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Research Article

IN-VITRO ANTIBACTERIAL ACTIVITY OF *AZADIRACHTA INDICA* AGAINST HUMAN PATHOGENS

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Abstract: The antibacterial activity of the plant parts extracts (stem, root, leaf, flower and whole plant) of *Azadirachta indica* was studied against *Escherichia coli, Klebsiella pneumoniae* and *Proteus vulgaris* (Gram-negative), *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive) by the agar well diffusion method. The ethanol and methanolic extracts displayed broad spectrum activity against all the test organisms but root extracts of chloroform and Petroleum ether showed no activity against Gram-negative bacteria. The antibacterial activity of the extracts was compared to the drug Tetracycline. The minimum inhibitory concentration (MIC) of the ethanol and methanol extracts of leaf and whole plant determined by the agar dilution method ranged between 1.96-19.5 and 1.96 with that of *Staphylococcus aureus* being the least. Phytochemical screening of the plant revealed the presence of tannins, alkaloids, flavonoids and saponins. The results of this study support the traditional use of *Azadirachta indica* whole plant as an antibacterial agent.

Key**words:** *Azadirachta indica* **,** Solvent extracts, Antibacterial activity, Minimum inhibitory concentration, Tetracycline

INTRODUCTION

Man always been surrounded by countless microorganisms. The disease producing microbes are playing a very important role in human life. Pathogenic microorganisms are always trying to develop resistance to the various antimicrobial agents used for their control. Therefore, the chemotherapy of infectious diseases has proved to be a continuous struggle. Scientists are always in search of new antimicrobial agents to control the ever increasing menance of the microbes. Thus it is of paramount importance for the microbiologists to develop new resistant strains. Therefore, medicinal plants are gifts of nature to cure limitless number of diseases among human beings [1]. The abundance of plants on the earth's surfaces has led to an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents [2].Researchers have shown that all different parts of the plants which include; stem, root, leaves, flowers, seeds etc possess antimicrobial property [3]. Recently several workers have reported antibacterial activities of local plants [4,5,6].

Azadirachta indica belongs to the family of Meliaceae and commonly known as Vepa in Telugu, Nim, Nimgachh in Bengali, Danujhada, Limbado, Limbra, Limdo in Guajarati, Nim, Nimb in Hindi ,Arista, Nimba, Nimbah, Picumarda in Sanskrit , Indian Lilac , Margosa tree, Neem tree in English , Bemu, Bevinamara,Bivu, Kaybevu in Kannada and Bakam, Drekh, Nim in Punjabi .The plant has been considered as a Nature's Drugstore, distributed throughout the tropics and sub tropics. It has been extensively used in Indian traditional medicine as abortifacient, analgesic, anthelminthic, antiyeast, antiulcer, antifertility, antifilarial, antifungal, antihyperglycemic, anti-inflammatory, antiviral, antimalarial, diuretic, antinematodal, antipyretic, antispasmodic, insecticidal, antispermatogenic, antitumor, hypercholesteremic, hypoglycaemic, immunomodulator. Moreover, it possesses antibacterial activity.

The plant yielded interesting compounds like alkaloids, lavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones, biologically most active compound is azadirachtin, it is actually a mixture of seven isomeric compounds labelled as azadirachtin A-Gand azadirachtin E is more effective. Other compounds that have a biological activity are salannin, volatile oils, meliantriol , nimbidin and nimbin. Present study has been designed to determine the role of plant extracts of *Azadirachta indica* both in aqueous and solvent extracts against pathogenic bacteria.

MATERIALS AND METHODS

Plant material and extract preparation:

Different parts of *Azadirachta indica* (root, stem, leaf, flower and whole plant) were collected from different localities of Bangalore and its nearby areas and washed thoroughly with distilled water. The cleaned plant parts are then allowed for the complete shade drying and then made to fine powder with a mechanical grinder and stored in an airtight container. A powdered plant parts were extracted successfully with the aqueous and organic solvents with increasing of polarity by using Soxhlet apparatus. The extraction was carried out for 24 hours at room temperature with mild shaking. The extracts were filtered and concentrated at 45^0 C using rotary vacuum evaporator. The obtained extracts were vacuum dried and used for further investigation.

Preparation of dilutions of crude extract for antibacterial assay:

The methods of Akujobi et al.[1] and Esimone et al.[8] were adopted. The crude extracts were dissolved in 30% dimethyl sulphoxide (DMSO) and further diluted to obtain of each

extracted sample of 100 mg/L concentration was used for the determination of antibacterial activity by agar well diffusion method.

