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Studies on some Pharmacognostic profiles of PitheceIl'obium dulce Benth. Leaves (Leguminosae)

M. SUGUMARAN, T. VETRIC HELVAN AND D. VENKAPAYYA,
Department of Pharmaceutical chemistry, Adhiparasakthi College of Pharmacy,
Melmaruvathur - 603319, Tamil Nadu.

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ABSTRACT: The macroscopical characters of the leaves, leaf constants, physico-chemical constants, extractive values, colour, consistency, pH, extractive values with different solvents, micro chemical test, fluorescence characters of liquid extracts and leaf powder after treatment with different chemical reagents under visible and UV light at 254mn, measurement of cell and tissues were studied to fix some pharmacognostical parameters for leaves of *Pithecellobium, dulce Benth* which will enable the future investigators for identification of the plant. Preliminary phytochemical study on different extracts of the leaves were also performed.

INTRODUCTION

Pithecellubium dulce Benth. (Leguminosae)[1] is a small to *medium sized,* evergreen, spiny tree up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andamans. It is known as Vilayati babul in Hindi and Kodukkapuli in Tamil. The bark of the plant is reported to be used as astringent in dysentery, febrifuge and it is also useful in dermatitis and eye inflammation. The leaves have been reported to possess astringent, emollient, abortifacient and antidiabetic properties. A steroid saponin, lipids, phospholipids, glycosides, glycolipids and polysaccharides have been reported from the seeds[2-5]. The bark contains 37% of tannins of catechol type. Quericitin, kaempferol, dulcitol and afezilin have been reported from the leaves[6,7]. Roots have been reported to possess estrogenic activity[8]. Studies on alkylated resins from seed oil has been reported recently[9].

The present investigation deals with the studies on some important pharmacognostical characteristic of the leaves of *Pithecellobium dulce* as whole and its powdered form.

MATERIALS AND METHODS

Plant Materials:

The plant material was collected from Sembulam Village at Kancheepuram Dist. in the month of January 2005. The plant was identified by local people of that village and authenticated by Dr. P. Jayaraman, Director, plant anatomy research centre (PARC), Chennai. A herbarium specimen of the plant (C-3) was preserved in the Department of pharmacognosy of our Institute *for* further reference. The leaves were separated and dried under shade, pulverized by mechanical' grinder, passed through 40 mesh sieve and stored in a closed vessel for further use.

Reagents:

All the reagents used were of analytical grade obtained from S.D. Fine chemicals

Ltd., Mumbai and Qualigens fine chemicals, Mumbai.

Methods:

The macroscopic characters of the leaves were observed. Measurements of the cells/ tissues were made with the help of micrometer under a compound microscope[10]. The ash values, alcohol soluble and water soluble extractives values of leaves were determined as per the Indian pharmacopoeial methods[11] The crude fibre content was done by Dutch process[12] Loss on drying was determined by using infrared balance[13] Other extractive values were determined successively starting from petroleum ether (60-80°), benzene, chloroform, acetone, alcohol, distilled water by using soxhlet extraction apparatus[14] For this purpose the powder (100g) was successively hot extracted with 300ml of above solvents for 72h. Before switching over to the next solvent, the powder under extraction (marc) was dried to remove the traces of earlier solvent. The dried extractives were obtained after evaporation of solvent under reduced pressure. Further the colour, consistency and pH of extracts were also noted. The behaviour of the powdered leaves with different chemical reagents was studied[15]. The fluorescence characters of the various extracts and powdered leaf with different chemical reagents were observed under day light and UV light at 254nm, by following procedure reported by Kokoshi et al.[16] Quantitative microscopy was determined by methods prescribed by Trease and Evans[17] Preliminary phytochemical tests of different extracts were performed by using specific reagents through standard procedures[18-21]

RESULTS AND DISCUSSION

The morphological characters of leaves were studied and recorded in Table 1.

