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Standardization & Antibacterial Activity of *Thevetia neriifolia Juss.*

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ABSTRACT

Thevetia neriifolia Juss. beloning to family Apocynaceae, an important medicinal plant was subjected to phytochemical screening, pharmacognosy and antibacterial investigations. Preliminary phytochemical screening of extracts revealed the presence of the bioactive compounds, such as alkaloids, anthroquinones, flavonoids, phenolic compounds, saponins, steroids and tannins. Pharmacognostical studies such as extractive values and fluorescence analysis brought out the standardized data in the quality control of this drug. Petroleum ether, Benzene, chloroform, ethyl acetate, ethanol, & water extracts of drug showed significant antibacterial activity against the tested human pathogens. Petroleum ether and ethanol extracts exhibited maximum activity against the tested human pathogens, while extracts ethyl acetate & water extract showed minimum activity against some pathogens. The bioactive compounds responsible for these antibacterial activities could be isolated and identified to develop a new drug of pharmaceutical interest. Powdered drug analysis after treatment with 15different reagents emitted various colour radiations under UV and visible light which may provide a lead in identification of the drug in powder form. The study revealed specific identities for *Thevetia neriifolia* Juss. This may play a key role in identification of plant and can be useful in standardization of the herbal drugs.

Keywords: *Thevetia neriifolia* Juss., Pharmacognosy, Phytochemical, Antibacterial activity, Apocynaceae

1. Introduction

Nearly 80% of the world population some plant accessions collected from all parts depends upon traditional systems of health care [1]. In recent years secondary metabolites and phytochemicals, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [2]. Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of many bacterial infections [3]. The major chemical substances of interest in these surveys were the alkaloids, steroids and saponins, however other diverse are naturally occurring phytocomponents such as flavonoids, tannins, unsaturated steroids, triterpinoids, and essential oils [4]. *Thevetia nerifolia* is used medicinally in Philippine Islands, Guiana, Brazil and Gold Coast. *Thevetia nerifolia i*s used to treat various inflammatory and cardiovascular diseases, beside the antiviral and antifungal properties. Generally, *Thevetia nerifolia* are applied in cardiac disorder, fever, ringworms, wasp sting [5]. Leaves are willow – like linear, lanceolate & glossy green in colour. Stem is green turning silver/grey. Flowers are 3 inches (7.6 cm) long, have 5 overlapping petals that open in a spiraled pin wheeled. It is long funnel shaped sometimes fragrant yellow in colour. Flowers bloom from summer to fall. Flowers contain the triterpene alcohols, lupenol, α-β-amyrin, and taraxerol. The broken foliage oozes toxic white latex sap. Fruit is deep red –black in colour. Roots of the plant bearing white coloured. The plants are spread by seeds. Generally, it contains thevetin B & digitoxigenin-β-gentionbiosyl (1→ 4)- α-L- acoprioside: 19- carboxy digitoxigenin-β- gentiobiosyl-(1→4)-α-L thevetoside, thevetin A , cannogenin – β-gentiobiosyl-(1→4)-α-L- acofrioside,& cannogenin –α- L- rhamnoside, uzarigenin-β-gentiobiosyl-(1→4)-α-L-thevetoside & thevetogenin-β-gentiobiosyl-(1→4)-α-L-thevetoside & thevetogenin-β-gentiobiosyl-(1→4)-2-O-acetryl-α-L-thevetoside, thevetogenin-β-gentiobiosy

-(1→4) –α-L acofrioside, thevetogenin-β- glucoside (1→4)-α-thevetoside. It also contains peruvoside so far reported from seeds only and lupeol acetate [6].

2. Material and methods

2.1 Plant material and Preparation of the Extracts

Fresh plant material are collected from Botanical garden of Rakshpal Bahadur college of Pharmacy, Bareilly, U.P., were preserved in 70 % ethyl alcohol for histological studies. Voucher herbarium sample along with the voucher crude drug sample (voucher number- 0456/S.R.) is preserved at the herbarium of Department of Botany, MJP Rohilkhand University, Bareilly Free hand sections were used for histological studies. Quantitative microscopy was done as per the standard methods described by TE Wallis [7].

The collected plant material were chopped into small pieces, shade dried and coarsely powdered with suitable pulverizer. The coarse powder was subjected to successive extraction with warm organic solvents like petroleum ether, Benzene, chloroform, ethyl acetate, ethanol, & water by soxhlet method [8].

