

## EVALUATION OF ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF *Thevetia peruviana* (KANER) LEAVES

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### ABSTRACT

*Thevetia peruviana* is an evergreen tropical shrub or small tree. Its leaves are willow-like, linear-lanceolate, and glossy green in colour. The antimicrobial potential of the 50% ethanolic extract of *Thevetia peruviana* (kaner) leaves has been evaluated against some micro organisms like *Staphylococcus aureus*, *Rhizobium* sp., *E.coli* and *Streptococcus* sp. Results indicated that the phytochemical extracts of *T. peruviana* exhibited significant activity at different varying dosages (50-150 mg/ml). However, the present study depicts that the 50% ethanolic extract of *Thevetia peruviana* leaves can be used as a potential source of novel antibacterial agents against *E.coli* and *Staphylococcus aureus*, where as *Streptococcus* sp. shown resistance against extracts of *T. peruviana* and *Rhizobium* sp. slightly inhibited at high dosages.

**KEYWORDS:** *Thevetia peruviana*, antimicrobial, phytochemical, *E.coli* and *Staphylococcus aureus*

Medicinal plants are abundantly available at relatively low cost in Chhattisgarh. The plant drugs prove relatively nontoxic, safe and even free from serious side effects<sup>1</sup>. On the other hand, some toxic plants have life threatening properties. *Thevetia peruviana* Schum. (Family-Apocynaceae), commonly known as yellow oleander, it is a small ornamental shrub, which grows up to a height of about 3 to 4 meters. *Thevetia peruviana* is a plant probably native to Central and South America but now frequently grown throughout the tropical and sub-tropical region of the world. It is an evergreen small shrub that bears yellow, trumpet like flowers and its fruit is deep red/black in color encasing a large seed that bears some resemblance to Chinese "lucky nut"<sup>2,3</sup>.

The absorption of the equivalent of two *T. peruviana* leaves may be sufficient to kill a 7.5 kg dog bitch. Many cytotoxic compounds have been investigated in the leaves of *T. peruviana*, such as Thevetin A and B, Thevetoxin, Peruvoside, Ruvoside and Nerifolin<sup>4</sup>. It has immense medicinal properties. This plant is particularly known for its ability to produce cardiac glycosides; flavonol glycoside from leaves. *Thevetia peruviana* has inhibitory effect against HIV-1 reverse transcriptase and HIV-1 Integrase<sup>5</sup>. It has also been regarded as a potential source of biologically active compounds, namely insecticides, rodenticides, and bactericide<sup>6</sup>. *Thevetia peruviana* plant extracts have also been reported to have antifungal properties against *Cladosporium cucumerinum*<sup>7</sup>. The presence of unsaturated linoleic

acid in Yellow oleander oil, which has drying properties<sup>8,9</sup> makes Yellow oleander oil suitable for making a surface coating such as paint.

In Nigeria and Ghana, the bark is used as an antipyretic, however, it is both emetic and poisonous in excess<sup>10</sup>. Insecticidal, molluscidal and antibacterial properties of the leaves and seed oil have been reported<sup>11,12</sup>. Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections<sup>13</sup>.

The frequency of animal life threatening infections caused by pathogenic micro-organisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised animals in both developing and developed countries. In developing countries such as Bangladesh, Nepal and Nigeria, irrational use of antimicrobial agents is a major cause of antibiotic resistance.

The purpose of the present study was to find out the antibacterial screening activity of ethanol-extracted leaves of *T. peruviana*.

### MATERIALS AND METHODS

**(I) Collection of Plant materials:** The leaves of *Thevetia peruviana* were collected from in and around garden area of Bhilai Mahila Mahavidyalaya, Bhilai, India. All these plants were authenticated and the voucher specimens were deposited in Herbarium

of Department of Botany, Bhilai Mahila Mahavidyalaya, Bhilai, India. Later leaves of these plants were subjected to surface sterilization using 50% alcohol and then shade dried for further analysis.

**(II) Preparation of the Extracts:** The 100g leaves of *Thevetia peruviana* in a soxhlet

apparatus were extracted with 50% alcohol at 60 - 80°C. The extracts were collected in a separate container and concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature (40 - 50°C) and note down the yield of crude extracts.

**(III) Microorganisms Used:** *Staphylococcus aureus*, *Streptococcus* sp., *Escherichia coli*, and

*Rhizobium* was collected from the standard stock cultures of Microbiology and Biotechnology Departmental Laboratory, Bhilai Mahila Mahavidyalaya, Bhilai, India. The bacterial cultures were maintained on nutrient agar medium respectively, and were stored at 4°C for determining antimicrobial activity of *Thevetia peruviana* plants leaves.