The physical constant values include total ash, acid insoluble, water soluble ash and sulphated ash; loss on drying, crude fibre content; alcohol soluble extractive and rater soluble extractive were reported in Table 2. Acid insoluble ash value is higher than that of water insoluble ash value. Quantitative microscopical studies (Table 3) also give valuable information regarding specific leaf constants such as vein islet, vein termination number, palisade ratio, stomatal number and stomatal index The leaf constants, stomatal number and stomatal index were scanned both in upper and lower epidermis, where abundant stomata were observed in the lower surface, but no stomata was present in the upper surface of the leaf. After successive extraction with each solvent, the percentage of dry extract was calculated in terms of air-dried weight, reported in Table 4.

The chloroform extract show minimum whereas water extract shows maximum extractive value. The water soluble extractive is more as compared with alcohol (90 %) soluble extractive indicating presence of more polar constituents in leaf extract.

The fluorescence characteristic of the powdered leaf, when treated with various chemical reagents (Table 5) and its extracts (Table 6) have been extensively studied. Like wise the behaviour of leaf powder (Table 7) upon the treatment with different Fluorescence studies on extracts revealed different shades of green fluorescence under UV light at 254nm. The size of cell elements like trichomes, starch grains, parenchyma cells, fibres and xylem vessels were shown in table 9. The various qualitative chemical tests (Table 10) have shown the presence of phytosterols, triterpenoids, flavonoids, glycosides, phenolic, tannins, saponins in huge amount whereas, alkaloids, aromatic acids, fixed oils, volatile oils were totally absent in leaf extract of this plant.

The above mentioned parameters are helpful for the future identification and decide authentification of the plant in herbal industry / factories. The physicochemical standards such as ash values, extractive values, crude fibre content and fluorescence analysis will be useful to identify the authenticity of the drug even from the crushed or powdered plant materials. It will serve as a standard data for the quality, control of the preparations containing this plant in future. The leaf constants can be included as microscopical standards in Indian herbal pharmacopoeia. Phytochemical study is also useful to isolate the pharmacologically active principles present in the drug. The information obtained from the ash values and extractive values are useful during the' time of collection and also during extraction process. Using these standards, the plant can be differentiated from the other related species.

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Table 1 ; Macroscopical characters of *pithecellobium dulce benth. Leaves.*

Colour	Green
Odour	Characteristic
Size	1 to 4 cm long and 1 to 1.5 cm broad.
Shape	Obliquely ovate – oblong
Taste	Slightly bitter
Surface	Smooth
Texture	Fibrous
Apex	Acute
Margin	Entire
Venation	Reticulate
Type	Simple
Petiole	1 cm long
Base	Thin spines are in pairs at the base of leaves and range from 2 to 15 mm in length
Orientation	Isobilateral

Table 2 : Physico – Chemical Constants of Leaves of *Pithecello Bium Dulce*

Parameters	* Values in % W / W
Alcohol soluble extractive	16.72
Water soluble extractive	17.65
Loss on drying	7.14
Total ash	13.71
Acid insoluble ash	8.94
Water insoluble ash	6.61
Sulphated ash	4.71
Crude fibre content	29.68

*** Each values is an average of three determinations**

Table : 4 Extractive values of the leaves of *pithecellobium dulce*

Yield in Percentage					
Petroleum ether	**Chloroform**	**Benzene**	**Acetone**	**Ethanol**	**Water**
6.68	1.72	3.24	2.69	17.93	18.58

Table 5 : Fluorescene characters of the powdered leaves of *pithecellobium dulce* under UV and visible light.

Treatment	Colour Developed	
	UV Light (254 nm)	**Visible Light**
Powder as such	Dark green	Green
IN HNO_3	Greenish blue	Yellowish brown
IN NaOH in water	Pale green	Greenish yellow
	Green	Pale green
IN HCL	Brown	Yellowish green
		Dark yellowish
50 % HNO_3	Dark Brown	Green
		Red
Acetic acid	Brownish	Brown
Picric acid	Red	
50% $Fecl_3$	Yellowish	Green
	Brown	
N / 50 Iodine	Light green	Greenish blue
	Grey	Dark grey
50% H_2SO_4	Yellowish	
	Green	Green
Ethanol	Green	
IN NaOH in ethanol	Greyish white	Grey
Methanol	Yellowish	Yellowish Green
	Green	
Powder mounted with nitro cellulose	Yellow	Greenish yellow
	Brown	Black
Powder treated with NaOH in methanol, dried and	Brown	Yellow
mounted with nitro cellulose		
Powder treated with HCL, dried & mounted with		
nitro cellulose		
Powder treated with NaOH in water & mounted		
with nitro cellulose		
Powder treated with Antimony tri chloride	White	Greenish yellow
Powder treated with HNO_3, dried & mounted with		
nitro cellulose		

