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Full Length Research Paper

Antimicrobial activity of some traditional medicinal plants

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The antimicrobial activity of the extracts of *Andrographis paniculata* Nees; *Phyllanthus niruri* Linn.; *Terminalia bellerica* Roxb.; *Terminalia chebula* Retz.; and *Vitex negundo* Linn., was studied against four gram negative and one gram positive bacteria. The results showed that the minimum inhibitory concentration (MIC) of *P. niruri* leaf extract was 50 µg/ml against *Salmonella typhi* and *Staphylococcus aureus*, where as, the MICs of *T. bellerica* fruit extract against *Escherichia coli* and *S. aureus* were 50 and 200 µg/ml respectively. However, the leaf extracts of the *Andrographis paniculata*, *T. chebula* and *V. negundo* have not shown any antimicrobial activity in the tested concentrations.

Key words: Traditional medicinal plants, antimicrobial activity.

INTRODUCTION

Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases (Stary and Hans, 1998). The plant extracts have been developed and proposed for use as antimicrobial substances (Del Campo et al., 2000). Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases. Medicinal herbs practiced in traditional folk medicine in India were screened for the presence of antibacterial activity. Ghosh et al. (2007) studied antibacterial activity of *Terminalia bellerica, Terminalia chebula, Emblica officinalis, Punica granatum* and *Lawsonia inermis*. Amongst them, the highest antibacterial potentiality was exhibited by the methanolic leaf extracts of *T. chebula* followed by the aqueous fruit extracts of *T. bellerica.*

Organic solvent extracts of leaves and bark of *Vitex negundo* L. revealed the promising antibacterial activity on *Escherichia coli* and *Staphylococcus aureus.* Inhibition on the growth of both was recorded from the leaf extracts of ethanol and methanol **(**Panda et al., 2009). The entire plant extracts of *Phyllanthus niruri* was tested for its antibacterial effect on *E. coli*, *S. aureus* and *Salmonella typhi* (Ekwenye and Njoku, 2006). Both aqueous and ethanol extracts were inhibitory to the above said organisms.

The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential to evaluate plants of medicinal value systematically for various ailments that are used in traditional medicine. Hence, there is a need to screen medicinal plants for their promising biological activity. Antibacterial activities of various plant extracts were documented in the previous scientific reports using number of organic solvents like ethanol, methanol and petroleum ether. Our experiment was carried out with the organic solvent dimethyl sulphoxide with a view to find out the potentiality of five different traditional medicinal plants such as *Andrographis paniculata* Nees., *P. niruri* Linn., *T. bellerica* Roxb., *T. chebula* Retz. and *V. negundo* Linn. on five selected bacterial strains. *A. paniculata* is an annual herb of about a foot in length, occurs in plains, a member of Acanthaceae. The leaves are used in the treatment of dysentery, jaundice and fever. *P. niruri* is an herb. It belongs to the family Euphorbiaceae. Infusion of the plant is used in the treatment of jaundice, dysentery, skin diseases and fever. *V. negundo* (Verbenaceae) is a shrub with ash colour digitate leaves. It is used in the treatment of jaundice, uterine infections and dysentery. Both *T. bellerica* and *T. chebula* are trees.

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They belong to the family Combretaceae. *T. bellerica* is used in the treatment of diarrhoea, jaundice, cough and sore throat. *T. chebula* is used as laxative and expectorant (Rustomjee and Nanabhai, 1999).

MATERIALS AND METHODS

Plant material

The plants were collected from Kolli Hills, Namakkal District, Tamil Nadu. The plants were taxonomically identified with the Flora of Tamil Nadu Carnatic, The Rapinat Herbarium, St. Joseph's College (Autonomous) Tiruchirappalli -2, Tamil Nadu, South India. The plant parts like leaves and fruits were detached and washed with clean water. Materials were air dried on a clean sheet for one week at room temperature.

Preparation of plant extract

The dried leaves of *A. paniculata*, *P. niruri, V. negundo* and the dried fruits of *T. bellerica* and *T. chebula* were powdered and sieved through a 40-mesh screen. The fine powder was stored in air tight containers and left in the refrigerator.

For each plant material, 1% stock solution was prepared with 0.1% dimethyl sulphoxide solution (1gram powder was soaked in 100 ml of 0.1% dimethyl sulphoxide) for one week. The extract was filtered using membrane filter. The extracts obtained were stored in a refrigerator at 4°C until required for use.

