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Phytochemical screening, ultra violet and infra-red spectroscopy of ethanolic leaf extract of *Thevetia peruviana* (Pers.) *Thevetia* yellow

Krati Ghavri and Madhavi Adhav

Abstract
Thevetia peruviana (Pers.) is one of the important medicinal as well as ornamental plant belongs to the family Apocynaceae and commonly called as yellow oleander. In phytochemical screening the ethanolic leaf extract shows the presence of alkaloids, flavanoids, glycosides - cardiac glycosides, phenolic compounds, tannins, phytosterols, carbohydrates, proteins and amino acids. The fixed oils, fats, gums and mucilages were totally absent. The UV and IR spectroscopy of ethanolic leaf extract of *Thevetia* yellow shows the presence of carbonyl group, β - diketones, aldehydes, ketones, amides, lactams, sulfur compounds, aromatic compounds, nitro compounds, indole alkaloids, chalcones, flavones, flavanol, quercetin, NaQSA (sodium salt of quercetin 5'sulfonic acid), myricetin types of flavonoids.

Keywords: *Thevetia peruviana* (Pers.), phytochemical screening, Ultra violet and Infra-red spectroscopy

Introduction
Nature is a source of medicinal agents for thousands of year and an impressive number of modern drugs have been isolated from natural sources. According to (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.
Thevetia peruviana (Pers.) is a small tree, 15-20 ft. high belongs to the family Apocynaceae originally a native of America and West Indies. Leaves are simple, linear – lanceolate, whorled. Flowers medium, yellow, solitary or in few flowered cymes. (Fig No. 1).All parts of this plant abound in a milky juice which is highly poisonous. (Chopra *et al*., 1984) [2]. The plant 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, 1984). The leaves are emetic and purgative, leaf decoction is given to prevent conception. (Ambasta, 1986; Kaushik and Diman, 1999; Retnam and Martin, 2006) [1, 16, 23]. The cardiac glycosides obtained from bark, kernals and flowers are useful for heart diseases (Prajapati *et al.*, 2007) [22].

Material and Methods
Plant material (Leaves) of *Thevetia peruviana* (Pers.) were collected from Devi Ahilya Vishwavidyalaya campus, Indore. The collected plant material were identified with the help of Flora of Madhya Pradesh. (Mudgal *et al*., 1997) [21].
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) [19, 18].
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) [8, 9, 11]. To find out the alkaloids, flavanoids, chromophoric groups and functional groups present in the extract. Spectral studies were carried out by Ultra violet and Infra-red Spectroscopy. (Dyer, 1994; Dutta, 2000; Harborne *et al.*,1975; Hencezkowski *et al.*, 2001; Manske, 1960; Silverstein *et al*., 1991; Silverstein and Webster, 2012) [6, 5, 10, 12, 20, 25, 26].

Observations and Discussion
Phytochemical screening
The leaf extract of *Thevetia* yellow reveals the presence of alkaloids, flavanoids, glycosides-

cardiac glycosides, phenolic compounds, tannins, phytosterols, carbohydrates, saponins, proteins and amino acids was noted in the observation Table, while fixed oils, fats, gums and mucilages were found absent. (Table No. 01).

Fig 1: *Thevetia peruviana* (Pers.) *Thevetia* Yellow

Table 1: Preliminary phytochemical screening of ethanolic leaf extract of *Thevetia peruviana* (Pers.) *Thevetia* Yellow

