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THE BACTERICIDAL ACTION OF THE EXTRACTS OF *Vitex nigundo* (NIRGUNDI) LEAVES AGAINST THE DERMATOPHYTES

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ABSTRACT

The authors investigated the bactericidal action of *Vitex nigundo* (Nirgundi), an endemic aromatic shrub against the dermatophytic bacteria *Staphylococcus aureus* and *Streptococcus pyogenes* using disc diffusion method. The different extracts of the leaves of *Vitex nigundo* have been found to inhibit the growth of bacterial strains. Petroleum ether and alcoholic extracts revealed that the moderate bactericidal action but the aqueous extract showed that the bactericidal action only at higher concentration.

KEYWORDS: Bactericidal; *Vitex nigundo,* dermatophytes*, Staphylococcus aureus, Streptococcus pyogenes*

Vitex nigundo, a common aromatic shrub, up to 4.5 meter in height or sometimes a small, slender tree with quadrangular branch lets, is found all over India. Its local name is Nirgundi. Local people use the genus *Vitex* belongs to family Verbenaceae, in folk medicine. It often occurs gregariously in wastelands and is widely planted as a hedge plant along the roads and fields. The leaves are usually with five leaf lets (rarely three) which are palmately arranged. It is also found in Afghanistan, Burma, China, Malaysia, Pakistan, Philippines, Sri Lanka and Tropical Africa. The plant is used as commercial drug in the indigenous system of medicine. The leaves of *Vitex nigundo* are reported to possess pesticidal, antifungal, antibacterial properties (Chopra et al., 1956) and anti-inflammatory and analgesic activities (Dharmasiri et al., 2003). Though almost all plant parts are used, the extracts from leaves and the roots is the most important in the field of medicines and is sold as drugs as well as the leaf extracts is used in Ayurvedic and Unani systems of medicines (Chadha, 1976). This paper describes the comparative study of the antibacterial properties of leaf extracts of *Vitex nigundo* and attempts were made to find out the sensitivity of all the extracts against the test microorganisms *Staphylococcus aureus* and *Streptococcus pyogenes*.

MATERIALS AND METHODS

Plant Materials

Mature fresh leaves (MFL) of *Vitex nigundo* were collected in February 2012, at Behra Baba Ghat, near the bank of river Betwa, Vidisha, M. P., India and authenticated by Dr. S. K. Jain, Department of Botany, S. S. L. Jain P. G. College, Vidisha, M. P., India. Voucher specimen has been deposited in the Herbarium of the Department of Botany, St. Mary's P. G. College, Vidisha, M. P., India.

Preparation of Extracts

Five hundred grams of MFL were air dried at room temperature and extracted with several solvents according to polarity i. e. petroleum ether, ethanol and distilled water respectively (Harborne, 1984) using the Soxhlet apparatus. Each extract was first filtered through Whatman Filter Paper No. 1 to clarify and then through a 0.45 µm membrane filter. After this, an excess was evaporated under reduced pressure in vacuum evaporator. The dried crude extracts were sterilized overnight by UV radiation and then stored at room temperature in amber color glass vials.

Bacterial Strains

The test microorganisms *Staphylococcus aureus* (NCIM,2079) and *Streptococcus pyogenes* (NCIM,2608) were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune, India.

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Preparation of Concentration and Antibiotic Discs

All the three crude extracts each 100 mg, were dissolved in 1 ml of dimethyle sulphooxide (DMSO) and prepared the different concentrations. The following doses were prepared to observe antibacterial activity in vitro 20 mg ml^{-1}, 40 mg ml^{-1}, 60 mg ml^{-1}, 80 mg ml^{-1} and 100 mg ml^{-1} by using the standard formula.

$$\text{X ml of the stock solution} = \frac{\text{Desired concentration X Final volume}}{\text{Concentration of stock solution}}$$

Where,

X ml of the stock solution = Quantity of the stock solution to make the desired concentration.

Desired concentrations = 20 mg ml^{-1}, 40 mg ml^{-1}, 60 mg ml^{-1}, 80 mg ml^{-1} and 100 mg ml^{-1}

Final volume = 1 ml.

