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PRELIMINARY PHYTOCHEMICAL AND STANDARDIZATION OF THE PLANT *DREGEA VOLUBILIS., BENTH*

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Abstract: The plant extract of *Dregea volubilis* was screened for *invitro and exvivo* activities. Phytochemicals, chemical compounds that occur naturally in plants, are responsible for color and organoleptic properties, such as deep purple of blue berries and smell of garlic. The term is generally used to refer to those chemicals that may have biological significance but are not established as essential nutrients. Hence, medicinal plants have been receiving great attention worldwide researches because of the safe utility. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. Phytochemical screening of the plant revealed the presence of alkaloids, glycosides etc. In addition the values of percentage extractive and ash values, results of fluorescence analysis and phytochemical data will be helpful for the standardization and quality control of precious indigenous drug. The study scientifically validates the use of plant in traditional medicine.

Keywords: *Dregea volubilis*, Asclepiadaceae, Fluorescence-analysis, Hexane, Chloroform.

INTRODUCTION

Natural products of plant origin have played a vital role in the development of new therapeutic agents. These natural products have primarily been isolated from plants used in folklore and traditional medicinal systems of various regions and countries. The curative properties of different medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occurs as secondary metabolites. Medicinal chemistry plays a vital role and forms a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals. The plant may be considered a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by men, but also for a multitude of compounds like glycosides, alkaloids, volatile oils, etc. That exerts a physiological effect. *Marsdenia volubilis* is a medicinal member of the family Asclepidaceae, which is cosmopolitan in distribution. It contains dregein alkaloids. The studied plant is being used very specifically in the indigenous systems of medicine such as Ayurveda, Siddha and Unani. It was carried out to characterize the therapeutically active constituents of the *Dregea volubilis.*

MATERIALS AND METHODS

Plant material:

Fresh leaves of *Dregea volubilis* were collected from Chennai, Tamil Nadu and further identity was confirmed by tallying with herbarium specimens at the plant anatomy research center by Prof. Jayaraman, Tambaram, Chennai. The leaves were shade dried and coarsely powdered and used for extraction.

Extraction of Plant Material:

The plant material, leaves were collected, shade dried, and coarsely powdered in a blender. The coarse powder was successively extracted in a soxhlet extractor with hexane and chloroform by hot percolation method. The solvent was removed by distillation over boiling water bath and remaining under reduced pressure. The residue was stored in the desiccator and used for phyto-chemical studies.

Preliminary phytochemical analysis:

Preliminary phytochemical investigation was conducted as per procedure described by (Kokate, 1994). The chemical tests for various phytoconstituents in different extracts was carried out as described below and the results were recorded (Evans,) (Table no. 1)

Determination of alkaloids: To the extract, few drops of acetic acid were added, followed by Dragendroff's reagent and shaken well. Formation of orange red precipitate indicates the presence of alkaloids.

The substance was mixed with little amount of dil. hydrochloric acid and Mayer's reagent. Formation of white precipitate indicates the presence of alkaloids. (S.J.Somolenski)

Glycosides: The extract was mixed with a little anthrone on a watch glass. One drop of concentrated sulphuric acid was added and made into a paste, warmed gently over water bath. The presence of glycosides was identified by dark green coloration.

Determination of flavones (Shinado's Test): To the extract in alcohol few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes and observed for red coloration for the presence of flavones (Zhishen J).

To the extract in alcohol, 10% sodium hydroxide solution and ammonia was added dark yellow color indicates presence of flavones.

Steroids (Liebermann - Burchard test): 1 mg of the extract was dissolved in a few drops of chloroform, 3 ml acetic anhydride, 3 ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube and observed for bluish green color for the presence of steroid.

