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Pharmacognostic and Preliminary Physiochemical Investigations of *Thevetia peruviana* (Pers.) K. Schum Flowers

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ABSTRACT:

Flowers of Plant *Thevetia peruviana* (Pers.) K. Schum.were reported to possess good medicinal value in traditional system of medicine, the present investigation deals with Macroscopic, Microscopic and preliminary phytochemical investigation of flowers of *Thevetia peruviana* which includes pharmacognostical parameters, physiochemical parameters like ash values, extractive values and moisture content. The total ash, acid insoluble ash, water-soluble ash values and sulfated ash were observed to be 3.50%, 1.60%, 1.30% and 1.20% w/w respectively. Alcohol soluble, water-soluble and Ether soluble extractive values of the flowers were observed to be 8.30%, 4.80%, and 3.70% respectively. Powdered flowers were also subjected to fluorescence analysis with different chemicals. Phytochemical investigation of powdered drug revealed the presence of Alkaloids, glycosides, tannins, phenolic compounds, proteins, essential oils, gums, mucilage and fixed oils. The main aim of the present investigation is to study the macro, microscopic and some other pharmacognostic characters and physicochemical standards of flowers of *Thevetia peruviana* Pers. which could be used to prepare a monograph for the proper identification of the plant.

Keywords: *Thevetia peruviana*, Phytochemical, Fluorescence analysis, Physical evaluation.

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INTRODUCTION:

Thevetia peruviana (Pers.) K. Schum. is a small evergreen plant of 2–6 meter in height with a broad geographical and ecological distribution[1], and its convinced parts are used as medicinal resources in Chinese folk remedy[2]. *Thevetia peruviana* is drought-tolerant evergreen plant of the Family *Appocyanaceae* that originate from Mediterranean countries[3]. All parts of the yellow oleander plant are toxic to humans, animals and certain insects[4].

This species generate derivative metabolites[5], some of which are of pharmacological significance. It is usually grown-up as an ornamental plant in warm clement and subtropical regions, due to its profuse and long durable flowering and temperate durability[6-7]. It is used for screens, equivocation alongside highways, planting along beaches and in town areas by removing suckers and leaving just a few stems, it can also be shaped into very attractive small trees. In Northern regions it may be grown-up as an interior or terrace plant. Oleander has supple branches with green, flat bark ultimately turning to dark grey.

On cutting or broken branches emanate a thick, white sap[8-9]. The leaves are 5 to 15 cm long, thin, acuminated or sharp in the apex, shortly petiolate, with a coriaceus dark-green cutting edge. Flowers are shaped in fatal heads and their colors differ from deep yellow, white, purple and orange[10]. Each flower is about 5 cm in diameter with five petals. The fruit consists of a thin follicle 7.5 to 18.5 cm long which opens to separate feathery seeds. Oleander can be propagated by seed[11] but, being allogamous and highly heterozygous, it shows huge unpredictability in seedling populations. Phytochemical Analysis revealed that *Thevetia peruviana* contains a new cardiac glycoside called Digitoxigenin[12], Thevetin A and B, theveridoside, cerberin[12,13], galactouronic acid, rhamnose, aucubin, ursolic acid, cardenolides[13], quercetin, alpha and beeta amyrin, and lupenyl acetate[14], as prime phytoconstituents.

MATERIAL AND METHODS:

Plant material collection and Authentication:

The flowers of plant *Thevetia peruviana* Pers. were collected from the local area Jaipur Dist. Rajasthan, India, in the month of June-July 2010 and were positively identified and confirmed by the botanist, Mr.A.sharma, Department of Botany, University of Rajasthan, Jaipur. a voucher specimen has been deposited (RUBL 20856) in the herbarium of the botany department of the University of Rajasthan. The fresh mature flowers were used for the study of macroscopic and microscopic characters, whereas the dried uniform flower powder was used for the determination of ash value, extractive Values, loss on drying and phytochemical investigation.

Drying and Pulverization:

Flowers of *Thevetia peruviana* were collected and cut into small pieces .it was shed dried and pulverized to mash size 22 and stored in air tight container for further use.