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

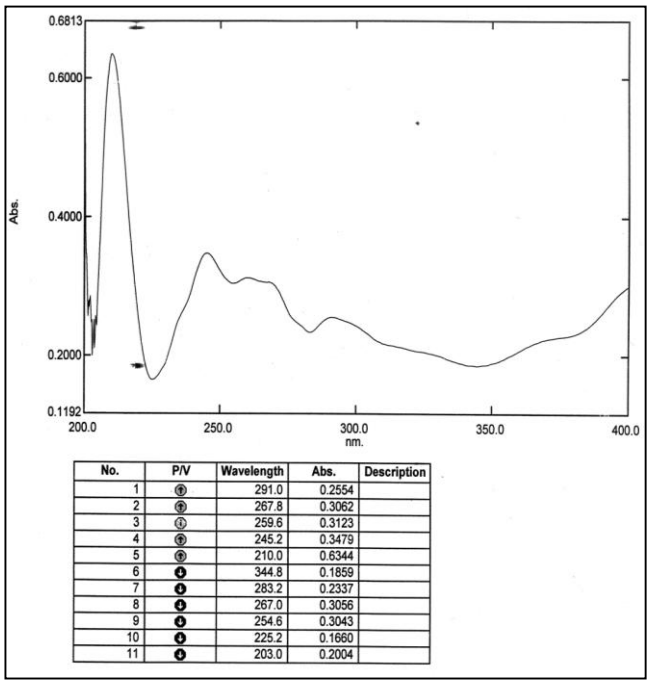
+ = Present, - = Absent

UV Spectroscopy of ethanolic leaf extract of morphotypes of *Thevetia peruviana* (Pers.).*Thevetia* Yellow

The UV spectrum of *Thevetia peruviana* (Pers.) (yellow leaf) shows the absorption band at 291 nm which indicates the presence of thiocarbonyl compound, C=S, sulfur containing group, (Indole alkaloid-vincamajine) and dihydrokamferol type of flavanoid. The band at 268 nm reveals the presence of C=S, sulfur containing group, thiophene,1- phenyl,3-butadiene. Flavones and chalcones type of flavanoids and reserpine type of Indole alkaloid.

The shoulder band at 260 nm confirms the presence of β-diketones, C=S sulfur containing group, α,β -unsaturated ketones, pyridine,(quercetin and flavanol type of flavanoids) and gelsemicine type of Indole alkaloid. The intense band at 245 nm indicates the presence of β-diketones, α,βunsaturated ketones and aldehydes, carbonyl group. Flavone type of flavanoids and aricine type of Indole alkaloid. Appearance of strong band at 210 nm shows the presence of amides, lactams, S=O, sulfoxide, di-n- butyl sulfide, aromatic compounds, acrolein, ketones and aldehydes.

UV spectroscopy shows the presence of four types of Indole alkaloids viz. vincamajine, reserpine, gelsemicine, aricine and five types of flavonoids viz. dihydrokamferol, quercetin, flavone, flavanol and chalcones. (Fig No. 2, Table No. 2).



No.	P/V	Wavelength	Abs.	Description
1	↑	291.0	0.2554	
2	↑	267.8	0.3062	
3	↓	259.6	0.3123	
4	↑	245.2	0.3479	
5	↑	210.0	0.6344	
6	↓	344.8	0.1859	
7	↓	283.2	0.2337	
8	↓	267.0	0.3056	
9	↓	254.6	0.3043	
10	↓	225.2	0.1660	
11	↓	203.0	0.2004	

Fig 2: UV spectrum of *Thevetia* yellow

Table 2: UV spectroscopy of ethanolic leaf extract of ethanolic leaf extract of *Thevetia peruviana* (Pers.) *Thevetia* Yellow

S. No.	Wavelength nm	Abs.	Types of Alkaloids	Chromophoric group	Types of Flavonoids
1.	291	0.2554	Vincamajine	Thiocarbonyl compound, C=S, sulfur containing group	Dihydrokamferol
2.	268	0.3062	Reserpine	C=S,sulfur containing group,thiophene,1- pheny l,3- butadiene	Flavone, Chalcones
3.	260	0.3123	Gelsemicine	C=S sulfur containing group,α,β -unsaturated ketones, pyridine	Quercetin, Flavanol
4.	245	0.3479	Aricine	β-diketones,α,βunsaturated ketones and aldehydes, carbonyl group	Flavone
5.	210	0.6344	–	Amides, lactams, S=O, sulfoxide, di-n- butyl sulfide, aromatic compounds, acrolein, ketones and aldehydes.	–

IR Spectroscopy of ethanolic leaf extract of *Thevetia peruviana* (Pers.) *Thevetia* Yellow

