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Original Article

SYSTEMATIC SCREENING FOR PHYTOCHEMICALS OF VARIOUS SOLVENT EXTRACTS OF*THEVETIA PERUVIANA* SCHUM. LEAVES AND FRUIT RIND

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ABSTRACT

Thevetia peruviana (S) (yellow oleander) is a tropical evergreen shrub or small tree that is in the Apocynaceae family as and closely related to *Nerium oleander*, is mainly known for its cardiac glycosides, which was traditionally used as antipyretic [10], as emetics, diuretics and tonics [12].In addition, its extensive use reported for antitumor therapy [11] and therefore it has important medicinal values.

Objective: The present investigation deals with thorough phytochemical analysis of leaf and fruit rind of *Thevetia peruviana* (S). This study represents a first report of quantitative and qualitative determination of phytochemicals of *T. peruviana* (S).

Methods: Plant leaves and fruit rind material extracted by using solvents of increasing polarity and the quantity of crude extracts obtained. The ethanol extract has shown highest percentage of yield in both the samples followed by water in fruit rind, than hexane and chloroform extracts. Furthermore, pilot solubility tests performed for the selection of vehicle solvents.

Results: The qualitative phytochemical studies have clearly demonstrated that the plant *Thevetia peruviana* (S)is a rich source ofalkaloids,flavonoids, saponins, cardiac glycosides, anthroquinone glycosides, coumarins, phenols, tannins, steroids, oils and fats.Further quantitative determination confirmed that *T. peruviana* (S) is very rich in alkaloid 52.92 ± 0.06 mg/g (leaf) and 59.28 ± 0.04 mg/g (fruit rind), saponins 7.97 ± 0.36 mg/g (leaf) and 14.27 ± 1.02 mg/g (fruit rind)followed by flavonoid and phenolics.

Conclusion: Among the extracts, the ethanolic extract has found to be rich in phytoconstituents, which gives a very strong reason to select this plant for cytotoxic evaluation and other pharmacological properties.

Keywords: *Thevetia peruviana* (S), Yellow oleander, Phytochemical, Alkaloids, Cardiac glycosides,

INTRODUCTION

Plants produce a vast array of secondary metabolites as defense against environmental stress or other factors like pest attacks, wounds, and injuries. The complex secondary metabolites have found various therapeutic uses from time immemorial. The early history of modern medicine contains descriptions of plant-derived phytochemicals, many of which are still in use. Some examples are the discovery of cardio tonics from foxglove, salicylic acid from willow bark, and morphine from poppies [1]. Various terpenoid compounds synthesized in plants as secondary metabolites are proving their potential in modern scientific studies against inflammatory diseases and cancer [2].

The Apocynaceae family has approximately 5000 species and classified in five subfamilies consisting of 415 genera [3] including *Cascabela*R*, Cerbera*L., and *Thevetia* L. These genera share a close morphological relationship. It includes most of the well-known tropical ornamental plants (Oleander, Frangipani, Allamanda, Mandevilla). The sap of most plants is milky latex, which is often having economic importance or medicinal benefits. This sap is often toxic [4].

Plant genus *Thevetia* comprises several species, among them only a few explored substantially for their medicinal properties using approved pharmacological parameters. Most of the other species have remained scientifically untouched despite that they have reported to possess some curative abilities in traditional medicine.

Thevetia peruviana (S) (yellow oleander) is a tropical evergreen shrub or small tree that is in the same family Apocynaceae and closely related to *Nerium oleander*, commonly known as oleander. Yellow oleander will grow to 20-30' tall in its native habitat. It is an upright shrub that features willow-like, linear-lanceolate, glossy green leaves (to 6-7" long) with distinctive midribs and large 3" long funnel-shaped, fragrant yellow (less commonly apricot) flowers in few-flowered terminal clusters (cymes). Flowers bloom from summer to fall and give way to black seedpods, each containing 1-2 nut-like seeds.

Thevetia peruviana (S) grown largely in parts of West Africa as an ornamental plant [10]. In Nigeria and Ghana, the bark is widely used as antipyretic. Further reports suggest its use as emetics, diuretics, tonics and antitumor [11][12]. Since the margin between effective and toxic doses is not very distinct, local people therefore exercise some degree of caution in taking such medicines, restricting their use mainly to topical applications. As with many of the Apocynaceae family members, plant stems exude a milky sap when cut and all parts of the plant are poisonous if ingested. Plant saps can cause allergic skin reactions in some people. Smoke from burning plant material can also be toxic.

