

STUDY TO ASSESS THE ANTIMICROBIAL ACTIVITIES OF *AZADIRACHTA INDICA* (NEEM) PLANT EXTRACTS

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Abstract

The aim of present work is to evaluate the antibacterial property of Neem plant parts. The methanolic extracts of Neem leaves and stem were investigated for antibacterial activity against *Bacillus species* and *Escherichia coli* using agar well diffusion method. It was reported in the study that the methanol extract of Neem plant parts possess remarkable antibacterial property compared with the Streptomycin as a standard. Therefore, it is suggested that the leaf and stem extracts of *Azadirachta indica* is recommended for the treatment of human infections associated with these organisms and Neem plant parts can be used more and more for commercial purpose..

Keywords: Antibacterial Property, *Azadirachta indica*, Neem, Well Diffusion Method, *Bacillus species* and *Escherichia coli*.

1. Introduction

The biologically active compounds present in the plants which have latent for development as medicinal agents. In the developing countries, Herbal medicines already form the basis of beneficial use, however recently, there has been rise in the use of herbal medicines in the developed country also. [1] A plenty of plants reputed in traditional medicine which hold protective and therapeutic properties. [2] Therefore, plants provide an alternate approach for new treatments, as they are valued source of new molecules which may after possible chemical manipulation provide new and improved drugs. [3] The use of plant extracts in the treatment of diseases has become an important interest over the years. This is as a result of the fact that microorganisms are developing resistance to many drugs and as such created situation where some of the common and less expensive antimicrobial agents are losing effectiveness. [4]

In view of this, there is an urgent need to find the alternative to chemotherapeutic drugs in disease treatment particularly those of plants origin which are easily available and have

considerably less side effects,[5] safer, and more effective agents that can be used to fight infectious diseases. [6]

The better understanding of medicinal potentials of plants could give great scientific and medical insights towards strategic and effective approach to combat drug resistant microbes. Plants, unarguably serve as major source of food for most herbivores. It is however important that not only can plant provide the dietary requirements for living organisms, some plants are found useful in folk medicine for combating several health challenges.

Neem belongs to *Meliaceae* family, also known as a Limbo, Nim, Nimba, Medusa and Vempu. It is also called "village pharmacy" of South Asia because of its enormous medicinal properties. Every part of Neem is so useful for the treatment of human disease. The Neem (*Azadirachta indica*) is an evergreen tree, used in traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical countries in India, Africa, Central America, South America, North America and Australia. Its twigs provide a chewing stick and are widely used in the Indian sub-continent. [7] Earlier studies on Neem have showed that it contains active substances with multiple medicinal properties. [8]

Each part of Neem tree has various medicinal properties. Non woody part of Neem such as leaf, bark, oil, flowers, fruits and seed show great properties that is Antiallergic, Antifungal, Antibiotic, Antidermatic, Antibacterial, Antiinflammatory, Insecticidal, Larvicidal, Antimalarial, Antiulcer and other biological activities. [9] *Azadirachta indica* in folklore medicine for the treatment of Diabetes and show the potential role of anti-diabetic activity [10]. Aqueous extract of Neem leaf extract has a good therapeutic potential as anti hyperglycemic agent in IDDM and NIDDM as well as hypolipidemia and hypotensive activities. [11]

The ability of some plants to be used for medicinal purposes have been linked to the existence of certain chemically active substances in various parts of these plants and their extracts. These active substances are known as phytochemicals and are capable of producing specific physiological action on target organisms. [12], [13] The phytoconstituents alkaloids, glycosides, flavanoids and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens. [14]

The purpose of the present study was to investigate the antimicrobial activity of Neem plant parts against human pathogenic bacteria, including *Bacillus species* and *Escherichia coli*.

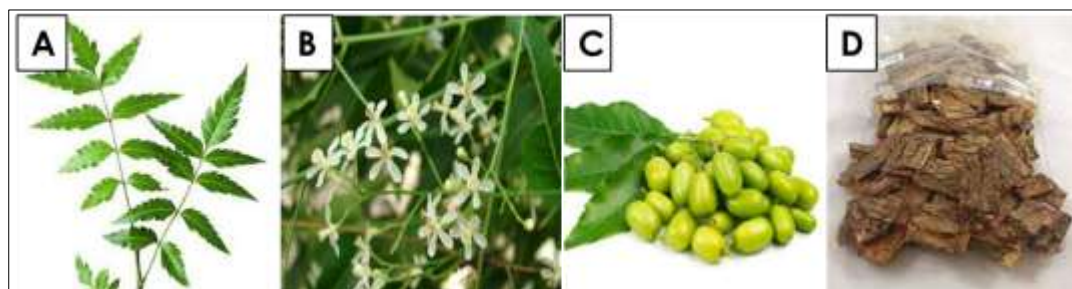


Fig. 1 Morphology of different parts of *Azadirachta indica* A. Leaves, B. Influrescence (Flowers), C. Fruits, D. Stem (Bark)

