

IN-VITRO ANTIMICROBIAL SCREENING OF LEAF AND STEM EXTRACTS OF *VITEX PEDUNCULARIS* WALL. EX SCHAUER

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ABSTRACT

Leaf and stem extracts of *Vitex peduncularis* Wall. ex Schauer., were screened for antimicrobial activity using different organic solvents such as methanol, chloroform and water against fourteen bacterial pathogens such as *Salmonella typhi*, *Salmonella typhimurium*, *Shigella flexneri*, *Shigella boydii*, *Pseudomonas* sp., *Enterococcus* sp., *Enterobacter aerogenes*, *Escherichia coli*, *Chromobacterium violaceum*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella* sp., and six fungal pathogens such as *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Candida* sp., *Rhizopus* sp., and *Sclerotium* sp. It was observed that the methanolic extracts of both leaf and stem showed maximum inhibitory response against all the test pathogens which are known to be associated with Urinary Tract Infections. Maximum antibacterial activity was found in methanolic extract against *S. aureus* (34.1mm). Maximum antifungal activity was observed against *Candida* and *Rhizopus* sp. showing a clearance zone of inhibition of 26.2 and 30.1 mm, respectively. A control and standard set of experiment was also carried out with the standard antibiotic discs. From the result, it was concluded that both parts of the plant was found to possess antibacterial and antifungal properties apart from numerous ethno medicinal uses. Hence, efforts should be made for detail investigation on pharmacological aspects and clinical trials to evaluate the efficacy of the potent drug.

Keywords: Antimicrobial activity, *Vitex peduncularis*, Organic solvents, Urinary Tract Infections.

INTRODUCTION

India is rich in medicinal plants and over 75% of the folk population still relies on traditional knowledge and use of herbal remedies for curing various ailments (1). Plants are known to produce certain bioactive molecules which react with other organisms in the environment and in turn cause the inhibition of bacterial or fungal growth (antimicrobial activity). Medicinal plants that have been traditionally used omit to produce a variety of compounds with known therapeutic properties (2, 3). The demand for more and more drugs from plant sources is continuously increasing; therefore it is essential for a systematic evaluation of plants used in traditional medicine for various ailments.

The genus *Vitex* belonging verbenaceae family includes more than 270 species, predominantly shrubs and trees and is restricted to tropical and subtropical regions. The plants of this genus has a plethora of ethanopharmacological uses and has been used to treat a range of human ailments related to insects, bacteria, fungi, snakes and poisonous spiders and diseases associated with menstruation and gynecological problems. Traditionally, the boiled bark extract of *Vitex peduncularis* is used as a drink to treat joint ache. According to Suksamran and Bheemsankarrao, leaves of *Vitex peduncularis* contain compounds like peduncularaside, iridoid anguside, vitexin, triterpenoids and flavonoids which act as anti-inflammatory properties (4, 5). It is also known to promote cardiovascular health by improving blood and nutrient flow to the heart muscles. Though the traditional system of medicine has a long history of use but they lack the scientific documentation, particularly in light of modern scientific knowledge (6).

A very little work has been carried out on the antimicrobial activity of *Vitex peduncularis*. Hence, in the present study, an attempt has been made to evaluate the antimicrobial activity of chloroform, methanol and aqueous extracts against various human pathogenic bacteria and fungi.

METHODS AND MATERIALS

Collection of Plant sample

The plant material such as leaves and stem of *Vitex peduncularis* were collected from Suruguda village, Tutamala forest range, Lefripada, Sundargarh district, Orissa and its identity was confirmed through consulting the herbarium at Institute of Minerals and Material Technology (RRL), Bhubaneswar following "The flora of Orissa", Volume III (Saxena and Bramham, 1995) (7).



Fig. 1: Flowering twig of *Vitex peduncularis*

Preparation of plant extract

The collected samples were washed with distilled water properly for removing adhered soil and other particles and shade dried for about 7 to 10 days. The dried material was finely powdered and stored. Then the powder was subjected for Soxhlet extraction using solvents such as water, methanol and chloroform. The extracts were concentrated and dried in a rotary evaporator and then placed in a desiccators and was allowed to dry completely. Once completely dry, the extracts were used for antimicrobial assay.

