WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 03, 1443-1453. Research Article ISSN 2277–7105

PRELIMINARY PHYTOCHEMICAL AND MICROSCOPIC CHARACTERIZAION OF GOOD LUCK TREE (*THEVETIA PERUVIANA)*

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Article Received on
20 Dec. 2017,
Revised on 10 Jan. 2018,
Accepted on 31 Jan. 2018
DOI: 10.20959/wjpr20183-10943

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ABSTRACT

Thevetia peruviana is a medium evergreen tree found distributed throughout tropical parts of India. *Thevetia peruviana* plant leaves are used as cardiotonic and diuretic and also reported to possess good medicinal value in traditional system of medicine. Glycosides are present in large amount in this plant which has played a major role in human health. The study of macroscopic and microscopic examination of *Thevetia peruviana* has been proved that this plant has linear and thick surface. In TLC method there were 4 and 5 spots were observed which indicates that flavonoid is present in good quality this fact is also supported by quantitative analysis of flavonoid for *Thevetia peruviana*. The main aim of the present investigation is to study the macro, microscopic and some other pharmacognostic standards of *Thevetia peruviana* Plant.

KEYWORDS: *Thevetia peruviana*, Phytochemical, Anti-inflammatory and Anti-microbial.

INTRODUCTION

Thevetia peruviana belongs to the family Apocynaceae & it commonly known as Yellow oleander & Lucky nut. *Thevetia peruviana* called Manjarali in Tamil Nadu. It is a small evergreen tree (3-4 m high) cultivated as an ornamental plant in tropical & subtropical regions of the world including India, Australia and China. Fruit contains 2-4 flat gray seeds, which yields about half a litre of oil from 1 kg of dry kernel. It has also been regarded as a rich source of biologically active compounds such as insecticides, fungicides & bactericides,

that shows *Thevetia peruviana* plant extract have also been reported have Anti-microbial properties.[1]

Properties and uses of *Thevetia peruviana*

Pharmacological Activity and Antimicrobial Activity

The antimicrobial activity of ethanol extract obtained from *Thevetia peruviana* was tested against bacterial species of *Escherichia coli.* Better antimicrobial activity was observed with the extracts showed maximum activity against E.coli. Antifungal light-dependent activity was observed for some of the fractions and both crude extracts. The most photoactive fraction was analysed by capillary gas chromatography with mass spectrometry in order identify its constituents.

Pesticidal Activity

The leaf and bark of *Thevetia peruviana* plant was administered for 24 h to the freshwater fish Catlacatla (Hamilton) to evaluate their pesticidal activity in laboratory and cemented pond condition. So, the biochemical analysis is taken only acetone leaf and bark extract of *Thevetia peruviana* plant in laboratory condition.

Antispermatogenic Activity

This study was conducted to evaluate the antifertility potential of *Thevetia peruviana* in male albino rats with their phytochemical evaluations. Phytochemical examination showed that plant is rich in active constituents i.e. amyrin acetate, lupeol acetate, amyrin, lupeol and thevetigenin.

Anti-inflammatry Activity

Thevetia peruviana seed contain glucosides of neriifolin, acetylneriifolin and therein. Seed oil distillates of *Thevetia peruviana* have been found to contain anti-bacterial activity. In the present work the fresh flowers of *Thevetia peruviana* were subjected to phytochemical studies. The result of the study showed that the flower contain quercitine, kaempferol and quercitin-7-o-galactoside.The anti-inflammatory nature of the isolated compound was tested by in vitro method and the result of the study revealed that isolated compound showed a biphasic property.

Anti-diarrhoeal Activity

The study screened the antidiarrhoeal and cytotoxic effect of ethanol-extracted leaves of yellow oleander. The extract was tested against castor oil included diarrhoea in a model of albino rats and showed significant antidiarrhoeal activity. Disc diffusion method are used for test the in vitro antibacterial activities of the extract and exhibited poor antibacterial activities against both gram + and gram –bacteria. Ethanol extract leaves of yellow oleander showed narrow zone of inhibition in the bacteria lawns *Shigella flexneri*, *S. Typhi* and *S. Aureus*.[2]

Anti-termite Activity

Thevetia peruviana seed oil was used to make a surface coating with antifungal, antibacterial and anti-termite properties. The paint exhibited inhibitory activity against *E. coli, S. aureus, Bacillus substalis* and *Candida albicans* in a concentration dependent manner. The repellent action of paint against subterranean termites was significant. From these result, it was concluded that the *Thevetia Peruviana* based oil plant was substantially protected wood from subterranean termite attack.

