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PHARMACOGNOSTIC AND PHYTOCHEMICAL SCREENING OF *DESMODIUM TRIFLORUM* LINN.

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ABSTRACT: The plant of *Desmodium triflorum* Linn (Fabaceae) are reported to have great medicinal value. The aim of this study to evaluate pharmacognostic evaluation including examination of morphological characters, ash value, powder analysis, and extractive values were carried out. Phytochemical screening including chemical examination and chromatographic study were also carried out. This would help to scientifically justify its pharmacological activities of particular chemical constituents in different extracts.

INTRODUCTION: Botanical Description:

Desmodium triflorum is an ornamental plant commonly known as Jangali Methi, belonging to family Fabaceae. A small perennial much branched prostrate trailing herb with slender stems rooting at the nodes; leaves trifoliate, leaflet membranous, obovate, cuneate, truncate, or emarginate or rarely rounded; flowers pink or white, 1-5 fascicled in the axils of the leaves; fruits pods, with straight upper edge and indented lower edge reticulately veined¹.

Chemical Constituents:

Leaf contains alkaloids (0.01-0.15%), beta phenylethylamine, tyramine, hypaphorine, and flavonoids. Root contains (0.01-0.02%) alkaloids. Ursolic acid, vitexin, genistin (Chio and Huang et al.1995), fucosterol, 2-O- β -xylosylvitexin (Sreenivasan et al., 1984) and a rare diholosylflavone, 2-O-glucosylvitexin had been isolated from DF.

The alkaloids of DF include hypaphorine, N, N-dimethyltryptophan, betaine, choline, β -phenethylamine (a minor constituent), N, and N-dimethyltryptamine oxide².

Ethnobotanical uses:

The plant is acrid, sweet, cooling, expectorant, and galactagogue, and used in vitiated condition of pitta, cough, bronchitis, wounds, abscess, sores, pruritus, dysentery, flatulence and burning sensation¹. This plant also used in ache (stomach), dermatosis, dysentery, abscess, diarrhea, ophthalmia, rheumatism, sore, tonic, diuretic, and tumor³.

Reported Activities:

Antioxidant and antiproliferative activity⁴, analgesic and anti-inflammatory activity⁵, anthelmintic action⁶, anticonvulsant activity⁷, antibacterial Activity⁸, antinociceptive Activity⁹.

Classification of *Desmodium triflorum* plant:

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

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Division: Magnoliophyta
 Class: Magnoliopsida
 Order: Fabales
 Family: Fabaceae
 Genus: Desmodium
 Species: *D. triflorum* L.



FIG.1: *DESMODIUM TRIFLORUM* PLANT

MATERIAL AND METHODS:

The present section deals with the detailed description of various methods and techniques employed for carrying out different studies categorized in to following heading.

Collection and authentication of the plant leaves:

The leaves of *Desmodium triflorum* were collected from outfield medicinal garden near to Gwalior (M.P.) that show the green color with rough surface. The plant leaves was washed thoroughly in tap water, dried in shade, finely powdered and used for extraction. Plant was identified and herbarium specimen was submitted in Department of Pharmacognosy for future references.

Extraction of Plant Material:

Extraction is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures. The extraction was done by following general procedure. Powdered material (leaves) was packed in soxhlet apparatus. The drug was defatted with petroleum ether (60-80°C) for about 30-35 complete cycles. Defatted material was subjected to further extraction process

by dichloromethane, methanol and water. All the extracts were concentrated under vacuum. After completion of total, the extracted powder was discarded and the extracts so obtained were further processed. The excess solvent in the extracts were removed by distillation and the concentrated extracts so obtained were further dried at a temperature not exceeding 40°C in water bath. The extracts were then collected kept in Petri dish and stored in desiccators at room temperature. The yield values and other physical properties were observed¹⁰.

