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# ANALYSIS OF PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITIES OF ETHANOLIC LEAF EXTRACT OF *THEVETIA PERUVIANA* (PERS.) K. SCHUM [*THEVETIA* WHITE]

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**ABSTRACT : *Thevetia peruviana* (Pers.) K. Schum is one of the important medicinal as well as ornamental plant belongs to the Dogbane family Apocynaceae. It is commonly known as yellow oleander. The plant is popular in traditional medicine especially for treatment of heart problems. The leaves are emetic and purgative. Leaf decoction is given to prevent conception. The ethanolic leaf extract of *Thevetia* White was tested for antimicrobial activity against human pathogenic bacteria. *Thevetia* White leaf extract showed strong antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The phytochemical analysis reveals the presence of alkaloids, flavanoids, cardiac glycosides, phenolic compounds, phytosterols and tannins, which are mainly contributed to antimicrobial activity and medicinal utility of the plant.**

**Key words :** *Antimicrobial activity, Cardiac glycosides, Phytochemical analysis, Thevetia peruviana (Pers.) K. Schum.*

## INTRODUCTION

Medicinal plants are the local heritage with global importance. The medicinal plants play an important role in the development of potent therapeutic agents. They have curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary metabolites in one or more parts of these plants. Over the last decade there has been a growing interest in drugs of plant origin. According to (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.

*Thevetia peruviana* (Pers.) K. Schum is a small tree, 15-20 ft. high belongs to the Dogbane family Apocynaceae. It is a native of South America and West Indies. Leaves are simple, linear-lanceolate and whorled (Fig.1). All parts of this plant abound in a milky juice which is highly poisonous. (Chopra *et al.*,1984). The plant (*Thevetia* Yellow) 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 cardiac glycosides obtained from bark, kernals and flowers (*Thevetia* Yellow) are useful for heart diseases (Prajapati *et al.*,2007). The root of this plant are made into a paste and applied to tumours (Singh and Dey,2005). The leaves are emetic and purgative. Leaf decoction is given to prevent conception. The purified glycosides thevetin extracted from the seed is prescribed as a cardio tonic drug. Seeds used as an abortifacient and purgative in rheumatism and dropsy; also used as an alexeteric. Diluted latex is given to treat irregular menstruation. (Ambasta,1986; Kaushik & Dhiman,1999 and Retnam & Martin,2006).

## MATERIAL AND METHODS

**Collection of plant material :** Plant materials (*Thevetia* White leaves) of *Thevetia peruviana* (Pers.) K. Schum were collected from Nehruvangram, Indore. The collected plant materials were identified with the help of Flora of Madhya Pradesh (Mudgal *et al.*,1997).

**Extraction :** To obtain ethanolic extract 100 gm 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).

**Preliminary phytochemical screening :** 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 and Harborne *et al.*,1979).

**Antimicrobial testing :** Each extract sample was tested for antimicrobial activity against human pathogenic bacteria by 'Cup Borer Method (Kavanagh,1963 and Cheesbrough, 1993). The cultures of bacteria have been obtained from Microbial Type Culture, Gene Bank, Chandigarh.

**The name and culture number of bacteria are as follows :**

| **Gram positive bacteria** | **Gram negative bacteria** |
|---|---|
| *B.subtilis* ATCC 6633 | *E.coli*, MTCC 739 |
| *S.aureus* ATCC 9144 | *K.pneumoniae* ATCC 33495 |
| | *S.typhi* ATCC 10749 |
| | *P.aeruginosa* ATCC 25668 |
| | *P.vulgaris* MTCC 1771 |

## RESULTS AND DISCUSSION

**Phytochemical screening :** The leaf extract of Thevetia White reveals the presence of alkaloids, flavanoids,

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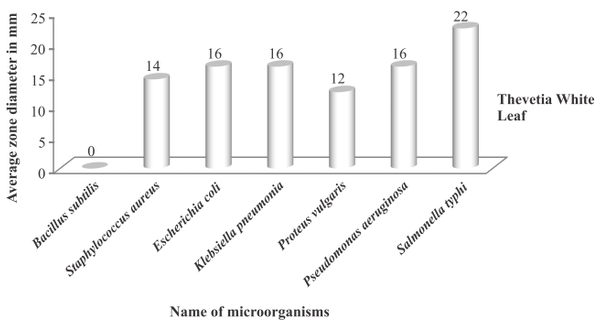
**Table. 1 Phytochemical screening of ethanolic leaf extract of *Thevetia peruviana* (Pers.) K. Schum *Thevetia* White.**

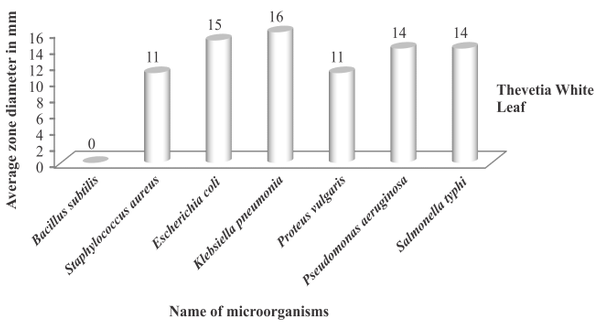
| S. | Plant constituents test/reagents | Results |
|---|---|---|
| 1. | **Alkaloids** | |
| | Mayer's reagent | + |
| | Dragendorff's reagent | + |
| | Hager's reagent | + |
| | Wagner's reagent | + |
| 2. | **Carbohydrates** | |
| | Molish's reagent | + |
| | Benedict's reagent | + |
| | Fehling solution | + |
| 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 | + |
| 1. | **Cardiac glycosides** | |
| | Killer kiliani test | + |

