepared by dissolving the test extracts with water. Then it was hydrolyzed with 1 volume of 1 N-HCL and subjected to following chemical test.

Molisch's test: Test solution with few drops of Molisch's reagent and 2 ml of conc. H_2So_4 added slowly from the sides of the test tubes. It showed a purple ring at the junction of two liquids.

Barfoed's test: 1 ml of test solution is heated with 1 ml of Barfoed, s reagent on water bath, if red cupric oxide is formed, monosaccharide is present. Disaccharides on prolong heating (about 10 min.) may also cause reduction, owing to partial hydrolysis to monosaccharide.

Benedict's test: Test solution treated with Benedict' reagent and after boiling on water bath, it showed reddish brown precipitate.

Fehling's test: The test solution when heated with equal volume of Fehling's A and B solution, gave orange red precipitate, indicating the presence of reducing sugars

Flavonoids

The flavonoids are all structurally derived from the parent substance called flavones. The flavonoids occur in the free from as well as bound to sugars as glycosides. For this reason, when analyzing flavonoids it is usually better to examine the flavonoids in hydrolyzed plant extracts.

Preparation of test solution: To a small amount of extract added equal volume of 2 M HCL and heated in a test tube for 30 to 40 min at 100^oC. The cooled extract was filtered, and extracted with ethyl acetate The ethyl acetate was concentrated to dryness, and used to test for flavonoids. **Shinoda test:** Test solution with few fragments of magnesium ribbon and conc. HCL showed pink to magenta red colour. To a small quantity of test solution when lead acetate solution was added, it formed yellow colored precipitate.

Alkaline reagent test: Test solution when treated with sodium hydroxide solution showed increase in the intensity of yellow colour, which becomes colorless on addition of few drops of dilute acid.

Glycosides

Preparation of test solution: The test solution was prepared by dissolving extract in the alcohol or hydro- alcoholic solution.

Test for Cardiac glycosides:

Kedde' test: Add one drop of 90% alcohol and 2 drops of 2 % 3, 5- dinitro benzoic acid in 90% alcohol. Make alkaline with 20 % sodium hydroxide solution, purple colour is produced. The colour reaction with 3, 5- dinitro benzoic acid depends on the presence of α , β -unsaturated lactones in the aglycone.

Baljet's test: The test solution treated with sodium picrate gave yellow to orange colour.

Raymond's test: Test solution treated with hot methanolic alkali, violet colour is produced.

Bromine water test: Test solution dissolve in bromine water give yellow precipitate.

Keller-killani test for digitoxose: The test solution treated with few drops of Fecl3 solution and mixed, then H₂So₄ containing Fecl3 solution was added, it formed two layers. Lower layer reddish brown, upper layer turns bluish green.

Legal's test: Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gave pink to red colour.

Test for anthraquinone glycosides:

Borntrager's test: Boiled powdered drug with 5 ml of 10 % sulphuric acid for five minutes. Filtered while hot, cooled the filtrate shaken gently with equal volume of benzene. Benzene

layer was separated and then treated with half of its volume solution ammonia (10%). Allowed to separate it. The ammonical layer acquired rose pink colour due to presence of anthraquinones.

Proteins

Preparation of test solution: The test solution was prepared by dissolving the extract in water. **Millon's test:** Test solution was treated with millon's reagent and heated on a water bath. The proteins were stained red

Biuret test: Test solution was treated with 40% sodium hydroxide and dilute copper sulphate solution gave blue colour.

Xanthoproteic test: Test solution was treated with conc. HNO₃ and boiled which gave yellow precipitate.

Modified Borntrager's test: C-glycosides of anthraquinones require more drastic conditions for hydrolysis. Hydrolysis of the drug was carried out with 5 ml of dilute of HCL and 5 ml of 5 % solution of Fecl₃. For hydrolyzed extract procedure was carried out as described under Borntrager's test.

Test for steroids

Preparation of test extract solution: The extract was refluxed separately with alcoholic solution of potassium hydroxide till complete saponification. The saponified extract was diluted with water and unsaponificable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (saponificable matter) was subjected to the following test by dissolving the residue in the chloroform.

Salkowski test: To the test extract solution add few drops of conc. H₂SO₄ shaken and allowed to stand, lower layer turned red indicating the presence of steroids.

Libermann - Burchard test: The test solution treated with few drops of acetic anhydride and mixed, when conc. H_2SO_4 was added from the sides of the test tubes, it showed a brown ring at the junction of the two layers and the upper layers turned green. Added few drops of concentrated H_2SO_4 . Blue colour appeared.

Sulphur test: Sulphur test when added in to the test solution, it sank it.

Tannins and phenol compound

To 2-3 ml of alcoholic or aqueous extract, added few drops of following reagents.

5% Fecl₃ solution: Deep blue- black colour.

Lead acetate solution: White precipitate.

Bromine water: Discoloration of bromine water

Acetic acid solution: Red colour solution.

Dilute iodine solution: Transient red colour.

One drop of NH₄OH, excess 10% AGNO₃ solution. Heated for 20 min in boiling water bath. White precipitate was observed, then dark silver mirror deposited on wall of test tube.

