

Antimicrobial activity of *Vitex negundo* leaf extracts

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Abstract

Methanol, diethyl ether and acetone extracts of leaf of *Vitex negundo* were tested for their antibacterial activity against two human pathogenic bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa* and one fungus *Aspergillus niger* using the disc diffusion method. It was found that all the extracts produced inhibitory effect but the methanol extract of leaves exhibited a superior level of antimicrobial activity. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) was determined by broth dilution method. The results were compared with the reference antibiotics.

Keywords:- Antibacterial activity, *Vitex negundo*, leaf extract, MIC

Introduction

Infectious diseases are a serious problem worldwide and account for high proportion of health problems in the developing countries (Sashikumar *et al.*, 2003). On the other hand some of the drugs currently in use result in adverse side effects (Covington, 1988). Therefore the search for new antimicrobial substances exhibiting minimal side effects is warranted (Kandil *et al.*, 1994). One of the most promising area in the search for new biologically active compounds are the plants used in traditional medicine (Alonso *et al.*, 1995).

Vitex negundo (verbenaceae), a large aromatic shrub upto 4-5 meters in height is found throughout the greater part of India growing upto an altitude of 1500m in the outer Himalayas. In addition to India, it is also found in Sri Lanka, Burma, China, Pakistan, Afganistan, Malaysia, tropical Africa and the Philippines. It can be propagated readily by vegetative cutting. The leaves of *Vitex negundo* are reported to possess

pesticidal, antifungal and antibacterial properties. The plant is also used as a commercial drug in the indigenous system of medicine (Anon, 1976).

Materials and Method

The present investigation was conducted to evaluate the antimicrobial potential of leaf extracts of *Vitex negundo* against three pathogenic microorganisms *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Aspergillus niger* which was obtained from Institute of Microbial Technology (IMTECH), Chandigarh. Local isolates were obtained from B.H.E.L Hospital, Haridwar.

The matured leaves of *Vitex negundo* were collected from Haridwar and its adjoining areas, dried in shade and crushed in mortar. The crushed leaves were extracted with methanol, diethyl ether and acetone for 24h using Soxhlet apparatus. The solvent was removed in rotary evaporator and the crude extract was used. Three different dilutions of the extracts will be prepared in dimethyl sulphoxide (DMSO). The working concentrations of the extracts were 200, 400 and 800 mg mL⁻¹ respectively.

The antibacterial activity was tested *in vitro* by disc diffusion assay (Bauer *et al.*, 1966) on nutrient agar medium (NAM) and Sabouraud's dextrose agar medium (SDA) by taking Whattmann no 42 filter

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paper discs which was saturated with 10 μ l of different dilutions of different extracts. DMSO as negative control and ciprofloxacin, gentamycin and clotrimazole (500ppm) were used as positive control for *S. aureus*, *P. aeruginosa* and *A. niger* respectively. The plates were incubated at 37°C for

Table 1: Inhibition zones of the leaf extracts of *Vitex negundo* against *Staphylococcus aureus*

Extract	Concentration (mg/ml)	*Effective zone of inhibition	
		<i>S. aureus</i> (MTCC)	<i>S. aureus</i> (local)
Methanol	800	17.6** \pm 0.57	13.6** \pm 0.57
	400	14.6** \pm 0.57	12.0** \pm 0
	200	11.3** \pm 0.57	9.6** \pm 0.57
Diethyl ether	800	17.3** \pm 0.57	13.3** \pm 0.57
	400	12.0** \pm 1.0	11.3** \pm 0.57
	200	9.6** \pm 0.57	9.3** \pm 0.57
Acetone	800	17.0** \pm 0	12.0** \pm 1.0
	400	14.3** \pm 0.57	10.6** \pm 0.57
	200	10.0** \pm 0	9.3** \pm 0.57
Ciprofloxacin	500ppm	15** \pm 1.0	12** \pm 1.0
DMSO	100%	-	-

Note: Values are the average of 3 replicates \pm SD, *Effective zone of inhibition=Total zone of inhibition-diameter of the disc(5mm), **Significant at 0.05 %level

24 h in case of bacteria and at 28 °C for 5 to 7 days in case of fungi. The activity was measured in terms of diameter of inhibition zone appearing around the filter paper discs saturated with leaf extracts. The tests were made in triplicates.

