



REVIEW OF RESEARCH

ISSN: 2249-894X

IMPACT FACTOR : 5.7631(UIF)

VOLUME - 13 | ISSUE - 1 | OCTOBER - 2023



ANTIBACTERIAL ACTIVITY OF VITEX NEGUNDO (NIRGUDI) PLANT EXTRACTS

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ABSTRACT :

Vitex negundo is commonly known as Nirgundi. It belongs to family Verbenaceae. Its plant is branched, deciduous shrub found in all parts of India. Nirgundi possess various medicinal properties. It is also well known to possess antibacterial properties. The aim of present work is to evaluate the antibacterial property of Nirgundi plant parts. The methanol extract of Nirgundi stem, leaves and flowers were investigated against *Escherichia coli* and *Staphylococcus aureus*. It was reported in the study that the methanol extract of Nirgundi plant parts possess potent antibacterial property compared with the Streptomycin as a standard. Therefore, it is suggested to develop alternative antimicrobial drugs for the treatment of infectious diseases and Nirgundi plant parts can be used more and more for commercial purpose.



KEYWORDS : Antibacterial Property, *Vitex negundo*, Nirgundi, Well Diffusion Method, *Escherichia coli* and *Staphylococcus aureus*.

1. INTRODUCTION

Microbes i.e. viruses, bacteria, parasites are present everywhere among which some are beneficial for our health and on the other hand some are harmful. The infectious diseases cause 26 per cent of annual deaths worldwide, nearly 30 per cent of 1.49 billion disability-adjusted life years (DALYs) are lost every year to diseases of infectious origin [1]. Antibiotics are the medicine that inhibits the growth or destroys the microorganisms. Because the bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents, [2] for there is a need to develop a new drug which serves the purpose.

Sushruta and *Vaghbata* had recognized the number of diseases as *Sankramaka* or infectious. They have also indicated the association of microorganisms with some such diseases. For that, so many antimicrobial drugs have been mentioned in Ayurveda text sinceages on the name of *Krumighna* or *Rakshoghna*. [3] [4]

Vitex negundu is a medicinal plant commonly known as Nirgundi. It belongs to family Verbenaceae. It is woody aromatic shrub bearing 3-5 leafs in branches. Its flower is bluish purple in color[5]. With its medicinal importance plant is also used as food crop and source of timber [6]. The plant is used in home remedies for various diseases such as cold, pneumonitis, pain etc.[7]It is used in Ayurveda as analgesic and anti-itching agent internally and externally[8]. It also has various medicinal properties such as anti-inflammatory activity, [9] [10] drug potentiating activity [11]. The leaf extract

shows anticancer activity. The fresh leaf extracts are used to relieve body joint pains. The leaf extract of *Vitex nigundu* shows significant activity against pathogenic bacteria such as *E.coli*, *Proteus* and *Pseudomonas*[12].

Resistance developed by pathogenic microorganisms against antibiotics along with their side effects are potentially toxic for host. These reasons having given rise to an urgent need of developing new antimicrobial drugs which possess less adverse effect as compare to synthetic drug, hence the present study was undertaken to evaluate antimicrobial activity of various parts of Nirgundi.

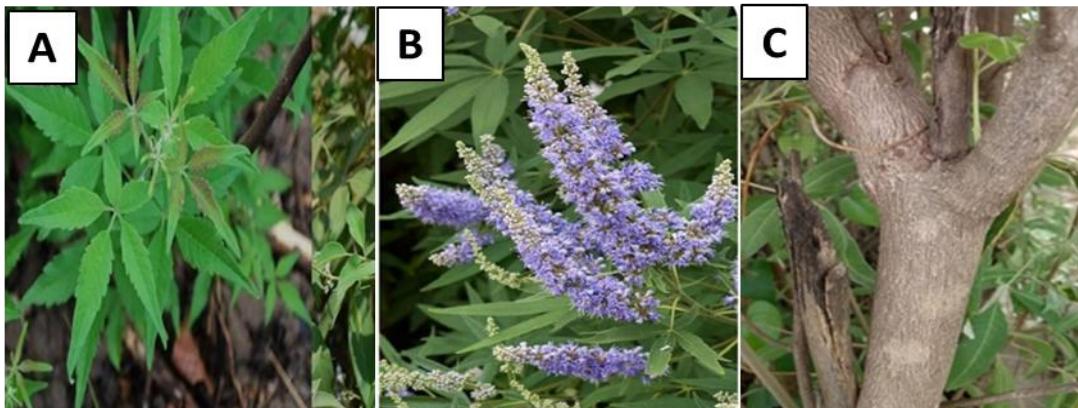


Fig. 1 Morphology of different parts of *Vitex negundu* A. Leaves, B. Inflorescence (Flowers), C. Stem (Bark)

In the present study, an attempt has been made to test methanol extract of Nirgundi stem, leaves and flowers for *invitro* antibacterial activity against two bacteria such as *Escherichia coli* and *Staphylococcus aureus* using Streptomycin as standard.