The *N. oleander* plant contains toxic properties due to the presence of digitoxin like steroidal glycosides such as oleandrin. It is estimated that as many as 100 novel chemical substances are present in various parts of the oleander plant [5]. In 1957, the National Cancer Institute showed that three compounds in the plant, namely, oleandrin, adynerin and ursolic acid had significant anti-cancer activities on various cancer cell lines. Since then several new chemical compounds have been identified from the methanolic or ethanolic extracts of the plant [6]. Several, reports have revealed that a member of the genus *Thevetia* called *Thevetia thevetiodes* known in the Mexicancordilleras as the joyote, and has been used for immediate relief of pain, presumably by amelioration of the muscle spasm [7]. Recently it has been shown that, the cardiotonic properties are exploited therapeutically as they produce digitalis-like action on the heart, disrupt normal neuro-motor and neuromuscular functions and cause diuresis [8]. Similarly, the leaves and seed oil reported for their insecticidal, molluscicidal and antibacterial properties [9].

In view of the above reports, it is very much clear that members of Apocynaceae family are of high medicinal importance possessing several pharmacological properties. However, the selected plant *Thevetia peruviana* (S) the reports on the phytochemical investigations are scanty and the present investigations were under taken with a presumption that it may possess important bioactive compounds.

MATERIALS AND METHODS

Plant material collection

Unripe fruits and leaves of *Thevetia peruviana* (S) were collected from the surroundings of Kuvempu University, Shankarghatta, Dist. Shimoga, and Karnataka, India. Prof. V. Krishna, Taxonomist, Dept. of Biotechnology, Kuvempu University authenticated the plant and the specimen was deposited at Department. The fruits material processed by separating the epicarp (fruit rind) and the leaf and fruit rind material was shade dried and powdered.

Chemicals

Hexane, chloroform, ethanol, dimethyl sulfoxide (DMSO), quercetin and all the chemicals used for phytochemical analysis were purchased from Merck and Himedia. The chemicals and solvents used were of analytical grade.

Organoleptic Evaluation

Organoleptic evaluation refers to the assessment of the selected plant drug by colour, odour, taste and texture, etc. The organoleptic characters of the samples were evaluated according to the methods of Jackson and Snowdown 1968 [13].

Fluorescence analysis

Many substances such as alkaloids like quinines and berberine in diluted sulphuric acid when suitably illuminated emit light of different wavelengths or colour from that which falls on them. This emitted light (fluorescence) ceases when the exciting light is removed [14]. Fluorescence analysis of the powdered drugs were performed which helps to detect the adulteration, because phytoconstituents exhibits characteristic fluorescence under ultraviolet light when they mixed with the reagents. The fluorescence exhibited by the mixture was attributed to the chemical constituents present in the crude drug. Prior to the phytochemical screening a rough estimation of phytoconstituents was done by the behaviour of powder drug with different chemical reagents in which powdered drug showed different colours when it got mixed with the particular reagents which indicates the presence of phytochemicals in accordance with the colours obtained.

The fluorescence characteristics were studied by adopting the method proposed by (Kokoshi*et al.,* 1958) [15]. The behaviour of the samples with different chemical reagents and solvents and fluorescence characters were analysed on UV light at 254nm and 366nm.

Soxhlet Extraction

Successive extraction was done using 500 g of powdered material of leaf and fruit rind in soxhlet apparatus. The solvents hexane (2L, 50^0C ˜ 15 cycles) chloroform (2 L, 45^0C ˜15 cycles) and ethanol (2 L, 70^0C, ˜15–17cycles) were used. All the extracts were concentrated *in vacuo*, followed by the cold extraction of fruit rind material using 9:1 ratio of water and ethanol respectively for 24 h with frequent agitation. Water extract was set for the filtration and concentrated *in vacuo*. The yield of each dried extract was calculated.

Interestingly, it was observed that the fruit rind ethanolic extract showed three different fractions. Based on that the fractions were separated as bottom residue (BR), middle crystals (MC) and upper liquid (UL), all the dried extracts were used for the further analysis.

Pilot solubility Tests of plant extracts

Solubility tests were carried out for the analysis of solubility of crude extracts in different solvents like, hexane, chloroform, acetone, DMSO, ethanol, methanol, water, 1N NaOH, and 1N HCl.