Test pathogens

Various bacterial pathogenic strains such as *Salmonella typhi*, *Salmonella paratyphi* A, *Salmonella paratyphi* B, *Salmonella typhimurium*, *Shigella flexneri*, *Shigella boydii*, *Pseudomonas* sp., *Enterococcus* sp., *Enterobacter aerogenes*, *Escherichia coli*, *Chromobacterium violaceum*, *Staphylococcus aureus*, *Proteus mirabilis* and *Klebsiella* sp. and fungal pathogens such as *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Candida* sp., *Rhizopus* sp. and *Sclerotium* sp. were collected from Kalinga Institute of Medical Science, Bhubaneswar and maintained in Nutrient agar slant and Potato Dextrose agar slant for bacteria and fungi, respectively.

Antimicrobial assay

Preliminary screening of the extracts was carried out by disc diffusion method (8). Freshly grown liquid culture of the test

pathogens were seeded over the nutrient agar plates with a sterile swab. Sterile filter paper discs were soaked with different concentration of extracts of individual solvents and were placed on the plates at equidistance. Then the plates were incubated at 37°C for 18-24 hrs. Clearance zone formed around the discs indicates a positive antimicrobial activity and were measured. Each experiment was carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the antibacterial activity of the extracts.

RESULTS AND DISCUSSION

Preliminary screening of antimicrobial activity was evaluated by using disc diffusion methods against fourteen human pathogenic bacteria and six pathogenic fungi were presented in the Figure 1-4.

During antibacterial activity test of leaf, methanol extract was found to possess maximum activity than other two extracts. Methanol extract was found to have maximum activity against *S. aureus* (34.1mm) whereas minimum activity was found against *S. flexneri* (12.9mm). In chloroform extract, maximum activity was found

against *Klebsiella* sp. (25.1mm) whereas minimum activity was found against *S. paratyphii* A (12.1mm). In case of aqueous extract of leaf, maximum activity was found against *S. paratyphii* A (15.6mm) whereas minimum activity i.e. 7.8mm against *S. boydii*. Aqueous extract was found to be less active than other two extracts. In case of stem, antibacterial activity was found to be maximum against *Enterobacter aerogenes* (25.9mm) and least against *Klebsiella* sp. i.e. 15.3mm in case of methanol extract. In chloroform extract, maximum i.e. 22.6mm and minimum i.e. 14.5mm was found against *S. typhimurium* and *Pseudomonas* sp. respectively. No activity was observed against *S. paratyphii* A and *S. paratyphii* B. In aqueous extract, maximum and minimum activity has been observed against *S. paratyphii* A (15.6mm) and *S. boydii* (7.8mm), respectively. The leaf extract was found to have more antibacterial activity than stem extracts of *Vitex peduncularis* (Figure.1). According to many workers, a very low or no antibacterial activity of the *V.negundo* was found against *B.subtilis*, *S.aureus*, *S.epidermidis*, *S.typhimurium*, *Proteus vulgaris*, *E.coli*, and *P.aeruginosa* (9, 10, 11, 12).

