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(Research Article)

In Vitro Evaluation of Antimicrobial activity of *Vitex Negundo* Leaf Extract

Jayasree Tirumalasetty[1,*], Anuradha Basavaraju[2], Chandrasekhar Nutalapati[3], Naveen Aalasyam[4]

[1,3,4] Department of pharmacology, Mamata Medical College,Khammam-507002, Andhra Pradesh, India.
[2]Department of Microbiology, Mamata Medical College,Khammam-507002, Andhra Pradesh, India.

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ABSTRACT

The main objective of this work was to study the antibacterial activity of Vitex negundo leaf extract [MEVNL] against two Gram (-) and two Gram (+) bacterial strains using agar disk diffusion. The methanol extract of Vitex negundo leaf extract [MEVNL]) was evaluated for its antibacterial activity against two Gram (-) and two Gram (+) bacterial strains using agar disk diffusion and micro dilution methods MEVNL at 2 mg/disk showed broad spectrum of growth inhibition activity against both groups of bacteria. This study revealed that the methanol extract of leaves of Vitex negundo exhibited significant antibacterial activity and may provide the scientific rationale for its popular use as antibacterial agent in folk medicines.

Key Words: *Rauwolfia densiflora,* Densiflorine, Apocynaceae, Phytochemical, Pharmacological.

INTRODUCTION

Vitex negundo is native to tropical Eastern and Southern Africa and Asia. It is widely cultivated and naturalized elsewhere [1]. *Vitex negundo* are commonly found near bodies of water, recently disturbed land, grasslands, and mixed open forests [2]. *Vitex negundo* is generally known as Negundo in India. It is also known as the five-leaved chaste tree, is a large aromatic shrub with quadrangular, densely whitish, tomentose branch lets. It is widely used in folk medicine, particularly in South and Southeast Asia. It belongs to family Verbanaceae and is found throughout India. *Vitex negundo* has been used for various medicinal purposes in Ayurveda and Unani systems of medicine. Various medicinal properties are attributed to it particularly in the treatment of anti-inflammatory, fungal diseases, antioxidant, and hepatoprotective disorders [3, 4] and as anti microbial [5, 6]. The leaves and whole plant is used as an anti-inflammatory, antiseptic, antipyretic diuretic and also as antibiotic. In our study the methanol extract of leaves of *Vitex Negundo* is evaluated for antibacterial activity against two Gram (-) and two Gram (+) bacterial strains which revealed significant antimicrobial action and may provide the scientific rationale for its popular use as antibacterial agent in folk medicines.

MATERIAL AND METHODS

The study was done during January 2012 to February 2012. The leaves of *Vitex negundo* was obtained from a vegetable garden near Khammam. The identification and authentication of the leaves was done at the department of Botany, Government Degree College. The leaves are shade dried and extraction was done with 95% ethanol by soxhelts apparatus in department of Pharmacology. The extract was dried under vacuum, stored at room temperature and protected from direct sunlight.

Phytochemical Analysis

Preliminary photochemical analysis was made for presence of alkaloid, flavonoids, Carbohydrates, glycosides, proteins and amino acids, steroids, vitamin C, fat and fixed oil [7].

Organism Used

The test organisms included four clinical isolates of *staphylococcus aureus, enterococci, E.coli* and *Klebsiella* which were used in the present study and an attempt has been made to test the in vitro antibacterial activity of *Vitex negundo* leaf extract against *staphylococcus aureus, enterococci, E.coli, Klebsiella.* Clinical isolates of these organisms isolated during the study period from pus for staphylococcus aureus and from urine for *enterococci, Escherichia coli* and *Klebsiella* were utilized for this study.

Preparation of Inoculums

The four test organisms chosen were grown in sterile peptone water over night. Then the turbidity of the test organisms was matched with Mc Farland standards.

Preparation of Agar Medium

Muller-Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing [8].

Preparation of Sensitivity Disc

Whatman No.1 filter paper was punched using paper puncher of which discs of 6.0mm in diameter was obtained.

These were placed in sterile capped bijou bottles and sterilized in a hot air oven at 160°C for one hour. Preparation of concentrate for sensitivity test was done by taking two gramms (2g) of each plant extract and dissolved in 2ml of appropriate diluents to arrive at 1.0g/ml (1000000ug/ml) which serves as stock solution [9]. From the stock four concentrations were prepared. 0.3ml was added to 0.7ml of diluents, 0.2ml to 0.8ml, 0.1ml to 0.9ml, 0.01ml to 0.99 making 1ml each. 100 sterilized discs were placed into each bottle which gave disc potencies of 3000ug/disc, 2000ug/discs, 1000ug/discs, and 100ug/disc respectively. Clinical isolates of these test organisms isolated during the study period were utilized for this study. The stock culture was maintained on nutrient agar at 4°c in a refrigerator in accordance with procedure [10].

Standardization of Inoculums

Each culture of the isolates was standardized by culturing on nutrient agar for 24hrs at 37°C. The overnight culture were diluted in normal saline (0.5 w/v) until turbidity marched with 0.5 McFarland standard to give a mean of 3.3*106 Cfu/ml [11].Standard disc diffusion method [12,13] was employed to screen for antimicrobial activity of the plant extracts. Muller Hilton agar plates were used for the inoculation of organism. The test organisms were streaked evenly on the surface of the agar plates with the use of sterilized wire loop. With the aid of sterile pair of forceps, impregnated paper discs containing the extracts of materials at different concentrations 3000µg/discs, 2000 µg /discs, 1000 µg /disc and 100 µg /disc were arranged radially and pressed slightly and firmly to the inoculated agar surface to ensure even contact. The plates were incubated at 35°C for 24hrs. The degree of sensitivity was determined by measuring the diameter in millimeter of the visible zone of inhibition of the microbial growth produced by the diffusion of the extracts [14].

