RESEARCH ARTICLE

Antimicrobial activity of Rhizospheric Bacteria of *Azadirachta indica* Producing Metabolites against Human Bacterial Pathogens

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ABSTRACT

Medicinal plants are widely used all over the world for natural medicines. *Azadirachta indica* are known as "mother medicine of nature"; they have chemical compounds for curing and preventing diseases. These plants have valuable antimicrobial resources and can produce a large number of metabolites which having antibacterial properties, regulating their own growth and development to encourage other organism beneficial to them and suppress organisms that are harmful. Soil microorganism provides an excellent resource for isolation and identification of therapeutically important products; Antimicrobial metabolites were produced by different bacteria present in soil. In present study 21 rhizospheric soil samples of *Azadirachta indica* were collected from western Vidharbh region of Maharashtra state and were analyzed for presence of bacteria which can produce metabolites, isolation of desired bacteria were carried out by serial dilution method, Total 27 bacteria have been isolated from rhizospheric soil samples and out of 27 only 3 were potent isolates whose have been characterized on the basis of antibiogram test that revealed the activity of isolates, further characterization was done by following the Bergey's Manual of Systematic Bacteriology. Accordingly *Azadirachta indica* rhizospheric characterized isolates were *Sporosarcina*, *Micrococcus luteus* and *Staphylococcus epidermis*. These potent isolates could be further exploited for the production of metabolites in production media.

Keywords: *Azadirachta indica*, Medicinal plants, Metabolites, Rhizospheric.

INTRODUCTION

Medicinal plants are part of human society to combat disease, from the down of civilization. *Azadirachta indica* is well known in India and its neighboring countries for more than 2000 years as one of the most versatile medicinal plant having a wide spectrum of biological activity. *A. indica* and *M. azedarach* are two closely related species of *Meliaceae*. The former is popularly known as Indian neem, and the latter as the Persian lilac. Neem is an evergreen tree cultivated in various part of the Indian

subcontinent. A very part of the tree has been used as traditional medicinal for house hold remedy against various human ailments (Biswas *et al.,* 2002).

India has one of the richest medical cultures in the world. Indian literature incorporated in remarkably broad definition of medicinal plant and considers all plants are potential sources of medicinal substance. The plants containing medicinal substance which substances which can be use as antifungal, antibacterial, anticancerous etc. are term as medicinal plant. The world health organization (WHO) has listed 21000 plants which are used for medicinal purposes around the world. Among this 2500 species are in India, out of which 150 species are use commercially on a fairly, large scale, India is the largest producer of medicinal herbs and is called as botanical garden of the world. Plants are primary source of medicine, among the plants known for their medicinal values (Yadav *et al*., 2013)

Medicinal plants are considered to be very rich sources of metabolites. Microorganisms live in a world of chemical signals; they use small molecular weights compounds known metabolites, to regulate their own growth and development, to encourage other organisms that are helpful. Microbial metabolites are exquisitely selective; others are broadly active against many species. Organisms resistant to the effects of metabolites thrives microbes use metabolites to regulate the environment in which they live and form this platform they control the function.

The plant chemicals are classified as primary and secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. In higher plants such compounds are offer concentration in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolites. Primary metabolites obtain from higher plants for commercial use is high volume-low value bulk chemical (e.g. vegetable oil, fatty acid, carbohydrates etc) (Wink *et al.,* 2005).

Secondary metabolites are classically organic compounds produce from micro-organism during the alteration of primary metabolites synthesis. Secondary metabolites have a role in the growth and development of microbes and are usually form in the stationary phase. Many among secondary metabolites have ecological function; which include defense mechanism also function as antimicrobial agents or antibiotics and by producing various pigments. Antibiotics are one of the most important and wide employed secondary metabolites produce by bacteria. The soil microbes are a major source of antibiotics various bacterial strains are selected for antibiotics production as its isolation, maintenance and strain improvements is easy (Pande and Malviya 2014).

Azadirachta indica, commonly known as neem, has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. Neem has been broadly used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally multifaceted. More than 140 compounds have been isolated from diverse parts of neem. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used conventionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been verified to exhibit immunomodulatory, antiinflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. (Biswas *et al*., 2002). The presence study is carried out by the antibacterial activity of rhizospheric bacteria of *Azadirachta indica* producing metabolites against human bacterial pathogens.