Anti-cancer Activity

"Researcher conducted a screening program targeting TRAIL resistance-overcoming activity against human gastric adenocarcinoma (AGS) cells using thevefolin isolated from *Thevetia peruviana*, that induced DR5 expression at both the mRNA, protein level and real-time PCR study showed that thevefolin enhanced mRNA expression of DR4 and DR5 in AGS cells, suggesting that up-regulation of death-receptor expression may be related to TRAIL resistance-overcoming activity of thevefolin". The activity of extracts from *Thevetia peruviana* in inhibiting cell replication capacity is analyzed. The test of cytotoxicity showed inhibition of cell replication in the three tumor cells, more effectively in the type HL-60, showing a dose-dependent correlation with major action in the conc. of 200µg/mL. In HEP-G2 and in PC-12, the dose-dependence correlation was not observed but obtained significant inhibitions.[3]

MATERIALS AND METHODS

Apparatus

Test tube, Graduated cylinder, Funnal, Beaker, Pestle and Mortar, Water Bath, Methanol, Measuring cylinder, Soxhlet apparatus, Beaker, Whatman paper, Distilled water, funnel, epindrop tube, Flask, Measuring cylinder, Rotary shaker apparatus, Crucible and Water bath.

Plant material

The present study was carried out on *Thevetia peruviana*. This plant can be cultivated in waste lands. It requires minimum water when it 30 is in growing stage. It starts flowering after and half year. Phytochemical activity was identified in *Thevetia peruviana* Leaf.

Method

Collection of plant

After selection of plant it is must to collect the plant parts for the research purpose. Throughout India the plant *Thevetia peruviana* is available. After the collection of sample it needs to be dried to make the sample extract. In general the plant material should be dried at temperature below 30^0C to avoid the decomposition of thermolabile compounds. Shade dried the sample and powder was prepared with the help of the blender.

Standardization

Macroscopic Examination

(1) Size: A graduated ruler in millimetres was used for measurement of the length, width of crude materials.

(2) Colour: Untreated sample was examined under diffused daylight.

(3) Surface characteristics, texture: The material was touched to determine if it is soft or hard ; bended and ruptured to obtain information on brittleness and the plant material were fractured to observe whether material is fibrous, smooth, rough and granular.

(4) Odour: The material was powdered and the strength of the odour was determined whether (weak, distinct, strong) and then sensation of odour whether (aromatic, fruity, musty, mouldy, rancid etc) was observed.

(5) Taste: The small amount of both plant materials was tasted and observation was taken.

Microscopic Examination

Microscopy of the leaf

Stomata, trichomes and epidermal cells are important identifying characteristics of the leaf. In transverse section, their exact nature can't be studied. Hence, exposure of surface/epidermis becomes important for the detail microscopical study.

Procedure

The piece of leaf was cleared off by boiling with chloral hydrate and the upper layer of the epidermis was peeled out. The section of epidermis was kept on slide and mounted in

glycerine water. Various features of leaf were examined. In case of stomata, stomatal number and stomatal index was taken out by arranging the camera Lucida and drawing board for making the drawing to scale. 1mm of square was drawn by means of stage micrometer and then cleared leaf was placed on the slide. The epidermal cells and stomata were traced and then the number of stomata present in the area of 1sq.mm was counted and stomatal index was calculated by the formula.

I=S/E+S*100

Where, **I=** Stomatal index

S= No. Of stomata per unit area

E= No. Of epidermal cells in the same unit area

Microscopy of the stem

Microscopy of fresh stem was studied. For microscopy transverse section of stem were taken and stained with saffranin. Photomicrographs were obtained of the sections. Histochemical analysis was done by staining the hand cut sections with different reagents. The stem was treated with chloral hydrate solution followed by staining in 1% saffranin for 5 – 10 minutes and mounted in 50% glycerine.[4]

Preliminary screening of secondary metabolites

Extraction

The shade dried leaves material was powdered using mixer grinder and subjected to soxhlet Extraction with methanol for 18 hrs. The solvent was evaporated by using steam water bath and extract was weighted. The condensed extract was used for preliminary screening of phytochemical.