Concentration of stock solution = 100 mg ml^{-1}

These concentrations were filtered by using membrane (pore size 0.47 µm) and the discs of 6 mm diameter (Sterile blank, HiMedia) were impregnated into the final concentration of the each extracts i. e. 20 mg ml^{-1}, 40 mg ml^{-1}, 60 mg ml^{-1}, 80 mg ml^{-1} and 100 mg ml^{-1}. The final impregnated discs used for the sensitivity test were from 20 mg $disc^{-1}$ to 100 mg $disc^{-1}$. These impregnated discs were dried in incubator at 37 ºC for 18 24 hours.

Antibacterial Assay

For the sensitivity test, disc diffusion method of Bauer et al, 1966 was used. Firstly, the liquid broth cultures of *Staphylococcus aureus* and *Streptococcus pyogenes* at log phase, were used as inoculums for spreading onto Mueller Hinton agar (pH 6.8 - 7.2) (HiMedia) plates. Sterile impregnated discs (20 mg $disc^{-1}$ to 100 mg $disc^{-1}$) were placed on the petri plates. Discs of chloramphenicol (30 g $disc^{-1}$, HiMedia), ciphalothin (30 g $disc^{-1}$, HiMedia) and gentamycin (30 g $disc^{-1}$, HiMedia) were used, as a comparative and positive control and blank disc impregnated with DMSO were used as a negative control. All test plates were incubated at 37˚C for 24 hours and the diameter of zones of inhibition were measured in mm.

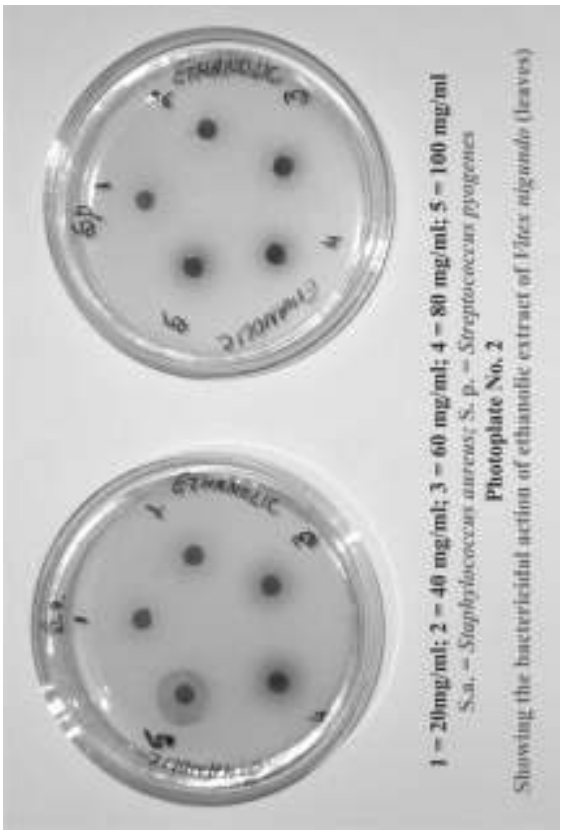
RESULTS

Table,1 shows the growth inhibition produced by leaf extracts of *Vitex nigundo* toward two bacterial strains. Inhibition zones were measured in mm at 24 hours with the size of disc (6 mm) after incubation. The results indicated that all extracts completely inhibited the growth of both the bacterial species except the aqueous extract assayed at the highest dose (Photoplate No. 1, 2, 3 and 4). Growths of both the bacteria were 100 % inhibited at 20 mg ml^{-1} in all extracts, except in aqueous extracts, which shows the antibacterial activity only on higher concentration i. e. 80 mg ml^{-1} and 100 mg ml^{-1} for *Staphylococcus aureus* and 100 mg ml^{-1} for *Streptococcus pyogenes.* DMSO showed no activity against any of the bacterial strain tested, whereas chloramphenicol, ciphalothin and gentamycin showed the activity of all the tested strains.

