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Antibacterial activity of *Vicoa indica* and *Tridax procumbens* against Multi-Drug Resistant (MDR) clinical isolates

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Abstract

The antibacterial activity of aqueous and ethanolic extracts of *Vicoa indica* and *Tridax procumbens* were tested against multi-drug resistant clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *and Klebsiella pneumoniae*. The Minimum Inhibitory Concentrations (MIC) of 4mg / ml (Tube dilution) and 2mg / ml (Disc Diffusion) of ethanolic extract of *Vicoa indica* were active against drug resistant *Staphylococcus aureus*, while a MIC of 5mg / ml(Tube Dilution) and 3mg / ml (Disc Diffusion) of ethanolic extract of *Tridax procumbens* were active against drug resistant *Escherichia coli*. There was no observable activity by both the extracts of both the herbs against *K. pneumonia* and *P. aeruginosa.*

Keywords: antibacterial activity, anti drug resistant herbs, bioactivity, *Tridax procumbens, Vicoa indica*

INTRODUCTION

Anti-microbial agents may be defined as substances of natural, semi synthetic or synthetic origin that kill or inhibit the growth of bacteria, but cause little or no host damage. For many centuries antibiotics played a great role in treating and controlling vast array of infections and each year many new drugs with improvised action are available. However, the gradual emergence of populations of antibiotic-resistant bacteria resulting from use, misuse and outright abuse of antibiotics has today become a major public health problem globally (Komolafe, 2003). The endless search for newer anti bacterial drug is attributed to the drug resistance which is a major problem well discussed, however not monitored by proper antibiotic policies (Wang *et al.*, 2002). It may be noted that, the problem of drug resistance has reached a stage, which has pushed for search of newer anti-microbial agents, either synthetic or from herbal sources. Furthermore, the significant development of non-formal drug therapies has initiated global scientists to work on authentication of existing herbs for anti-microbial property. This paper documents the anti-bacterial activity of two herbs *viz.*, *Vicoa indica* and *Tridax procumbens* against selected drug resistant clinical isolates.

MATERIALS AND METHODS

Collection of plants

Vicoa indica is a perennial herb distributed throughout central Asia, northeast tropical Africa and *Tridax procumbens*, the procumbent herb, is found commonly in tropical Africa, Asia and Australia. (Murugesamudhaliyar, 1998). Specimens of *Vicoa indica* and *Tridax procumbens* were collected from the social forest areas in around Chennai, Tamil Nadu, South India.

The leaves of *Vicoa indica* was demonstrated to have anti-inflammatory effect (Krishnaveni *et al*., 1997) and anti microbial activity (Gopal, *et al*., 1992). A component 4,5,6-Trihydroxy 3,7-Dihydroxy flavone, isolated from *Vicoa indica*, is known to possess anti-inflammatory and analgesic effects. (Krishnaveni *et al*., 1997). The leaves of *Tridax procumbens* are used in the treatment of bronchial cattarach, dysentery and diarrhea (Taddei and Romero, 2000). Its crushed leaves are used as topical application on wounds and cuts; however there is no documented literature evidence for its wound healing properties. It has been reported to have antibacterial activity and anti fungal activity as well (Leach *et al.*, 1998).

Bacterial cultures

The drug resistant isolates of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *and Klebsiella pneumoniae* were obtained from Chennai city hospitals and were identified using recommended bacteriological biochemical tests. The cultures were grown in Muller Hinton broth at 37^0C and maintained in Nutrient agar slopes at 4^0C. Antibiotic sensitivity patterns were determined by Kirby Baur method (Baur *et al*., 1966).

The antibiotic sensitivity pattern of the four clinical isolates are shown in table-1.

