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Analysis of phytochemical components and anti-microbial activity of the toxic plant -*Thevetia Peruviana*

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Abstract

Medicinal plants are widely distributed in the country. Among these, the toxic plant *Thevetia peruviana* also comes under the medicinal plants category and it has many medicinal uses. The leaves of *Thevetia peruviana* were extracted with ethanol and then it was purified. Proteins and phenols were confirmed by performing TLC (Thin layer chromatography). Glycosides were confirmed by Borntrager's test. The purified sample was used to analyze the phytochemical compounds by GC/MS (Gas chromatography/Mass spectroscopy). The amount of glycosides present in the leaf extract was quantified using Spectrophotometer. Antimicrobial was tested against Escherichia coli, *Salmonela typhi, Staphylococcus, Vibrio cholera* and *Shigella*. The result showed the presence of proteins, phenols and glycosides in the leaf extract. Eleven phytochemical components were obtained GC/MS.

Keywords: Phytochemistry; Medicinal plants; *Thevetia peruviana*; Toxic-plant; Chromatography.

Introduction

Thevetia peruviana (Usman, 2009) is a plant native to Mexico and Central America and a close relative to *Nerium oleander*. It contains a milky sap containing a compound called thevetin that is used as a heart stimulant but in its natural form is extremely poisonous, as are all parts of the plants, especially the seeds. Its leaves are long, lance shaped and green in colour. Leaves are covered in waxy coating to reduce water loss (typical of oleanders). Its stem is green turning silver /gray as it ages.

Yellow Oleander is a completely different species from the plant just called Oleander. The two bushes belong to the same family, the Dogbane Family or *Apocynaceae,* but they reside in entirely different genera.

Yellow Oleander flower is funnel-shaped - a narrow tube below that flares out above. A top the narrow basal tube is an area known as the "throat," and in the Yellow Oleander flower, the throat is where the flower manages its pollination strategy (Thilagavathi *et al*., 2010).

All parts of the plant, particularly the seeds are poisonous owing to the presence of cardiac glycosides or cardiac toxins, which act directly on the heart. Ingestion of these plant parts could lead to death. The whole plant exudes in a milky juice, which is very poisonous. It is a small ornamental tree, which grows to about 10 to 15 feet high. The leaves are spirally arranged, linear and about 13 to 15cm in length. Flowers are bright yellow and funnel - shaped with 5 petals spirally twisted. The fruits are somewhat globular, slightly fleshy and have a diameter of 4 to 5cm. The fruits, which are green in colour, become black on ripening. Each fruit contains a nut, which is longitudinally and transversely divided (Shaw and Pearn, 1979). All parts of the plant contain the milky juice. Grown is as an ornamental tree in gardens. This plant is native of Central & South America, but now frequently grown throughout the tropical and sub - tropical regions. All parts of the plant are poisonous, especially the kernels of the fruit. The absorption of the equivalent of two *Thevetia peruviana* leaves may be sufficient to kill a 12.5kg child (Eddleston *et al*., 1998).

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Due to the diverse medicinal properties attributed to the plant, the present investigation was undertaken to analyze:

(i) To analyze the phytochemical compounds
(ii) To extract cardenolides glycosides from leaf extract
(iii) To check Antimicrobial activity

Materials & Methods

Sample collection

The Plant Material *Thevetia peruviana* (yellow oleander) was collected from Pallavaram.

Extraction method

7g of fresh *Thevetia peruviana* leaves were collected, weighed and allowed to dry. Then it was powdered using motor and pestle. The powdered leaves were homogenized with 20ml of ethanol. Along with 20ml of ethanol, 50ml of ethanol was added and 50ml of Distilled water was added and poured into the separating funnel. The separating funnel was kept overnight to separate the extract into aqueous phase and solid phase. Then the aqueous phase was collected, filtered and centrifuged. The centrifuged sample was allowed to evaporate for further use.

Thin layer chromatography

The TLC plate was kept in Hot Air Oven for 1 hour at 90-100˚c to activate the plate. Then 18g of Silica gel was weighed and dissolved using distilled water. The silica gel past was then poured evenly on the TLC plate to form a thin layer. The TLC plate was kept in hot air oven for 1-hour at 90-100˚c to dry. The evaporated sample was loaded in the gel and allowed to run in the solvent-ethanol.

The sample, which was runned in the TLC was removed. Ethanol was added to the powdered form of sample that contains silica gel. Then it was centrifuged at 5,000rpm for 10 minutes. The supernatant was collected to obtain the purified sample.

Protein Analysis

The extracts were analyzed for the presence of protein by using TLC. The evaporated sample was loaded in the thin layer formation of silica gel in the TLC plate. Then, it was placed in the beaker which containing ethanol and allowed to run. After running, the ninhydrin was sprayed over the sample and again the plate was kept in hot air oven for 20 minutes to observe the colour change. Pink or purple colour formation indicates the presence of protein.