PHARMACOGNOSTIC STUDY

Macroscopic studies:

Whole fresh flowers of ***Thevetia peruviana*** was examined for colour, odour, taste, shape and structure, size and touch were determined.

Microscopic studies:

Under microscopical examination histological characters of flower were studied, different sections of flower made and observed under Compound microscope. Foe the microscopical study, all flowers were collected and washed with distilled water properly and cut thin sections of flower and also different parts like **Petals, Sepals, ovary** etc. with the help of blade and sweeped the sections with the fine camel hair brush and suspended in distilled water in watch glass. After that selected thin section placed on cleaned glass slide and added one drop of glycerin and covered with cover slip carefully, wiped out excess of mountant on slide with filter paper and observed under compound microscope for various **histological characters** of flower parts.

Powder of the dried flowers was used for the observation of powder microscopical characters. The powdered drug was separately treated with phloroglucinol and HCl solution. Glycerin and iodine solution were used to determine organoleptic properties and the presence of Basal cells of trichomes, epidermal cells, oil globules, vein termination, fibers and starch grains[15,16].

Physiochemical Investigation:

The moisture content, total ash, water-soluble ash, acid-insoluble ash, sulphated ash, alcohol, water-soluble and ether soluble extractive values were determined as a part of its physiochemical parameters. The Powdered flowers parts were subjected to analysis under day/visible light and ultra violet light after treatment with various chemical as a part of Fluorescence analysis[15]**.**

RESULT AND DISCUSSION:

The Flowers of *Thevetia peruviana* Pers. were observed to be Narrow funnel shaped with swird petals and dark green sepals, 6-7 X 2-3 cm size, Yellowish green to dark yellow color **(Fig. 1, 2, 3)** with pleasant and agreeable smell, sweetish taste, and soft smooth touch.**(Table 1).**


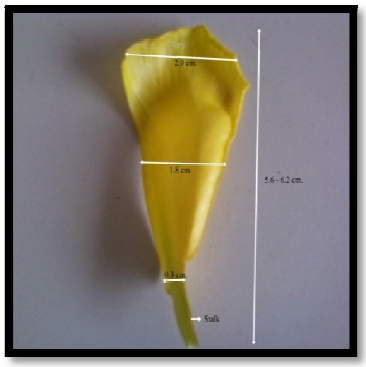
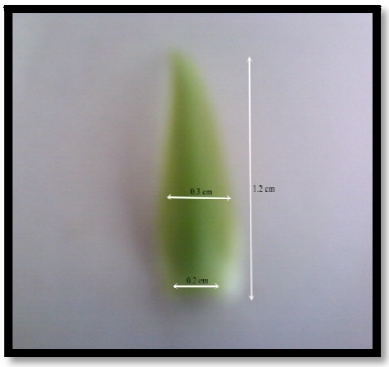

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Figure 1, 2, 3: Morphological Features of Flower, Petal, Sepal

Table 1: Morphological properties of flowers of *Thevetia peruviana*

S.NO.	OBSERVED FOR	FRESH FLOWER
1.	**Shape & Structure**	Narrow funnel shaped with swird Petals,
2.	**Color**	Yellowish green, Dark yellow, Yellowish
3.	**Taste**	Sweetish, agreeable
4.	**Odour**	Characteristic, Pleasant smell
5.	**Touch**	Smooth, Soft
6.	**Size**	6-7 × 2-3 cm

In the microscopic studies, the flower shows all the typical characteristics of flower **(Fig. 4)**. The sepal shows the presence of upper and lower epidermis, centrally vascular bundles as phloem surrounds with the xylem, cortex, parenchyma and also trichomes **(Fig. 5)**. The petal shows the presence of upper and lower epidermis, centrally vascular bundles as phloem surrounds with the xylem, cortex **(Fig. 6)**. The cross section of ovary shows the presence of trichomes, vein trace, locule, ovule and placenta. **(Fig. 7)**

Microscopic study of powder flower revealed the presence of basal Cell of the Trichomes, epidermal cell, stomata, vein – islets, vein – termination. **(Fig. 8)**

Physiochemical tests of powdered drug of *Thevetia peruviana* (Pers.) K. Schum. Flower for the presence of secondary metabolites and color observation shows the following results when treated with different reagents. **(Table 2)**