Microorganisms

Pure isolates of *E. coli* (3 strains), *Klebsiella pneumoniae* (2 strains), *Salmonella paratyphi* A (3 strains), *S. typhi* (4 strains) and *S. aureus* (3 strains) were obtained from the Department of Clinical Microbiology, K.A.P.V. Government Medical College, Tamil Nadu, S. India and stored in a semisolid medium at 4°C until needed.

Standardization of inoculum

Organisms from the semisolid nutrient medium were inoculated into peptone water. After 6 h of inoculation, a loop full of peptone water with inoculum was streaked on Muller Hinton agar to check the purity. About 3 - 5 pure colonies of each organism were inoculated into normal saline and the turbidity was adjusted to the McFarlands scale (150×10^6 cfu/ml).

Preparation of medium

Muller Hinton Agar was prepared and bottled in a screw capped (universal) container and autoclaved (121°C) for 20min. Then the medium was allowed to equilibrate in a water bath to a constant temperature (50°C).

Preparation of dilutions of antibacterial agents

Dilutions of antibacterial agents from selected plant parts were prepared from stock solution (1 ml = 10,000 µg). The concentrations used for the study were 50, 100, 200 and 400 µg/ml. Different concentrations of the plant extracts were mixed thoroughly with Muller Hinton Agar. About 20 ml of medium per plate was poured into each Petri plate and was left to solidify. Control plates without antimicrobial agents were also prepared.

Inoculation of the media

The agar surface of the plates containing the antimicrobial agents was inoculated with replicator with sixteen 4 mm stainless steel screws as prolongs (like "Steer's Replicator"). About 16 holes with equal distance were made on the stainless steel plate. With the help of this device, 16 different samples were inoculated at the same time. Inoculated agar plates were incubated at 37°C for 24 h.

RESULTS

Results of the present investigation were depicted in Table 1 and in Plates1 - 5. It was noteworthy that the lowest concentration of the leaf extracts of (50 µg/ml) *P. niruri* was found to be very effective in inhibiting the growth of all the selected strains of *S. typhi* (4 strains) and *S. aureus* (3 strains), where as, *P. niruri* has no inhibitory effect on the other 3 bacterial strains of *E. coli, K. pneumoniae* and *S. paratyphi* A even at 400 µg/ml.

The fruit extracts of *T. bellerica* inhibited the growth of all the 3 strains of *S. aureus* at 50 µg/ml. The same fruit extracts exhibited growth inhibition of *E. coli* at 200 µg/ml only. *T. bellerica* has no inhibitory effect on the other 3 bacterial strains of *K. pneumoniae, S. paratyphi* A and *S. typhi* even at 400 µg/ml. The other three plants did not show any inhibitory effect on the growth of the various strains of bacteria used in the present study.

DISCUSSION

The results of the present study revealed that the dimethyl sulphoxide extracts of leaves of *P. niruri* possess appreciable potentiality of inhibiting the growth of all the strains of *S. typhi* and *S. aureus* at 50 µg/ml. Results of the present study are in line with the scientific investigations of Ekwenge and Njoku (2006). The aqueous and ethanolic extracts of *P. niruri* showed high inhibition against *S. aureus, E. coli* and *S. typhi.* Different organic solvents and aqueous extracts of the leaves and bark of *V. negundo* exhibited significant antibacterial activity against *E. coli* and *S. aureus* which resulted in the complete inhibition of the growth of bacteria in the experiments conducted by Panda et al. (2009).

The bacterial strains of *E. coli* was found to be sensitive and were inhibited by the dimethyl sulphoxide extracts of fruit of *T. bellerica* at high concentration only (200 µg/ml). The same extract showed remarkable inhibition of all the strains of *S. aureus* at the lowest concentration (50 µg/ml) experimented in the present investigation.

From the present study, it is evident that the dimethyl sulphoxide leaf extracts of *P. niruri* had profound and highly significant effect on *S. typhi* and *S. aureus* at the lowest concentration (50 µg/ml) examined. Thus *P. niruri* may provide a possible cure for typhoid and Staphylococcal diseases. This justifies the need why it is

Table 1. Antimicrobial activity of the extracts of different medicinal plants.