Triterpenoids

Preparation of test extract solution: The test extract solution was prepared by dissolving extract in the chloroform.

Salkowski test: Few drops of concentrated sulphuric acid were added to the test solution, shaken and on standing lower layer turned golden yellow.⁸⁻¹⁰

ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Activity:

2ml of an alcoholic solution of DPPH (0.05 mM) was added to an equal volume of ethanolic extract DC (100-500 μ g/mL) and ascorbic acid(25mg) dissolved in 250 ml of methanol to get 500 μ g/ml stock solution separately. Small concentrations of ascorbic acid and extract (2, 4, 6, 8, 10 μ g/ml of both) were prepared by serially diluting stock solutions. For determination of free radical-scavenging activity of the extracts, stable DPPH radical was used. 0.1 mM solution of DPPH in methanol (22.2 mg in 1000 ml) was freshly prepared. IC₅₀ values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. The following formula was used to calculate Radical scavenging activity¹¹:

% Radical Scavenging Activity = Acontrol- Asample / Acontrol * 100

Where, A_{control} = Absorbance of control

A_{sample} = Absorbance of sample

The inhibition curve had been plotted for duplicate experiments and represented as % of mean inhibition \pm standard deviation.

By extraction of aerial parts of *Desmodium gangeticum* the percentage yield was calculated and given in table no.1 and figure no.1

Sr. No.	Extract	Wight of	Weight of	% yield
		powder drug (g)	extract (g)	
1.	Petroleum ether extract	200	5.369	2.68
2.	Benzene extract	193	3.068	1.58
3.	Chloroform extract	187	5.644	3.08
4.	Acetone extract	175.5	4.224	2.40
5.	Ethanol extract	168	6.645	3.95
6.	Aqueous extract	160	21.003	13.12

Table 1: Percentage yield of aerial parts of Desmodium gangeticum



Figure 1: Percentage yield of aerial parts of Desmodium gangeticum

Various standardization parameter of *Desmodium gangeticum* were determined and shown in table no.2 and figure no.2

 Table 2: Ash Value of powdered drug of Desmodium gangeticum

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S.No	Ash values	NMT 10 % w/w	Observation	in (w/w)
1	Total ash		3.65 %	
2	Acid insoluble ash		2.15 %	
3	Water soluble ash		1.65 %	
	Obse 1.65% 2.15%	rvation in (mg/g	m NMT 10%) I Total ash I Acid insoluble ash I Water soluble ash

Figure 2: Ash Value of powdered drug of Desmodium gangeticum By phytochemical analysis of various extracts from aerial parts of *Desmodium gangeticum* different phytoconstituents was present in it and shown in table 3.

S. No	Extract	Observation
1	Petroleum ether	Alkaloid, Protein, Amino acid and Carbohydrate.
2	Benzene	Tannins and Flavanoids.
3	Chloroform	Alkaloid and Carbohydrate.
4	Acetone	Carbohydrate and Alkaloid.
5	Ethanol	Carbohydrate, Protein, Amino acid, Flavanoids and
		Carbohydrate.
6	Aqueous	Alkaloid, Tannins, Flavonoids, Carbohydrates,
		Amino acid and Protein.

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Table 4: 50% inhibition (IC₅₀) for EtoH extract of DC by DPPH method

S.No	Sample	IC50 (µg/ml)
1.	Ascorbic acid	3.17
2.	Ethanolic extract of DC	76.2

RESULTS AND DISCUSSION

It is seen from the literature that *Desmodium gangeticum* is a very important plant for its large number of medicinal properties. The plant shows many pharmacological activities like Antiinflammatory, Anti-nociceptive, Analgesic, Anti-amnesic, Anti-diabetic, Anti-oxidant, antiulcer, batter CVS activites, CNS depressant, Antibacterial, Wound Healing & antipyretic¹². The phytochemical analysis of various extracts of Desmodium gangeticum aqueous extract and ethanolic extract exhibited maximum percentage yield (21.003 gm and 6.645 gm) w/w respectively followed by petroleum ether (5.369gm), chloroform (5.644gm), acetone (4.224gm), benzene (3.068gm). The standardization parameters were loss on drying at 100- $105^{\circ}C$ (5.8% w/w), total ash value (3.65%, w/w), acid insoluble ash value (2.15%, w/w), water soluble ash value (1.65 % w/w). Water soluble extractive value (1.498%, w/w) and alcohol soluble extractive value were found to be (0.544%, w/w) respectively. The phytochemical analysis showed that the various extract of *Desmodium gangeticum* contained Alkaloid, Protein, Amino acid and Carbohydrate in (petroleum ether), Tannins and Flavanoids in (Benzene), Alkaloid and Carbohydrate in (Chloroform), Carbohydrate and Alkaloid present (Acetone), Carbohydrate, Protein, Amino acid, Flavanoids and Carbohydrate in (Ethanolic exract), Alkaloid, Tannins, Flavonoids, Carbohydrates, Amino acid and Protein in (Aqueous extract) respectively. The antioxidant potential was determined by DPPH method. In the present study, the IC50 values of ethanolic DC extract was found to be 76.2 µg/ml.

CONCLUSION

The present study may be useful to supplement information in regard to its characterization and identification of plant.

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