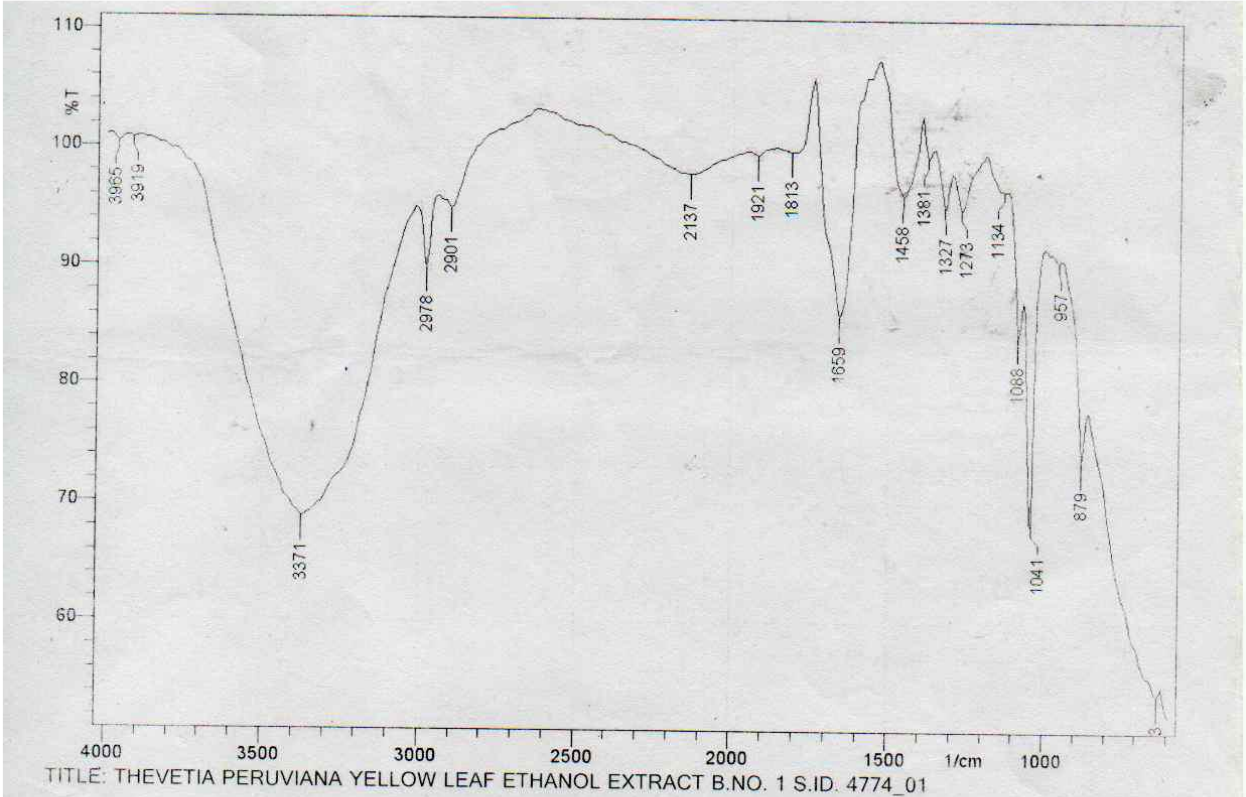
The IR spectrum of yellow leaf shows weak peak at 632 cm^{-1}.The sharp peak at 879 cm^{-1} indicates the presence of aromatic substitution, C-H bending vibration and gem disubstituted olefinic group and quercetin type of flavanoid.The small peak at 957 cm^{-1} shows the presence of alkene, C-H bending.

The very sharp peak at 1041 cm^{-1} reveals the presence of sulfur compounds, sulfoxides, S=O stretching, thiocarbonyl group and sodium salt of quercetin 5' sulfonic acid (NAQSA).The shoulder peak at 1134 cm^{-1} shows the presence of sulfur compounds, sulfones, aromatic, C-H bending and thiocarbonyl group. The peak at 1273 cm^{-1} is due to the presence of aromatic, C-H bending, amines, C-N vibration and ketones. The small peak at 1327 cm^{-1} is corresponding to aromatic, C-H bending, gem – dimethyl group, sulfur compound, sulfones and sulfonamides, amines, C-N vibration and nitro compounds and myricetin type of flavanoid.

The shoulder peak at 1381 cm^{-1} again confirms the presence of gem - dimethyl group, C- CH_3 bending, nitro/sulfur compound, sulfates, sufites and S=O stretching. The peak at 1458 cm^{-1} only shows the presence of alkane,-CH_2 and –CH_3 and quercetin type of flavanoid. The sharp peak at 1659 cm^{-1} indicates the presence of amides, carbonyl stretching vibration and quinines. The peak at 1813 cm^{-1} only indicates carbonyl group. The peak 1921 cm^{-1} shows weak absorption of aromatic, alkenes. The presence of overtone at 2137 cm^{-1}reveals the presence of monosubstituted alkyne, -N=C-N stretching vibration and diamides. Another overtone at 2901 cm^{-1} and sharp peak at 2978 cm^{-1} shows the presence of β – diketones, O-H stretching, carboxylic acid / intermolecular hydrogen bonding, C-H stretching and aldehydes.