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Table 1. Antibiotic susceptibility pattern of tested microorganisms

S.No	Symbol	Name of the antibiotic	Tested organisms			
			S.aureus	***K.pneumoniae***	***E.coli***	***P.aeruginosa***
1	A	Ampicillin	NA	Resistant	Resistant	NA
2	Ac	Amoxyclov	NA	Resistant	Resistant	NA
3	Ak	Amikacin	NA	Sensitive	Resistant	NA
4	Ca	Ceftazidime	NA	Resistant	Resistant	Resistant
5	Ce	Cephotoxime	NA	Resistant	Resistant	Resistant
6	Cf	Ciproflaxicin	NA	Resistant	Resistant	Resistant
7	Cu	Cefuroxime	NA	Resistant	Resistant	NA
8	Cz	Cefazolin	NA	Resistant	NA	NA
9	G	Gentamycin	Sensitive	Resistant	Resistant	Resistant
10	I	Imipeneum	NA	Sensitive	Sensitive	Resistant
11	Na	Nalidixic acid	Resistant	Resistant	Resistant	NA
12	Nf	Nitrofurantoin	Sensitive	Intermediate	Resistant	NA
13	Nt	Netillin	NA	Resistant	Intermediate	NA
14	Nx	Norfloxacin	NA	Resistant	Resistant	Resistant
15	P	Penicillin	Resistant	Resistant	Resistant	NA
16	R	Rifampicin	Resistant	NA	NA	NA
17	Va	Vancomycin	Resistant	NA	NA	NA

Key- NA: Not Applicable

Extract Preparation

The collected leaves were cleaned, shade dried and pounded to coarse powder in a blender. For the preparation of aqueous and ethanolic extracts, 10 g of the leaf powder is mixed with 100ml of water or ethanol, respectively. The homogenate was kept in orbital shaker for 48hrs and filtered through muslin cloth. The supernatant was dried at 55^0C and stored in air tight vessel at 4^0C. The sediments were re-extracted and processed as described above. The working solutions were prepared by dissolving 10mg / 1ml of the extract dissolved in PBS for aqueous extract and 0.25% DMF in case of ethanolic extract. The extracts were screened for antimicrobial activity by disc diffusion (Joan, 1972; Mitchell, 2000) and tube dilution methods (Manavathu *et al.,* 1996; Nakamura *et al.,* 1999). For disc diffusion, known concentrations of the extract were poured into the small discs of filter paper and dried. In tube dilution technique the appropriate amounts of extract were added to the testing medium (Muller Hinton Broth) to make the final concentration from 1 mg / ml to 10 mg / ml. Appropriate controls were included in the assay.

Disc diffusion method

In this method the organisms were surface swabbed with 0.1 ml of the logarithmic phase bacteria at a density adjusted to 0.5 McFarland turbidity standard (10^8 cfu / ml) in the medium (Muller- Hinton agar) and prepared discs were placed into the medium with 2 cm spacing. The plates were incubated at 37^0C for 24-48hours. The lowest concentration that prevented visible growth as zone of clearance is taken as Minimum Inhibitory Concentration (MIC). The plates were observed for zone of inhibition that was measured by standard techniques (Nakamura *et al.,* 1999).

Tube dilution Method

The extracts of concentrations ranging from 1 mg / ml to 10 mg / ml were dissolved in 2.6 ml of Muller Hinton broth to make a final volume of 3.6 ml. The initial OD were measured at 590nm, to which 0.4ml of the bacterial suspension containing $1X10^6$ cfu / ml was added to 3.6ml of the susceptibility test broth. The test was performed in duplicates. The Final OD of the incubated plates at 37^0C for 24- 48 hours was measured calculated. The differences between the initial and final OD were assessed. The concentrations showing fall in OD compared with positive control is taken as the Minimum Inhibitory Concentration (MIC) of the drug (Manavathu *et al.,* 1996).

RESULTS AND DISCUSSION

The results of both disc diffusion and tube dilution methods of the aqueous and ethanolic extracts of *Vicoa indica* and *Tridax procumbens* over the four organisms is summarized in tables 2 and 3 and shown in figures 1- 4.

The results clearly show that the ethanolic extract of *Vicoa* was active against drug resistant *S. aureus* at the MIC of 4mg / ml in the tube dilution method and at MIC

Table 2. Effects of different concentrations of aqueous and ethanolic extracts of *Vicoa indica* and *Tridax procumbens* against multi-drug resistant clinical isolates by disc diffusion method.