Test Microorganisms:

The microorganisms such as *Escherichia coli* (MTCC-B2401), *Klebsiella pneumoniae* (MTCC-B2405) and *Proteus vulgaris* (MTCC-B1771), *Bacillus subtilis* (MTCC-B2274) and *Staphylococcus aureus* (MTCC-B1144) were obtained from Microbial type culture collection (MTCC), IMTECH, Chandigarh, India. They were re dissolved and the pure cultures sub cultured on Nutrient agar slants. They were stored at 4^0 C until required for the study.

Antibacterial assay:

The agar well diffusion method as described by Esimone et al.[8] was used in the antibacterial screening procedure. Mueller-Hinton (MH) agar (Biolab) plates were prepared using sterile 90 mm petridishes. MH agar at 48^0 C was inoculated with a MH broth culture (10^6-10^8 bacteria CFU ml^{-1}) of each bacterial species and poured over the agar plates to form a homogenous layer. Four wells (holes) were made in the plates (about 6 mm diameter) using a sterile cork borer and 50 µl of plant extract (100 mg/ml) was tested in triplicate (four wells per plate), with a Tetracycline (30 µg/ml) well as a reference or positive control; 50 µl of each solvent was also poured on agar wells and used as negative control. The plates were evaluated after incubation at 37^0 C for 24 hours after which the zones of inhibition around each well were measured. The ratio between the diameter of the inhibition zones (mm) produced by plant extracts and the inhibition zone around the well with tetracycline (mm) was used to express antibacterial activity.

Determination of minimum inhibitory concentration (MIC):

The determination of minimum inhibitory concentration of the alcoholic extracts were carried out using the agar dilution method described by Lajubutu et al.[11] Different concentrations of the extracts were prepared to give a final concentration in the range of 1.96 to 19.5 mg/ml. 2 ml of each dilution was mixed with 18 ml of Mueller-Hinton agar, poured into petridishes and allowed to set. The agar was streaked with an overnight broth culture of the test organisms and incubated overnight. The lowest concentration inhibiting growth was regarded as the minimum inhibitory concentration of the extracts.

RESULTS

The antibacterial activity of aqueous and solvent extracts (ethanol, methanol, chloroform and Petroleum ether) of different plant parts of *Azadirachta indica* (root, stem, leaf, flower and whole plant) against human pathogenic bacteria both Gram-positive and Gram-negative bacteria are presented in Table-1. The results revealed that among five pathogenic bacteria *Bacillus subtilis* and *Staphylococcus aureus* belongs Gram-positive bacteria showed higher susceptible for leaf, flower and whole plant extracts, than root and stem extracts. In Gram-positive bacteria *Staphylococcus aureus* showed maximum inhibition where as Gram-negative bacteria showed least susceptible for leaf, flower and whole plant, while root and stem extracts did not show any activity. The different solvents viz. ethanol, methanol, chloroform and petroleum ether of root, stem, leaf, flower and whole plant extracts were tested for antibacterial activity. Among the four solvent extracts revealed that ethanol and methanol extracts showed highly significant activity against both Gram-positive and Gram-negative bacteria. Other solvent extracts viz. chloroform and petroleum ether showed less significant activity when compared with tetracycline (Table-1). Among tested bacteria *Staphylococcus aureus* and *Bacillus subtilis* were sensitive to ethanol and methanolic extracts of whole plant, leaf and flower, while moderately sensitive to chloroform and petroleum ether respectively.

In microdilution, minimum inhibitory concentration (MIC) values of ethanolic, methanolic extracts of leaf, whole plant of *Azadirachta indica* against five human pathogenic bacteria were shown in Table-2. The results of the tests for minimum inhibitory concentration revealed that the *Staphylococcus aureus* and *Bacillus subtilis* (between 1.96-3.90) were lower than that of *Klebsiella pneumoniae, Proteus vulgaris* and *Escherichia coli* (13.6-19.5). This is means that higher doses of the antibacterial agent will be needed in the treatment of infections caused by *Klebsiella pneumoniae, Proteusvulgaris* and *Escherichia coli* provided they are not toxic to the tissues.

DISCUSSION

The presence of antibacterial substances in the higher plants in well established [16]. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines of it can be the base for the development of a medicine, a natural blueprint for the development of a drug[17]. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study the plant extracts by ethanol and methanol provided more consistent antibacterial activity compared to those extracted by water. The results of antibacterial activity of all plant parts of *Azadirachta indica* against the investigated bacterial strains are shown in Table-1.

The present results showed that the whole plant and leaf extracts are more effective than the flowers, stem and root extracts. This may be due to whole plant and leaves contain more number of secondary metabolites which may interfere with the antibacterial activity of the extracts. Out of the five solvents used for extraction the ethanol and methanol extracts showed the highest activity against the test organisms followed by aqueous, chloroform and petroleum ether extracts. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent ethanol and methanol extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest antibacterial activity and three extracts (ethanol, methanol and aqueous) were more active against the Gram-positive bacterial strains (*Staphylococcus aureus* and *Bacillus subtilis*) than the Gram-negative bacterial strains.

