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PRELIMINARY PHYTOCHEMICAL AND PHARMACOGNOSTICAL STANDARDIZATION OF AERIAL PARTS OF *MERREMIA TRIDENTATA* (LINN) HALLIER.F. CONVOLVULACEAE

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ABSTRACT

The aerial parts of *Merremia tridentata* (Linn) Hallier.f**.** of Convolvulaceae is used for hair growth promotion in folklore medicine. In the present investigation a detailed pharmacognostical study of aerial parts of *Merremia tridentata* L. is carried out as a preliminary step to standardize the raw materials for the purpose of identification of adulterants and substitute from original drug as well as which could be useful to lay down development of Pharmacopoeial monograph. The study includes macroscopical studies, physico- chemical properties, fluorescence analysis, preliminary phytochemical screening and TLC study.

Keywords: *Merremia tridentata*, Pharmacognostical, Phytochemical and TLC study.

INTRODUCTION

Herbal medicines are promising choice over modern synthetic drugs. They show minimum/no side effects and are considered to be safe. *Merremia tridentata* (Linn) Hallier.f**.** Family (Convolvulaceae), commonly known as prasarini in (Hindi), arrow-leaf Morning Glory in English and in tamil it is called as mudivalarthi [1-2].

M.tridentata traditionally used for Skin disease, Dandruff, Diabetics, Diarrhea, used to improve hair growth. It is also used in treatments of inflammations, wound healing and Hemorrhoids [2-3]. In the present study morphology, physicochemical standards, Fluorescence analysis, preliminary phytochemical screening and thin layer chromatography were performed.

MATERIALS AND METHODS

Collection and authentication of plant material

The plant was collected in the surroundings of Kallapattu village, Villupuram district, Tamil nadu in the month of December-2012 and it was authenticated by the taxonomist of French Institute, Puducherry and the herbarium was preserved in the Department museum for future reference with an Accession No. HIFP.026653.

Preparation of powdered drug

The collected plants were washed by using tab water and dried under shade then the dried materials were made into powder by a wearing blender. The powdered drug was used for further studies.

MACROSCOPICAL STUDIES

Morphological features were studied directly in the field and were photographed. The macroscopical study includes the evaluation of organoleptic characters and external features of the various parts of selected plant material [4]
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PHYSIO-CHEMICAL ANALYSIS

Determination of Ash Values

The ash was determined by four different methods which measures total ash, acid-insoluble ash, water soluble ash and sulphated ash. Acid-insoluble ash value of a crude drug is always less than Total ash value of the same drug [5-6].

Total ash

2 to 3 g of the air-dried crude drug is weighed in a

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tarred platinum or silica dish and it was incinerated at a temperature not exceeding 450^{0}C until free from carbon, then the residue was allowed to cool in suitable desiccators for 30 minutes and then it was weighed without delay. The content of total ash was calculated in mg per gram of air-dried material. Percentage of total ash was calculated by using the formula-

$$\% \text{ of total ash} = \left(\frac{\text{weight of ash obtained}}{\text{weight of air dried drug taken}}\right) \text{x } 100$$

Acid insoluble ash

The ash was boiled with 25 ml of 2 M hydrochloric acid for 5 minutes, insoluble matter was collected on an ashless filter paper, washed with hot water, ignited, cooled in desiccators and weighed. The % of acid-insoluble ash was calculated with reference to the air-dried drug.

Water-soluble ash

The ash was boiled with 25 ml of water for 5 minutes, insoluble matter was collected on an ash less filter paper, washed with hot water, ignited for 15 mints, cooled in desiccators and weighed. The % of water-soluble ash was calculated with reference to the air-dried drug.

Sulphated Ash

The silica or platinum crucible was heated to redness for 10 minutes, and then it's allowed to cool in desiccators and weighed. Unless otherwise specified in the individual monograph 1 g of the substance was transfered to the crucible being examined. The crucible and the contents were weighed accurately, and then it was ignited gently at first, until the substance is thoroughly charred. The residue was cooled and moistens with 1 ml of sulphuric acid, and then it was heated gently until the white fumes are no longer evolved and ignited at 800±25^{0}C until all black particles have disappeared. The crucible was allowed to cool, and a few drops of sulphuric acid were added and heated, ignited as before, allowed to cool and weighed.