Herb	Nature of extract	Name of the Microorganism	Zone of inhibition in mm					
			10mg/ml	5mg/ml	4mg/ml	3mg/ml	2mg/ml	1mg/ml
V.indica	Aqueous	*S.aureus*	No zone	No zone	No zone	No zone	No zone	No zone
		K.pneumoniae	No zone	No zone	No zone	No zone	No zone	No zone
		E.coli	No zone	No zone	No zone	No zone	No zone	No zone
		P.aeruginosa	No zone	No zone	No zone	No zone	No zone	No zone
	Ethanolic	*S.aureus*	**20**	**14**	**12**	8	3	No zone
		K.pneumoniae	No zone	No zone	No zone	No zone	No zone	No zone
		E.coli	No zone	No zone	No zone	No zone	No zone	No zone
		P.aeruginosa	No zone	No zone	No zone	No zone	No zone	No zone
T.procumbens	Aqueous	*S.aureus*	No zone	No zone	No zone	No zone	No zone	No zone
		K.pneumoniae	No zone	No zone	No zone	No zone	No zone	No zone
		E.coli	No zone	No zone	No zone	No zone	No zone	No zone
		P.aeruginosa	No zone	No zone	No zone	No zone	No zone	No zone
	Ethanolic	*S.aureus*	No zone	No zone	No zone	No zone	No zone	No zone
		K.pneumoniae	No zone	No zone	No zone	No zone	No zone	No zone
		E.coli	**21**	**13**	9	4	No zone	No zone
		P.aeruginosa	No zone	No zone	No zone	No zone	No zone	No zone

Table 3. Effect of different concentrations of aqueous and ethanolic extracts of *Vicoa indica* and *Tridax procumbens* against multi drug resistant clinical isolates by tube dilution method

Herb	Nature of extract	Name of the Microorganism	Concentration of the extract and growth					
			10mg/ml	5mg/ml	4mg/ml	3mg/ml	2mg/ml	1mg/ml
V.indica	Aqueous	*S.aureus*	0.46	0.55	0.56	0.54	0.51	0.42
		K.pneumoniae	0.49	0.57	0.52	0.58	0.49	0.44
		E.coli	0.52	0.59	0.56	0.48	0.51	0.56
		P.aeruginosa	0.51	0.56	0.49	0.46	0.42	0.43
	Ethanolic	*S.aureus*	**0**	**0**	**0**	0.18	0.24	0.56
		K.pneumoniae	0.47	0.45	0.43	0.45	0.46	0.39
		E.coli	0.46	0.48	0.43	0.41	0.52	0.51
		P.aeruginosa	0.59	0.56	0.52	0.48	0.49	0.53
T.procumbens	Aqueous	*S.aureus*	0.57	0.54	0.51	0.52	0.48	0.46
		K.pneumoniae	0.56	0.54	0.52	0.51	0.48	0.43
		E.coli	0.56	0.51	0.52	0.53	0.49	0.51
		P.aeruginosa	0.51	0.52	0.49	0.46	0.48	0.49
	Ethanolic	*S.aureus*	0.42	0.46	0.48	0.44	0.42	0.46
		K.pneumoniae	0.44	0.43	0.41	0.42	0.51	0.49
		E.coli	**0**	**0**	0.15	0.27	0.38	0.46
		P.aeruginosa	0.46	0.52	0.51	0.54	0.48	0.52

Note : Growth is measured by difference in OD at 590 nm. CV = initial OD - final OD