Preliminary Phytochemical Screening of plant extracts

Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analyses like carbohydrates, saponins, oils, fats, flavonoids, terpenoids, alkaloids etc. A brief experimental procedure for the various phytochemical screening methods for the secondary metabolites were performed with some modifications from the method of Harborne (1973) [16].

Quantitative Estimation of Phytochemicals

Total Alkaloid Estimation

Alkaloid determination using Harborne method [16]. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and precipitate was collected, washed with dilute ammonium hydroxide and then filtered. The crude alkaloids were dried and weighed.

Total Saponin Estimation

Saponin determination was done using Obadoni and Ochuko (2001) method [17]. 10 g of sample powder was taken into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C.

The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously for purification. The aqueous layer was recovered, the purification process was repeated. Then 60 ml of n-butanol was added, the combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride where the solvent layer was recovered and heated on a water bath. After evaporation the samples were oven dried to a constant weight and the total saponin content of *T. peruviana* (S) leaf and fruit rind material was calculated as percentage yield per 1g.

Total Phenolic content estimation

The concentration of total phenolics in the *T. peruviana* (S) extracts were determined according to the protocol described by Chandler and Dodds (1993) [18]. 1mL of each extract was mixed in a test tube containing 1mL of 95% ethanol, 5mL of distilled water and 0.5mL of 50% Folin–Ciocalteu reagent. The resultant mixture was allowed to react for 5min and 1mL of 5% sodium carbonate was added, mixed thoroughly and placed in dark for 1 h. The absorbance was red at 725 nm using the UV–VIS spectrophotometer. The total phenolic contents in *T. peruviana* (S) were expressed as Gallic acid equivalents in microgram per gram of the extract.

Total Flavonoid Estimation

Aluminium Chloride Colorimetric Method of Woisky*et al.,* (1998) [19] was followed for the determination of total flavonoid concentration of different extracts. 10 milligrams of quercetinwas dissolved in 80% ethanol.Diluted to 100 to 500 µg/ml to make the calibration curve. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M Potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm.

The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of different extracts which have shown positive results in preliminary phytochemical analysis were reacted with aluminium chloride for determination of flavonoid content as described above.

Total Carbohydrate estimation

Total carbohydrate was estimated by Anthron method [20]. Dextrose was used for standard gradient preparation of 100- 500 µg. 400µl of all the extracts were taken and the volume was made up to 1 ml to which 4 ml of anthron reagent was added. The reaction mixture was incubated in boiling water bath for 10 min. The absorbance was red at 620 nm.

Statistical analysis

Data are expressed as Mean ± S.E. All the assays were analysed by one-way analysis of variance. (ANOVA)

RESULTS

Organoleptic Characters Studies

The organoleptic characters of the plant materials were determined and the results are shown in table 1. The aromatic and pleasant smell indicates the presence of more prominent secondary metabolites in leaf than in the fruit rind. The sweet taste of fruit material indicates the presence of abundant amount of sugars or glycol moiety.

Fluorescent analysis of plant material

The behaviour of the samples with different chemical reagents was studied and fluorescence characters were analysed on UV light at 254 nm and 366nm.

The fluorescence characteristics of plant materials were studied under UV light and results are tabulated in (table 2). This analysis is a rapid method for identification of groups present and selection of appropriate solvent for extraction.

Table 1: Organoleptic study of the plant material

Parameters	Leaf	Fruit Rind
Observations		
Condition	Dried	Dried
Colour	Dark Green to brown	Green to black
Odour	Aromatic	Pleasant
Taste	Bitter	Slightly sweet
Texture	Smooth	Crumpled
Size	2-3 mm	1-2 cm

Table 2: Fluorescent analysis of aerial parts of *Thevetia peruviana* (S),

S. No.	Particulars of the treatment	Leaf		Fruit	
		Normal light	UV light (366 nm)	Normal light	UV light (366 nm)
1.	Powder as such	G	G	B	B
2.	Powder + 1N NaOH (aqueous)	Y	G	RY	LG
3.	Powder +1N NaOH (alcoholic)	G	O	T	C
4.	Powder + 50% HCl	Y	GR	LR	C
5.	Powder + 50%H2SO4	YG	R	T	LG
6.	Powder + 50%HNO3	O	R	Y	LG
7.	Powder + Ammonia	G	G	T	C
8.	Powder + Iodine	Br	B	O	O
9.	Powder + 5% FeCl3	O	B	O	O
10.	Powder + Nitric acid+ Ammonia	Y	G	LY	LG
11.	Powder + Petroleum ether	T	M	T	T
12.	Powder + Chloroform	G	FM	LR	LBr
13.	Powder + Methanol	G	MR	LG	R
14.	Powder + Hexane	LG	M	T	T
15.	Powder + Ethyl Acetate	G	O	T	T
16.	Powder + Ethanol	G	R	T	T

G: Green, Y: Yellow, YG: Yellowish Green, O: Orange, Br: Brown, LG: Light Green, GR: greenish red, R: Red, B: Black, M: Magenta, FM: Fluorescent magenta, MR: Magenta red, RY: Reddish yellow, LR: Light red, T: Transparent, LY: Light yellow, LBr: Light brown, C: Cream.