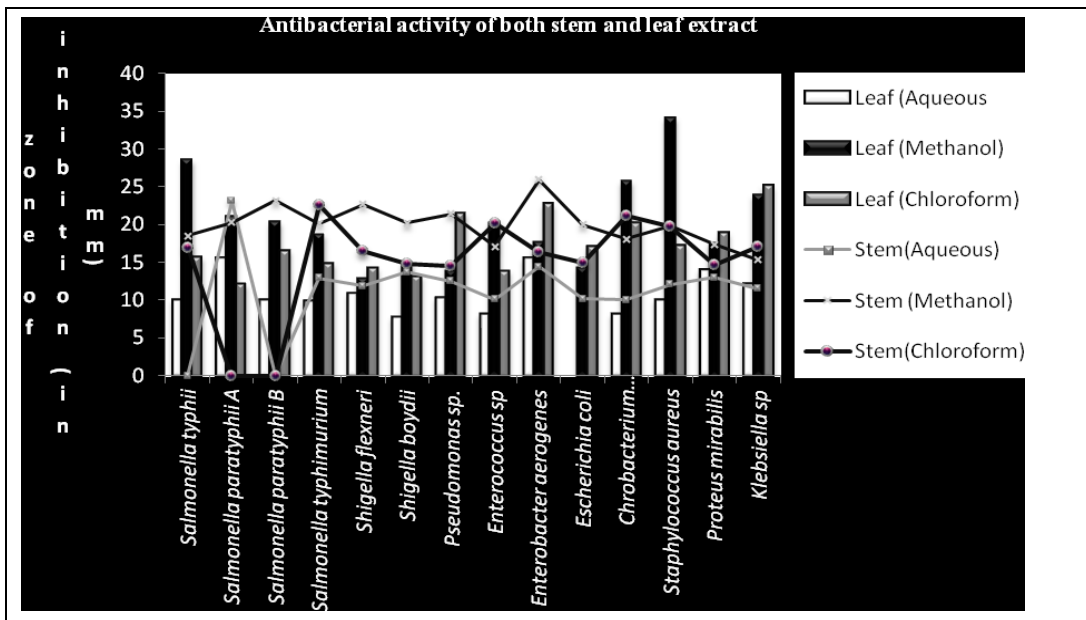


Fig. 1: Inhibition zone of both leaf and stem extract (methanol, aqueous, chloroform) of *Vitex peduncularis*

Antifungal activity was evaluated against six human pathogenic fungi as shown in Figure 2 and 3. The response shown by pathogenic fungi was very clear and distinct, the *Candida* and *Rhizopus* species being most sensitive showing clear zone of inhibition of 26.2 and 30.1 mm, respectively in both methanol and chloroform extract. Similarly, from figure 3, it can be concluded

that the methanolic extract of stem show maximum activity against *Aspergillus flavus* compared to other fungal strains and in case of chloroform extract, the antifungal activity was maximum in *Rhizopus sp.* and *Sclerotium sp.* which constitutes about 18 %. No antifungal activity was observed in case of aqueous extract of both leaf and stem.

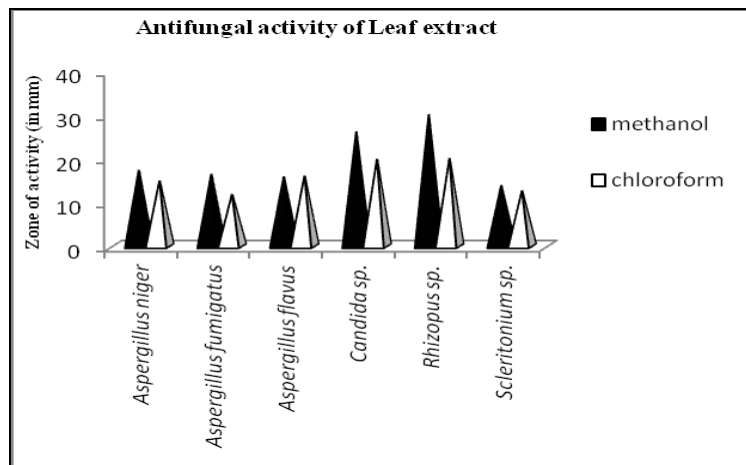


Fig. 2: Antifungal activity of leaf extract of *Vitex peduncularis*

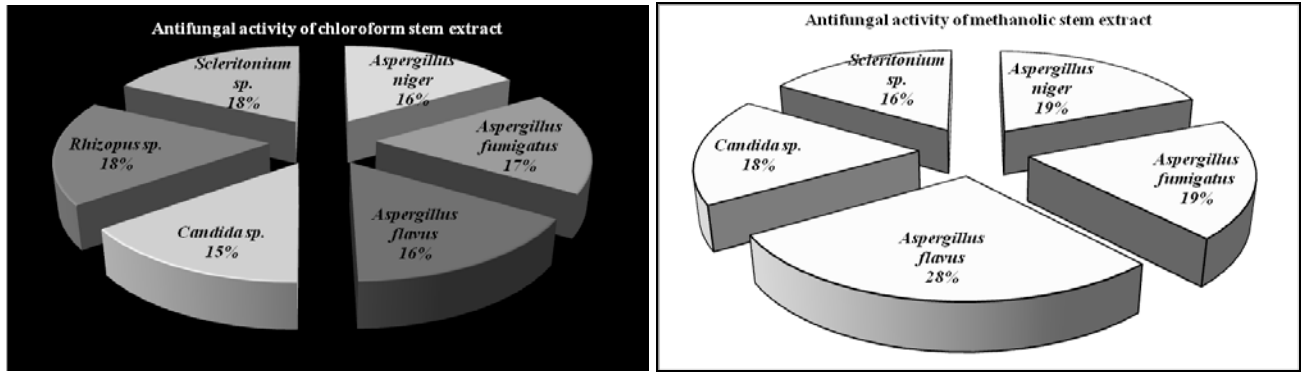


Fig. 3: Antifungal activity of stem extract (methanol and chloroform) of *Vitex peduncularis*