Extractive value

The extractive values were done by two methods

1. Batch process
2. Successive process

Batch process

Macerate 5 g of the air-dried, coarsely powdered drug, with 100 ml of respective solvent of the specified strength in a closed flask for 24 hrs, shaking frequently during the first 6 hrs and then it was allowed to stand for 18 hrs. Then it's filtered and evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish dried at 105^{0}C and weighed. The air-dried samples were macerated with various solvents like petroleum ether (40-60°C), chloroform, acetone, methanol, ethanol, and water.Then, finally the % of extractive value was calculated for each solvent with reference to the air-dried drug [7-10].

Successive process

Macerate 5 g of the air-dried, coarsely powdered drug, with 100 ml of solvent of the specified strength in a closed flask for 24 hrs, shaking frequently during the first 6 hrs and then it was allowed to stand for 18 hrs. Then filtered rapidly and evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish, dried at 105^{0}C and weighed. The air-dried sample was macerated with various solvents like petroleum ether (40-60°C), chloroform, methanol and water successively. The % extractive value was calculated for each solvent with reference to the air-dried drug.

Foaming index

About 1g of the plant material was size reduced (coarse powder), weighed accurately and transferred to a 500ml conical flask containing 100ml of boiling water. Maintained at moderate boiling for 30 minutes, cooled and filtered into a 100ml volumetric flask and added sufficient water through the filter to dilute to volume. Poured the decoction into 10 stoppered test tubes (height-16cm, diameter-16mm) in successive portions of 1ml, 2ml, 3ml, etc. up to 10ml and adjusted the volume of the liquid in each tube with water to 10ml. Stoppered the tubes and shake them in a lengthwise motion for 15 seconds, at the rate of two shakes per second. Allowed to stand for 15 minutes and measured the height of the foam. The results were assessed as follows [11].

- If the height of the foam in every tube is less than 1cm, the foaming index is less than 100
- If a height of foam of 1cm is measured in any tube, the volume of the plant material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.
- If the height of the foam is more than 1cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilutions of the decoction in order to obtain a result.

The foaming index was calculated by using the following formula

$$FOAMING\ INDEX = \frac{1000}{A}$$

Loss on drying

A glass stoppered, shallow weighing bottle that has been dried under the same conditions was weighed the sample was weighed and transferred into the shallow bottle and the weight of the sample along with the bottle was taken. The sample was distributed as evenly as possible by gentle sidewise shaking to a depth not exceeding 10 mm. The bottle was placed in the drying chamber (oven or desiccator), the stopper was removed and left in the

chamber. The sample was dried until a constant weight was obtained. After drying is completed, the bottle was closed promptly and allowed to cool at room temperature (where applicable) in a desiccator before weighing. The bottle and its content were weighed. The percentage of loss on drying was calculated.

Crude fibre content

The leaf powder was defatted with lipid solvent before processing. 2g of powdered drug was treated with 50ml of 10% v/v HNO_3. Then boiled with constant stirring (till about 30 seconds after boiling starts).and filtered through fine cotton cloth on a Buchner funnel. The residue was washed with boiling water (suction may be used) and the residue was transferred from the cloth to a beaker and 50ml of 2.5% v/v NaOH solution was added then it was boiled continuously with constant stirring. Then strained and washed with hot water as mentioned earlier. For quantitative determination, residue was transferred to a cleaned and dried crucible. Weighed the residue and the percentage of crude fibres was determined [12].

FLUORESCENCE ANALYSIS

For fluorescence analysis the plant powder and methanolic extract of plant was treated with different acids and alkali (50% H_2SO_4, 50% HCL, 50% HNO_3, 1N NAOH, 5% KOH.) separately and then these extracts were subjected to fluorescence analysis in visible/day light and UV light at short and long wavelength [13].