Soxhlet Extraction

500 gram of the powdered material of leaves and fruit rind was refluxed separately with 1/10 (w/v) hexane, chloroform and ethanol in a soxhlet apparatus for 48 h in batches of 250 g each. The percentage yield of hexane, chloroform and ethanol and water extract from leaf and fruit rind were calculated. Where leaf ethanolic extract had maximum percentage of yield of 10.47%.

However, in fruit rind, water extract showed maximum percentage of yield of 25.36% followed by ethanolic extract with 16.669%. Chloroform showed least yield in both samples with 1.439% in leaves and 1.668% in fruit rind. The Hexane extract had moderate yield of 3.813% and 2.319% in leaf and fruit rind respectively. The results are tabulated in table 3 and 4.

Table 3: Percent yield of *Thevetia peruviana* (S) leaf extracts (w/v)

Solvents	Material (g)	Nature of the extract	Yield %
Hexane	500	Greenish sticky natured	3.813
Chloroform	500	Dark green powder	1.439
Ethanol	500	Greenish brown sticky mass	10.47

Pilot Solubility Test

Solubility tests of all extracts were performed using different solvents. Where all the extracts showed maximum solubility in DMSO followed by methanol, ethanol, chloroform, ethyl acetate, hexane, 1N NaoH, 1 N HCl and water respectively. Among all the tested solvents, least solubility was observed in distilled water. The results are tabulated in table 5.

Selection of vehicle solvents:

Among the solvents tested for pilot solubility analysis, those solvents that have fewer effects in *in-vivo* system wereconsider as vehicle solvents for further drug formulations as represented in Table 6. LH, LC, FH and FC were dissolved in DMSO whereas LE, FUL was dissolved in ethanol. Furthermore, FBR, FMC and FW extracts were dissolved in water only.

Table 4: Percent yield of *Thevetia peruviana* (S) fruit rind extracts (w/v)

Solvents	Material (g)	Nature of the extract	Yield %
Hexane	500	Light yellowish green solid	2.319
Chloroform	500	Dark brown powder	1.668
Ethanol Upper Liquid		Dark Black particulate solid	7.885
Ethanol Bottom Residue	500	Dark brown sticky mass	5.150
Ethanol Middle Crystals		Reddish crystals later turned into sticky mass	3.634
Water Extract	500	Dark brownish black mass	25.36

Table 5: Pilot solubility tests of extracts of *T. peruviana* (S).

T. peruviana (S) Extracts	Solvents								
	H	C	A	D	EOL,	M	D. W	NaOH	HCl
LH	++	++	++	+	-	++	-	-	-
LC	++	++	++	++	+ Δ	+	-	-	-
LE	-	-	-	+ +	++	++	-	+	+
FH	+	++	+	+ Δ	+	-	-	-	-
FC	-	++	+ Δ	++ Δ	+ Δ	-	-	-	-
FUL	+ Δ	+ Δ	-	++ Δ	++ Δ	++	-	+ Δ	+ Δ
FBR	-	-	-	++	+	+	++	++	++
FMC	-	-	-	++ Δ	-	++	++	++	++
FWE	-	-	-	+	-	-	++	++	++

H: hexane, C: chloroform, A: acetone, D: DMSO, EOL: ethanol, M: methanol, W: water, 1N NaOH, and 1N HCl, -:Insoluble,+: Partial soluble, ++: Complete soluble, +**Δ**: soluble on heating.

Table 6: Solvents preferred for solubility of extracts for pharmacological evaluation:

Leaf Extracts	Solvent	Fruit Rind Extracts		Solvent
Hexane	DMSO	Hexane		DMSO Δ
Chloroform	DMSO	Chloroform		DMSO Δ
Ethanol	Ethanol	Ethanol	Upper Liquid	Ethanol
			Bottom Residue	Water
			Middle Crystals	Water
		Water		Water

Table 7: Fluorescent analysis of *Thevetia peruviana* (S) extracts.