Plant name (Antimicrobial agent)	Part used	Name of the organism and no. of (n) isolates		MICs (µg)			
				50	100	200	400
A. paniculata	Leaves	*E. coli*	n = 3	-	-	-	3
		K. pneumoniae	n = 2	-	-	-	2
		S. paratyphi A	n = 3	-	-	-	3
		S. typhi	n = 4	-	-	-	4
		S. aureus	n = 3	-	-	-	3
P. niruri	Leaves	*E. coli*	n = 3	-	-	-	3
		K. pneumoniae	n = 2	-	-	-	2
		S. paratyphi A	n = 3	-	-	-	3
		S. typhi	n = 4	4	-	-	-
		S. aureus	n = 3	3	-	-	-
T. bellerica	Fruits	*E. coli*	n = 3	-	-	3	-
		K. pneumoniae	n = 2	-	-	-	3
		S. paratyphi A	n = 3	-	-	-	2
		S. typhi	n = 4	-	-	-	2
		S. aureus	*n = 3*	3	-	-	-
T. chebula	Fruits	*E. coli*	n = 3	-	-	-	3
		K. pneumoniae	n = 2	-	-	-	2
		S. paratyphi A	n = 3	-	-	-	3
		S. typhi	n = 4	-	-	-	4
		S. aureus	n = 3	-	-	-	3
V. negundo	Leaves	*E. coli*	n = 3	-	-	-	3
		K. pneumoniae	n = 2	-	-	-	2
		S. paratyphi A	n = 3	-	-	-	3
		S. typhi	n = 4	-	-	-	4
		S. aureus	n = 3	-	-	-	3

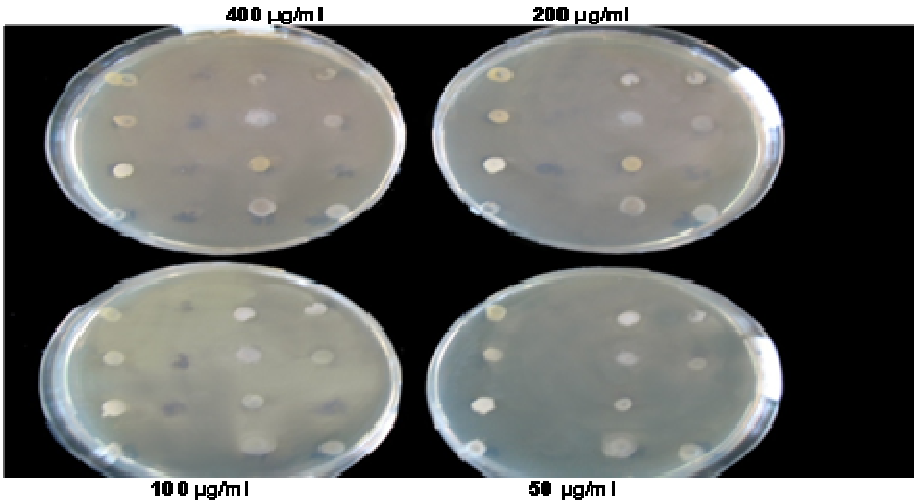

Plate 1. *P. niruri* showing antimicrobial activity against *S. typhi* and *S. aureus at* 50 µg/ml.