MATERIALS AND METHODS

Sample Collection*:* Soil samples were collected from rhizospheric region of *Azadirachta indica* plant located in different region in Akola city western Vidharbh region of Maharashtra. Total 21 rhizospheric soil samples were collected in the sterilized polythene bag containing soil sample were transfer immediately to laboratory.

Isolation of Bacteria: The collected Rhizospheric soil samples of *Azadirachta indica* were weight 1 gm aseptically and immediately transfer to 9 ml saline suspension that is called as Stock culture. After the rhizospheric soil was added to prepare stock solution further Serial dilution method was performed to get reduce number of bacteria. Dilution was made up to 10^{-8} to reduce the load of bacteria for better isolation of colonies. After inoculating and incubation period different colonies were observed on Nutrient agar plates and Selective medium plates. Colony characteristics were observed and noted. Single colony

was streak on nutrient agar slant for the isolation of pure culture.

Isolation of crude extracts producing antimicrobial substances: For the isolation of antimicrobial crude extract the test bacterial sample was inoculated in nutrient broth fermentation medium & incubated at 37^0c for 48 hrs. Generally the antimicrobial substances produced by bacteria in their maximum stationary phage so after incubation period. The fermented broth was then treated to separate the biomass from broth. The broth was then centrifuged at 5000 rpm on for 15 minutes and then subjected to extraction with ethyl acetate by solvent extraction procedure equal volume of ethyl acetate was added to the filtrate and mixed well by vigorous shaking for 10 minutes. Tubes were allowed to settle for 5 minutes till two clear immiscible layers are formed. The upper layer containing the extracted compounds was separated and collected in another tube. This filtrate extract was evaporated to dryness in hot air oven. The extract residue was dissolved in dimethyl sulfoxide (DMSO) and stored at 4^oc to be used as stock solution for antimicrobial assay.

Antibiogram test: Microorganisms are found in their natural habitat and are in constant exposure of undesirable chemicals, which may have antimicrobial activity against various microbes other than itself. To check the resistivity or sensitivity of a microbe against the various pathogens antibiotic sensitivity test is used to perform. This test is also termed as Antibiogram test. Nutrient agar plates were prepared. 20 µl of selected test pathogens (*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*) were spread on to the solidified nutrient agar plates. Three wells were made at appropriate distance onto the agar plate with the help of gel puncture and filled using different concentration like 25 µl, 50µl, and 75 µl of the bacterial isolates' broth extracts obtained from different strains. Petri plates were incubated at 37ºC for overnight (Malviya and Pandey 2014). Then the diameter of the zone of inhibition was measured in mm and noted. The antimicrobial activity was determined by measuring the clear zone around the wells.

Identification: Different techniques and tests were performed such as Simple staining, Gram staining, Endospore staining, Motility, Acid fast staining, Biochemical Test, Sugar Fermentation Glucose, Lactose, Mannitol, IMViC test, Indole test, Methyl Red test, Vogas proskauer test, Citrate utilization test, Enzymes test Catalase, Oxidase, Urease, NO3 Reduction, H_2S Production, Starch hydrolysis, etc. for the identification of potent isolates (Pandey and Singh, 2013).

RESULTS AND DISCUSSION

Isolation, Purification and characterization of rhizospheric soil sample of *Curcuma longa.*

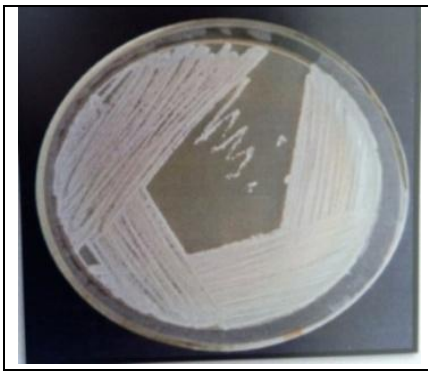
Soil samples of the *Azadirachta indica* rhizosphere regions were collected from the different region in Akola City, Western Vidharbh region of Maharashtra. The bacterial culture from the soil samples were collected by the serial dilution and spread plate technique. The total 27 culture have been isolated from the soil samples and out of total 27 only 3 have been characterized which are potent isolates.