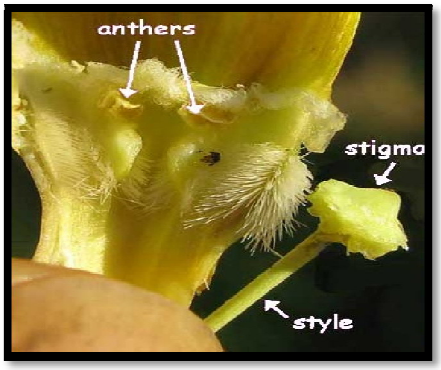


Figure 4: L. S. of Flower

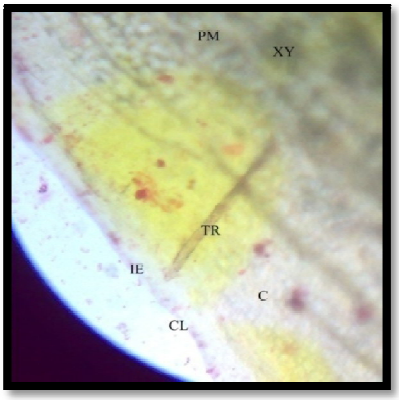


Figure 5: T. S. of Petal

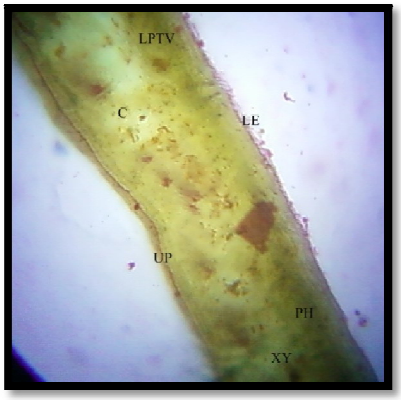


Figure 6: T. S. of Sepal

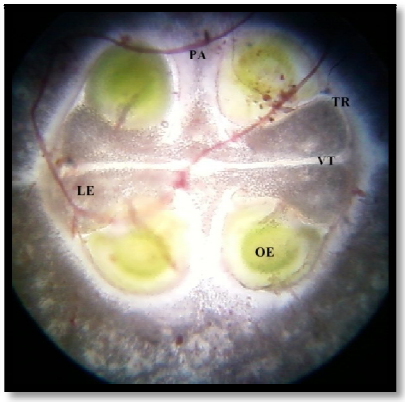
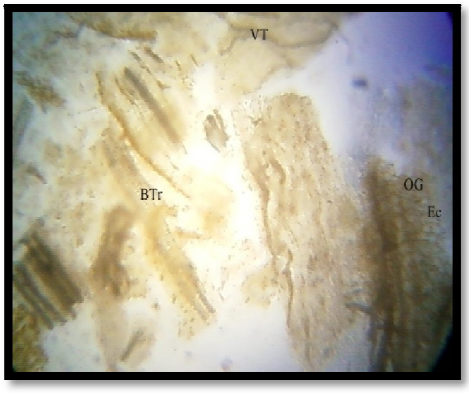


Figure 7: T. S. of Ovary

IE- inner epidermis, **LE-** lower epidermis, **PH-** phloem, **PC-** parenchyma, **CL-** cuticle, **XY-** xylem, **UP-** upper epidermis, **LPTV-** leaf parallel trace vein, **C-** cortex, **TR-** trichomes, **VT-** vein trace, **LE-** locule, **OE-** ovule, **PA-** placenta.



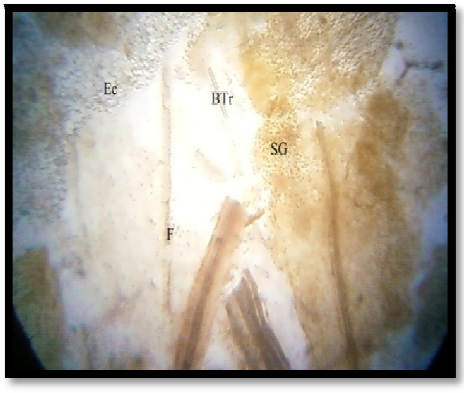


Figure 8, 9: Powder Microscopy of flower

BTr – Basal cell of the Trichomes, **Ec** – Epidermal cells, **VT** – Vein termination, **OG –** oil globules, **SG –** starch grains, **F –** fibers.