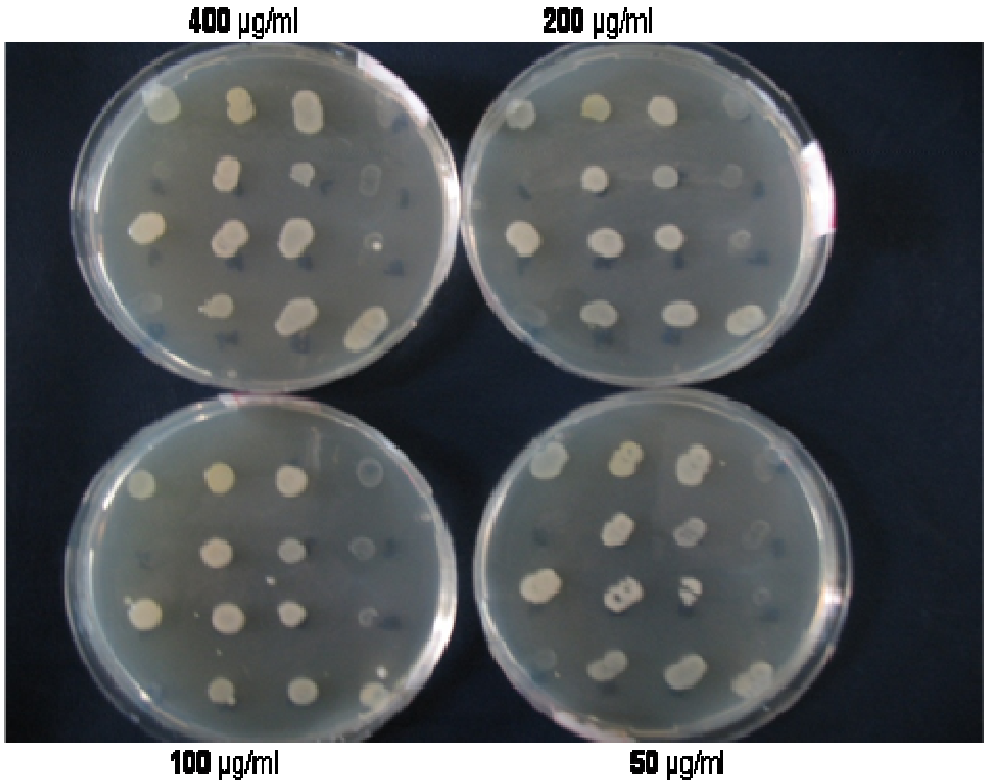

Plate 2. *T. bellerica* showing antimicrobial activity against *S. aureus* and against *E. coli* at 50 µg/ml and 200 µg/ml.

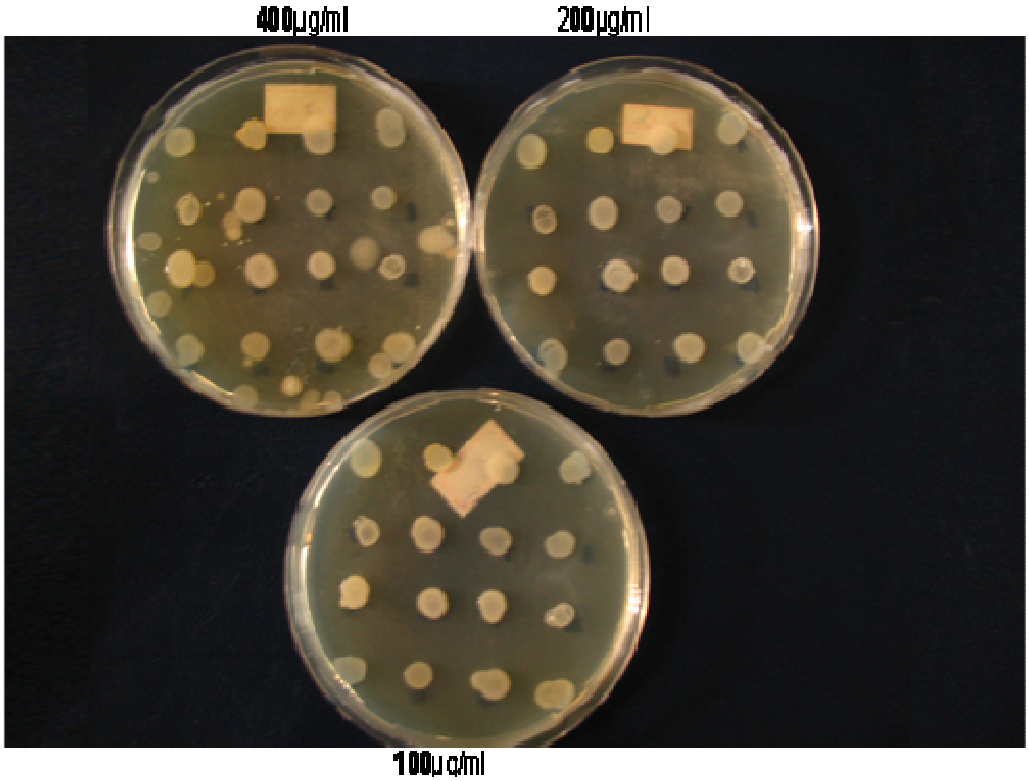

Plate 3. *A. paniculata* not showing antimicrobial activity even at 400 µg/ml.