2.2 Preliminary Phytochemical Screening [9]

2.2.1 Test for alkaloids: To the test solution in 10 ml menthol, add 1 % (w/v) HCl and any of Mayors reagents, Wagner's reagent or Dragendroff reagent (6 drops). A creamish or brownish red or orange precipitate indicated the presence of alkaloids.

2.2.2 Test for Anthraquinones: To the test solution add a benzene drop and ammonia drop, a pink colour indicates the presence of anthraquinons.

2.2.3 Test for Flavonoids:

1) 5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentratedH_2SO_4. A yellow colour in each extract indicated the presence of flavonoids. The yellow colour disappeared on standing. Few drops of 1% ammonia solution were added to portion of each filtrate. A yellow colour indicates the presence of flavonoids.
2) A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colour indicates opposite test for flavonoids.
3) To the test solution in 10 ml of ethanol add conc. HCl-Mg ribbon developing a pink –tomato red colour indicates the presence of flavonoids.

2.2.4 Test for Coumarins: To the test solution add a drop of sodium sulphate developing yellow colour. Indicates the presence of coumarines.

2.2.5 Test for Phenols: To the test solution add a drop of ferric chloride. Developing of intense colour develops.

2.2.6 Test for Saponins: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrates was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth, which indicates the presence of saponins.

2.2.7 Test for steroids: 2 ml of acetic anhydride was added to the solution along with 2 ml of conc. H_2SO_4. The colour changed from violet to blue or green in some samples. This indicates the steroids.

2.2.8 Test for terpenoids: 5mlof each extract mixed with 2ml of choloform, and 3 ml concentrated H_2SO_4 was carefully added to form a layer reddish brown colour of the interface was formed to show positive results for the presence of terpenoids.

2.2.9 Test for Tannins: About 0.5 g of the leaves was dried and powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colouration.

2.2.10 Test for amino acids and proteins: Take 2-3 ml of sample solution in a test tube .Add 3-4 drops of ninhydrine solution and heat. Appearance of purple or violet indicates the presence of proteins.

2.2.11 Test for carbohydrates: Add 1ml of Benedicts reagents to test tube and heat the mixture to boiling in a water bath for 2mins .the formation of an orange red colour precipitate due to formation of a copper (I) oxide indicates the presence of reducing sugars.

2.3 Physicochemical parameters

The percent of loss on drying, total ash, acid insoluble ash, water soluble ash, pH of 1 % w/v solution of aqueous extract and swelling index has been shown in Table 1.

Table 1: Physicochemical parameters of *Thevetia neriifolia* Juss.

S. No.	Parameters	Average Values
1	Total ash (%)	5.15
2	Acid-insoluble ash (%)	0.65
3	Water soluble ash (%)	5.92
4	pH (1% w/v aqueous extract)	7.83
5	Swelling index	4.32
6	Loss on Drying (%)	4.63
7	Petroleum ether extractive value (%)	1.25
8	Benzene (%)	2.65
9	Chloroform (%)	5.6
10	Ethyl acetate (%)	3.28
11	Ethanol (%)	8.9
12	Distilled water (%)	4.80

A known quantity of dried leaf powder was extracted in a soxhlet apparatus with petroleum ether (60-80ºC), benzene, chloroform, ethyl acetate and ethanol (95 %) and finally macerated with distilled water for 24 hours successively and the % of respective extractive values have been shown in Table 1. The successive extracts were tested for different constituents.

Table 2: Preliminary phytochemical screening of various extracts of *Thevetia neriifolia* Juss.

S.No	Test	Pet.Ether	Benzene	Chloroform	Ethyl acetate	Ethanol	Water
1	Alkaloids	- ve	+ ve	- ve	+ ve	+ ve	- ve
2	Aminoacids	- ve	+ ve	- ve	+ ve	- ve	+ ve
3	Coumarin	- ve	- ve	- ve	- ve	- ve	- ve
4	Flavonoids	+ ve	- ve	+ ve	+ ve	+ ve	+ ve
5	Steroid	+ ve	- ve	- ve	- ve	+ ve	+ ve
6	Phenol	- ve	- ve	- ve	- ve	+ ve	+ ve
7	Tannins	- ve	- ve	- ve	+ ve	- ve	- ve
8	Glycosides	+ ve	+ ve	+ ve	- ve	+ ve	- ve
9	Terpenoids	+ ve	- ve	- ve	- ve	- ve	- ve
10	Proteins	- ve	+ ve	+ ve	+ ve	- ve	+ ve
11	Saponin	- ve	+ ve	+ ve	+ ve	+ ve	+ ve

Where: +ve – Present and –ve- Absent

Table 3: Fluorescence analysis of powder of *Thevetia neriifolia* Juss.

