



# Thin Layer Chromatographic analysis for detecting various bioactive compounds in the ethanolic leaf extracts of *Thevetia peruviana* (Pers.) K. Schum [Thevetia White]

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**Abstract:** The present study is aimed to investigate the bioactive compounds present in the tested plant by using Thin Layer Chromatography (TLC). *Thevetia peruviana* (Pers.) K. Schum is one of the important medicinal as well as ornamental plant belongs to the Dogbane family Apocynaceae. It is commonly known as yellow oleander. The plant is popular in traditional medicine especially for treatment of heart problems. The leaves are emetic and purgative. Leaf decoction is given to prevent conception. Thin layer chromatographic analysis reveals the presence of different bioactive compounds like alkaloids, flavanoids, cardiac glycosides, phenolic compounds, phytosterols and tannins, which are mainly contributed to medicinal utility of the plant, hence, Thevetia White ethanolic leaf extract can be used for drug preparation in pharmaceutical industries.

**Keywords:** Thin Layer Chromatography, cardiac glycosides, phytochemical analysis, *Thevetia peruviana* (Pers.) K. Schum.

## INTRODUCTION

The plant kingdom contributed immensely to human health when no synthetic medicines were available, and when no concept of surgery existed. India is endowed with a rich wealth of medicinal plants. These plants have made a good contribution to the development of ancient Indian Materia Medica.

It is estimated that about 2, 50,000 to 5, 00,000 plant species present on the earth possess medicinal properties, which are used to cure a number of disorders and diseases. In most part of Asia, traditional plants have been part of their culture. (Prajapati *et al.*, 2007; Kaushik and Dhiman, 1999).

*Thevetia peruviana* (Pers.) K. Schum is a small tree; 15-20 ft. high belongs to the Dogbane family Apocynaceae. It is a native of South America and West Indies. Leaves are simple, linear – lanceolate and whorled. (Fig No. 1). All parts of this plant abound in a milky juice which is highly poisonous. (Chopra *et al.*, 1984).

The plant (Thevetia Yellow) is bitter, pungent, acrid, astringent to the bowels, useful in urethral discharges, worms, skin diseases, leucoderma, wound piles, eye trouble, itching, fever and bronchitis. (Kirtikar and Basu, 1981). The cardiac glycosides obtained from bark, kernels and flowers (Thevetia Yellow) are useful for heart diseases. (Prajapati *et al.*, 2007) The root of this plant are made into a paste and applied to tumours. (Singh and Dey, 2005).

The leaves are emetic and purgative. Leaf decoction is given to prevent conception. The purified glycosides thevetin extracted from the seed is prescribed as a cardio tonic drug. Seeds used as an abortifacient and purgative in rheumatism and dropsy; also used as an alexeteric. Diluted latex is given to treat irregular menstruation. (Ambasta, 1986; Kaushik and Dhiman, 1999; Retnam and Martin, 2006).



**Fig 1: *Thevetia peruviana* (Pers.) K. Schum [Thevetia White]**

## MATERIAL AND METHODS

### 1 .Collection of Plant Material

Plant materials (*Thevetia* White leaves) of *Thevetia peruviana* (Pers.) K. Schum were collected from Nehruvngram, Indore. The collected plant materials were identified with the help of Flora of Madhya Pradesh. (Mudgal *et al.*,1997).

### 2. Extraction

To obtain ethanolic extract 100gm. of shade dried plant material was extracted with 500 ml. of ethanol (95%) in “Soxhlet Extraction Apparatus. Finally the prepared plant material was macerated with water for 24 hrs. to obtain aqueous extract. Each extract was concentrated by distilling off the solvent (Kokate, 1994 and Kokate *et al.*, 1993).

### 3. Preliminary Phytochemical Screening

The extract thus obtained was than subjected to preliminary phytochemical screening for identification of various plant constituents by methods suggested by (Finar, 1962; Farnsworth, 1966; Harborne, 1973; Harborne *et al.*,1979).

### 4. Thin Layer Chromatography (TLC)

Each ethanolic extract was than subjected to Thin Layer Chromatography by methods suggested by Kokate (1994) Stahl (1969) , Wagner *et al.*(1984) ,Braithwaite and Smith (2009), Indhumathi and Mohandas (2013). The absorbent silica gel GF<sub>256</sub> was coated to a thickness of 0.3 mm on clean TLC plates by commercial spreader.

The plates were activated at 105°C for 30 minutes and used. Rf values were calculated. Various solvent systems were used to detect the phytochemical constituents. The selection of mobile phase depends upon, type of constituents to be analyzed.. Here (8) different mobile phases were used.