The % Yield of the Petroleum ether, dichloromethane, Methanol, & Aqueous extract of was calculated by using the following formula.

$$\% \text{ Yield} = \frac{\text{Net weight of powder in gram after extraction}}{\text{Total weight of leaf powder in gram taken for extraction}} \times 100$$

Determination of Physico-Chemical Parameters:

Various physicochemical parameters were analyzed for the confirmation of identity & purity. The extractive values with alcohol and water were also determined.

Moisture content:

Moisture is an inevitable component of crude drugs, which must be eliminated. The moisture content should be minimized in order to prevent decomposition of crude drugs either due to chemical changes or microbial contamination.

The powdered sample of *H.rosa-sinensis* and *C.gigantea* was weighed accurately and kept in IR moisture balance. The loss in weight was recorded as percentage moisture with respect to air-dried sample of crude drug¹⁰⁻¹¹.

$$\% \text{ Moisture} = \frac{(Fw-Pw)}{W} \times 100$$

Where: Fw = Final constant weight of drug along with container

Pw = Weight of empty container

W = Total weight of drug taken

Ash values:

I. Total ash value:

For the determination of total ash, 2 gm of the air dried crude drug was weighed in the tarred silica dish and incinerated at a temperature 450°C until free from carbon in Muffule furnace and then was cooled and weighed. The residue was collected on an ash less filter paper and then incinerated until the residue is white or nearly so. The percentage of ash was calculated with reference to the air-dried drug.

II. Acid insoluble ash value:

The ash obtained from the previous process was boiled with 25ml of 2M HCl for 5 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited, cooled in a dessicator and weighed. Percentage of acid insoluble ash was calculated with reference to the air-dried drug.

III. Water soluble ash:

The ash was boiled with 25ml of water for 5 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited for 15min. at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and this represents the water-soluble ash. Percentage of water-soluble ash was calculated with reference to the air-dried drug.¹⁰⁻¹³

Phytochemical Screening:

Preliminary phytochemical screening was performed for all the extracts¹⁴⁻¹⁵.

Detection of Carbohydrate:

500 mg of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Molisch's Test:

To 1 ml of filtrate, 2 drops of Molisch's reagent was added in a test tube and 2 ml of concentrated sulphuric acid added carefully along the side of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrate.

Fehling's Test:

To 1 ml of filtrate, 4 ml of Fehling's solution was added in a test tube and heated for 10 minutes in a

water bath. Formation of red precipitate indicates the presence of reducing sugar.

Detection of Glycosides:

0.5 gm of extract was hydrolyzed with 20 ml of dilute hydrochloric acid (0.1 N) and filtered. The filtrate was used to test the presence glycosides.

Modified Borntrager's Test:

To 01 ml of filtrate, 02 ml of 1% ferric chloride solution was added in a test tube and heated for 10 minutes in boiling water bath. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half of its volume of ammonia solution. Formation of rose pink or cherry colour in the ammonia layer indicates the presence of glycoside.

Killer Killiani Test:

Small portion from the respective extracts was shaken with 1 ml glacial acetic acid containing a trace of ferric chloride. 1 ml of conc. sulphuric acid (H₂SO₄) was added carefully by the sides of the test tube. A blue colour in the acetic acid layer and red colour at the junction of the two liquids indicate the presence of glycosides.

Detection of Alkaloids:

0.5 gm of extract was dissolved in 10 ml of dilute hydrochloric acid (0.1 N) and filtered. The filtrate was used to test the presence of alkaloids.

Mayer's Test:

Filtrates were treated with Mayer's reagent; formation of yellow cream coloured precipitate indicates the presence of alkaloids.

Dragendorff's Test:

Filtrates were treated with Dragendorff's reagent; formation of red colored precipitate indicates the presence of alkaloids.

Hager's test:

Filtrates were treated with Hager's reagent; formation of yellow coloured precipitate indicates the presence of alkaloids.

Detection of phytosterols and triterpenoids: 0.5 gm of extract was treated with 10 ml chloroform and filtered. The filtrate was used to test the presence of phytosterols and triterpenoids.