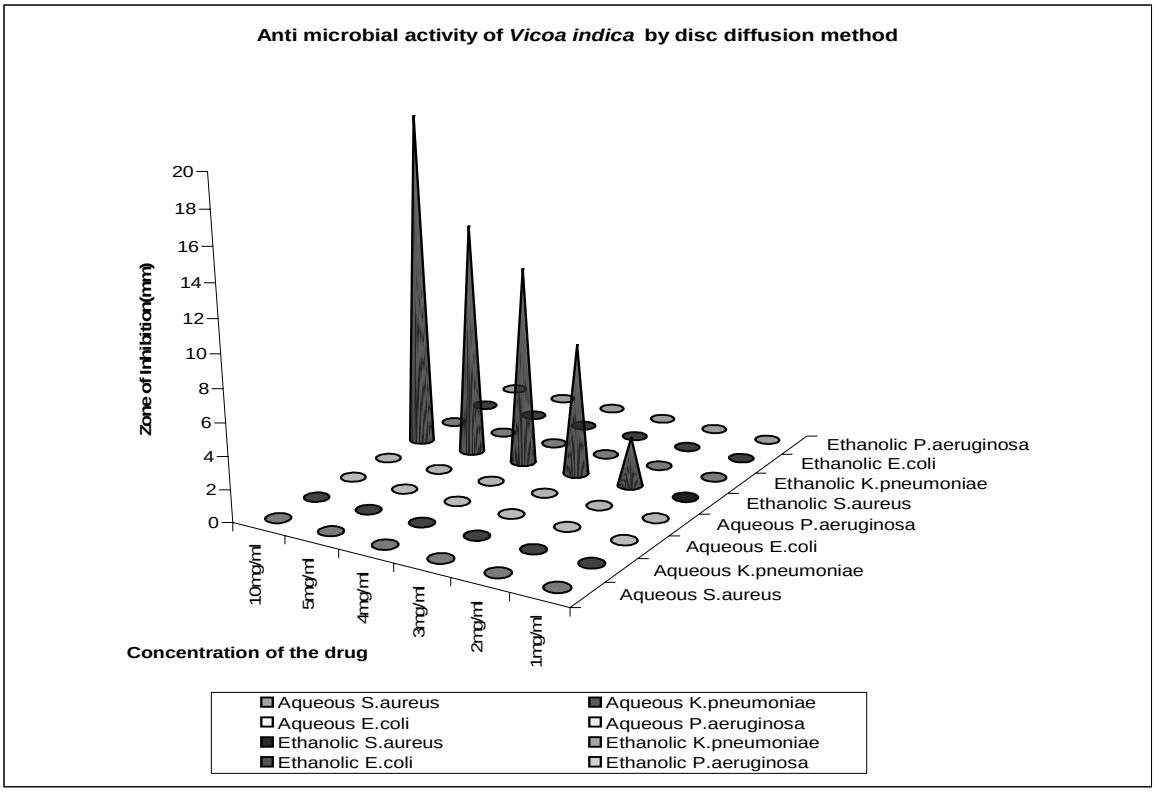

Figure 1. Effect of aqueous and ethanolic extracts of *Vicoa indica* by disc diffusion method against the four Clinical isolates.