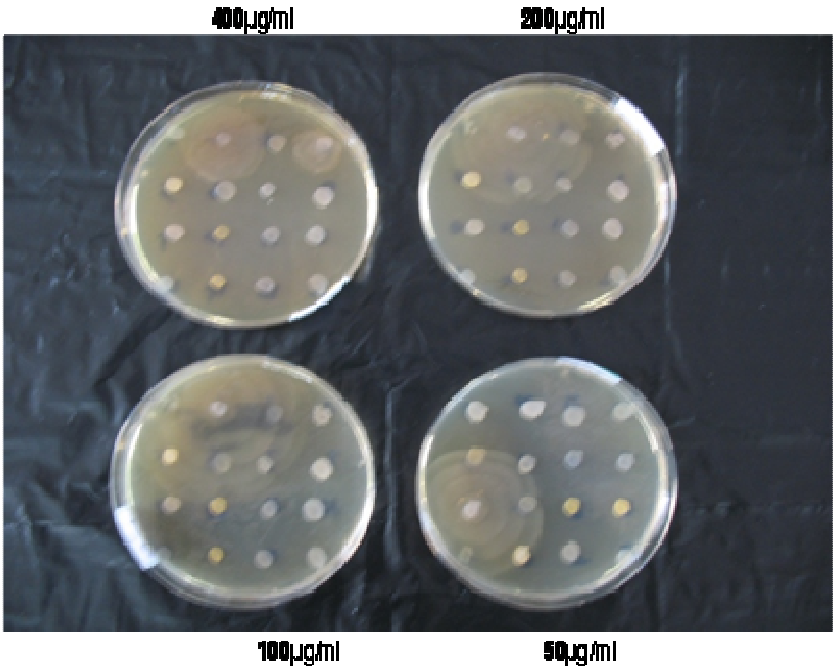

Plate 4. *T. chebula* not showing antimicrobial activity even at 400 µg/ml.

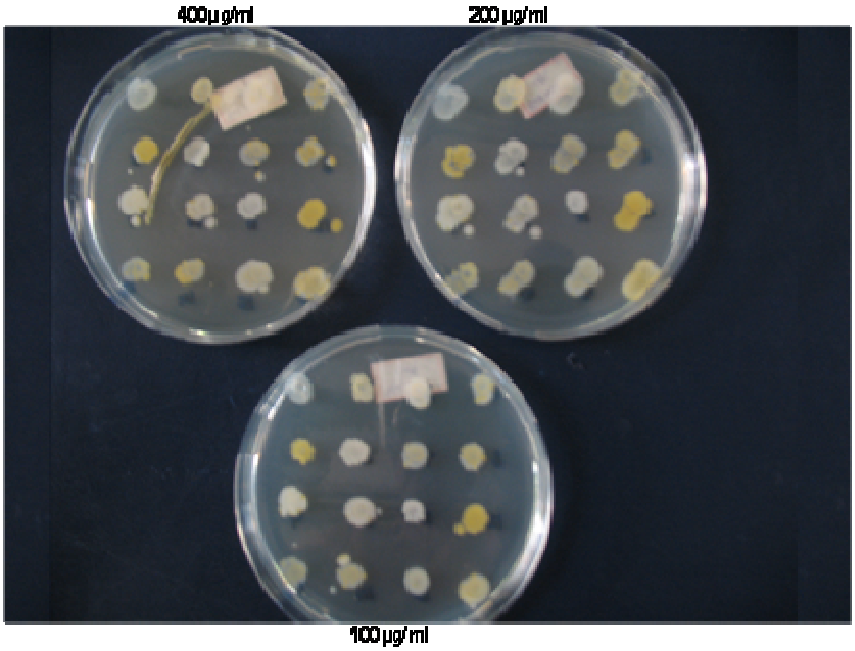

Plate 5. *V. negundo* not showing antimicrobial activity even at 400 µg/ml.

used in folk medicine as curative plant for typhoid fever and as intestinal anesthetic (Krishnamurty, 1993).

Conclusion

Our experimental results provide additional information regarding the organic solvent used for the extraction of plants. It was highlighted in our study that the dimethyl sulphoxide extracts of *P. niruri* and *T. bellerica* showed remarkable antibacterial effect against *S. typhi, E. coli* and *S. aureus.* Hence, it may be recommended that these two plants could be used in the treatment of human diseases caused by the above mentioned organisms.

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