Iodine test for phenol

Thin layer was formed with the help of silica gel on TLC plate. The sample was loaded in the gel and allowed to run in acetic acid and chloroform (1:9). After 10 minutes, it was removed and place in a glass beaker containing iodine crystals. The colour change indicates the presence of phenols and alkaloids.

Borntrager's test

To the extract, the chloroform was added and mixed well. To the separated layer of chloroform, the ammonia solution was added. The colour change indicates the presence of glycosides.

GC-MS [Gas Chromatography-Mass Spectrometry]

GC/MS is a technique that can be used to separate volatile organic compounds (VOCs). We can combined GC or MS with other separation and analytical techniques. The Gas Chromatography/ Mass Spectrometry (GC/MS) instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). It is one of the most accurate tools for analyzing environmental samples. The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. A "library" of

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known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector.

The purified sample was used for GC/MS to analyze the compounds present in the sample.

Conditions

Column Oven Temperature	: 70˚c
Injector Temperature	: 200˚c
Injection Mode	: split
Split Ratio	: 40
Flow Control Mode	: Linear Velocity
Column Flow	: 1.51ml/min
Carrier Gas	: Helium 99.9995%
purity	

Column Oven Temperature Program

Rate (minute)	Temperature	Hold-Time
-	70	2
10	300	10 (35.0 min)

Estimation of glycosides

1g of sample was extracted with 3ml of 70% ethanol and filtered. To these extracts lead acetate and Di-sodium hydrogen phosphate was added to purify the sample. Baljet's Reagents (9.5ml picric acid and 0.5ml 10% aqueous NaoH) was added to the sample (El Olemy *et al.,* 1997) and the OD Value was taken at 495 nm using Spectrophotometer to know the amount of Glycosides present in the sample.

Anti-microbial assay

Preparation of microbes

The test microbes were collected and maintained in freshly prepared Nutrient agar Slants. Then it was incubated at 37˚C for overnight. A loopful of bacterial strain was added to a 50ml of L. B Broth in a conical flask and it was incubated at 37˚C for overnight.

Agar- well diffusion method

Fig.1. Ninhydrin Test

Agar well diffusion method was used to detect the antimicrobial activity of leaf extract against various organisms (*E.coli, S.typhi, Staphylococcus, V.cholerae* and *Shigella*). The culture of bacteria was spread on to the agar plates using L- rod. The wells were cut using gel puncture and to it, 100µl of extract was added. The plates were then incubated at 37˚C for 24 hours. After incubation the zone of bacterial inhibition was measured (*Sharma*, 1992).

Results and discussion

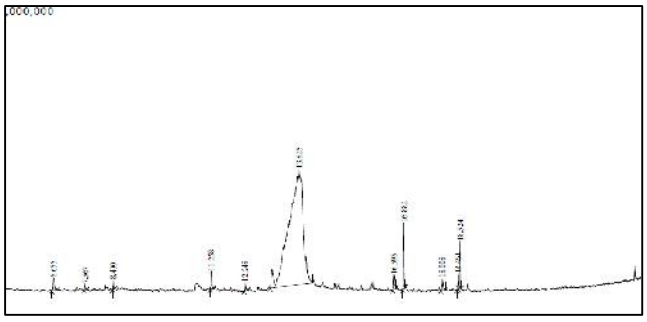

Fig.2a. GC/MS Analysis

Peak Report TIC

PEAK#	R.TIME	AREA	AREA%	NAME
1	6.655	213432	0.85	2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRAN-4-ONE
2	7.567	69299	0.28	3 Methyl 1 heptanol
3	8.400	87407	0.35	3,8-Dimethylundecane
4	11.258	213934	0.85	TETRADECANE
5	12.248	107598	0.43	Lauric acid
6	13.825	22656059	90.22	MOME INOSITOL
7	16.593	184917	0.74	PALMITIC ACID
8	16.882	648982	2.58	ETHYL PALMITATE
9	18.008	230786	0.92	Phytol
10	18.461	153823	0.61	ETHYL (9Z,12Z) 9,12 OCTADECADIENOATE
11	18.524	547841	2.18	Ethyl linolenate
		25113078	100.00	

Fig.2b. GC/MS Analysis

The extract obtained from the leaves of *Thevetia peruviana* were analyzed for the presence of phytochemical components. The results show

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that the presence of phenols and proteins in the leaf extract. Purple or pink colour change indicates the presence of protein (Fig.1). The dark yellow colour was observed in the TLC plate and it shows the presence of phenols. Glycosides were confirmed by performing Borntrager's test.