The minimum inhibitory concentration (MIC) was determined in sterile microtiter plates each having 24 wells (Forbes et al., 1998). 1 μ l of methanol extract was taken from the stock solution having a concentration of 800mg mL⁻¹ and serially diluted using nutrient broth. 0.1 μ l of the bacterial and

Table 2: Inhibition zones of the leaf extracts of *Vitex negundo* against *P. aeruginosa*

Extract	Concentration (mg/ml)	*Effective zone of inhibition	
		<i>P. aeruginosa</i> (MTCC)	<i>P. aeruginosa</i> (local)
Methanol	800	12.3** \pm 0.57	10.6** \pm 0.57
	400	10.3** \pm 0.57	8.0** \pm 1.0
	200	8.3** \pm 0.57	5.3** \pm 0.57
Diethyl ether	800	11.6** \pm 0.57	9.0** \pm 1.0
	400	9.3** \pm 0.57	6.3** \pm 0.57
	200	6.6** \pm 1.15	3.3** \pm 0.57
Acetone	800	10.3** \pm 0.57	9.6** \pm 0.57
	400	8.3** \pm 0.57	7.6** \pm 0.57
	200	5.6** \pm 0.57	6.0** \pm 0
Gentamycin	500ppm	11** \pm 1.0	9** \pm 1.0
DMSO	100%	-	-

Note: Values are the average of 3 replicates \pm SD, *Effective zone of inhibition=Total zone of inhibition-diameter of the disc(5mm), **Significant at 0.05%level

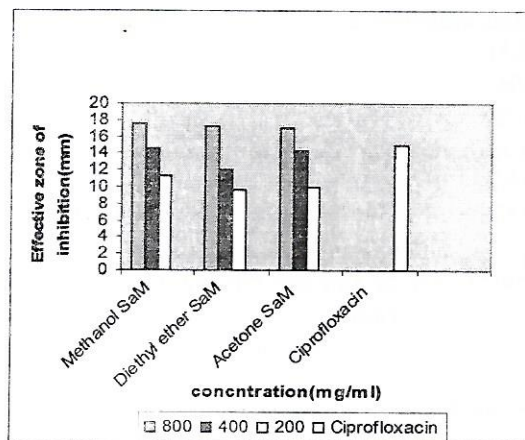


Fig. 1. Comparative analysis of the activity of leaf extract of *V. negundo* and that of antibiotic control against *S. aureus*



Table 3: Inhibition zones of the leaf extracts of *Vitex negundo* against *A. niger*

Extract	Concentration (mg/ml)	*Effective zone of inhibition	
		<i>A. niger</i> (MTCC)	<i>A. niger</i> (local)
Methanol	800	10.3**±0.57	7.6**±0.57
	400	7.6**±0.57	5.3**±0.57
	200	5.3**±0.57	3.6**±0.57
Acetone	800	9.3**±0.57	7.0**±1.0
	400	5.6**±0.57	5.3**±0.57
	200	4.0**±1.0	3.6**±1.15
Diethyl ether	800	6.6**±0.57	5.0**±1.0
	400	5.0**±0	3.6**±0.57
	200	3.6**±0.57	2.0**±0
Clotrimazole	500ppm	10**±1.0	14±0.47
DMSO	100%	-	-

Note: Values are the average of 3 replicates ± SD, *Effective zone of inhibition=Total zone of inhibition-diameter of the disc(5mm), **Significant at 0.05% level

Table 4: MIC (Minimum inhibitory concentration) of methanol extract of *Vitex negundo* against *S. aureus*

