



International Journal of Medicine and Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijmpr



Research Article

Open Access

Medicinal Plants Rhizosphere – A Boon for Inhabiting Antibiotic Producing Microorganisms

Nisha Kashyap¹ and Nisha Thakur*²

¹Department of Microbiology, Abhilashi University, Mandi (H.P.) 171005, India

²Department of Biotechnology, Dr. YS Parmar University of Horticulture and Forestry, Solan (H.P) 173212, India

ABSTRACT

To explore the production of antibiotics from soil microbes residing in the rhizosphere of medicinal plants, a study was carried out at Himachal Pradesh, a hilly state situated in northern Himalayas. Microbes were isolated from the rhizospheric region of three medicinal plants viz., *Aloe vera*, *Mentha* and *Salvia*. Obtained isolates were compared with each other and the isolate having high cfu (colony forming unit) in a particular rhizosphere was selected for further study. For antibiotic sensitivity test (AST) the obtained isolate was clinically screened against three pathogenic microorganisms which were obtained from IGMC (Indira Gandhi Medical College, Shimla) through regular isolations at Cary-Blair transport medium.

These four isolates were identified through morphological and biochemical studies and then were fed into ABIS (Advanced Bacterial Identification Software) which identified these as *Bacillus* from *Aloe vera* rhizosphere and 3 other pathogenics as *Staphylococcus*, *Pseudomonas* and *E.coli*. Results obtained during AST were expressed in terms of the diameter of the inhibition zone caused by *Bacillus* against clinical isolates. Highest zone diameter of 26mm was observed in *Pseudomonas* followed by *Staphylococcus* and *E.coli*

Keywords: rhizosphere, *Aloe vera*, *Mentha* and *Salvia*, *Bacillus*

ARTICLE INFO

CONTENTS

1. Introduction	100
2. Materials and Methods	100
3. Results and discussion.	101
4. Conclusion.	102
5. References.	102

Article History: Received 21 May 2017, Accepted 02 July 2017, Available Online 10 August 2017

*Corresponding Author

Nisha Thakur
Department of Biotechnology,
Dr. YS Parmar University of Horticulture
and Forestry, Solan (H.P) 173212, India
Manuscript ID: IJMPR3269



PAPER-QR CODE

Citation: Nisha Thakur, Medicinal Plants Rhizosphere – A Boon for Inhabiting Antibiotic Producing Microorganisms. *Int. J. Med. Pharm. Res.*, 2017, 5(4): 99-103.

Copyright© 2017 Nisha Thakur, This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

1. Introduction

In day to day language the word “antibiotic” means “against life. However, in science we use the word to describe a set of chemicals that inhibit or kill bacterial pathogens [1]. The British scientist Alexander Fleming is credited with being the first to notice that one bacteria could inhibit the growth of other one by releasing some antibiotics. The plants having medicinal properties and have potential use as antimicrobial, antipyretic, anti-cancerous agents etc., are termed as medicinal plants [2and 3]. Many medicinal plants viz., *Aloe vera*, *Mentha*, *Salvia* leaf bears many medicinal properties like reduction in overproduction of perspirations and also used in treatment for depression, memory loss, and Alzheimer’s diseases [4].

Literature cited that many microbiologists isolated different types of microorganisms from rhizosphere of these medicinal plants [5and 6]. Members of the *Bacillus* genus are generally found in soil and represent a wide range of physiological abilities, allowing the organism to grow in every environment and compete desirably with other organisms within the environment due to its capability to form extremely resistant spores and produce metabolites that have antagonistic effects on other microorganisms [6]. Also species from the genus *Bacillus* are considered as safe microorganisms and they possess remarkable abilities to synthesize many substances that have been successfully used in clinical purposes [7].

The secondary metabolites produced by several species and strains of the genus *Bacillus* have been found to show antibacterial activity against different pathogenic microorganism. The genus *Bacilli* has been found is produce high preparation of antimicrobial compound including bacitracin, polymyxin, gramicidin, tyrocidine, subtilin, bacilysin and also have bactericidal effects on both gram positive and gram negative bacteria, therefore used as biocontrol agent [8]. The paper therefore highlights the isolation of bacteria from rhizospheric soils of medicinal plants and the isolated one was affectively screened against pathogenic bacterial isolates.