S. No.	Extract	Normal Light	UV Light Short Wavelength	UV Light Long Wavelength
01.	LH	Dark Green	Greenish Black	Magenta Red
02	LC	Dark Green	Black	Orange Deep Red
03.	LE	Dark Green	Black	Red and Green
04.	FH	White and Green	Cream	White
05.	FC	Brown	Light Green	Cream
06.	FUL	Black	Light Green	Cream
07.	FBR	Brownish Black	Black	Black
08.	FMC	Brownish Black	Black	Black
09.	FWE	Brownish Black	Black	Black

Table 8: Fluorescent analysis of *Thevetia peruviana* (S)extracts in vehicle solvents.

S. No.	Extract	Normal Light	UV Light Short Wavelength	UV Light Long Wavelength
01.	LH	Brownish Green	Dark Green	Dark Red
02	LC	Blackish Green	Black	Dark Red
03.	LE	Yellowish Brown	Dark Green	Dark Red
04.	FH	White	White	Cream
05.	FC	Light Yellow	Light Green	White
06.	FUL	Blackish Brown	Black	Light Green
07.	FBR	Blackish Brown	Black	Black
08.	FMC	Brown	Dark Green	Light Green
09.	FWE	Black	Black	Black

Fluorescent studies of successive extracts and extracts with vehicle solvents:

Fluorescent features of successive extracts and extracts dissolved in vehicle solvents were analysed as described above, and the results are tabulated in table (7 and 8).

Preliminary Phytochemical Screening:

Generally, the curative properties of medicinal plants can be attributed to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, sterols, coumarins etc. Therefore, the preliminary screening is essential for the detection of

chemical groups which may further lead to characterization of compounds. The results revealed the presence of carbohydrates, saponins, oils and fats, alkaloids, flavonoids, cardiac glycosides, coumarins and anthraquinone glycoside in plant leaf as well as fruit extracts (table. 9). Among the crude extracts, the LH contains flavonoid, phenolics, cardiac glycosides, oils and fats, whereas, the LC extract is very rich in steroids, carbohydrates, flavonoids and tannins. Similarly, LE extract contains alkaloids, carbohydrates, proteins, flavonoids, tannins, phenolics, cardiac glycosides and coumarins. Furthermore, in the fruit rind extracts the FH contains carbohydrate, phenolics, oils and fats. Whereas, the FC is rich in flavonoid and phenolics. The ethanolic extract fractions in which the FUL is having carbohydrate, protein, alkaloids, flavonoids, cardiac glycosides and phenolics. Similarly, FBR, FMC, and FWE have shown fever components like cardiac glycosides, phenolics, proteins and carbohydrates.

Quantitative Estimationof Phytochemicals:

The quantitative estimation of phytoconstituents was carried out by employing various standard methods. During analysis it has been found out thatalkaloids in leaf and fruit rind are present at 52.92 ± 0.06 mg/g and 59.28 ± 0.04 mg/g concentrations respectively. Similarly, total content of flavonoid was high in leaf ethanol extract (188.01 ± 0.02 mg/ g) followed by leaf hexane (78.975 ± 0.15 mg/ g), leaf chloroform (71.125 ±0.16 mg/ g), fruit chloroform (62.95 ± 0.09 mg/ g) and FUL (55.96 ±0.06 mg/ g). The estimation of total phenolic content has revealed the quantities in descending order in the LC (105.325 ± 0.41 mg/ g) LE (98.15 ± 0.2 mg/ g) and FH extracts (96.825 ± 0.4 mg/ g) and other extracts have shown less quantity of phenolics. Similarly, the quantification of saponins revealed that the fruit rind bears more saponins 14.27 ± 0.04 mg/g than in leaf 7.97 ± 0.05 mg/1g. In the same way the carbohydrates content was more in all the ethanolic extracts of fruit rind with 831.33 ± 0.01, 995.5 ± 0.4, 919.79 ± 0.06 mg/g in FUL, FBR, FMC respectively. Followed by water 356.82 ± 0.05 mg/g and others as shown in figure 1 and 2

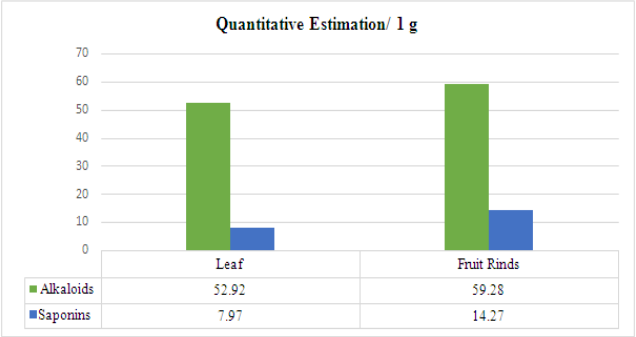

Fig. 1: Quantitative analysis of alkaloids and saponins in *T. peruviana* (S) leaf and fruit rind.