Rhizosphere microorganisms increase root exudation through production of plant hormones or more directly by physically damaging the roots (Grayston *et al*. 1996). In general, the nutrient-rich rhizosphere is naturally colonized by many beneficial or pathogenic bacteria which may have a considerable impact on plant growth, development and productivity. The numerous interactions between bacteria and roots may have beneficial, harmful or neutral effects on the plant, the outcome being dependent on the type of symbiotic interaction and the soil conditions.

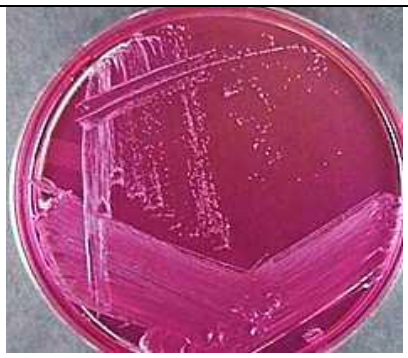
In the present study, medicinal plant *Azadirachta indica* has been selected, the rhizospheric region have been targeted to take the soil sample. The rhizosphere is the region adjacent to the plant root. The root exudates and the secondary metabolites secreted by the micro flora of the soil may affect each other and also to the plant health. There are total 27 cultures were isolated from these soil samples in which only 3 cultures have been screened. These 3 isolates are active against the selected pathogens, *E. coli*, *P. aeruginosa* and *S.aureus.* The characterized 3 cultures were *Sporosarcina, Micrococcus luteus and Staphylococcus epidermis*. To characterize these cultures, Bergey's manual has been followed. According to this, gram's staining, Catalase test, Endospore test, acid fast staining, glucose fermentation test, Mannitol fermentation test, lactose fermentation test, citrate utilization test, Oxidase test, glucose oxidation test, and nitrate reduction test have been performed. The analysis of antibiogram of the

entire characterized isolates has been observed before identification against selected pathogens. This has shown the drastic change in the activity of the isolates. The potent isolates were found able to produce metabolites on the basis of their specificity and hence the metabolites have shown the many fold increment in the activity of the isolates. Further, the isolates have been tested for the activity to inhibit the growth of the selected human pathogens by antibiogram test (Malviya and Pandey, 2014).

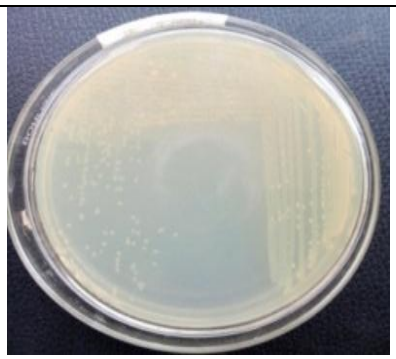
There are two potent isolates have been found, which has shown the best activity against selected pathogens. Those isolates are N2 (*Micrococcus luteus*) has shown zone of the inhibition 16 mm against *E. coli* and N3 (*Staphylococcus epidermis*) has shown zone of the inhibition 16 mm against *S.aureus*. The N1 (*Sporosarcina*) is the much more potent culture in comparison to other one. This culture has shown the best result against *S. aureus* (16.5 mm) in contrast to other pathogens. Between both the potent isolates, the N1 culture has the maximum activity against all the selected pathogens. This has been observed by comparing all the isolates activity of *Azadirachta indica* rhizospheric soil sample.



(Fig 1.1). Tryptic Soya Agar



(Fig 1.2). Mannitol Salt Agar



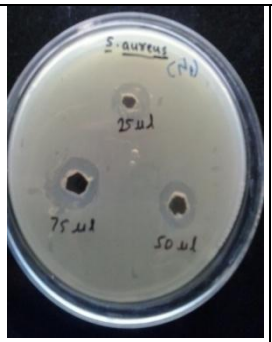
(Fig 1.3). Nutrient Agar

Fig 1: Showing results of isolated colonies on different selective media

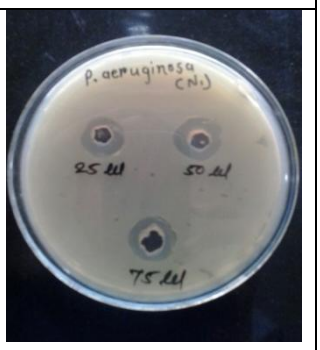
(Fig 4). Isolated pure culture in form of slants