Table 2: Physiochemical analysis of powdered drug of flower of *Thevetia peruviana*

S.NO.	REAGENTS	COLOUR OBSERVED
1.	Powder as such	Light black to Yellowish
2.	Powder + Conc. HCl	Brownish black
3.	Powder + Conc. HNO_3	Light brownish
4.	Powder + Conc. $H_2 SO_4$	Light brick red
5.	Powder + Glacial acetic acid	Light brownish
6.	Powder + 5% NaOH	Light brownish
7.	Powder + 5% KOH	Light brownish
8.	Powder + 5% Ferric chloride	Yellow-brownish
9.	Powder + Picric acid (Aq. Sol.)	Yellowish
10.	Powder + Ammonia solution	Light brownish

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. The ash values, extractive values and moisture content of flowers were determined .The results are depicted in **(Table 3).**

Table 3: Physical parameters of flowers of *Thevetia peruviana*

S. No.	PARAMETER	VALUES (%)w/w
1.	**Loss on Drying**	9.5
2.	**Extractive Values**	
	Ethanol soluble extractive	8.3
	Water soluble extractive	4.8
	Petroleum ether soluble Extractive	3.7
3.	**Ash Values**	
	Total Ash	3.5
	Water soluble ash	1.3
	Acid insoluble ash	1.6
	Sulphated ash	1.2

The result of fluorescence studies of flowers powder using different reagents is given in **Table 4**. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation.

Table 4: Fluorescence analysis of powder of flowers of *Thevetia peruviana*

S.NO.	TREATMENT	OBSERVATION (under long wavelength)	OBSERVATION (under short wavelength)
1.	Powder as such	No fluorescence	Yellowish black
2.	Powder + 1 N NaOH in methanol	No fluorescence	Greenish black
3.	Powder + 1 N NaOH in water	No fluorescence	Yellowish black
4.	Powder + 50% HCl	No fluorescence	Light yellowish
5.	Powder + 50% HNO_3	Reddish black	Yellowish
6.	Powder + 50% H_2SO_4	No fluorescence	Dark brownish
7.	Powder + Petrolium ether	Light brown	Light yellowish
8.	Powder + Chloroform	No fluorescence	Light yellowish
9.	Powder + Picric acid	Light yellowish	Yellowish
10.	Powder + 5% Ferric chloride solution	No fluorescence	Greenish-yellowish
11.	Powder + 5% iodine solution	No fluorescence	Light brown yellowish
12.	Powder + Methanol	No fluorescence	Light brown
13.	Powder + HNO_3 + NH_3	No fluorescence	Yellowish black

Preliminary phytochemical screening of powdered drug of flowers indicated high concentration of glycosides, alkaloids along with other constituents like flavonoids, essential oils and steroids. These secondary metabolites are known to posses various pharmacological effects and may be responsible for various pharmacological effects of *Thevetia peruviana* flowers. **(Table 5)**

Table 5: Preliminary Phytochemica screening of powder of *Thevetia peruviana* flowers

S.NO.	TEST FOR	TESTS	POWDERED DRUG
1.	CARBOHYDRATES	Molisch's test	- VE
		Iodine test	-VE
		Fehling's test	+VE
		Barfoed's test	-VE
2.	PROTEIN & AMINO ACIDS	Ninhydrin test	+VE
		Biuret test	+VE
		Millon's test	+VE
		Test with tannic acid	+VE
3.	FIXED OILS & FATS	Spot test	+VE
		Saponification test	-VE
4.	ALKALOIDS	Dragendroff's test	+VE
		Mayer's reagent test	+VE
		Wagner's reagent test	+VE
		Hager's reagent test	+VE
5.	GLYCOSIDES	Legal's test	+VE
		Borntrager's test	+VE
		Keller-killiani's test	+VE
6.	FLAVONOIDS	Ferric chloride test	+VE
		Shinoda's test	-VE
		Fluorescence test	-VE
7.	VOLATILE OIL	By Clevenger apparatus	+VE

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