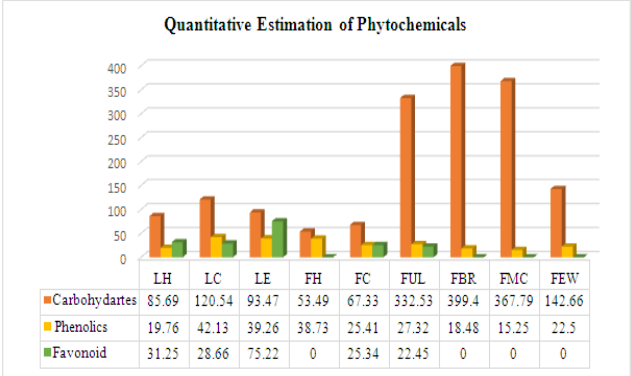

Fig. 2: Quantitative analysis of carbohydrates, phenolics and flavonoids in *T. peruviana* (S) leaf and fruit rind extracts.

DISCUSSION

The use of medicinal plant extracts for various diseases including cancer therapy is rapidly evolving, as they are affordable, with limited or no side effects. The active components present in such extracts have been shown to efficiently inhibit the diseases in a synergistic manner.

The plant *T. peruviana* (S) has been used traditionally for fever treatment as a purgative, emetic and as an anti-tumour medication. *T. peruviana* (S) has been claimed that all parts of these plants are toxic, and contain a variety of cardiac glycosides, which have a relatively high therapeutic index [6]. Phytochemical analysis of its seed oil has revealed its richness in bioactive components [21]. The Flowers of this plant were reported to possess good medicinal value, the phytochemical investigation showed the presence of alkaloids, glycosides, tannins phenolic compounds, proteins, essential oils, gums, mucilage and fixed oils [22].

Although the leaves and stem are being used for few topical applications in traditional medicine, but lack of scientific reports on leaf and fruit rind prompted us to undertake a systematic phytochemical analysis of the plant. Primarily, the organoleptic studies of plant material helped for the confirmation of presence of some organic compounds due to its aromatic smell of the extracts and the sweet taste indicated the presence of sugars in fruit rind material.

The fluorescence analysis of material under different UV wavelength was done. Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent, hence it may often assess qualitatively some crude drugs using fluorescence, as it is the most important parameter of pharmacognostical evaluation [23]. The study revealed that the leaf powder when dissolved in solvents like hexane, chloroform and methanol shown deep reddish magenta colour under UV 366 nm. This may helped to select the solvent, which is more appropriate for extraction.

The successive solvent extraction performed using different solvents, resulted high yield of ethanolic extract as water extract showed high yield in fruit rind extracts followed by hexane and chloroform. The study further channelled into exploring the extracts for the presence of phytochemicals, in which the first step was to select proper vehicle solvent. In this study, around nine different solvents as well as solutions were used among which water, DMSO and ethanol were preferred as vehicle solvents for further drug formulation.

The phytochemical studies have clearly demonstrated that the plant *Thevetia peruviana* (S)is a rich source of alkaloids, flavonoids, saponins, cardiac glycosides, anthraquinone glycosides, coumarins, phenols, tannins, steroids, oils and fats as follows. Among the extracts, the ethanolic extract has found to have more phytoconstituents. It is presumed that the presence of these constituents together could be attributed to the presence of pharmacological properties. Currently, investigations are underway to isolate, and characterize bioactive compounds to evaluate the beneficial properties. Moreover, it is noteworthy that the various phytochemicals, which are identified in this plant, are also found to be present abundantly in the related species like *T. thevetoids*and *Nerium oleander* and these plants have been reported for their potential pharmacological activities [7][8].