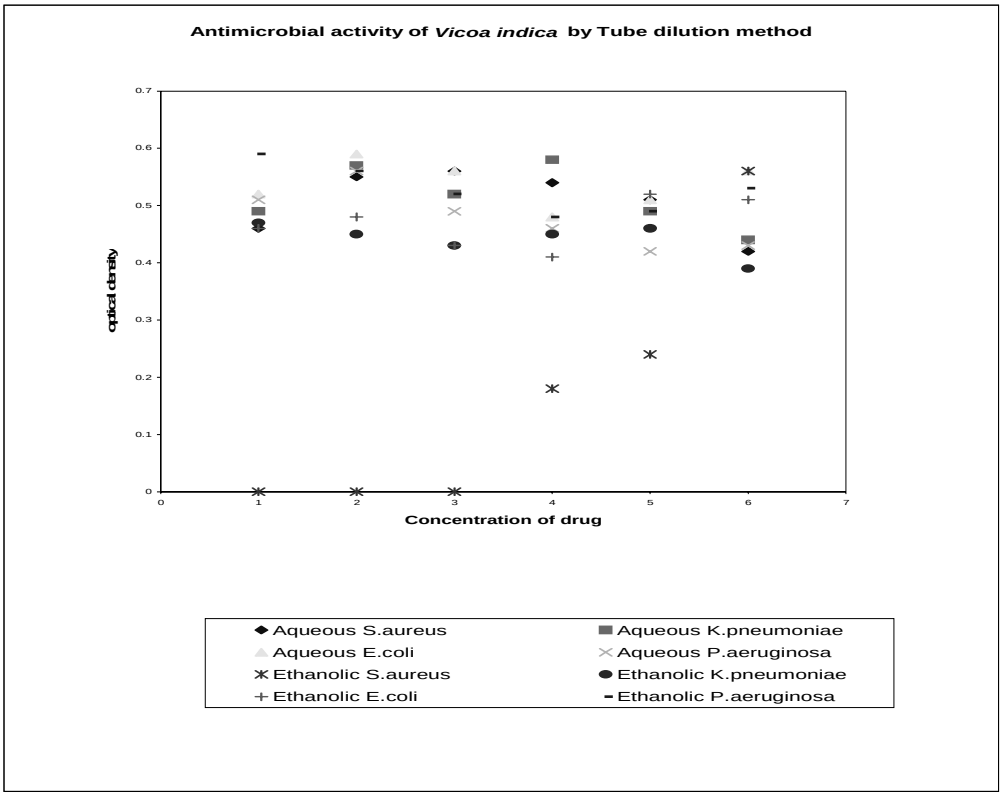

Figure 2. Antibacterial activity of *Vicoa indica* by tube dilution method

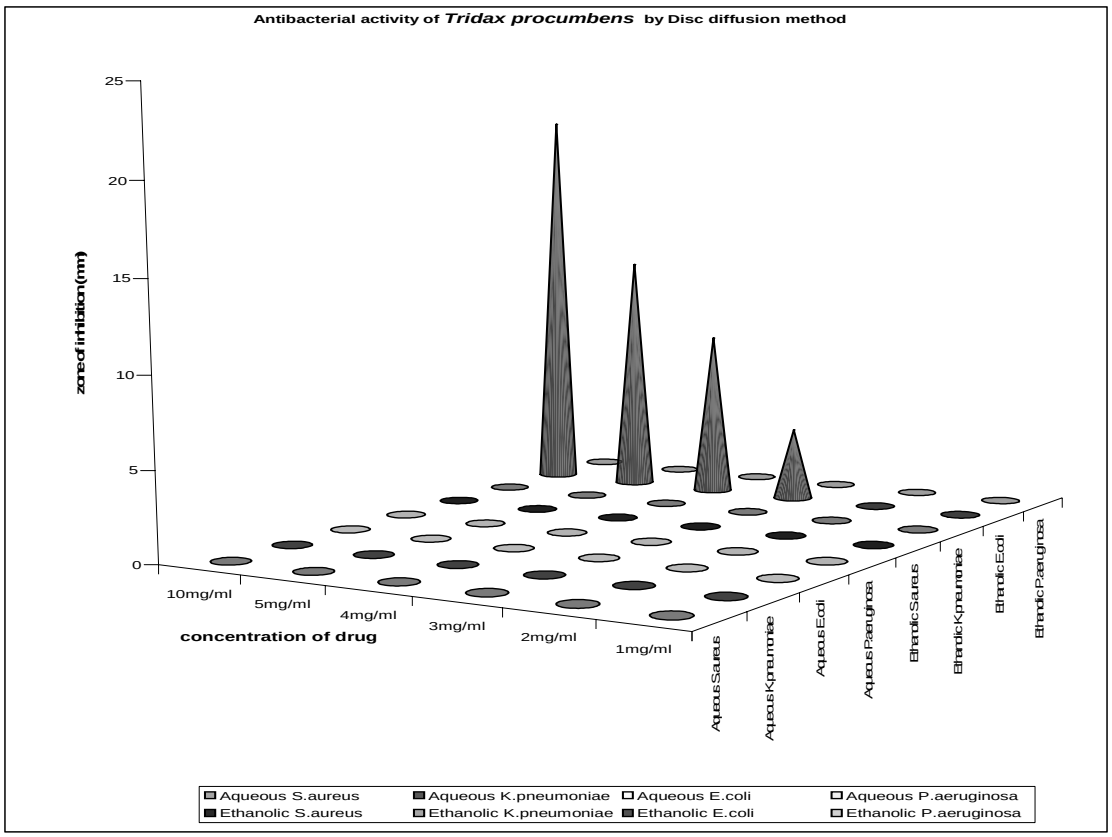

Figure 3. Effect of aqueous and ethanolic extracts of *Tridax procumbens* by disc diffusion method against the four Clinical isolates