Results showing zone of inhibition against pathogenic bacteria

(Fig 5). Against *E.coli*



(Fig 6). Against *S.aureus*



(Fig 7). Against *P.aeruginosa*

Table: 1: Antimicrobial activity of N1, N2, and N3 at different concentration

Test bacterial strains	Concentration /Diameter (µl/mm)								
	N1			N2			N3		
	25µl	50µl	75µl	25µl	50µl	75µl	25µl	50µl	75µl
E.coli	12	15	16	8	11	14	9	13	14
S.aureus	13	14	16.5	10	13	13	10	12	15
P.aeruginosa	12	13	16	11	12	13	11	12	14

❖ Abbreviations : N1- Sample No.1 | N2- Sample No.2 | N3- Sample No.3

Table 2: Morphological & Biochemical Characteristics

Sample	Gram Character	Motility	Endospore	Acid Fast	Sugar fermentation			IMVIC				Enzymes			No3 Reduction	H_2S prouction	Starch hydrolisis
					Glucose	Lactose	Mannitol	Indol	MR	VP	Citrate	Catalase	Oxidase	Urease			
N1	+	+	+	+	+	+	–	–	–	–	+	+	–	+	–	–	–
N2	+	–	–	NA	–	–	–	–	+	+	+	+	+	+	–	–	–
N3	+	–	–	NA	+	+	–	–	+	+	–	+	–	+	+	–	–

Where, +: Positive, – : Negative, MR: Methyl red, N1;N2 and N3: Sample Numbers
VP: Voges Proskaur, NA: Not Applicable

On the basis of cultural, Morphological and Biochemical characteristics.

The potent isolates were identified by using Bergey's manual of systematic Bacteriology.

N1: *Sporosarcina*, N2*: Micrococcus luteus* and **N3*: Staphylococcus epidermis***

CONCLUSION

The present study was an attempt to identify and pick out the versatile bacterial strains that display antimicrobial activity against variety of microbial pathogens intrinsically. Total 27cultures were isolated from rhizospheric region of *Azadirachta indica* out of 3 were potent isolates characterized as *Sporosarcina, Micrococcus luteus and Staphylococcus epidermis*.

The Rhizospheric bacterial crude extract of *Sporosarcina, Micrococcus luteus and Staphylococcus epidermis* were found to be more or less active against almost all tested pathogenic strains. Hence *Azadirachta indica* can be employed as source of natural antimicrobials that can serve as an alternative to conventional medicines. It was concluded that the best activity have been shown by the *Azadirachta indica* rhizospheric isolates (N1) which is of *Sporosarcina* against all three human pathogenic organisms *(E.coli , S.aureus , P.aeruginosa*). The activity of rhizospheric isolates was showing best results against *S. aureus*

The result of this study strongly supports that the bacterial isolates produces metabolites and may be used in the management of microbial infection and the present findings highlights the important for further investigation towards the goal of obtaining novel antimicrobial agent.

REFERENCES

Biswas Kaushik, Chattopadhyay I, Banerjee R and Bandyopadhyay (2002) Biological activity and medicinal properties of neem (*Azadirachta indica*) *Current Science*, 82(11): ??

Grayston, Susan J, Shenquing Wang, Campbell Colin D, Edwards Anthony C (1998) Selective influence of Plant species on microbial diversity in the rhizosphere. *Journal of Soil Biology and Biochemistry*, 30(3):369-378.

Malviya T and Pandey A (2014) Production of Antibiotics Isolated from Soil Bacteria from Rhizospheric and Non-Rhizospheric Region of Medicinal Plants, *Indian Journal of Applied Research*,4(8):??

Pandey A and Singh A (2013) A comparative study on secondary metabolites producing microbes isolated from rhizospheric and non rhizospheric region of *Azadirachta indica* and *Oscimum tenuiflorum*. *International Journal of Pharmaceutical Research and Allied Science*, 2(1):36-48.

Yadav Harikrishnan and Nanganuru (2013) Effect of Concentration of *Oscimum sanctum* Linn. (Tulsi) leaves extract on the α-Amylase, α-Glucosidase activity and Microorganism Growth, *International Journal of Engineering Science & Research Technology*, 2(2):2277-9655