**(IV) Antimicrobial activity:** Here the in vitro antibacterial activity was assayed by using agar well diffusion method<sup>14</sup>. The pure cultures of different

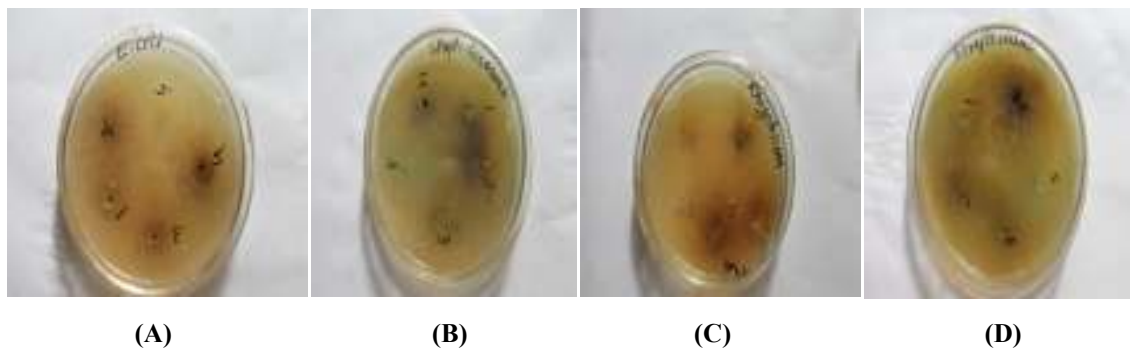
pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 hours. The 0.1ml of the culture was seeded on 25 ml of solidified nutrient agar plate for bacterial cultures. The wells were bored with 6mm borer in seeded agar and then the particular concentrations (50mg, 75mg, 100mg and 150mg/ml) 20µl/well plant extracts added in each separate well. Soon after the plates were then kept at 10°C for 30min. After it normalized to room temperature plates were incubated at 37°C for 24hr. Later, the zone of inhibition was measured and recorded<sup>15,16</sup>.

**RESULTS**

It is evident from the results that, the extracts of *Thevetia peruviana* plants leaves significantly inhibited the growth of *E.coli* and *Staphylococcus aureus*, the tested pathogens in a dose dependent manner (50- 150mg/ml). The test organisms like *Escherichia coli* and *Staphylococcus aureus*, were proved to be maximum level of susceptibility to *Thevetia peruviana* extract with respect to increasing doses. Whereas, same drug was effectively inhibited *Rhizobium* sp. only at higher doses (150mg/ml). It has also not showed resistance antibiotic activity against *Streptococcus* sp. (Figure-1 and Table-1). The obtained results were compared with negative control (0.1ml of 50% alcohol/ well).

**Table 1: Antimicrobial activity of alcoholic extracts of *Thevetia peruviana* leaves extract against four bacterial strains (A) *E.coli* (B) *Staphylococcus aureus* (C) *Rhizobium* sp. and (D) *Streptococcus* sp.**

Microorganisms Medicinal plant extract doses in (mg/ml) (20µl/well)	Zone of Inhibition (cm)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>Rhizobium</i> sp.	<i>Streptococcus</i> sp.
Control 50% alcohol	0.200	-	0.200	0.100
50	0.600	0.300	0.200	0.100
75	0.625	0.450	0.200	0.100
100	0.675	0.625	0.350	0.200
150	0.775	0.675	0.375	0.200



**Figure 1: Antimicrobial activity of alcoholic extracts of *Thevetia peruviana* leaves extract against four bacterial strains (A) *E.coli* (B) *Staphylococcus aureus* (C) *Rhizobium sp.* and (D) *Streptococcus sp.***

## DISCUSSIONS

The results imply that, the extract of *Thevetia peruviana* plant exhibited more or less pronounced antibacterial potencies affecting Gram positive, Gram negative bacteria used in the present study. Several earlier reports also supporting the present research study. The chloroform and methanolic extracts of Nerium oleander roots bark and leaves showed high antimicrobial activity against *Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*<sup>17</sup>. The alcoholic extract of *Thevetia nerifolia* showed antimicrobial activity in a range of 75-1200µg/ml<sup>18</sup>. Ethanol-extracted leaves of yellow oleander showed narrow zone of inhibition in the bacterial lawns of *Shigella flexineri*, *Salmonella typhi*, *Klebsiella sp.*, *Staphylococcus aureus* and *Shigella sonnei*<sup>19</sup>. Callus extract of *Thevetia peruviana* possess higher activity in comparison with leaf extract against gram positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*) and gram negative (*Pseudomonas aeruginosa*)<sup>20</sup>.

## CONCLUSION

In conclusion, the alcoholic extract of *Thevetia peruviana* leaves could be an excellent source of antibiotic drugs against antibiotic resistant *E.coli* and *Staphylococcus aureus* used in this study. Probably this might be happened because the extracts of plant may contain active antibiotic principles with strong inhibitory effects against *E.coli* and *Staphylococcus aureus* test organisms. These plants represent novel leads and future studies may allow the development of a pharmacologically acceptable antimicrobial agent or class of agents.

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