## OBSERVATIONS AND DISCUSSION

### Phytochemical screening

The leaf extract of *Thevetia* White reveals the presence of alkaloids, flavanoids, glycosides-cardiac glycosides, phenolic compounds, tannins, phytosterols, carbohydrates, saponins, terpenoids, proteins and amino acids was noted in the observation Table, while fixed oils, fats, gums and mucilages were found absent.(**Table No. 01**).

**Table No.1: Phytochemical screening of ethanolic leaf extract of *Thevetia peruviana* (Pers.) K. Schum [*Thevetia* White]**



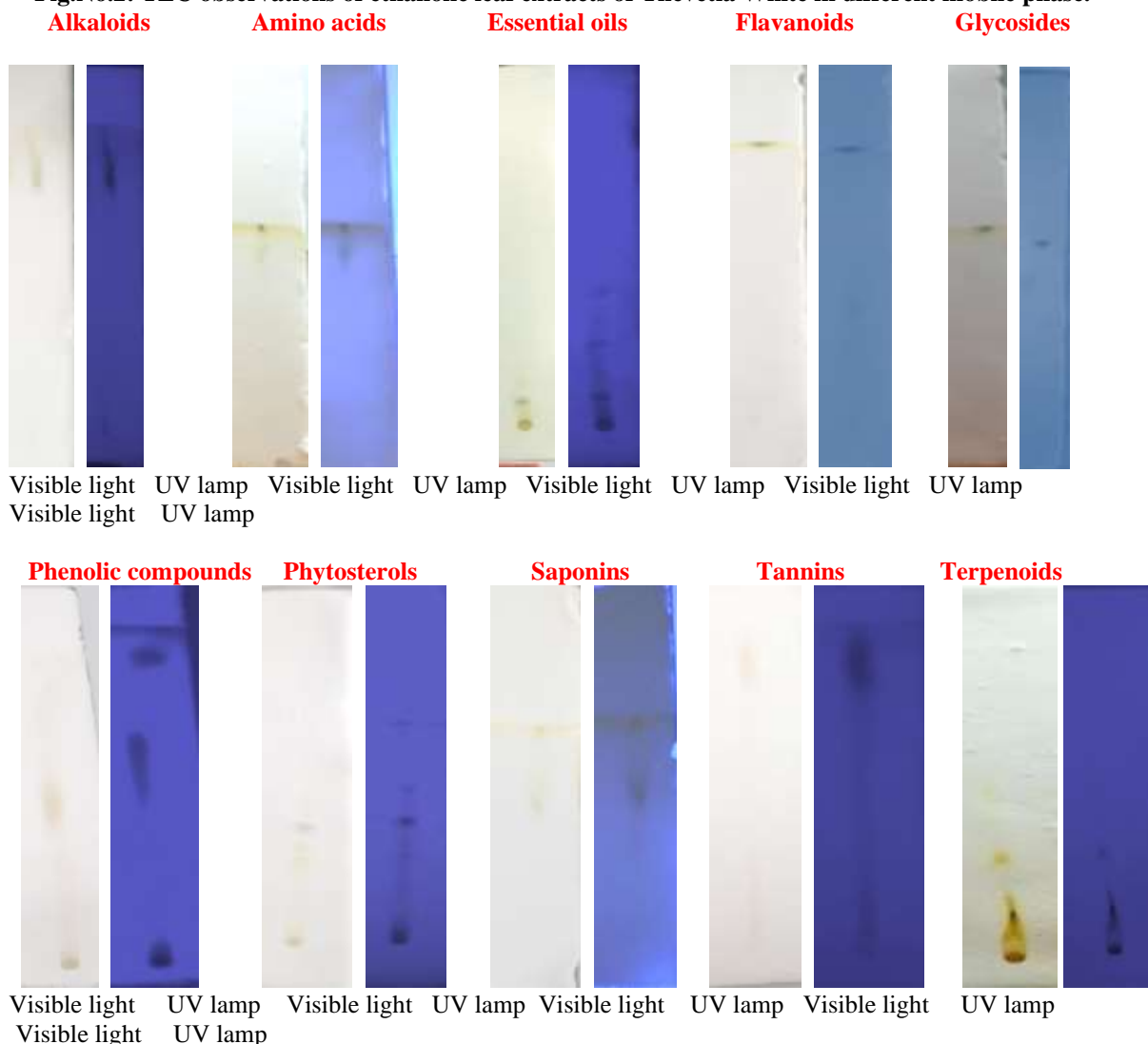
S. No.	Plant Constituents Test/Reagents	Results
1.	<b>Alkaloids</b>	
	Mayer's reagent	+
	Dragendorff's reagent	+
	Hager's reagent	+
	Wagner's reagent	+
2.	<b>Carbohydrates</b>	
	Molish's reagent	+
	Benedict's reagent	+
	Fehling solution	+
3.	<b>Types of Carbohydrates</b>	
	Glucose	+
	Fructose	+
	Galactose	-
	Lactose	+
	Starch	+
4.	<b>Phytosterols</b>	
	Liebermann-Burchard's test	+
5.	<b>Terpenoids</b>	
	Salkowski reaction	+
6.	<b>Fixed oils and fats</b>	
	Spot test	-
7.	<b>Saponins</b>	
	Foam test	+
8.	<b>Phenolic compounds</b>	
	Ferric chloride solution	+
9.	<b>Tannins</b>	
	Lead acetate solution	+
10.	<b>Proteins</b>	
	Biuret test	+
	Xanthoprotic test	+
11.	<b>Amino acids</b>	
	Ninhydrin reagent	+
12.	<b>Gums and mucilages</b>	
	Alcoholic precipitation	-
13.	<b>Flavanoids</b>	
	Shinoda test	+
	Lead acetate test	+
14.	<b>Cardiac glycosides</b>	
	Keller kiliani test	+