Replicates	Minimum inhibitory concentration (MIC in mg/ml)	
	<i>S. aureus</i> (MTCC)	<i>S. aureus</i> (local)
1	12.5	25
2	12.5	25
3	6.25	12.5
Mean	10.4	20.8
SD	±2.9	±5.8

Table 5: MIC (Minimum inhibitory concentration) of methanol extract of *Vitex negundo* against *P. aeruginosa*

Replicates	Minimum inhibitory concentration (MIC in mg/ml)	
	<i>P. aeruginosa</i> (MTCC)	<i>P. aeruginosa</i> (local)
1	25	50
2	25	50
3	12.5	25
Mean	20.8	41.6
SD	±5.8	±7.2

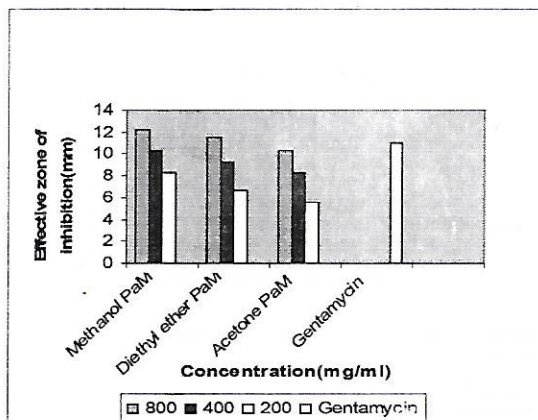


Fig. 2. Comparative analysis of the activity of leaf extract of *V. negundo* and that of antibiotic control against *P. aeruginosa*

fungal inoculum was added to each well and incubated. The tests were made in triplicates. The lowest concentration (highest dilution) of the plant extract preventing the turbidity is considered to be the MIC.

Results and Discussion

All the three dilutions of the leaf extract showed antimicrobial activity and the 800 mg/ml concentration was most effective and was

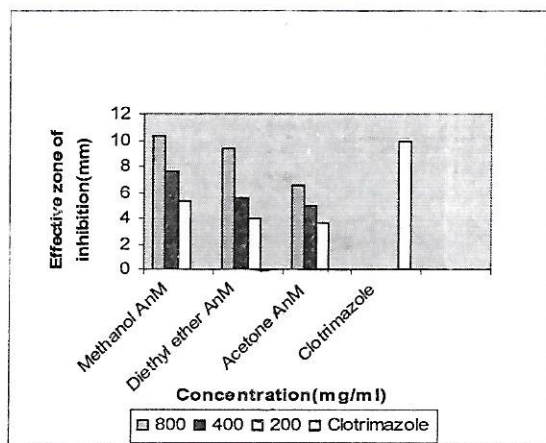


Fig. 3. Comparative analysis of the activity of leaf extract of *V. negundo* and that of antibiotic control against *A. niger*

Table 6: MIC (Minimum inhibitory concentration) of methanol extract of *Vitex negundo* against *A. niger*

Replicates	Minimum fungicidal concentration (MFC in mg/ml)	
	<i>A. niger</i> (M TCC)	<i>A. niger</i> (local)
1	200	200
2	200	400
3	400	400
Mean	41.6	333.3
SD	± 94.2	± 94.2

comparable to the one unit solution of antibiotic (Table 1, 2 and 3 and Fig. 1, 2 and 3). The minimum inhibitory concentration (MIC) of the methanol extract of *V. negundo* against *S. aureus*, *P. aeruginosa* and *A. niger* was found to be 10.4, 20.8 and 41.6 mg/ml respectively and the minimum bactericidal concentration (MBC) and

minimum fungicidal concentration (MFC) was found to be 41.6, 83.3 and 800 mg/ml respectively (Table 4, 5 and 6). The extract was found to be cidal in nature as there is no visible growth after subculturing the sample from the wells. Thus the results confirm the presence of antimicrobial components in the leaves of *Vitex negundo*.

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