Salkowaski Test:

To the test extract solution added few drops of conc. H_2SO_4 shaken and allowed to stand, lower layer turns reddish brown or golden yellow indicating the presence of triterpenes.

Detection of Protein and Amino acid

100 mg of each extract was taken in 10 ml of water and filtered. The filtrate was used to test the presence of protein and amino acids.

Millon's Test:

2 ml of filtrate was treated with 2 ml of Million's reagent in a test tube and heated in a water bath for 5 minutes, cooled and added few drops of Sodium Nitrate solution. Formation of white precipitate, which turns to red upon heating, indicates the presence of proteins and amino acid.

Ninhydrin Test:

To 2 ml of filtrate, 0.25% Ninhydrin reagent was added in a test tube and boiled for 2 minutes. Formation of blue color indicates the presence of amino acids.

Biuret test:

2 ml of filtrate was treated with 2 ml of 10% sodium hydroxide solution in a test tube and heated for 10 minutes. A drop of 7% copper sulphate solution was added in the above mixture. Formation of purplish violet colour indicates the presence of proteins.

Detection of Fixed oils and Fats:

Oily spot test:

One drop of each extract was placed on filter paper and solvent was allowed to evaporate. An oily stain on filter paper indicates the presence of foxed oil.

Detection of Phenolics and Tannins:

100 mg of each extract was boiled with 1 ml of distilled water and filtered. The filtrate was used for following tests.

Ferric chloride test:

To 2 ml of filtrate, 2 ml of 1% ferric chloride solution was added in a test tube. Formation of bluish black colour indicates the presence of phenolic nucleus.

Lead Acetate Test:

To 2 ml of filtrate, few drops lead acetate solution was added in a test tube. Formation of yellow precipitate indicates the presence of tannins.

Detection of Flavonoids:

Alkaline Reagent test:

To 100 mg of extract, few drops of sodium hydroxide solution were added in a test tube. Formation of intense yellow colour that becomes colorless on addition of few drops of dilute acid (HCl) indicates the presence of Flavonoids.

Detection of Saponin:

Foam Test:

Extracts were diluted with distilled water to 20 ml and Shaken in a graduated cylinder for 15 minutes. Formation of one cm layer of foam indicates the presence of Saponin.

Detection of Mucilage:

10 ml of the aqueous extract was tested for mucilage; the extract was added with 25 ml of 95% alcohol with constant stirring. The so formed precipitate was centrifuged and washed with alcohol, the dissolved in water (10 ml) and reprecipitated. After washing the precipitate was collected & dried in desiccators. On addition of a drop of water and allowed to stand for some time, it swelled to give a viscous mass which gave indication for presence of mucilage.

Chromatographic Study:

TLC of methanolic extract of *Desmodium triflorum*:

TLC for the separation of various bioactive compounds from bioactive extract, methanolic extract was developed to find out the probable number of compounds present in them. On the pre coated TLC plate, test samples (after dissolving in respective solvents) were applied in the form of spots with the help of fine capillary. Spots were marked on the top of the plate for their identification. Rectangular glass chambers were used for chromatography. To avoid insufficient chamber saturation and undesirable edge effect, a smooth sheet of filter paper was placed in TLC chamber and was allowed to be in the developing solvent. A number of developing solvent systems were tried during the study. Each time plate was

sprayed with Vanillin sulphuric acid and heated at 115°C for 5 minutes. The solvent system in which there is a satisfactory resolution was taken as a final solvent system. Solvent systems; Ethyl acetate: methanol: water (76:20:4) was found to be most satisfactory solvent system. After development of plates, they were air-dried and number of spots, color and R_f values were recorded¹⁰.

R_f value=Distance travelled by solute/Distance travelled by solvent

RESULTS AND DISCUSSION: Successive solvent extraction values in various organic solvent

were observed as n-hexane, dichloromethane, methanol, water as shown in (Table 1). The proximate analysis revealed the moisture content, total ash, acid insoluble ash, & water soluble ash values were observed to be 11.5%, 3.1 %, 9.2 %, 5.7 %, respectively as shown in (Table 2). The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolic compounds as shown in (Table 3).