PHYTOCHEMICAL SCREENING

Preparation of the extracts

The collected materials (aerial parts) were chopped into small pieces separately, shade-dried and coarsely powdered (sieve no. 40) using a pulverizor the coarse powders was extracted with various solvents like petroleum ether (40-60^{o}C), chloroform, acetone and methanol by Soxhlet method. The extracts were collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed *in vacuo*. The resulted extracts were used for phytochemical screening.

PRELIMINARY PHYTOCHEMICAL SCREENING

The extracts were subjected to qualitative chemical tests for the detection of various plant constituents like carbohydrates, glycosides, proteins and amino acids, fats, gums, mucilage, alkaloids, phytosterols, flavonoids, tannins and phenolic compounds and saponins [14-15].

THIN LAYER CHROMATOGRAPHY

The glass plates have to be carefully cleaned with acetone to remove grease. Then the slurry of silica gel (or other adsorbent) in water has to be vigorously shaken for a set time interval (e.g. 90 s) before spreading. Finally, plates after spreading have to be air dried and then activated by heating in an oven at 100-110^{0}C for 30 minutes. The solution of the sample in a volatile solvent is applied by using a capillary tube or a micropipette to a spot keeping 1.2 cm from the bottom of the TLC plate. The position of the sample spot is indicated by making an 'origin line' on the plate with the lead pencil. When the spot has dried, the plate is placed vertically in a suitable tank (which is paper-lined so that the atmosphere inside is saturated with the solvent phase) with its lower edge immersed in selected mobile phases. The solvent rises by capillary action, resolving the sample mixture into discrete spots [16-17].

Screening system

Ethyl acetate (10), Ethyl acetate: Hexane (5:5) and Methanol: Ethyl acetate: water (20:2.7:2)

RESULTS AND DISCUSSION

Macroscopic features

The macroscopic description helps in taxonomical identification of the selected plant and differentiating it from the related species. Leaves are linear-oblong, sessile; margin is entire or pedately lobed. The flowers are funnel shape, pale yellow in colour having 4 to 5 petals. Stems are green in colour, slender shaped. Fruits are pale yellow in colour having 4 seeds or fewer.

TLC

TLC was performed to separate and identify the single or mixture of constituents in methanolic extract. It was carried out for the identification of different components in the extracts qualitatively. The TLC of methanolic extract was done using various solvent systems, in that Ethyl acetate (10), Ethyl acetate:Hexane (5:5), Methanol: Ethyl acetate: water(20:2.7:2) shows maximum number of compounds with respective values.

Table 1. Macroscopic Studies of Aerial Parts of *M.tridentata*

Parameter	Leaf	Stem	Bud	Flower	Fruit
Colour	Green	Green	Green	Pale yellow	Pale yellow
Odour	None	None	None	None	None
Taste	Blank	Blank	Blank	Blank	Blank
Size	0.5-3cm	10-80cm	0.5-0.8cm	0.5-1cm	0.5-0.9cm
Shape	Linear-Oblong	Slender	Conical	Funnel	Globose
Touch	Soft	Rough	Soft	Soft	Rough

Table 2. Physicochemical evaluation of aerial parts of *M.tridentata*

S.no	Parameter	Result
1.	**Ash Value (%w/w)**	
	Total ash	8.8
	Acid insoluble ash	4
	Water soluble ash	5.6
	Sulphated ash	2.1
2.	**Extractive Values (%w/w)**	
	Batch process	
	Acetone	11
	Petroleum ether (40-60^o)	4.7
	Chloroform	10.4
	Methanol	24.76
	Ethanol	17.6
	Water	17.6
	Successive process	
	Petroleum ether (40-60^o)	4.8
	Chloroform	1.6
	Methanol	9.6
	Foaming index	Greater than 1000
4.	Loss on drying (%W/W)	9.1
5.	Crude fibre content (%W/W)	35.3

Table 3. Fluorescence analysis of powder of *Merremia tridentata*

Chemical reagents	Fluorescence colour		
	Day light	UV –light	
		254 nm	366 nm
Powder as such	Green	Brown	Green
50% H_2SO_4	Yellowish orange	Brown	Green
1N HCL	Pale Yellow	Brown	Light green
50% HNO_3	Pale yellow	Brown	Light green
1N NaOH	Pale Yellow	Brown	Light green
5% KOH	Yellowish orange	Brown	Dark green