2. MATERIALS AND METHODS

2.1 Collection of sample

The sample was collected from Kandalgaon near Solapur district, Maharashtra in the summer and the authentication of the sample is done by Department of Botany, D. B. F. Dayanand College of Arts and Science, Solapur, Maharashtra.

2.2 Preparation of sample

The collected samples of stem, leaves and flowers were dried under the shade at the room temperature. The processed samples were pulverized using electric grinder. The samples were extracted independently using 100g of selected solvents like methyl alcohol in the order of highest extraction yield. The residues were recovered after the extracting with the solvents from a rotary evaporator, after that obtained extracts were suspended in the appropriate solvent for investigation.

2.3 Production of extracts

In method of solvent extraction 30 g of dried powder of stem, leaves and flowers from *Vitex negundu*, were separately extracted for 48 hours with methanol using a Soxhlet device. The extracts were filtered using Whatman filter paper No.1 and utilized to determine antibacterial activity.



Fig. 2 A. Soxlet extraction, B. Stem extract, C. Leaves extract

2.4 Antibacterial activity

Table 1: Bacterial cultures used in study (Department of Microbiology, D. B. F. Dayanand College, Solapur, Maharashtra)

| Sr No. | Bacterial Pathogens | MTCC Number |
|--------|------------------------------|-------------|
| 1 | <i>Escherichia coli</i> | ATCC 8739 |
| 2 | <i>Staphylococcus aureus</i> | ATCC 6538 |

2.4.1 Preparation of nutrient broth slants and sub - culturing of microorganisms

Agar 1g, beef extract 500mg, peptone 500mg, and NaCl 250 mg were used to make nutrient agar medium and is dissolved in 50ml distilled water, boiled and then placed inside the test tubes, which was then closed with cotton plug and autoclaved at 15 pounds pressure for fifteen minutes. The test tubes which were containing the agar nutritional medium were placed in an inclined position for 30 minutes following sterilization. Thereafter, in an aseptic setting, pure cultures of *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538), were streaked over the surface of slants and the petri dishes were incubated at 370° C for 24 hours.

2.4.2 Production of growth medium for antibacterial sensitivity test:

20gm Agar, beef extract 10gm, peptone 10gm, and NaCl 5gm were mixed together and in 1000 ml boiling distilled water to create nutrient agar medium (pH 7.2). After that it was autoclaved in an autoclave at 15 pounds of pressure (1210 degrees Celsius) for exact 15 minutes. Following sterilization, the medium was allowed to cool to 450° C. before being placed into sterile Petri plates in a sterile manner, an amount of 20 - 25 ml of media poured into each petri plate. Medium from the petri plate was then kept aside to solidify at room temperature.

2.4.3 Inoculation of suspension of microbes on agar medium:

Sterilized, cotton plugs were dipped in to each standardized isolates (turbidity is adjusted so as to get consistent growth on the Petri plates) accompanied by whole petri plate surfaces were streaked with the swab three times exactly, the plates were rotated at 60° angle during streaking. After that the inoculums were dried for 1 - 5 min while covering during entire process. Then bore was punched on the

prepared plates by using sterile well (8mm). The 100 μ l dose of standard medicine Ciprofloxacin was loaded in each bore accordingly in sterile conditions using a sterile micropipette. Plates were kept at an ambient temperature for at least 30 min and then cultured at 37° C for at least 24 hours. The diameters of the zones of inhibition were calculated with scale in millimetres.

3. RESULTS AND DISCUSSION

The plant extract was tested against gram positive *Staphylococcus aureus* and gram negative bacteria *Escherichia coli*. Generally, gram negative bacteria are resistant than gram positive bacteria [13]. For the comparison, Streptomycin drug is used as standard with zone of inhibition 18 mm

Methanolic extract of stems, leaves and flowers of *Vitex negundo* had shown antimicrobial activity against *E. coli* with zones of inhibition 25 mm, 24 mm, 26 mm respectively. Maximum antimicrobial activity against *E.coli* was exhibited by flower extract of *Vitex negundo* with zone of inhibition-26 mm.

Methanolic extract of stem, leaves and flowers of *Vitex negundo* had also shown antimicrobial activity against *Staphylococcus aureus* with zone of inhibition 18 mm, 19 mm, 19 mm respectively. Maximum antimicrobial activity against *Staphylococcus aureus* was exhibited by leaves and flowers extract of *Vitex negundo* with zones of inhibition 19 mm. These zones of inhibition for various plant parts are higher while compared with the standard Streptomycin showing remarkable and potent activity.