DISCUSSION AND CONCLUSION

The discovery, development, and clinical use of antibiotics during the 20th century have decreased substantially the morbidity and mortality from bacterial infections. The antibiotic era began with the therapeutic application of sulfonamide drugs in the 1930s, followed by a golden period of discovery from approximately 1945 to 1970, when a number of structurally diverse, highly effective agents were discovered and developed. However, since the 1980s the introduction of new agents for clinical use has declined, reflecting both the challenge of identifying new drug classes and a declining commitment to antibacterial drug discovery by the pharmaceutical industry. The same period with a reduced rate of introduction of new agents has been accompanied by an alarming increase in bacterial resistance to existing agents, resulting in the emergence of a serious threat to global public health. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics (Chopra et al., 1997). Plant extracts are generally isolated from non woody plant material by distillation methods, usually steam or hydro distillation, and are variable mixtures of principally terpenoids and a variety of low molecular weight aliphatic hydrocarbons, nitrogen and sulphur containing compounds, coumarins and homologues of phenylpropanoids. Investigations into the antimicrobial activities, mode of action and potential uses of plant extracts have regained momentum. There appears to be a revival in the use of traditional approaches to protecting livestock and food from disease, pests and spoilage in industrial countries. This is especially true in regard to plant extracts and their antimicrobial evaluation, as can be seen from the comprehensive range of organisms against which plant extracts have been tested (Agrawal et al., 2012a; Agrawal et al., 2012b; Agrawal et al., 2007; Sumathi and Parvathi, 2010).

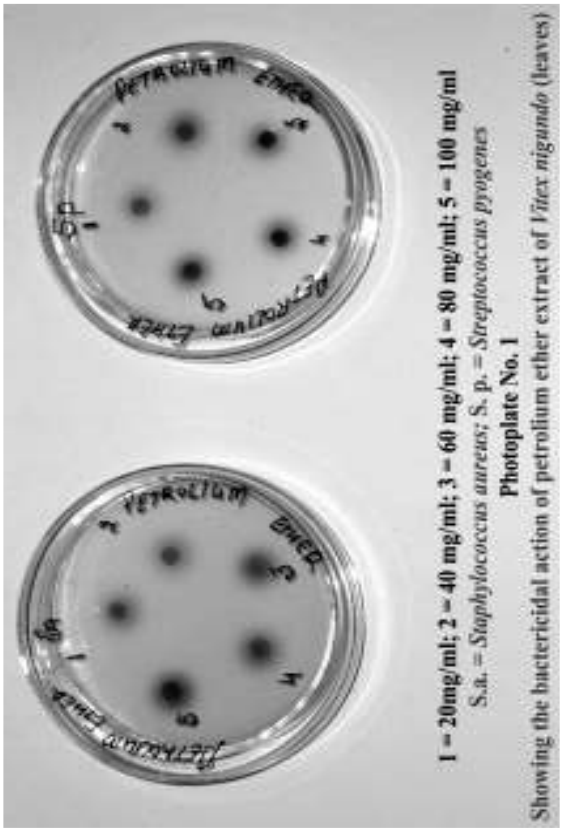
From the results there is variation in the degrees of antibacterial activities of the extracts on the isolates. The