Table 4. Fluorescence analysis of methanolic extract of *Merremia tridentata*

Chemical reagents		Fluorescence colour		
		Day light	UV –light	
			254 nm	366 nm
1.	Extract as such	Brown	No fluorescence	Green
2.	50% H_2SO_4	Brown	No fluorescence	Green
3.	1N HCL	Brown	No fluorescence	Fluorescent Green
4.	50% HNO_3	Brownish orange	No fluorescence	Green
5.	1N NaoH	Brown	No fluorescence	Green
6.	5% KOH	Brown	No fluorescence	Green

Table 5. Preliminary Phytochemical Screening

S.no	Phytoconstituents	Experiment	Observation			
			Pet. ether	$CHCL_3$	Acetone	CH_3OH
1.	Carbohydrates	Molisch's reagent	-	+	+	+
		Fehling's A&B	-	+	+	+
		Benedict's reagent	-	+	+	+
		Barfoed's reagent	-	+	+	+
2.	Protein and Amino acids	Biuret test	-	-	-	+
		Millon's reagent	-	-	-	+

3.	Alkaloids	Dragendroff's reagent	-	-	+	+
		Mayer's reagent	-	-	+	+
		Hager's reagent	-	-	+	+
		Wager's reagent	-	-	+	+
4.	Steroids	Libermann's reagent	+	-	+	-
		Salkowski test	+	-	+	-
5.	Phenols	Ferric chloride	-	-	+	+
		10% sodium chloride	-	-	+	+
6.	Tannins	10% sodium chloride	-	-	+	+
		Aqueous bromine solution	-	-	+	+
7.	Flavonoids	Shinoda's test	-	-	+	+
		Zinc hydrochloride test	-	-	+	+
8.	Saponins	Foam test	-	-	+	+
9.	Glycosides	Keller-killiani's test	-	-	-	-
		Borntrager's test	-	-	-	-
		Legal's test	-	-	-	-

(+)= present; (-) = absent

Fig 1. Leaves, Stems and Buds of *Merremia tridentata* (Linn) Hallier.f. (Convolvulaceae)