The purified compound was analyzed for the presence of phytochemical components using GC/MS. The results show that eleven different compounds were present (Fig.2a & 2b). They are:

(i) 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-one.
(ii) 3-methyl 1- hepatanol
(iii) 3,6-Dimethylundecane
(iv) Tetradecane
(v) Lauric acid
(vi) Mome inositol
(vii) Palmitic acid
(viii) Ethyl palmitate
(ix) Phytol
(x) Ethyl(9z,12z)-9,12- octadecadienoate
(xi) Ethyl linolenate.

Among these, three compounds results in high peaks (mome inositol, Ethyl palmitate, Ethyl linolenate). Apart from these 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-one compound has an anti-cancerous, anti-microbial, anti-inflammatory property. It is a Millard reaction product of glucose and glycine. Momo inositol - use of a compound that specifically inhibits the lipolytic activity of HSL, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of a disorder where a decreased level of plasma FFA is desired. The amount of glycosides compounds present in the sample was quantified using spectrophotometer. The results show 2.89% of glycosides present in 3ml of extract.

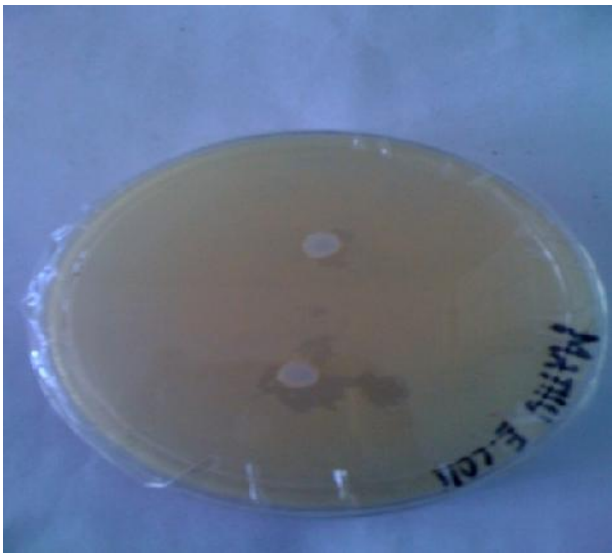

Fig..3a. Antibacterial activity (*E.coli*)

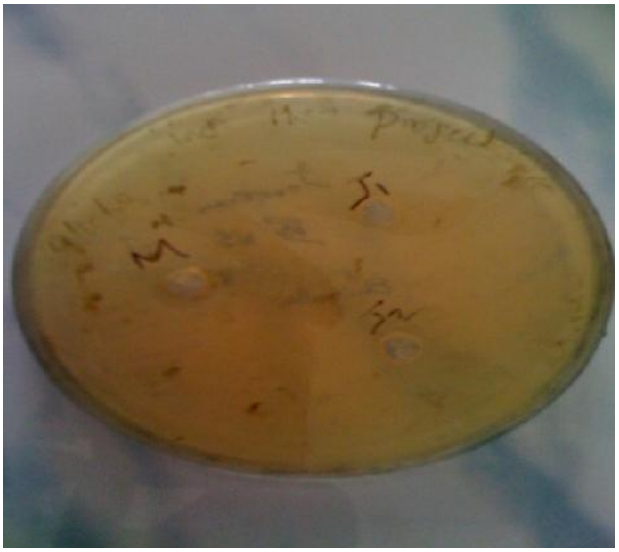

Fig. 3b. Antibacterial activity (*Shigella*)

The 100µl of leaf extract was tested against *E.coli, Shigella, Staphylococcus, Salmonella typhi,* and *Vibrio cholera.* The result showed little activity against *E.coli and Shigella* (Fig 3a & 3b). Good results can be obtained by increasing the concentration of the leaf extract. The seed extract of *Thevetia peruviana* was done previously by Omolara (2007), and the amount of cardiac glycosides was compared in dry seed meal and in extraction. The result showed that the protein content was increased in seed after treated with different quantities of alcohol and this protein amount was compared with untreated seed sample. The protein obtained from the treated sample can therefore serve as a protein source for animal feed formulation.

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The compound 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-one and mome inositol analyzed by GC/MS has an anti-cancerous and anti-proliferate property. This anti-proliferate and pro-apoptotic effects of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyranone through inactivation of NF-kappaB in human colon cancer cells was previously done and proved by Ban *et al.* (2007). These compounds can be isolated and used for further studies using MTT assay.

Conclusion

Present investigation suggests that leaf extract of Thevitia peruvaina contain higher amount of protein and lesser amount of Glycosides. GC mass analysis shows 11 novel compounds were present in leaf extract. Leaf extract showed antimicrobial activity against *E.Coli* and *Shigella*. Presence of 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4 proves its antibacterial activity. Among the 11 compound 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-one and mome inositol has an anti-cancerous and anti-proliferate property.

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