Alkaloids Test

Hager's test

Filterates were treated with Hager's reagent (saturated solution of picric acid solution). Formation of yellow precipitate indicates the presence of alkaloids.

Carbohydrates Test

Fehling's Test

Filterates were hydrolysed with dilute hydrochloric acid, neutrilized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugar.

Glycosides Test

Modified Borntrager's Test

Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of glycosides.

Saponins Test

Foam Test

Small amount of extract was shaken with little quantity of water. If foam produced persists for 10 min., indicates the presence of saponins

Phytosterols Test

Salkowski's Test

Extracts were treated with chloroform and filtered. The filterates were treated with few drops of conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpens.

Resins Test

Acetone-water Test

Extracts were treated with acetone. Then small amount of water was added and shaken. Appearance of turbidity would indicate the presence of resins.

Phenols Test

Ferric chloride Test

Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Flavonoids Test

Lead acetate Test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoid.

Tannins Test

Gelatin Test

To the extracts, gelatin solution (1%) containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Proteins Test

Xanthoproteic Test

The extracts were treated with few drops of conc. nitric acid solution. Formation of yellow colour indicates the presence of proteins.

Diterpenes Test

Copper acetate Test

Extracts were dissolved in water and treated with few drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.[5]

TLC (THIN LAYER CHROMATOGRAPHY)

Procedure: The sample was spotted on the plate and dried for few min. Then the solvent system was prepared and allowed to stabilize for 10 min. Then the plate was dipped in the solvent chamber and allowed to run up to three forth of the plate. Then it was removed and was air dried. The plate was examined visually.

RESULT AND DICUSSION

Results observed after performing various experiments were extreme good and indicated that *Thevetia peruviana* has extreme scope phytochemical components. Phytochemical characteristics verified with various test results given in fig-2. The preliminary phytochemical analysis indicated that Alkaloids**,** flavonoids, glycosides, saponins, resins and diterpenes were present in *Thevetia peruviana* plant extracts. But protein, tannin, phenol, phytosterol and carbohydrate were not present showing in table-3. Glycosides are present in large amount in this plant which has played a major role in human health. These secondary metabolites localized in leaf and collected in different parts of plant. Powder of plant has characteristic odour is citrus, bitter in taste.

Table 1: Nature and Percentage yield of extracts of *Thevetia peruviana.*

Sr. no.	Name of the extract	Nature	Colour	% Yield (w/w)
1.	Methanolic extract soxhlet	Shade	Green	2.25
2.	Methanolic extract with shaking	Shade	Green	2.33

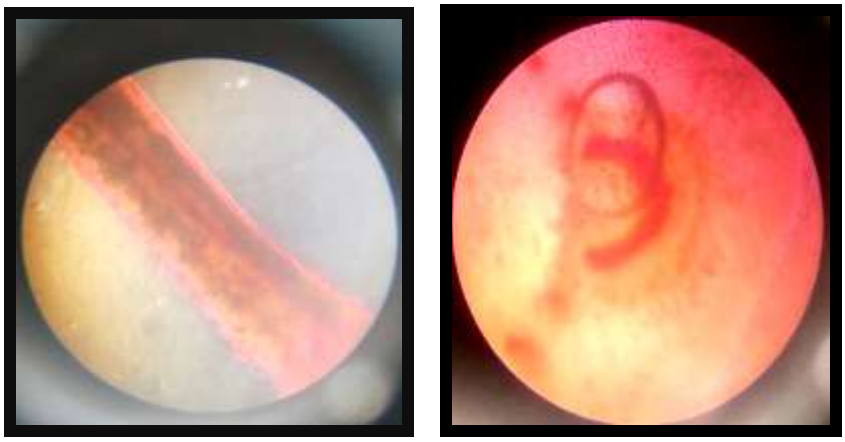
Standardization

Macroscopic Examination

Table 2: Macroscopic examination-Leaves.

