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In-Vitro Evaluation of Anti-Bacterial and Anti-Fungal Activity of *Vitex nigundo* (Verbenaceae)

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Abstract

Vitex negundo belongs to the family Verbenaceae. It is a large aromatic shrub distributed throughout the greater part of India up to an altitude of 1500 m in the outer Himalayas. It is widely planted as a hedge plant along the roads and between the roads. Traditionally it is having the flok claims like useful in treatment of rheumatism, insecticidal, antimicrobial, anticancer, tranquillizer, tonic, febrifuge, expectorant and diuretic properties. In the present study an attempt had been made to evaluate comparative antibacterial and antifungal principles from *Vitex nigundo* with some therapeutically used antibiotics. Different extracts of *Vitex negundo* leaves were investigated for its anti microbial and antifungal activity on five bacterial species and three fungal species these are *Staphylococcus aureus, Proteus vulgaris, Bacillus subtilis, E.coli, Pseudomonas aerugenosa and Aspergillus niger, Aspergillus flavon, Candida albicans respectively.* Among all extracts water-ethanol (50:50) extract showed maximum anti microbial and water extract showed maximum antifungal activity against all tested species.

Keywords: *Vitex negundo,* antimicrobial, minimum inhibition concentration.

Introduction

Vitex negundo belonging to family Verbenaceae is a large aromatic shrub distributed throughout the greater part of India up to an altitude of 1500 m in the outer Himalayas[3] It is a gregarious shrub that is found abundant along the banks or rivers, in moist situations, open waste lands and near the deciduous forests. It is widely planted as a hedge plant along the roads and between the roads. The leaves are tri-or pentafoliate, lateral leaflets are smaller and nearly glabrous. The shrub is one of the important plants used in Indian medicine. Almost all parts of the herb are useful as a drug but the leaves and roots are most important and sold as drugs[2].

Traditionally it is having the flok claims like useful in treatment of rheumatism, insecticidal,

antimicrobial, anticancer, tranquillizer, tonic, febrifuge, expectorant and diuretic properties.

Material and Methods

Plant material

Fresh leaves of *Vitex negundo* collected in the month of August to September from Amravati, Maharashtra and authenticated from Prof. Dr.Marathe (Taxonomist), Botany Department, Vidharbha Institute of Humanities and Science, Amravati, Maharashtra. The fresh leaves of *Vitex negundo* were dried under shade & powdered in a mixer grinder. The powder leaves packed in a paper bags & stored in air tight container until use.

Preparation of extracts

The powdered leaves material was first defatted with petroleum ether and further extracted with benzene, chloroform, ethanol, water-ethanol (50:50) and water .Cold Maceration technique used for water extract. Qualitative phytochemical analysis of all extracts was performed to know chemical constituents of extracts.[4-5]

Antimicrobial Study[6]

Microorganisms: All the strains of micro- organism were obtained from National chemical Laboratory; Pune. Table 1 is showing Gram-Positive, Gram-negative and fungal microorganisms used for the study.

Table 1: Gram-Positive, Gram-negative and fungal microorganisms.

S. No.	Name of micro organism	CODE
1.	*Staphylococcus aureus*	NCIM 2079
2.	*Proteus vulgaris*	NCIM 2813
3.	*Bacillus subtilis*	NCIM 2063
4.	*E.coli*	NCIM 2065
5.	*Pseudomonas aerugenosa*	NCIM 2036
6 7.	*Aspergillus niger* *Aspergillus flavon*	NCIM 545 NCIM-610
8.	*Candida albicans*	NCIM 3100

Antibacterial assay

*Agar-well diffusion method***:** The agar diffusion method was used to screen the antibacterial activity of various extract of leaves of. *Vitex negundo* . 0.2 ml of each of the seeded broth containing 10^{-7} test organisms was inoculated on the plates of solidified agar and spreaded uniformly. Then eight wells were cut in the agar layer of each plate with an aluminum bore of 6mm diameter. In every plate 6 different extracts of concentration 20 mg/ml dissolved in DMSO were added while in 7th and 8th well standard tetracycline and control DMSO was added. Then all plates were incubated at 37^0C ± 1 for 18 hrs. After the incubation period the mean diameter of the zone of inhibition in mm obtained around the well was measured which has been shown in Table 2.

*Antifungal assay***:** Anti-fungal study was carried out through same procedure as used in antibacterial study only difference was media used for antifungal study was Sabouraud dextrose agar media (SDA MEDIUM).[6, 7] Results are shown in Table no.2.