TABLE 1: DIFFERENT EXTRACTS WITH THEIR APPEARANCE AND % YIELD (IN gm)

Extracts	Color of dried extracts	Consistency of dried extracts	% Yield (W/W)
n- hexane extract of <i>Desmodium triflorum</i>	Dark Brown	Sticky	12 %
Dichloromethane extract of <i>Desmodium triflorum</i>	Dark Green	Dried Powder	18 %
Methanolic extract of <i>Desmodium triflorum</i>	Dark Orange	Sticky	10 %
Water extract of <i>Desmodium triflorum</i>	Dark Brown	Resinous	4 %

TABLE 2: EVALUATION OF PHYSICOCHEMICAL PARAMETER

S.N.	Parameters	Values obtained (%w/w) dry weight basis
1.	Moisture content	3.9
2.	Total ash	9.7
3.	Acid insoluble ash	3.4
4.	Water soluble extractive	5.2

TABLE 3: QUALITATIVE CHEMICAL ANALYSIS OF *DESMODIUM TRIFLORUM* BY CHEMICAL TESTS

S.No.	Test	n-hexane	Dichloromethane	Methanolic	Aqueous
1.	Carbohydrate				
	Molish test	-	-	-	+
2.	Felling test	-	-	-	+
	Glycosides				
3.	Bronteger test	-	-	+	+
	Alkaloid				
4.	Mayer test	-	+	+	-
	Hager test	-	+	+	-
5.	Phytosterol + Triterpinoids				
	Salkowaski test	-	+++	-	-
6.	Protein + Amino acid				
	Biuret test	-	-	-	-
7.	Ninhydrin test	-	-	-	-
	Phenolic test				
8.	Ferric test	-	-	+	+
	Lead acetate test	-	-	+	+
9.	Flavonoids				
	Alkaline test	-	-	+	+
10.	Saponin				
	Foam test	-	-	-	-

Note: + ve indicates positive result, whereas - ve indicates negative result

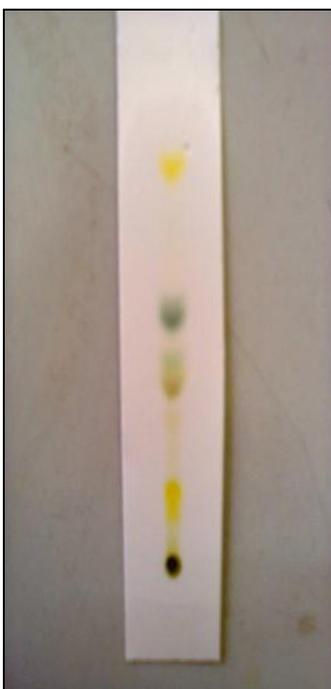
TLC of methanolic extract of *Desmodium triflorum*:

TLC study has shown the presence of different components present in methanolic extract of

Desmodium triflorum when the extracts were run in specific solvent system. Before reaching to most optimum solvent system a number of systems were employed as shown in (Table 4, Fig.2)

TABLE 4: SUMMARY OF TLC

S No.	Fractions	Solvent systems	Detecting reagents	Color	No. of spots	R _f value of Spots
1	Butanolic	Ethyl acetate: methanol: water (76:20:4)	Vanillin sulphuric acid, heated at 110 ^o C for 5 Min.	Green & yellow	6	0.20, 0.30, 0.53, 0.58, 0.68, 0.72

**FIG.2: TLC OF METHANOLIC EXTRACTS**

CONCLUSION: The preliminary pharmacognostic and phytochemical analysis revealed the successive solvent extraction value in different solvents, moisture content, total ash, acid insoluble ash, sulphatd ash, & water soluble ash, different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolic compounds, and TLC study of methanolic extract has shown the different components present in extract.

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