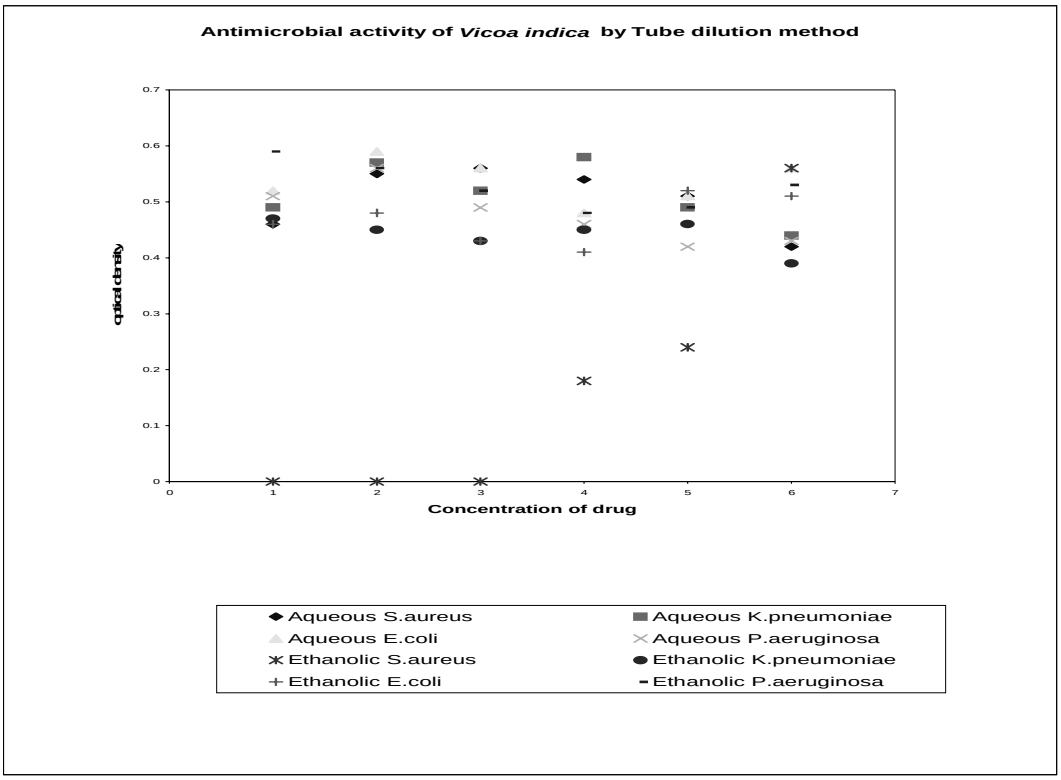

Figure 4. Antibacterial activity of *Tridax* by tube dilution method

of 2mg / ml in the disc diffusion method. These results were in accordance with the previous report by Gopal *et al.* (1992),who found the isolated vicolides to show activity against *S. aureus* but not against *E. coli* (Gopal, *et al.*, 1992). The ethanolic extract of *Tridax procumbens* was active against *E. coli* at the MIC of 5mg / ml in the tube dilution and 3 mg / ml in the disc diffusion method. Leach *et al.* (1988) have already reported antibacterial activity exhibited by *Tridax* but not against *E. coli*. From the results of the present study it may be inferred that the extracts of *Vicoa* is active against gram-positive organisms and *Tridax* against gram-negative bacteria. Similar results of specific activity of herbs towards particular group of bacteria were observed by Jansen *et al.* (1989) and Manavathu (1996), also. There was no observable antibacterial activity against *Pseudomonas aeroginosa*, and *Klebsiella pneumoniae* by both the herbs.

The antibacterial activity was clearly concentration dependent as extracts showed enhanced activity at higher concentrations as observed from the results of disc diffusion method of the present study. The aqueous extracts of these herbs did not show any observable bacteriostatic activity in both the methods. This might be due to the differences in the polarity of extraction as most of the indigenous preparations are of this nature. However, it is also to be highlighted that screening of more herbs will be useful in isolating new compounds to fight against drug resistance, to find economical alternative and overcome side effects.

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