Through the quantitative analysis it has been confirmed that *T. peruviana* (S) is very rich in alkaloid 52.92 ± 0.06 mg/g and 59.28 ± 0.04 mg/g.saponins 7.97 ± 0.36 mg/g and 14.27 ± 1.02 mg/g respectively in leaf and fruit rind material. Followed by flavonoid and phenolics, which gives a very strong reason to select this plant for future evaluation of cytotoxic studies and other pharmacological properties.

Thus, the data obtained in this investigation gives a clear indication of the presence of probable bioactive compounds in the form of, alkaloids, flavonoids, saponins, cardiac glycosides etc. Further, this is the first systematic analysis on the chemical constituents of the fruit rind of *T. peruviana* (S) as there are no reports available thus far.

Certainly, the future investigations would throw much light on the beneficial properties, which could open new avenues to economically exploit the plant as a rich resource of bioactive components for pharmaceutical industry.

Table 9: Preliminary qualitative tests for phytochemicals in the leaf and fruit rind extracts of *Thevetia peruviana* (S)

Tests	Leaf extracts					Fruit rind extracts			
	LH	LC	LE	FH	FC	FE			FW
						U L	M C	B R	
Alkaloids									
a) Dragendorff's Test	- ve	-ve	+ ve	- ve	- ve	+ve	-ve	-ve	-ve
b) Hager's Test	- ve	-ve	+ ve	- ve	- ve	+ve	-ve	-ve	-ve
c)Wagner's Test	- ve	-ve	+ ve	- ve	- ve	+ve	-ve	-ve	-ve
d) Mayer's Test	- ve	-ve	+ ve	- ve	- ve	+ve	-ve	-ve	-ve
Carbohydrates									
a) Fehling's Test	+ve	+ve	+ve	+ ve	+ve	+ve	+ve	+ ve	+ ve
b) Barfoed'sTest	+ve	+ve	+ve	- ve	- ve	+ve	+ve	+ ve	+ ve
c) Bail's Test	- ve	- ve	- ve	- ve	+ ve	+ ve	-ve	- ve	- ve
d) Selwinoff's Test	- ve	- ve	- ve	+ve	+ve	+ve	+ve	+ ve	+ve
Flavonoids									
a) Lead acetateTest	- ve	+ve	+ve	- ve	+ve	+ve	- ve	- ve	- ve
a) Alkaline Test	+ve	+ve	+ve	- ve	+ve	+ve	- ve	- ve	- ve
Proteins									
a) Biuret Test	-ve	-ve	+ ve	-ve	- ve	+ ve	+ve	+ve	+ ve
b) Million's Test	-ve	-ve	+ ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve
Saponins									
a) Foam Test	- ve	- ve	+ ve	- ve	- ve	- ve	- ve	- ve	+ ve
Steroids									
a)Salkowaski reaction	- ve	+ ve	-ve	-ve	-ve	-ve	-ve	-ve	- ve
b)Liberman and Burchard's Test	- ve	+ ve	- ve	- ve	-ve	- ve	- ve	- ve	- ve
Tannins and Phenolic									
a) $FeCl_3$ Test	+ve	+ ve	+ ve	+ ve	+ ve	+ ve	- ve	+ ve	+ ve
b) Lead Acetate Test	+ve	+ ve	+ ve	+ ve	+ ve	+ ve	- ve	+ ve	+ ve
Cardiac Glycosides									
a)Legal's Test	+ve	- ve	+ ve	- ve	- ve	+ ve	+ ve	+ ve	+ ve
b) KillarKillani's Test	+ve	- ve	+ ve	- ve	- ve	+ ve	+ ve	+ ve	+ ve
A. Glycosides									
a) Borntrager's Test	- ve	- ve	- ve	- ve	- ve	+ ve	+ ve	+ ve	+ ve
b) Modified Borntrager's Test	- ve	- ve	- ve	- ve	- ve	+ ve	+ ve	+ ve	+ ve
Coumarins	- ve	- ve	+ ve	- ve	- ve	+ ve	- ve	- ve	- ve
Oils and Fat	+ve	- ve	- ve	+ve	- ve	- ve	- ve	- ve	- ve

LH: leaf hexane, HC: leaf chloroform, LE: leaf ethanol, FUL: Fruit rind upper liquid, FBR: Fruit rind bottom residue, FMC: Fruit rind Middle Crystals FW: Fruit rind water extracts +: Presence, -: Absence,

CONCLUSION

It is opined that *T. peruviana* (S), is rich source of phytochemicals such as alkaloids, flavonoids, saponins, cardiac glycosides, anthraquinone glycosides, coumarins, phenols, and tannins. Presumably, that the presence of these constituents together could be attributed to the presence of pharmacological properties. Quantitative study further confirms the fact, that the abundance of secondary metabolites may definitely show promising results in further pharmacological activities.