Phenol: To the substance a few drops of alcohol and ferric chloride solution was added. Bluish green or red indicates the presence of phenol. (Evans)

Table-1: Preliminary phytochemical test for different extracts:

S. No	Test	Hexane	Chloroform
1	Alkaloid	-	-
2	Glycoside	-	+
3	Anthraquinone	-	-
4	Terpenoid	-	-
5	Steroid	+	+
6	Flavonoids	-	+
7	Phenols	-	-
8	Tannins	-	-
9	Sugars	-	+
10	Quinones	-	-
11	Saponins	-	-
12	Proteins	-	-
13	Resin	-	-

+indicates the presence of the active constituent
- indicates the absence of the active constituent

Fluorescence Analysis

Fluorescence analysis of the plant *Dregea volubilis* was observed in day light and UV light (long and short) using powdered drug and various solvent extracts of the drug.

Method: The drug Powder was treated with neutral solvents like hexane, chloroform, ethyl acetate and ethanol and acids like 1M hydrochloric acid or 50% sulphuric acid and alkaline solutions like aqueous sodium hydroxide or alcoholic sodium hydroxide. They were subjected to fluorescence analysis and the results were tabulated (Table no.2).

Table-2: Fluorescence analysis of the powder drug

S.No	REAGENTS USED	DAY LIGHT	Long UV (320-400nm)	Short UV (280-320 nm)
1	Powder Drug	Brown	Green	Black
2	Powder + 1 N Hcl	Pale Yellow	Pale Green	Pale Green
3	Powder + 1 N NaoH	Red	Greenish	Pale Green
4	Powder + 50% Hcl	Pale Yellow	Pale Green	Bluish Green
5	Powder + 50% H_2So_4	Brownish Black	Brown	Black
6	Powder + 50% HNo_3	Reddish orange	Pale Green	Pale Green
7	Powder + Methanol	Yellowish Orange	Emerald Green	Pale Green
8	Powder + Methanol + 1 N NaoH	Yellowish Brown	Emerald Green	Brownish Green

Physico Chemical Constants (I.P. 2007):

Ash Values:

1. Determination of Total Ash: Weigh accurately 2gm of the air - dried crude drug in a tarred platinum or silica dish and incinerate at a temperature not exceeding 450° until free from carbon, cool and weigh. Calculate the percentage of ash with reference to the air dried drug.

2. Determination of Acid Insoluble Ash: Boil the ash with 25ml of 2M hydrochloric acid for 5 minutes; collect the insoluble matter on an ash less filter paper. Wash with hot water, ignite, cool in a desiccator and weigh (Yulan rao). Calculate the percentage of acid insoluble ash with reference to the air dried drug.

3. Determination of Water Soluble Ash: Boil the ash, for 5 minutes with 25ml of water, collect the insoluble matter in a Gooch crucible (or) on an ash less filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450°. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash. Calculate the percentage of water soluble ash with reference to the air dried drug.

4. Determination of Sulphated Ash: A silica crucible was heated to redness for about 10 minutes, allowed to cool in a desiccator and weighed. About 1gm of powdered drug was accurately weighed and taken in the crucible. The crucible was cooled and the residue was moistened with 1ml of sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800°C until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool, few drops of sulphuric acid was added and again heated. The ignition was carried and as before, allowed to cool and weighed. The operation was repeated until two successive weighing did not differ by more than 0.5mg. The percentage of sulphated ash was determined with reference to the air dried sample.

Extractive Values:

The extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents.

1. Determination of water Soluble Extractive: Add 5gm of powdered drug to 50ml of water at 80°C in a stoppered flask shake well and allow standing for 10 minutes. Cool, add 2 gm of kiesulghur and filter. Transfer 5ml of the filtrate to a tarred evaporating dish 7.5 cm in diameter. Evaporate the solvent on a water bath, continue drying for 30 minutes, finally dry in a steam oven for 2 hours and weigh the residue. Calculate the percentage of water soluble extractive with reference to the air dried drug.

2. Determination of Alcohol Soluble Extractive: 5gm of the air dried and coarsely powdered drug has to be macerated with 100ml of ethanol of the specified strength in a closed flask for 24 hrs, shaking frequently during the first 6 hours and allowing standing for 18hrs. There after filter rapidly taking precautions against loss of ethanol evaporate 25ml of the filtrate to dryness in a tarred flat bottomed shallow dish, dry at 105°C and weigh. The percentage of ethanol soluble extractive with reference the air dried drug has to be calculated.