Several factors are known to influence the active principle present in the plant. Polarity of the extracting solvent greatly influences the antimicrobial property. The activity of plant extracts against both gram positive and gram negative bacteria may be an indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant [14][15]. Findings of the present study and previous works implicate the indication of the trial drug as a potent therapeutic agent for antibacterial property.

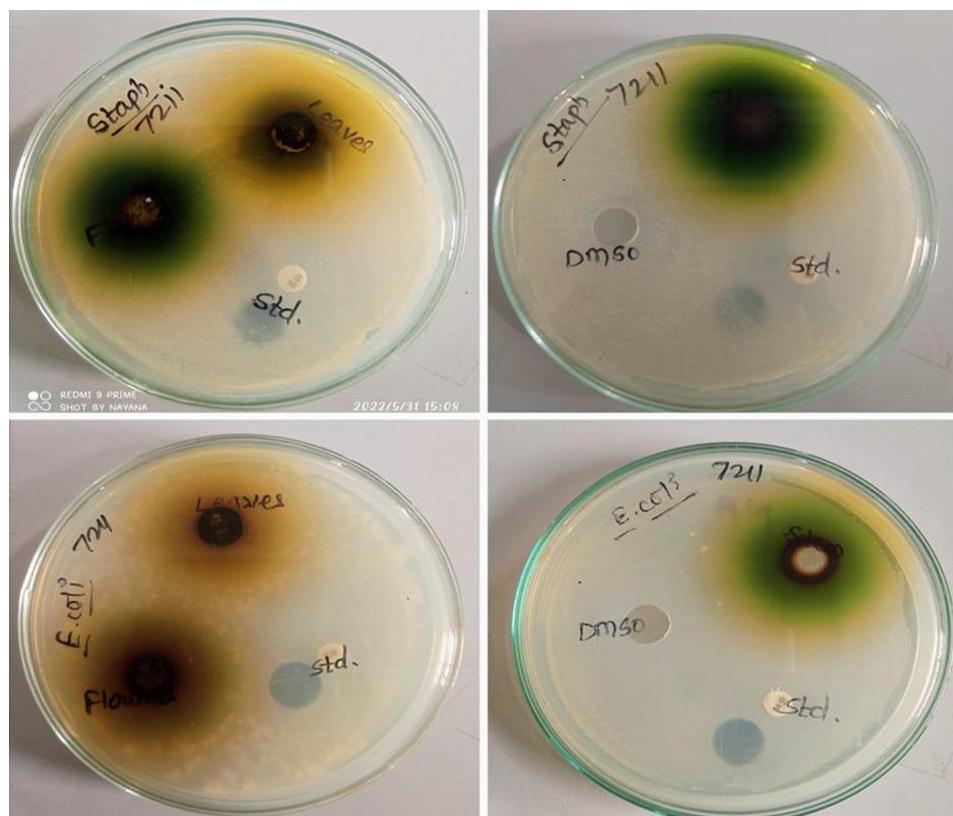


Fig. 3 Zones of inhibition

Table 2 Zone of inhibition for *Escherichia coli* and *Staphylococcus aureus*

| Sr. No. | Name of the microorganism | Diameter of zone of inhibition(mm) | | | | |
|---------|------------------------------|------------------------------------|--------|--------|--------------|------|
| | | Stem | Leaves | Flower | Streptomycin | DMSO |
| 1 | <i>Escherichia coli</i> | 25 | 24 | 26 | 18 | — |
| 2 | <i>Staphylococcus aureus</i> | 18 | 19 | 19 | 18 | — |

4. CONCLUSION

Methanol has stronger extraction capacity which could be helpful in extracting greater no of active constituents responsible for antibacterial activity. Our findings prove that, the stem, leaves and flowers of *Vitex negundo* plant have medicinal antimicrobial activities and can use against microorganism under study.

The results obtained in this study are promising which can be employed for commercial purpose. The ethno botanical use of plant origin is into existence since they were tested for their potentiality and also they were safe for human use. Thus the Sanskrit word for *Vitex negundo* i.e. Nirgundi describes its medicinal importance. "*Nirgundi shareeram rakshati roghhyah tasmat nirgund*" literally means that which protect the body from disease. Therefore it can be used as antibacterial supplement and for the development of new therapeutic agent.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Chemistry and management of D. B. F. Dayanand College of Arts and Science, Solapur, Maharashtra, India for providing funding under Mahatma Anand Swami Saraswati Research Start-Up Grant (DAYA/MAS-RSG/2021/08) and all the facilities to carry out the work.

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