CONFLICTS OF INTEREST

Authors declare that there are no con flicts of interest.

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REFERENCES

1. Rishton GM.. Natural products as a robust source of new drugs, drug leads:past successes and present day issues. Am J Cardiol 2008;101(10A):43D-49D.
2. Salminen A, Lehtonen M, Suuronen T, Kaarniranta K, Huuskonen J. Terpenoids:natural inhibitors of NF-kB signalling with anti-inflammatory and anticancer potential. J Cell Mol Life Sci 2008;65(19):2979-99.
3. Sennblad B, Bremer B. Classification of Apocynaceaes. L. according to a new approach combining Linnaean and phylogenetic taxonomy. J Syst Biol 2002;51:389-409.
4. Alvarado-Cardenas L. La Familia Apocynaceae en el Valle de Tehuacan-Cuicatlan (Puebla-Oaxaca). B.Sc. Thesis, *Facultad de Ciencias* UNAM. J Mexico 2003.
5. Siddiqui BS, Begum S, Siddiqui S, Lichter W. Two cytotoxic pentacyclic triterpenoids from *Nerium oleander*. J Phytochem.1995;39 (1):171-4.
6. Singh CU, Streeper R. Water soluble formulations of Digitalis Glycosides for treating Cell-proliferation and other diseases. J U S Pat Appl Publ 2005;0026849:A1.
7. Laudadio C, Devis M. Cardiac Glycosides for treatig muscle pain and spam. U S Pat Appl Publ 2003;0229029:A1.
8. Oji O, Madubuike FN, Nwalozie MC. Mortality in rats following dietary inclusion of *Thevetia neriifolia* seeds. J Fitoterapia 1993;64:137-9.
9. PanigrahiA, Raut SK. *Thevetia peruviana* in the control of slug and snail pests. J Mem Inst Oswaldo Cruz 1994;89(2):247-50.
10. Oliver-Bever B. Medicinal Plants in Tropical West Africa:Cambridge University Press Cambridge;1986. p. 366.
11. Richard W. Relationships between plant Folklore and Antitumor Activity:a historical review. J SIDA Contributions to Botany.2005;21(4):2205-41.

12. Rasoanaivo P, Petitjean A, Conan JY. Toxic and poisonous plants of Madagascar Đan ethno pharmacological survey. J Fitoterapia 1993;64(2):114-29.
13. Jackson, BP., Snowdown DW. Powdered vegetable drugs, Cheer Chil Ltd:London;1968.p.25.
14. Pratt RJ, Chase CR. Fluorescence of Powdered vegetable drug with particular reference to development of a system of identification. J Am Pharm Assoc 1949;38:324-33.
15. Kokoshi CJ, Kokoshi RJ. Fluorescence of powdered vegetable drugs under Ultraviolet radiation. J Am Pharm Assoc 1958;47:715-7.
16. Harborne JB. Phytochemical methods. Chapman and Hall, Ltd;1973.p.49-188.
17. Obdoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some Homeostatic plants in Edo and Delta States of Nigeria. Glo J Pure App Sci 2001;8:203-8.
18. Chandler SF, Dodds JH. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of Solanum laciniatum. J Plant Cell Rep1993;2:105-10.
19. Woisky R., Salatino A. Analysis of Propolis:Some parameters and procedures for chemical quality control J Api Res 1998;37:99-105.
20. Seifter S, Dayton S, Novic B, Muntwyler E. Quantitative detection of glycogen fractions by anthrone method. J Arch Biochem Biophys 1950;25:191.
21. Hammuel C, Abdullahi MS, Mankilik M, Anyinm BP, Adesina OB., Inekwe UV. *et al.* The Phytochemical and Antimicrobial Activities of Oil from the Seed of *Thevetia peruviana* Plant. J Appl Envi Bio Sci 2011;1(2):597-601.
22. Kumar P, Khatri P, Gopi J, Shukla P, Patel R. Pharmacognostic and Preliminary Physiochemical Investigations of *Thevetia peruviana* (Pers.) K. Schum Flowers International. J Res Pharm Sci 2012;2(1):100-8.
23. Ansari SH, Essentials of Pharmacognosy.1st Edn.Birla Publications Pvt. Ltd. New Delhi;2006.