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In vitro antimicrobial activity of ethanol extract of *Thevetia peruviana*

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ABSTRACT

The antimicrobial activity of ethanol extract obtained from *Thevetia peruviana* was tested against bacterial species of *Escherichia coli* ATCC 69314, *Streptococcus lactis* NCIM 50038, *Enterobacter aerogenes* NCIM 2340, *Alcaligenes faecalis* ATCC 15246, *Pseudomonas aeruginosa* NCIM 2200, *Proteus vulgaris* ATCC 6380 and *fungal species of Fusarium oxysporum* NCIM 1008, *Alternaria helianthii* ATCC 201540, *Curvularia lunata* ATCC 34477, *Aspergillus niger* NCIM 1207 and *Penicillium spp* NCIM 741. Better antimicrobial activity was observed with the extracts showed maximum activity against *E. coli, Enterobacter aerogenes, Alcaligenes faecalis*. Among different fungi tested *A. niger* and *Penicillium spp* were found to be more sensitive to crude extract when compared to others. A considerable reduction in the sporulation was also recorded.

KEYWORDS

Antibacterial;
Antifungal;
Ethanol extract;
Thevetia peruviana;
Food poisoning.

INTRODUCTION

Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species of India. Millions of rural households use medicinal plants in a self-help mode. Over one and a half million practitioners of the Indian System of Medicine in the oral and Codified streams use medicinal plants in preventive, promotive and curative applications. There are estimated to be over 7800 manufacturing units in India. In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. In recent years , secondary plant metabolities (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents[12]. Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections[2].

Thevetia peruviana belongs to the family Apocynaceae plant has been referred with different names as Digoxin, Lucky Nut, Nerium oleander, Yellow Oleander. This plant is native of Central & South America, but now frequently grown throughout the tropical and sub-tropical regions. It is a small ornamental

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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 color, become black on ripening. Each fruit contains a nut which is longitudinally and transversely divided. 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 oisonous. The absorption of the equivalent of two *Thevetia peruviana* leaves may be sufficient to kill a 12.5kg child. All parts of the plant contain the milky juice. many cytotoxic compounds have been investigated in *Thevetia peruviana are* Cardiac glycosides, Thevetin A & B, Thevetoxin, Peruvoside, Ruvoside and Nerifolin are found in *T.Peruviana*(Arnold et al., 1935). As part of our investigation on the antimicrobial properties of *Thevetia peruviana* plant, a photochemical study based on the *in vitro* screening of ethanol crude extract was done.

MATERIALS AND METHODS

The fruits of the plant *Thevetia peruviana* were collected from the plants grown in Kushavathi, Thirthahalli. Shimoga District, Karnataka. Further identified by DR. V. Krishna, Deparment of Biotechnology. Voucher specimens deposited at the same department, Kuvempu University, Karnataka, India.

Extraction of plant constituents

The dried seeds were powdered and soaked in the ethanol for about 10-15 days then this cold extract is subjected to distillation at low temperature under reduced pressure in rotary flash evaporator and concentrated on water bath to get the crude extract. Likewise, the powdered seed which is subjected to soxhlation is exhaustively extracted with ethanol for 48 hours. The solvent was distilled off at lower temperature under reduced pressure in rotary flash evaporator and concentrated on water bath to get the crude extract.

Disc diffusion method

The bioassay for bacterial strains was employed by disc diffusion method[4]. Filter paper discs(Whatman no. 1) of 5mm diameter were loaded with crude extracts. Discs were completely dried and sterilized. 100µl of cultures were spread on sterilized nutrient agar media; impregnated discs were placed on it and incubated for 24hrs at 37^0C. Streptomycin discs(10µg/disc) were used as a standard drug. The diameter of zone of inhibition in mm was recorded after incubation. The experiment was performed in triplicates and average diameter of zone of inhibition was obtained.

Screening of antifungal activity

The antifungal activity was determined by the poison food technique. The test fungus is allowed to grow on poisoned plate with ethanol extract. It was observed that reduction in colony diameter and extent of sporulation. The effect of sample on the fungal growth was determined by measuring the diameter of the colony obtained on poisoned plate.

RESULTS AND DISCUSSION

The disc diffusion method for antibacterial activity showed significant reduction in bacterial growth in terms of zone of inhibition around the disc. Among bacterial forms tested, *E.coli, Enterobacter aerogenes* and *Alcaligenes faecalis* were found to be more sensitive to crude extract. Other bacterial forms were inhibited by the extract. The zone of inhibition increased on increasing the concentration of extract in disc. This showed the concentration dependent activity.

TABLE 1: Antibacterial activity of *Thevetia peruviana*

Sl. no.	Organism	Plant extract used	Average inhibition zone in mm		
			Strep to mycin	Crude extract 5mg	Crude extract 10mg
1	*Escherichia coli*	Ethanol extract from seeds of thevetia peruviana	26.8	13.3	10.6
2	*Streptococcus lactis*		24.3	12.6	10.9
3	*Enterobacter aerogenes*		29.6	14.9	11.5
4	*Alcaligenes faecalis*		21.2	13.3	10.4
5	*Pseudomonas aeruginosa*		20.6	11.4	9.6
6	*Proteus vulgaris*		25.6	22.5	18.2

TABLE 2: Antifungal activity of *Thevetia peruviana*

Sl. no.	Test organism	Average colony diameter in mm in control plates	Average colony diameter in mm in plates with extract
1	*Fusarium oxysporum*	33	26
2	*Alternaria helianthii*	36	31
3	*Curvularia spp*	39	28
4	*Aspergillus niger*	24	12
5	*Penicillium spp*	29	14

The results of poison food technique revealed antifungal nature of the constituents present in the crude seed extract of Thevetia peruviana. Among different fungi tested

A.niger and *Penicillium spp* were found to be more sensitive to crude extract when compared to others. A considerable reduction in the sporulation was also recorded.

The antibacterial activity crude extract is shown in TABLE 1. The extracts showed maximum activity against *E.coli, Enterobacter aerogenes* and *Alcaligenes faecalis*. These data revealed that extracts of *T.peruviana* exhibited significant antibacterial activity. In testing, inhibition zone increased with increase in drug concentrations and thus exhibiting concentration dependent activity.

The plants are the vital source of innumerable number of antimicrobial compounds. Several phytocon stituents like flavanoids[10], phenolics and polyphenols[7], tannins[11], terpenoids[9], sesquiterpenes[5] etc., are effective antimicrobial substances against a wide range of microorganisms.

CONCLUSION

The antimicrobial activity of *T.peruviana* may be attributed to the various phytochemical constituents present in the crude extract. The purified components may have even more potency with respect to inhibition of microbes. The work carried was a basic approach to find out the antimicrobial activity in *T.peruviana*. Further works on the types of phytoconstituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit several pathogenic microbes.

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