Sr.no	Organoleptic characters	*Thevetia peruviana*
1.	Size	11-13cm
2.	Surface characteristics, texture	Spirally arranged ,linear and thick
3.	Taste	Bitter
4.	Colour	Green
5.	Odour	Citrus

Microscopic Examination



(a) **(b)**

Fig. 1: (a) Transverse Sectioning (TS) of the stem and (b) Transverse sectioning (TS) of leaves of *Thevetia peruviana.*

Table 3: Phytochemical constitute of *Thevetia peruviana*

S.No.	Phytochemical Name	Reach or reagents test	Observation	Test Result
1.	Alkaloids	Hagar's reagent	Yellow Colour Precipitates	++
2.	Carbohydrates	Fehling's test	Red colour Precipitates	+
3.	Glycosides	Modified Borntrager's test	Rose pink colour of ammonical layer	+++
4.	Saponins	Foam test	Foam produced	+
5.	Phytosterols	Salkowski's Test	Appearance of the golden yellow colour	-
6.	Resins	Acetone-water test	Appearance of turbidity	+

7.	Phenol	Ferric chloride test	Appearance of the bluish and black colour	-
8.	Flavonoids	Lead acetate test	Yellow Colour precipitates	+++
9.	Tannins	Gelatin test	White colour Precipitates	-
10.	Proteins	Xanthoproteic test	Apperance of the Yellow Colour	+
11.	Diterpenes	Copper acetate test	Appearance of emerald green colour	+

(Absent= -), (Present=+), (Medium concentration=++), (High concentration=+++)

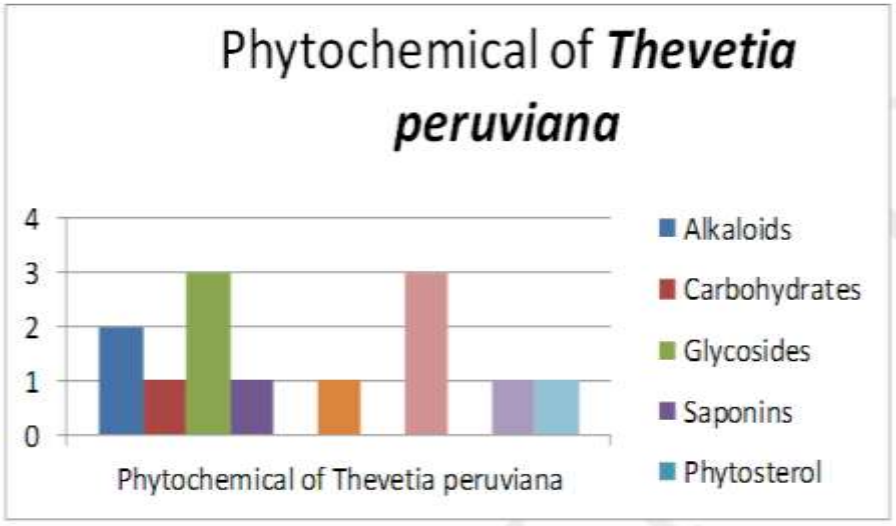


Fig. 2: Showing Phytochemicals of *Thevetia peruviana.*

THIN LAYER CHROMATOGRAPHY (TLC)

Table-4: TLC with solvent system I Chloroform: Methanol: nButanol: Water (10:10:1:6).

Sr.no.	Plant species	Extract	Distance travelled by solute (cm)	Distance travelled by solvent (cm)	Colour	Rf value
1.	*Thevetia peruviana*	Methanolic extract	1.7	6	Light green	0.28
			2.6	6	Mint green	0.43
			3.8	6	Fern green	0.63
			5	6	Lime green	0.83

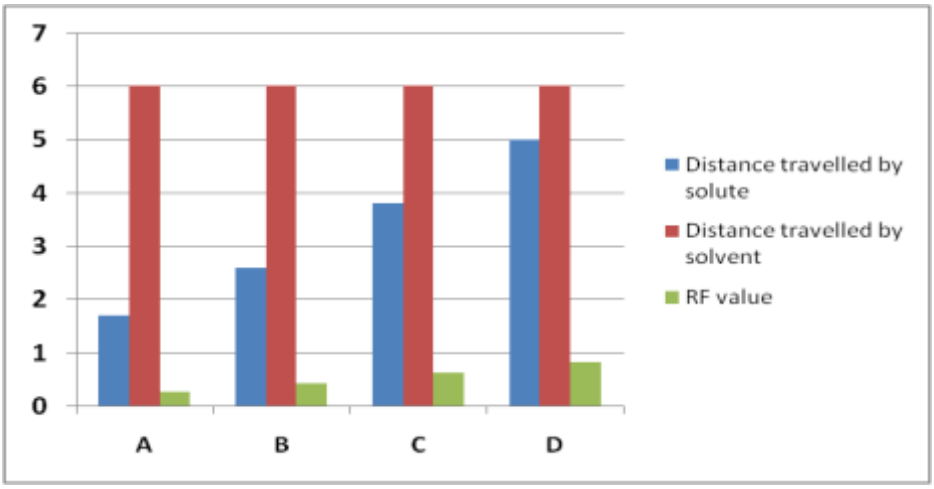


Fig. 3: TLC of flavanoids with solvent system I (Chloroform: Methanol: nButanol: Water (10:10:1:6).