Antibacterial activity of the standard Gentamycin disc against the bacterial pathogens has been carried out. Some pathogens showed better response to the extract than the standard antibiotic

Gentamycin e.g., *Staphylococcus aureus* showed a zone of inhibition of 34 mm in response to methanolic extract of leaf while only 23 mm was seen in response to Gentamycin as shown in the Table 1.

Table 1: Zone of inhibition shown by different bacteria in response to Gentamycin

Bacterial pathogens	Zone of inhibition (in mm)
<i>Enterococcus sp.</i>	23
<i>Shigella boydic</i>	22
<i>Chromobacterium violaceum</i>	30
<i>Proteus mirabilis</i>	7
<i>E.coli</i>	10
<i>Shigella flexineri</i>	20
<i>Staphylococcus aureus</i>	23
<i>Enterobacter aerogens</i>	24
<i>Salmonella paratyphii A</i>	27
<i>Salmonella paratyphii B</i>	17

From the Figure 4 it can be concluded that the antibacterial activity of both leaf and stem was higher as compared to antifungal activity. In methanol extract, *Enterobacter aerogens* and *Staphylococcus aureus* showed the highest activity of 7% and 8% of the total organism screened against the leaf and stem extract, respectively.

Hence to overcome the problem of antibiotic resistance ethnic medicinal plants have been extensively studied as an alternative treatment for diseases due to their ability to produce a variety of compounds of known therapeutic properties and much attention has been paid to plant extracts and their biologically active compounds(13, 14,15).

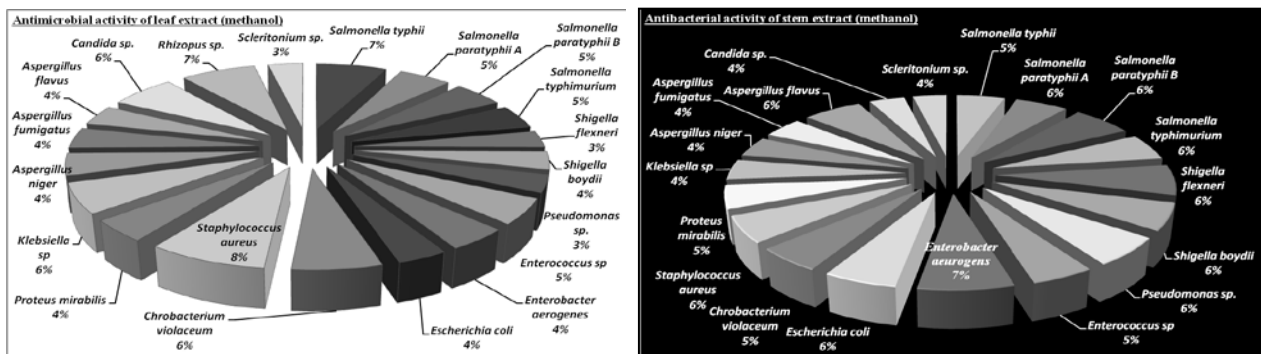


Fig. 4: Antimicrobial activity of leaf and stem extract (methanol) of *Vitex peduncularis*

CONCLUSION

From the result it can be concluded that the metanolic extract of both leaf and stem shows maximum activity against *Staphylococcus aureus* and *Enterobacter aerogens*, respectively. Similarly, maximum antifungal activity was found against *Candida* and *Rhizopus* species in methanolic extract. Polarity of the extracting solvent greatly influences the antimicrobial property. Traditional practitioners make use of water preliminary as solvent, but our studies showed that the methanol and chloroform extract of this plant were certainly much better and powerful as compared to aqueous extract. This may due to the better solubility of their active components in organic solvents. To summarize, since both leaf and stem of the plant have shown considerable antibacterial and

antifungal properties apart from numerous ethno medicinal uses, efforts should be made for advanced studies on the pharmacological aspects and clinical trials also should be conducted to evaluate the efficacy of the potent drug among various populations.

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