RESULTS AND DISCUSSION

Table 1 shows the diameter of the zone of inhibition of the extract of the plant material. The result shows that the extract exhibited antibacterial activity with the exception of the lowest concentration of extract. Methanolic extract showed greater activity against *E. coli* (16mm) at 3000ug/ml and *Staphylococcus aureus* (15mm) at 3000ug/ml than the remaining fractions. Activity was greater against *E.coli* in all the fractions (16mm, 15mm, and 12mm) at the highest concentration than *Staphylococcus aureus* (14mm, 11mm, and 9mm). Results shows that, antibacterial activity of the extracts was enhanced by an increase in the concentration of the extracts i.e. the higher the concentration of the plant extracts the greater the zone of inhibition. Augmentin and gentamicine were the antibiotics used as the control discs against the test organisms. These antibiotics showed greater activity than the crude extracts 30mm and 27mm respectively. The bioactive compounds like alkaloid, tannin, flavonoids, glycosides and saponins may be responsible for the antimicrobial action.

Traditional medicine in developing countries uses a wide variety of natural products in the treatment of common infection [15, 16]. In India, a large number of medicinal plants occur in the wild state.

Table 1: Zone of inhibition (mm) of Methanolic leaf extract of Vitex Negundo at various concentrations

Organisms	**Concentrations (µg/disc)**				
Staphylococcus aureus	Control	3000	2000	1000	100
	27	15	13	10	NI
Enterococci	25	13	11	10	NI
Klebsiella	29	15	13	12	NI
Escherichia coli	30	16	15	12	NI

NI - No inhibition, Control Discs = Gentamicine (*E. coli, Klebsiella*), Augmentin (*Staphylococcus aureus, Enterococci*)

Herbal medicines are an important part of the culture and traditions of many developing countries and depend on herbal medicines for their health care needs [17]. Presences of the phytochemical constituents such as alkaloids, flavanoides, tannin, and phenolic compounds have been reported to be important compounds in many other medicinal plants [18, 19] .The result of the present investigations, methanol extract of leaves of *Vitex negundo,* posses these compounds which might show antibacterial activity. Previously some reports concerning the antibacterial activity of *Vitex negundo* are present, but our findings support the efficacy. In another study [20]the antibacterial activity of *V. negundo* on bark and leaf of petroleum ether, chloroform, methanol and aqueous extracts against *B.subtilis, S.aureus, S.epidermidis, S. typhimurium, P.aeruginosa*, *V.cholerae*, and *V.alginolyteus* had little activity but inhibition was measured including disc and cup that measures 6mm indicates low activity moreover less concentration of extract was taken which does not give accuracy of results. So far the antibacterial activity on *Vitex negundo* was tested by two more research workers [21, 22] resulted in negative. On the other hand, one more study [23] reported response of four strains only. The antibacterial activity of *Vitex negundo* performed by Panda *et al*.in1999 on two parts (leaf and bark), that too at low concentrations of 50 µg /ml and moreover inhibition zones have been measured including cup or disc diameter, might give improper results. In one of the recent study [24] on ethanol and methanol extracts of leaf showed inhibition activity against both Gram (+) and Gram (-) bacteria.

In our study we have taken high concentrations of *Vitex Negundo* leaf extract which exhibited antibacterial activity against all the test organisms both Gram (+) and Gram (-) bacteria . This supports the previous study.[24] The activity showed by the plant extract may be due to the presence of active compounds. The zone of inhibition produced by the test organisms indicated the susceptibility to the plant extract. It was observed that the zone of inhibition observed by the bacterial isolates varied. According to another study [25] the effect of bioactive agent varies with target species. One more study [26] also reported that the position of the zone edge (diameter of the zone of inhibition) is determined by the initial population density of the organisms, their growth rate and diffusion of the antimicrobial agents, which clearly explains the difference in the zone of inhibition observed. Augmentin and gentamicine were the antibiotics used as the control discs against the test organisms. These antibiotics are well refined industrial products so they naturally showed greater activity than the crude extract.

CONCLUSION

Vitex negundo leaf extract have shown antibacterial activity against all the test organisms. This reveals that, the plant contains some bioactive compounds which justify their traditional usage in ethno medicine. As there is upsurge of bacterial resistance to antimicrobial drugs, the plant may be used as promising candidate for drug development. There is increased zone of inhibition as the concentration of extract is increased. The exact phytochemical constituents responsible for such activity can be identified by various methods like structural elucidation techniques and by the ethano pharmacological research. Development of novel antibacterial agents is the need of the hour and if they are from a natural source they have several advantages which includes low cost of the drug and most important is prevention of multi drug resistance in pathogenic bacteria. The Ethanopharmacological research can utilize the common plants of our country for novel drug development and can improves the traditional health care system.

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Corresponding Author:
Dr. Jayasree Tirumalasetty*,
Professor and Head,
Department of pharmacology, Mamata Medical College, Khammam-507002, Andhra Pradesh, India.
Email: drtjayasreemd@gmail.com