Table-5: TLC with solvent system II nButanol: Ethanol: Water (4:1.5:5).

Sr.no.	Plant species	Extract	Distance travelled by solute (cm)	Distance travelled by solvent (cm)	Colour	Rf value
1.	*Thevetia peruviana*	Methanolic extract	1.3	6	Amber green	0.21
			2.1	6	Spice green	0.35
			3.4	6	Clay green	0.56
			3.9	6	Rust green	0.65
			4	6	Cider green	0.66

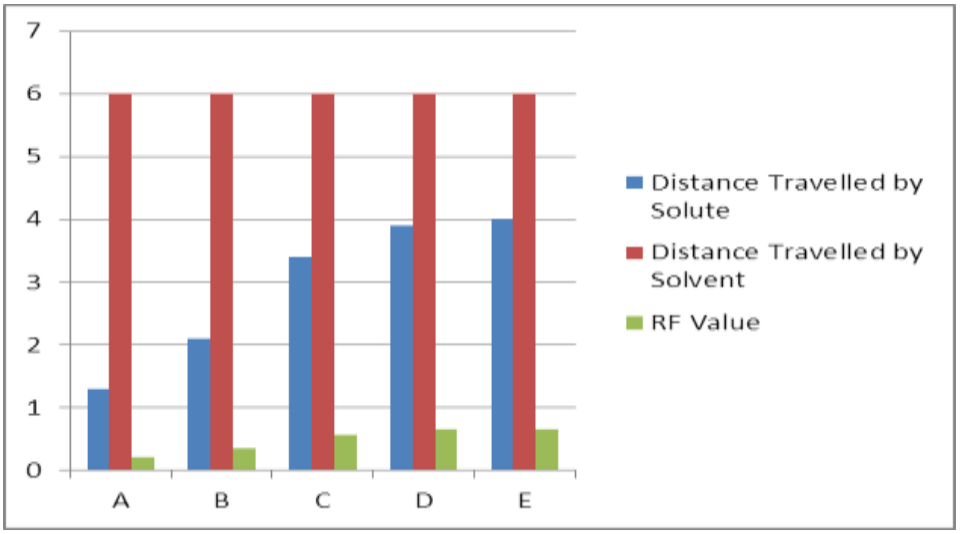


Fig. 4: TLC of flavonoids with solvent system II (nButanol: Ethanol: Water 4:1.5:5).

In TLC analysis, with solvent systemI, there was 4 spots were observed after the visualization process, which are having RF values 0.28, 0.43, 0.63 and 0.83 respectively. Then with solvent system II, there was 5 spots were observed after the visualization process, which having RF values 0.21, 0.35, 0.56, 0.65 and 0.66 respectively.

CONCLUSION

Thevetia peruviana plant or its individual part can be used for the treatment of various disorders in human being such as diabetes, liver toxicity, fungal infection, microbial infection, inflammation and relive pain. *Thevetia peruviana* is a plant which contains so many phytochemical properties, medicinal uses for various therapeutic purposes. Not only therapeutic effect but also other properties are included which may have application to normal life such as anti-fungal, anti-microbial and anti-termite effects also. The present study concluded that above applications and activities is due to the presence of various phytochemicals and metabolities in parts of *Thevetia peruviana.* It also has a vast application in the area of biodiesel product, which has specific area in manufacturing of many useful industrial products.

ACKNOWLEDGEMENT

We take this opportunity to acknowledge sincere thanks to our respected chairman, Dr S.C. Kulshrestha, Hon. Executive Director Dr B.K Tyagi, Director Dr. R.S Saxena, Shri Ram Group of Colleges Muzaffarnagar, U.P. India for providing necessary facility and tools to carry out the research dissertation work for post graduate students of MSc Biotechnology.

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