+Present – Absent

#### Thin Layer Chromatography (TLC)

In leaf extracts maximum separation was found in **Chloroform: Methanol: Water (7:4:1)** mobile phase which is used for the detection of phytosterols. The result of TLC analysis reveals the presence of alkaloids, amino acids, essential oils, flavanoids, glycosides, phenolic compounds, phytosterols, saponins, tannins and terpenoids.

**Fig.No.2: TLC observations of ethanolic leaf extracts of *Thevetia White* in different mobile phase.**



**Table No. 2 : TLC observations of different phytoconstituents from ethanolic leaf extracts of *Thevetia peruviana* (Pers.) K. Schum. [*Thevetia White*]**

S. N o.	Name of the Phytoconstituents	Mobile phases	Eyes			UV light		
			Number of spots on TLC plates	Colour	Rf	Number of spot on TLC plates	Colour	Rf
1.	Alkaloids	CHCL <sub>3</sub> : Methanol : Glacial acetic acid (83:17:10)	1	Brown	0.88	1	Greenish blue	0.88
2.	Amino acids	n-Butanol :acetic acid :water (4:5:1)	1 2	Brown Green	0.85 0.98	1 2	Brown Greenish brown	0.85 0.98



3.	Essential oils	Hexane : Acetone (9:1)	1 2	Black Yellow	0.18 0.26	1 2 3	Violet Violet Violet	0.18 0.26 0.40
4.	Flavanoids	Ethyl acetate :Methyl ethyl ketone :Acetic acid : Water(5:3:1:1)	1	Green	0.97	1	Greenish brown	0.97
5.	Glycosides	Ethyl Acetate : Benzene (2:1)	1	Green	0.94	1	Brown	0.94
6.	Phenolic Compounds	n - Butanol : Acetic acid : Water (35 : 5 : 12)	1	Brown	0.67	1 2	Violet Violet	0.67 0.92
7.	Phytosterols	P. ether : Ethyl acetate (7 : 3)	1 2	Orange Black	0.59 0.72	1 2 3	Green Green Violet	0.59 0.72 0.98
8.	Saponins	Chloroform : Methanol : Water (7:4:1)	1	Orange	0.95	1 2	Black Brown	0.71 0.95
9.	Tannins	Chloroform: Ethyl acetate: Ethanol (6:4:4)	1	Brown	0.90	1	Brown	0.90
10.	Terpenoids	Ethyl acetate: Chloroform (6:4)	1 2	Brown Brown	0.22 0.36	1 2	Brown Brown	0.22 0.36

### CONCLUSION

This investigation has shown the preliminary informations to determine the chemical composition of the studied *Thevetia peruviana* (Pers.) K. Schum. plant . [Thevetia White] .Thin layer chromatographic analysis reveals the presence of different bioactive compounds like alkaloids, flavanoids, cardiac glycosides, phenolic compounds, phytosterols and tannins, which are mainly contributed to medicinal utility of the plant hence, Thevetia White ethanolic leaf extract can be used for drug preparation in pharmaceutical industries.

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