Treatment	Visible Light	UV(254 nm)	UV (366 nm)
Drug Powder	Light green	Dark green	Blackish Brown
Ammonia	Reddish brown	Dark reddish brown	Black
Silver nitrate	Greenish brown	Green	Black
Benzene	Brown Dark greenish	Green	Black
Chloroform	Dark green	Black	Black
Butane	Green	Dark Green	Black
H_2SO_4	Black	Black	Black
Conc.HCL	Green	Greenish yellow	Black
Toluene	Green	Dark Green	Black
Ethyl Acetate	Greenish brown	Buff	Black
10%NaOH solution	Dark green	Buff green	Dark black
Perchloric Acid	Black	Black	Dark Black

Table 4: Antibacterial activity of *Thevetia nerifolia* extracts against some organisms

Tested micro-organism	Zone of inhibition (mm)																							
Extract (mg/ml)	Petroleum ether				Benzene				Chloroform				Ethyl acetate				Ethanol				Water			
	100	50	25	Std	100	50	25	Std	100	50	25	Std	100	50	25	Std	100	50	25	Std	100	50	25	Std
AR12	14	11	9	26	17	14	13	23	-	-	-	36	-	-	-	28	26	21	19	36	9	7	3	36
AR22	17	11	13	24	16	14	9	26	-	-	-	34	9	6	5	26	21	18	17	32	11	9	5	28
AR32	16	13	11	24	16	14	11	24	-	-	-	32	-	-	-	29	24	21	29	34	11	9	6	33
AR42	14	12	8	22	14	11	7	25	-	-	-	32	13	11	9	24	22	19	17	35	9	7	3	31

Where: AR12- E.coli, **AR22-**Salmonella typhi**, AR32-**Staphylococcus pneumonia**, AR42-** Staphylococcus aureus

The petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water extracts revealed presence of alkaloids, glycosides, flavonoids, proteins and amino acids Table 2.

2.4 Fluorescence Analysis: The analysis of extract under daylight is unreliable due to lack of fluorescence. So it was evaluated under day light and UV light, it is shown in Table 3.

2.5 Antibacterial Activities: Petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water extracts whole drug were used to prepare different concentrations (100mg/ml, 50mg/ml & 25mg/ml).These concentrations were used for antibacterial studies against specific microorganism.

2.5.1 Test Microorganisms:
The following cultures of human pathogens used were procured from Microbiological Section of IVRI, Izatnagar, Bareilly, the gram negative bacteria [E.coli (AR12), Salmonella typhi(AR22)], also the gram positive bacteria [Staphylococcus pneumonia (AR32), Staphyococcus aureus (AR42)].

2.5.2 Determination of Antibacterial activity
Agar diffusion method [9] was applied to determine the antibacterial activity. Nutrient Agar plates were swabbed with 8 hrs old-broth culture of respective bacteria. Four well 8 mm diameter) were made in each of these plates using sterile cork borer. About0.3 ml of different concentrations of plant solvent extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 hrs. The plates were incubated at 37^{o}C for bacteria .Diameter of the inhibition zone was recorded. Triplicates were maintained and the experiment was repeated thrice and the average values were recorded for antibacterial.

3. Results
In this investigation, the active phytocomponents of *Thevetia neriifolia* was studied and further the antibacterial activity of the plant extracts was assayed *in vitro* by agar well diffusion method
Against 2 gram positive & 2 gram negative bacterial species Table 4, summarizes the bacterial growth inhibition of petroleum ether (60-80ºC), benzene, chloroform, ethyl acetate, ethanol (95 %) and water extracts of plant. Successive solvent extraction values in various organic solvents were observed as petroleum ether (60-80ºC) 1.25%, benzene 2.56%, chloroform 5.6% , ethyl acetate 3.28% , ethanol (95 %) 8.9% and water 4.80% as shown in Table 1. Petroleum ether shows the presence of steroids, flavonoids, terpenoids and glycosides, benzene shows alkaloids, aminoacids, glycosides, proteins and saponin, Chloroform shows flavonoids, and glycosides, proteins and saponin, ethyl acetate shows alkaloids, aminoacids, glycosides, proteins and saponin, ethanol shows alkaloids, steroids, flavonoids , phenol, glycosides and saponin shows aminoacid, flavonoids, steroid, phenol, proteins and saponin. All the phytochemical constituent showing in Table2, presence showing by (+) & absent showing by (-). The chloroform extract show did not activity against E.coli, Salmonella typhi, Staphylococcus pneumonia and Staphylococcus aureus bacteria.

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