Which is conformity with earlier studies [18,19,20]. The higher resistance of Gram-negative bacterial to plant extracts of *Azadirachta indica* might have an effective permeability barrier, comprised of thick murein layer in this outer membrane, which restricts the penetration of amphipathic compounds and multidrug resistance pumps that extrude toxins across the barrier. It is possible that the apparent ineffectiveness of plant antimicrobials is largely due to the permeability barrier.

Table-1: Antibacterial activity of aqueous and alcoholic extracts of *Azadirachta indica* (well in agar method)

		Zone of inhibition(mm)				
Plant part	**Extracts**	***E.coli***	***K.pneumoniae***	***P.vulgaris***	***B.subtilis***	***S.aureus***
Root	Aqueous	-	-	-	6	8
	Ethanol	4	3	7	8	9
	Methanol	3	2	-	7	5
	Chloroform	-	-	-	4	3
	Petroleum ether	-	-	-	3	2
Stem	Aqueous	-	-	-	10	11
	Ethanol	7	6	10	12	14
	Methanol	6	5	9	10	12
	Chloroform	4	3	6	8	9
	Petroleum ether	3	-	5	6	7
Leaf	Aqueous	5	6	8	9	10
	Ethanol	12	9	16	20	24
	Methanol	10	8	14	18	22
	Chloroform	7	5	9	12	15
	Petroleum ether	5	4	7	10	13
Flower	Aqueous	4	5	7	8	9
	Ethanol	11	8	14	17	19
	Methanol	9	6	12	15	16
	Chloroform	6	4	7	11	13
	Petroleum ether	6	3	6	10	12
Whole plant	Aqueous	17	9	10	11	12
	Ethanol	14	10	18	23	26
	Methanol	12	8	16	20	24
	Chloroform	8	6	11	14	16
	Petroleum ether	6	4	8	13	14
Tetracycline	30µg/ml	20	18	23	25	28

Table-2: Minimum inhibitory concentration (MIC) of leaf and whole plant extract of *Azadirachta indica*

Organism	**Leaf extract**		**Whole plant**	
	Ethanol(mg/ml)	Methanol(mg/ml)	Ethanol(mg/ml)	Methanol(mg/ml)
E.coli	16.8	17.6	15.2	15.9
K.pneumoniae	14.6	15.2	13.6	14.0
P.vulgaris	18.6	19.5	16.2	17.8
B.subtilis	3.16	3.90	2.30	3.60
S.aureus	2.06	2.86	1.96	2.30

In the present study in broth dilution technique, the ethanolic and methanolic extracts of *Azadirachta indica* successfully control the *Bacillus subtilis* and *Staphylococcus aureus*. MIC value also revealed that almost all tested bacterial strains were sensitive to the ethanolic and methanolic extracts of our study plant. From the earlier studies it is also revealed that the organic solvent extract is better than aqueous extracts[20]. *Staphylococcus aureus* was the most susceptible bacteria amongst all the bacterial strains investigated in the present work. The highest MIC values of *Staphylococcus aureus* is an inhibition that either the plant extracts are less effective on some Gram-negative bacteria or that the organism has the potential of developing antibiotic resistance, while low MIC values for other

bacteria is an indication of the efficiently of the plant extraction. Now the present study revealed that the ethanolic and methanolic extracts were effective control the bacterial growth than the aqueous extract. This probably indicates that there are bioactive ingredients that are able to inhibit the growth of these common pathogens. According to this study, plant based antibacterial drug have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobial agents. The present results revealed that the extract of *Azadirachta indica* was effective against both Gram-positive and Gram-negative bacteria. Presence of chemical compounds viz. alkaloids, tannins and flavanoids of *Azadirachta indica* may inhibit the bacterial growth. Traditionally, *Azadirachta indica* was employed using/mixing with aqueous for treating the antibacterial and other infections. Naturally, the biological active compounds whose activity can be enhanced in the presence of ethanol and methanol could have been produced number of active compound responsible for antibacterial activity. The present study provides the scientific information about the plant extract of *Azadirachta indica* and supports the usage of this plant for curing many bacterial diseases by traditional healers. Further, phytochemical separation and immunological studies of this plant is in under progress.

CONCLUSION

The results of this study have shown that the leaf and whole plant extraction of *Azadirachta indica* have great potential as antibacterial agents in the treatment of infectious organisms. Further, detailed investigation of the active compounds of the plant for the exact mechanism of action will contribute greatly to the development new pharmaceuticals.

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