In the present study, an attempt has been made to test methanol extract of Neem leaves and stem for *in vitro* antibacterial activity against two bacteria such as *Bacillus species* and *Escherichia coli* 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 100 g 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 leaves and stem from *Azadirachta indica*, 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>Bacillus species</i>	-----
2	<i>Escherichia coli</i>	ATCC 8739

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

Agar 1g, beef extract 500 mg, peptone 500 mg, and NaCl 250 mg were used to make nutrient agar medium and is dissolved in 50 ml distilled water, boiled and then placed inside the test tubes, which was then closed with cotton plug and autoclaved at 15 pounds pressure (121°C) 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 *Bacillus species* and *E. coli* (ATCC 8739), were streaked over the surface of slants and the petri dishes were incubated at 37° C for 24 hours.

2.4.2 Production of growth medium for antibacterial sensitivity test:

20gm Agar, beef extract 10 g, peptone 10 g, and NaCl 5 g 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 (121 degrees Celsius) for exact 15 minutes. Following sterilization, the medium was allowed to cool to 45° 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 (8 mm). The 100µl 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 37⁰ 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 *Bacillus species* 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.

Methanolic extract of leaves and bark of *Azadirachta indica* had shown antimicrobial activity against *Bacillus species* with zones of inhibition 17 mm and 16 mm respectively **Fig. 3, Table 2** exhibiting similar antimicrobial activity. These zones of inhibition for various plant parts are moderate while compared with the standard Streptomycin with (zone of inhibition 28 mm) showing remarkable activity.

Methanolic extract of leaves and bark of *Azadirachta indica* had also shown antimicrobial activity against *E. coli* with zones of inhibition 19 mm and 18 mm respectively **Fig. 3, Table 2** exhibiting similar antimicrobial activity. These zones of inhibition for various plant parts are similar while compared with the standard Streptomycin with (zone of inhibition 22 mm) 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 [15][16]. Findings of the present study and previous works implicate the indication of the trial drug as a potent therapeutic agent for antibacterial property.

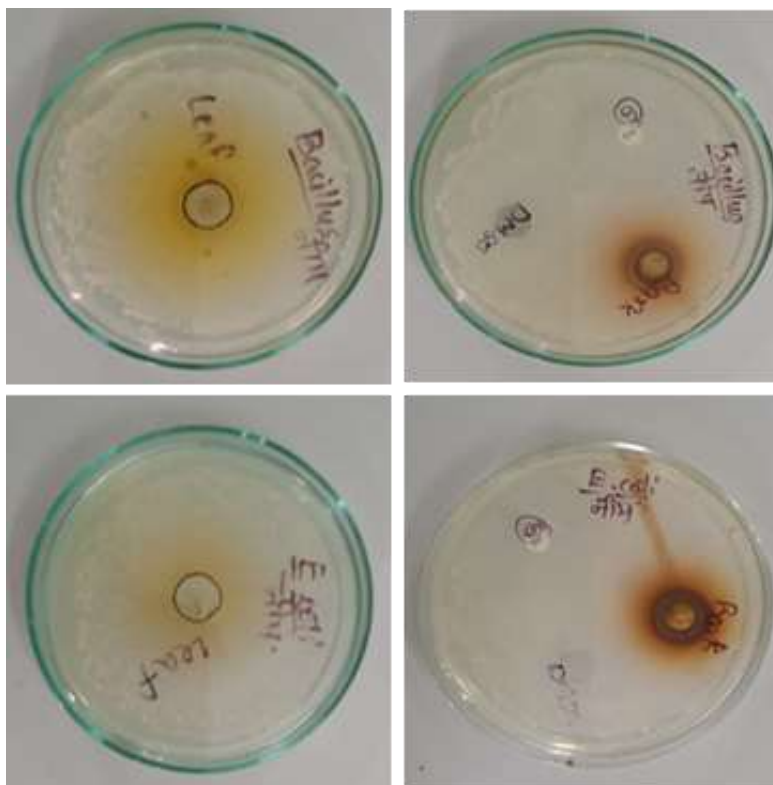


Fig. 3 Zones of inhibition

Table 2 Zone of inhibition for *Bacillus spp* and *Escherichia coli*

Sr. No.	Name of the microorganism	Diameter of zone of inhibition(mm)			
		Leaves	Bark	Streptomycin	DMSO
1	<i>Bacillus species</i>	17	16	28	–
2	<i>Escherichia coli</i>	19	18	22	–

4. CONCLUSION:

Methanol has stronger extraction capacity which could be helpful in extracting greater number of active constituents responsible for antibacterial activity. Our findings prove that leaves and stem of *Azadirachta indica* 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. Neem plant is considered as clinically effective and safer alternatives to the synthetic antibiotics. Extensive research in the area of isolation and characterization of the active principles of Neem plant is essential so that better, safer and cost effective drugs for curing various diseases and infections can be developed. 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. Therefore, it can be used as antibacterial supplement and for the development of new therapeutic agent.

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