+ = Present, - = Absent

**Fig. 1 *Thevetia peruviana* (Pers.) K. Schum Thevetia White.**




**Graph. 1 Antimicrobial activity of ethanolic leaf extract of *Thevetia peruviana* (Pers.) K. Schum against gram negative and gram positive bacteria (Quantity of extract used-0.17 ml).**




**Graph. 2 Antimicrobial activity of aqueous leaf extract of *Thevetia peruviana* (Pers.) K. Schum against gram negative and gram positive bacteria (Quantity of extract used-0.17 ml).**

glycosides-cardiac glycosides, phenolic compounds, tannins, phytosterols, carbohydrates, saponins, proteins and amino acids was noted in the observation, while fixed oils, fats, gums and mucilages were found absent (Table.1 & Fig.1).

**Table. 2 Antimicrobial activity of leaf extracts (ethanolic and aqueous) of *Thevetia* White against gram positive and gram negative bacteria**

| S. | Extract used | Quantity of extract in ml | Gram positive bacteria | | Gram negative bacteria | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | ***Bacillus subtilis*** | ***Staphylococcus aureus*** | ***Escherichia coli*** | ***Klebsiella pneumonia*** | ***Proteus vulgaris*** | ***Pseudomonas aeruginosa*** | ***Salmonella typhi*** |
| | | | **Average diameter of zone of inhibition in mm** | | | | | | |
| **1.** | **Ethanolic** | .05 | No Zone | No Zone | No Zone | No Zone | No Zone | No Zone | 14 |
| | | .08 | No Zone | 11 | 12 | No Zone | No Zone | 10 | 16 |
| | | .11 | No Zone | 12 | 13 | 12 | No Zone | 12 | 18 |
| | | .14 | No Zone | 13 | 14 | 14 | 10 | 13 | 20 |
| | | .17 | No Zone | 14 | 16 | 16 | 12 | 16 | 22 |
| | **r** | **NA** | **0.832** | **0.85** | **0.933** | **0.886** | **0.864** | **1** | |
| **2.** | **Aqueous** | .05 | No Zone | No Zone | No Zone | No Zone | No Zone | No Zone | 8 |
| | | .08 | No Zone | No Zone | 12 | No Zone | No Zone | 10 | 9 |
| | | .11 | No Zone | No Zone | 13 | No Zone | No Zone | 12 | 10 |
| | | .14 | No Zone | 9 | 14 | 14 | 9 | 13 | 12 |
| | | .17 | No Zone | 11 | 15 | 16 | 11 | 14 | 14 |
| | **r** | **NA** | **0.888** | **0.824** | **0.882** | **0.888** | **0.864** | **0.985** | |

r = Correlation coefficient, r = +1 Perfect positive correlation, r = -1 Perfect negative correlation.


**Fig. 2 Antimicrobial activity of ethanolic leaf extract of *Thevetia peruviana* (Pers.) K. Schum *Thevetia* White against gram positive and gram negative bacteria**

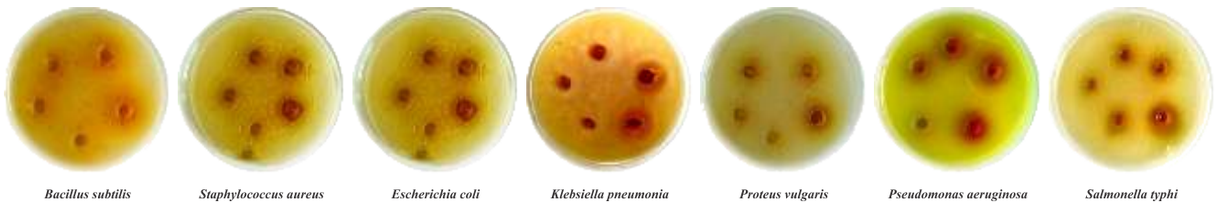



**Fig. 3 Antimicrobial activity of aqueous leaf extract of *Thevetia peruviana* (Pers.) K. Schum *Thevetia* White against gram positive and gram negative bacteria.**

**Antimicrobial testing :** The ethanolic and aqueous leaf extracts of Thevetia White exhibits strong antimicrobial activity against *S.aureus*, *E.coli*, *K.pneumoniae*, *Proteus vulgaris*, *P.aeruginosa* and *S.typhi* while it does not shows response against *B.subtilis* (Table.2; Figs.2&3 and Graphs.1&2).

This investigation has shown the preliminary information to determine the chemical composition and it is useful in establishing quality profile of a crude drug. The leaf extract showed strong antimicrobial activity against tested gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The presence of alkaloids, flavanoids, cardiac glycosides, phenolic compounds, tannins, phytosterols and saponins are mainly contributed in medicinal utility of plant. The results of the present study indicate that the leaves of *Thevetia* White have a medicinal potential to develop into a new drug of pharmaceutical interest. Thus, *Thevetia* White can be used as a substitute for Thevetia Yellow in curing diseases.

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