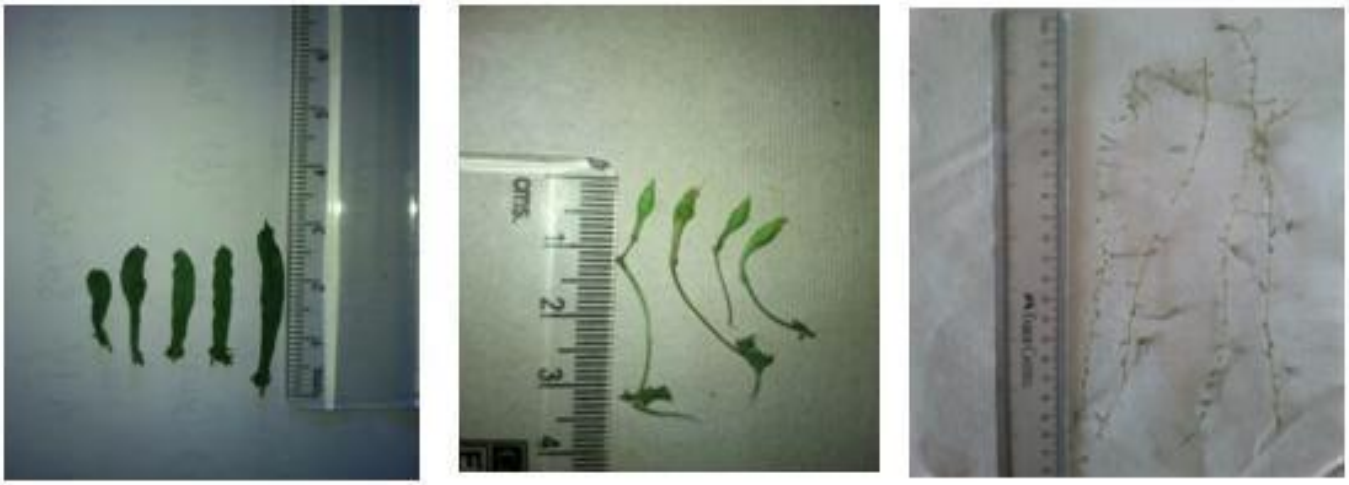
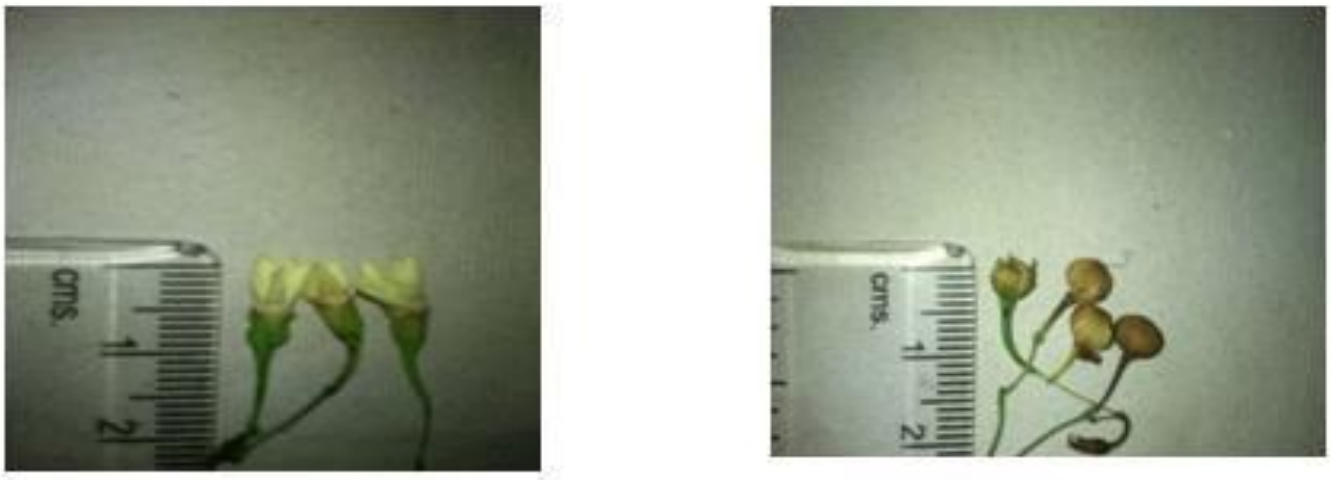


Fig 2. Flowers and Fruits of *Merremia tridentata* (Linn) Hallier.f. (Convolvulaceae)



Ethyl acetate 10	Ethyl Acetate: Hexane 5:5	Methanol: Ethyl Acetate: water 20:2.7:2
		

Preliminary phytochemical screening

Preliminary phytochemical screening was performed initially with different chemical reagents to detect the phytoconstituents present in each extract. The petroleum ether extract showed the presence of steroids. Chloroform extract was found to contain carbohydrate. Acetone extracts contains carbohydrates, alkaloids, steroids, phenols, tannins, and flavonoids. Finally methanol extract showed the presence of phenols, tannins, flavonoids, carbohydrates and alkaloids.

DISCUSSION AND CONCLUSION

The organoleptic evaluation provides the simplest and quickest means to establish the identity of a particular sample. The physicochemical parameters such as, ash value, extractive value of the selected drug was useful to establish pharmacognostical constants of the selected drug. The ash values are helpful in determining the quality and purity of a crude drug in the powdered form. The extractive values are useful for the estimation of specific constituents, soluble in that particular solvent used for the extraction. The loss on drying is designed to measure the amount of water and volatile matters in a sample, when the sample is dried under specific conditions. The determination of foaming index was carried out to estimate the saponins content of the drug. The crude fibre content is the method used to find out the percentage of fibres present in the drug. Fluorescence analysis of particular drug gives the information about the presence of chromophore. These kind of investigation could be useful to standardize the raw material from herbal origin to check their quality, purity and efficacy.

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