Determination of Minimum Inhibitory Concentration (MIC)

*Two fold serial dilution method***:** The test was carried out individually for different microorganisms with respect to the benzene, water-ethanol (50:50) extract which shows comparative more effective result than other solvent on bacteria. This testing was done in the seeded broth by two-fold serial dilution technique. The solutions of different extracts were prepared of concentration 20 mg/ml in DMSO. For both Gram positive and Gram-negative organism a series of 7 assay tubes for concentrations i.e. 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 mg/ml for each extract were used. While standard tetracycline concentration was taken as 5, 2.5, 0.625, 0.3125, 0.15625, 0.078072 mg/ml. One positive control for each microorganism was prepared by adding nutrient broth with respective microorganism inoculums. To each concentration test tube required volume of sterile nutrient broth and inoculums was added and kept for 24 hours incubation at 37^0C. After incubation period the growth of microorganism by considering turbidity was measured by using turbidometer [8] .Results are shown in Table 3.

Results and Discussion

Phytochemical investigations shows that *Vitex negundo* leaves contain essential oil and a component of the oil includes α-pinene, camphene, caryophyllene, citral, glycosides like negundoside, nishinadaside and other hydroxybenzoylmussaenosidic acid derivatives. The flavonoids reported from the leaves includes 5-hydroxy 3, 6, 7, 3', 4'-pentamethoxy flavone and 3, 5-dihydroxy-6, 7, 3', 4'-tetramethoxyflavonal.A new furanoerimophilane aldehyde have also been reported from leaves [1]

Table 2: Zone of inhibition in mm (Including bore diameter 6mm).

Extracts Microbial Strain 20mg/Ml	PT	BE	CH	ET	WT	WT:ET (50:50)	STD
Gram +ve							
Staphylococcus aureus	**8 ± 1.0**	**19.33 ± 0.58**	**14.331 ±1.53**	**16.33 ± 1.53**	**15 ±1.0000**	**18.66 ±0.5773**	22 ±1
Bacillus subtilis	**10 ± 1.0**	**17.33 ±2.08**	**16 ± 1.0000**	**16.33 ± 0.58**	**17.33 ±1.1547**	**19 ±1.0**	24.33 ±0.5
Gram –ve							
E. Coli	**9.33 ± 0.58**	**18.33 ±0.58**	**13 ± 1.0000**	**13± 1.0**	**13.66 ±0.5773**	**19 ±1.0**	23 ±1.7

P. aerugenosa	**9.33 ± 0.5773**	**17 ± 1.0**	**12.33 ± 2.0816**	**16.33± 1.1547**	**15 ± 1.00**	**18.33 ±0.5773**	24 ±1
Proteus vulgaris	**10 ± 1.0**	**17 ±1.00**	**14 ± 1.0000**	**17 ±1.0000**	**16 ± 1.00**	**17.66 ±0.5773**	24.33 ±0.5
Fungi							
Aspergillus niger	**15 ±1.15**	**19.33 ±0.57**	**14.66 ± 0.57**	**14.66 ±1.5**	**19.33 ±0.57**	**18.33 ±0.57**	28.33 ±0.57
Candida albicans	**12 ±1**	**20.33 ±0.57**	**16.33 ±1.5**	**16 ±1**	**17.66 ±0.57**	**17.33 ±0.57**	27 ±1
Aspergillus flavon	13 ± 1	19.33 ± 0.1	15.66 ±0.5	15 ± 2	19.33 ±1.52	18.33 ±0.57	29 ±1

Note: PE: Pet ether extract, BE: Benzene extract, CH: Chloroform extract, ET: Ethanol extract, WT: Water extract, WT: ET (50:50), STD: Standard drug

Antimicrobial studies by agar well diffusion method has shown that all extracts of leaves of *Vitex negundo* has considerable anti bacterial and antifungal activity against all microbial strains. Though all extract were found effective against bacteria and fungi, benzene and water-ethanol (50:50) extracts showed maximum inhibition against Gram +ve and Gram –ve bacteria. Petroleum ether extract showed less inhibition. MIC of water extract in average on both Gram +ve and Gram –ve was found to be 2.5 mg/ml while MIC of benzene and water extract was found to be in a range 2.5 to 5 mg/ml.

In anti-fungal study benzene and water: ethanol (50:50) extract showed good anti fungal activity. MIC of water: ethanol (50:50) extract on *A.niger, A.flavons, candida albicans* was found to be 2.5, 5, 10 mg/ml respectively and MIC of benzene extract was found to be 5, 5, 10mg/ml respectively .

Table 3: Results of Minimum inhibitory concentration.

Extracts / **Microbial Strain**	**BE**	**WT**	**WT:ET (50:50)**
	(mg/ml)		
Staphylococcus aureus	2.5	5	2.5
Bacillus subtilis	2.5	5	5
E. Coli	1.25	2.5	2.5
P. aerugenosa	5	5	2.5
Proteus vulgaris	5	10	2.5
Aspergillus niger	5	2.5	5
Candida albicans	10	10	10
Aspergillus flavon	10	5	5

Conclusion

As *Vitex negundo* belonging to family Verbenaceae is already a well known herb for its anti-inflammatory activity and from the above study we can also conclude that it also exhibits good antimicrobial and antifungal activity against various bacterial and fungal strains.

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