Determination of Moisture Content:

Add 20ml of dehydrated methanol to the titration vessel and titrate to the electrometric end point with the Karl Fischer reagent. Transfer quickly the prescribed amount of the substance being examined, accurately weighed, to the titration vessel. Stir for 1 minute and titrate again to the electrometric end point using the Karl Fischer reagent. The water content of the sample, in mg, is given by the expression S x F, in which S is the volume, in ml, of the Karl Fischer reagent used to titrate the sample, and F is the water equivalent factor.

Determination of Loss on Drying:

Place the prescribed quantity of the substance to be examined in a weighing bottle previously dried under the conditions prescribed for the substance to be examined. Dry the substance to constant mass or for the prescribed time by drying with diphosphorous pentoxide at atmospheric pressure and at room temperature in a desiccator. The above procedures were carried out as per Indian pharmacopoeia, British pharmacopoeia and United States pharmacopoeia. (Table no. 3)

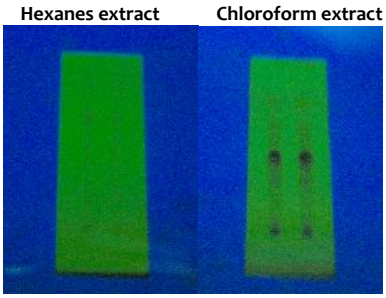
Table-3: Physico chemical constants

S. No	PARAMETERS	VALUE % w/w
1.	TOTAL ASH	18.00
2.	ACID INSOLUBLE ASH	0.75
3.	WATER SOLUBLE ASH	2.62
4.	SULPHATED ASH	5.67
5.	EXTRACTIVE VALUE	
	a. Water Soluble Extractive	4.12
	b. Alcohol Soluble Extractive	4.25
6.	MOISTURE CONTENT	11.25
7.	LOSS ON DRYING	6.27

TLC Studies:

The solvent system is taken in the TLC chamber and a filter paper is inserted in to chamber for saturation of chamber. The drug samples are spotted on the prepared TLC plate and kept in the chamber. After development of the spots the plate is taken out and observed in the UV chamber to detect number of spots developed. (Fig.1)

Figure.1: T L C Photo (under U V light)



RESULTS

Preliminary phytochemical analysis:

The results of preliminary phytochemical studies of Hexane and chloroform extracts of *Dregea volubilis* indicated in the presence of Alkaloids, glycosides, steroids, phenols, sugars.

DISCUSSION

Hexane extract shows the presence of sterols, chloroform extract shows the presence of glycosides, sugars and alkaloids. The ash of any organic material is composed of their nonvolatile inorganic components (metallic salts and silica). It usually represents the naturally occurring inorganic salts and organic matter added for the purpose of adulteration. Hence ash determination furnishes a basis for judging the identity and cleanliness of the drug.

Total ash involves the oxidation of the component of the product. A high ash value is an indicative of contamination, substitution and adulteration. The total ash usually consists of carbonate, phosphates, silicates and silica. The value of Total ash was found to be 18%.

The water soluble ash is the good indicator either previous extraction of water soluble salts. The value of water soluble ash was found to be 2.62%. The acid insoluble ash values indicate contamination with silicaceous materials like earth and sand.

The value was found to be 0.75%. Sulphated ash is obtained by treatment with dilute sulphuric acid where the oxides are converted to sulphates. The value was found to be 5.67%. Loss on drying determines the amount of volatile matter of any kind (including water) that can be driven off under condition specified.

The value of loss on drying was found to be 6.27 %. Moisture content determines the water molecules superficially bound to the chemical moiety in the plant drug. The moisture content was found to be 11.25%. The water soluble and the alcohol soluble extractives were found to be 4.12% and 4.25% respectively.

The hexane extract shows the presence of 5 spots with R_f values 0.92; 0.85; 0.79; 0.64; 0.52. The chloroform extract shows the presence of 4 spots with R_f value 0.88; 0.77; 0.74; 0.66.

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