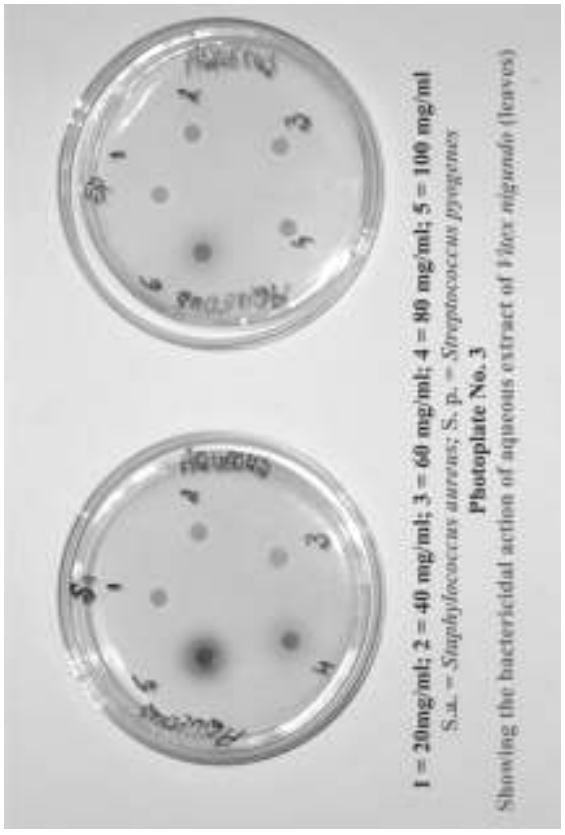
1 = 20mg/ml; 2 = 40 mg/ml; 3 = 60 mg/ml; 4 = 80 mg/ml; 5 = 100 mg/ml
S.a. = *Staphylococcus aureus*; S. p. = *Streptococcus pyogenes*
Photoplate No. 2
Showing the bactericidal action of ethanolic extract of *Vitex nigundo* (leaves)

Control
1- Chloramphenicol
2- Ciphalothin
3- Gentamycin
4- DMSO

Photoplate No. 4 Showing the bactericidal actions of the Controls



1 = 20mg/ml; 2 = 40 mg/ml; 3 = 60 mg/ml; 4 = 80 mg/ml; 5 = 100 mg/ml
S.a. = *Staphylococcus aureus*; S. p. = *Streptococcus pyogenes*
Photoplate No. 1
Showing the bactericidal action of petrolium ether extract of *Vitex nigundo* (leaves)



1 = 20mg/ml; 2 = 40 mg/ml; 3 = 60 mg/ml; 4 = 80 mg/ml; 5 = 100 mg/ml
S.a. = *Staphylococcus aureus*; S. p. = *Streptococcus pyogenes*
Photoplate No. 3
Showing the bactericidal action of aqueous extract of *Vitex nigundo* (leaves)

Table 1: The bactericidal action of the various extracts of *Vitex nigundo* and control

Extracts	Growth of Inhibition Zone (mm)[a] in different concentrations (mg ml^{-1}) of extracts									
	Staphylococcus aureus					*Streptococcus pyogenes*				
	01[b]	02	03	04	05	01	02	03	04	05
Petroleum ether	8.5[c]	9.4	10.2	11.7	12.0	8.1	9.0	9.8	10.7	11.4
Ethanolic	10.2	11.5	12.9	14.4	16.1	9.5	10.7	12.0	13.4	14.1
Aqueous	---[d]	---	---	7.4	8.9	---	---	---	---	7.2
Chloramphenicol[e]	20.9					17.5				
Ciphalothin	17.7					15.2				
Gentamycin	19.4					16.7				
DMSO	---					---				

[a] Each value is mean of the three replicates.
[b] 1 = 20 mg ml^{-1}, 2 = 40 mg ml^{-1}, 3 = 60 mg ml^{-1}, 4 = 80 mg ml^{-1}, 5 = 100 mg ml^{-1}.
[c] ± 0.2 mm.
[d] --- = No zone of inhibition.
[e] Chloramphenicol (30 g disc^{-1}), Ciphalothin (30 g disc^{-1}), Gentamycin (30 g disc^{-1}).

variation is presumed to be due to different active compounds present in these plants. Ethanol extracts of *Vitex nigundo* showed more antibacterial activity against *S. aureus* than *S. pyogenes*. There are number of naturally occurring compounds called secondary metabolites that possess plant protection properties. These compounds are effective against bacteria and showed the antibacterial activity (Ragasa et al., 1999). For this reason, three different extracts from leaves of *Vitex nigundo* were tested for antibacterial activity. The antibacterial assay showed that the various extracts of leaves of *Vitex nigundo* inhibited the growth of *Staphylococcus aureus* and *Streptococcus pyogenes* with good zone of inhibition. Ethanolic extract of *Vitex nigundo* were more effective as compared to other two extracts.

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