The broadening of peak (hump) at 3371 cm^{-1} is due to the presence of primary sulfonamides, aromatic, N-H stretching and amines.

From the IR spectroscopy it is concluded that three types of Flavonoids are present viz. Quercetin, Sodium Salt of Quercetin 5' Sulfonic Acid (NAQSA), Myricetin. (Fig No. 3, Table No. 3).



Peak table of THEVETIA PERUVIANA YELLOW LEAF ETHANOL EXTRACT B.NO. 1 S.ID. 4774_01:

PeakNumber	Begin [1/cm]	Max [1/cm]	End [1/cm]	Absolute Height [%T]	Width [1/cm]
1	617.18	632.61	856.33	54.01	239.16
2	856.33	879.48	910.33	73.79	54
3	948.91	956.62	979.77	90.65	30.86
4	1002.91	1041.48	1064.63	68.23	61.72
5	1064.63	1087.77	1118.63	84.37	54
6	1126.35	1134.06	1188.06	96.64	61.72
7	1195.78	1272.93	1296.07	94.95	100.29
8	1296.07	1326.93	1357.79	95.22	61.72
9	1357.79	1380.93	1396.36	99.34	38.57
10	1396.36	1458.08	1535.22	96.23	138.86
11	1535.22	1658.66	1743.52	85.99	208.3
12	1743.52	1812.95	1859.24	99.95	115.72
13	1859.24	1920.96	1944.1	99.61	84.86
14	1944.1	2136.97	2623	97.96	678.89
15	2623	2900.73	2939.3	94.94	316.3
16	2939.3	2977.87	3008.73	89.96	69.43
17	3008.73	3371.32	3895.92	68.9	887.19
18	3895.92	3919.07	3934.5	100.73	38.57
19	3934.5	3965.35	3988.5	100.48	54

Fig 3: IR spectrum of *Thevetia* Yellow

Table 3: IR spectroscopy of ethanolic leaf extract of *Thevetia peruviana* (Pers.) *Thevetia* Yellow

Peak No.	Peak cm-1	Types of flavanoid	Functional group
1.	632	-	-
2.	879	Quercetin	Aromatic substitution, C-H bending vibration and gem disubstituted olefinic group
3.	957	-	Alkene, C-H bending
4.	1041	NAQSA	Sulfur compounds, sufoxides, S=O stretching, thiocarbonyl group
5.	1088	NAQSA	Sulfur compounds, sulfones, aromatic, C-H bending and thiocarbonyl group
6.	1134	-	Sulfur compounds, sulfones, aromatic, C-H bending and thiocarbonyl group
7.	1273	-	Aromatic, C-H bending, amines, C-N vibration and ketones
8.	1327	Myricetin	Aromatic, C-H bending, gem – dimethyl group, sulfur compound, sulfones and sulfonamides, amines, C-N vibration and nitro compounds
9.	1381	-	Gem – dimethyl group, C- CH_3 bending, nitro/sulfur compound, sulfates, sufites and S=O stretching
10.	1458	Quercetin	Alkane, -CH_2 and –CH_3
11.	1659	-	Amides, carbonyl stretching vibration and quinines
12.	1813	-	Carbonyl group

13.	1921	-	Aromatic, alkenes
14.	2137	-	Monosubstituted alkyne, -N=C-N stretching vibration and diamides
15.	2901	-	B - diketones, O-H stretching, carboxylic acid / intermolecular hydrogen bonding, C-H stretching and aldehydes
16.	2978	-	B - diketones, O-H stretching, carboxylic acid / intermolecular hydrogen bonding, C-H stretching and aldehydes
17.	3371	-	Primary sulfonamides, aromatic, N-H stretching and amines
18.	3919	-	-
19.	3965	-	-

Conclusion

This study has shown the preliminary information to determine the chemical composition of *Thevetia* yellow leaf. The presence of chromophoric groups, functional groups, alkaloids, flavanoids, cardiac glycosides, phenolic compounds, tannins, phytosterols and saponins are mainly contributed in medicinal utility of plant, so the results of the present study indicate that the leaves have a medicinal potential to develop into a new drug of pharmaceutical interest.

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