Table 6 : Fluorescence Analysis of different extracts of *Pithe Cellobium Dulce*

Extracts	Day Light	UV Light (254 nm)
1. Petroleum ether	Light green	Greenish yellow
2. Chlorofom	Greenish brown	Dark green
3. Acetone	Dark green	Light green
4. Benezene	Blackish green	Black
5. Methanol	Yellowish green	Green
6. Water	Greenish buff colour	Dark green

Table 7 : Behavious of powdered leaves on tr eatment with different chemical reagents

Reagent	Colour Developed
Powder as such	Green
IN NaOH	Yellowish green
Picric acid	Yellow
Glacial acetic acid	Light green
IN Hcl	Light green
IN HNO_3	Yellow
5% Iodine	Light green
40% NaoH + few drops of 10% lead acetate	Yellowish white precipitate
HNO_3 + Ammonia solution	Light yellow
Con H_2SO_4	Light brown
5% $Fecl_3$	Light yellow
10% sodium hydroxide + copper sulphate	Dark green
Acetic acid + Con H_2SO_4	Green
Acetic acid + Ferric chloride + Con H_2SO_4	Black and then brown
Antimony tri chloride	Light green
Ammonia solution	Red

Table 8 : The colour, consistenc y and pH of the extracts of *pithocellobium dulce* leaf

S. No.	Extract	Colour	Consistency	pH
1	Petroleum Ether	Blackish Green	Semisolid	5. 75
2	Chloroform	Blackish Green	Semisolid	8.00
3	Benzene	Dark Green	Semisolid	7.25

4	Acetone	Dark Green	Semisolid	7.17
5	Ethanol	Greenish black	Viscous semisolid	7.06
6	Water	Greenish Brown	Viscous semisolid	4.75

Table 9 : Micro material measurement of cells / tissues of *Pithecello Bium Dulce* Leaves

Cells / Tissue	* Size in microns		
	Minimum	Average	Maximum
Diameter of starch grains	48	85.6	120
Length of Xylem vessels	120	124	156
Width of Xylem vessels	24	70.8	108
Length of Trichomes	96	125	168
Width of Trichomes	12	12	12
Length of Parenchyma Cells	204	252.4	312
Width of Parenchyma Cells	84	115.2	168
Length of Fibres	360	762.4	1020
Width of Fibres	36	63.2	108

*** Each values is an average of three determinations.**

Table 10 : Preliminary Phytochemical Screening of Various Extracts of *Pithe Cellobium Dulce*

S. No.	Plant Constituents	Petroleum Ether Extract	Chloroform Extract	Benzene Extract	Acetone Extract	Ethanol Extract	Aqueous Extract
1	Alkaloids	-	-	-	-	-	-
2	Carbohydrates	-	+	-	-	-	+
3	Glycosides	-	+	-	-	+	+
4	Saponins	-	-	-	-	+	+
5	Protein & Amino acids Phenolic	-	-	-	-	+	+
6	Compounds & Tannins	-	+	-	+	-	-
7	Gums & Mucilage	-	-	-	-	+	+
8	Flavanoids	-	-	-	-	+	+

9	Fixed Oils & Fats	-	-	-	-	-	-
10	Volatile Oils	-	-	-	-	-	-
11	Triterpenoids	-	+	+	-	-	-
12	Phytosterols	-	+	+	-	-	-
13	Aromatic Acids	-	-	-	-	-	-

(+) Presence of phytoconstituents, (-) Absence of phytoconstituents

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