2. Materials and Methods

Collection of soil samples:

Our sampling site Mandi, situated in India within Himachal Pradesh province in between 31°72'North latitude 76°92' East longitude with an elevation of 1044 metres (3,425 feet). With the help of sterile spatula soil samples were collected at a depth of 5cm from rhizospheric soils of *Aloe vera*, *Salvia* and *Mentha* in polythene bags. Samples were dried separately at 45° for 1 hour in a hot air oven, cooled to room temperature and further isolations were carried out.

Isolation and maintenance of microbial cultures:

One gm of each soil sample, suspended in 10ml of sterile saline water (0.86% NaCl) and vortexed. A series of dilutions from 10^{-1} to 10^{-7} were made, these dilutions were used in the spread plate method. The resulting serial dilutions of soil (0.1 ml) were pipette on nutrient agar (Himedia, India) media in triplicates. The plates were

incubated at 37°C for 24 h and observed intermittently. Maintenance of microbial cultures were done on nutrient agar slants and then preserved at 4°C.

Isolation of clinical isolates

Bacterial samples were also procured from Department of Microbiology, IGMC (Indira Gandhi Medical College, Shimla) Himachal Pradesh. Samples were isolated on Cary-Blair transport medium. The isolated pathogenic microorganisms were screened for antimicrobial sensitivity test.

Identification of bacterial isolates

For physiological culture identification, isolates were grouped on the basis of phenotypic characteristics such as colony colour, appearance, elevation, and margins on nutrient agar. Other characters such as endospore staining, capsule staining and Gram reaction (for cocci and bacilli detection) and cell arrangement were also recorded. The photos were slightly refined in sharpness and color tone with CorelDRAW X4. Routine biochemical tests such as indole; methyl red; Voges–Proskauer; citrate; the presence of oxidase and catalase; sugar utilization were assessed for each bacteria. Clustering of biochemical analysis was done with SXL software. The results obtained through morphological and biochemical tests were fed into ABIS (Advanced Bacterial Identification Software (<http://www.tgw1916.net/software.html>) to identify the isolates at genus level.

Production of bacitracin :

Bacterial growth scrapped aseptically from 48 hours old agar slants. Inoculated to 50ml sterilized basal medium in 250ml conical flask and shaken on rotatory shaker at 150rpm for 24 hours at 37°C. The collected supernatant was tested for antimicrobial activity against the test pathogens on nutrient agar plates incubated at 37°C for 24hrs by measuring the inhibition zones.

Antibiotic sensitivity test

Both Agar well diffusion and Disc diffusion method were used for the assessment of antimicrobial activity.

Agar well diffusion method

The aim of this experiment was to compare the antimicrobial effectiveness of the selected rhizobacterial isolate against pathogenic isolates. Different Muller Hinton agar Media plates of suitable media were prepared and inoculated with 1ml of respective pathogenic organisms by spread plate method. Wells measuring 3 mm in diameter were cut out under aseptic conditions using a stainless steel borer [9and10]. Each well was filled with 25 ul extract of bacitracin and incubate at 37°C. A clear zone of inhibition, which is more than 12 mm, was considered to be sensitive.

Disc diffusion method

7 mm filter paper discs (Whatman, no. 3) were impregnated with 1 ml of bacitracin . The discs were allowed to remain at room temperature until complete diluent evaporation and were placed onto the surface of the Muller Hinton agar Media agar inoculated with test microorganisms then incubated for 24 hours at 37°C in inverted position. Clear zones of inhibition were developed and were measured [11].

3. Results and Discussion

Collection and isolation: Total plate count of bacteria isolated from rhizospheric soil were recorded after 48 hours incubation. Table.1. Showed that maximum bacterial population was found in the rhizospheric soil of *Aloe vera* followed by *Mentha* and *Salvia*. Bacterial colonies obtained from *Aloe vera* rhizospheric soil were identified and designated as Sample no.1.

Table 1: Viable count (cfu/g) from the Rhizosphere Soil

Sr. No.	Soil isolates	Cfu 10 ⁶
1	<i>Aloe Vera</i>	8.02 x 10 ⁶
2	<i>Mint</i>	7.09 x 10 ⁶
3	<i>Salvia</i>	6.08 x 10 ⁶

Biochemical characterization of the isolates was done using various biochemical media. All isolates grew on a variety of sugars and isolates showing difference in biochemical treatments as shown in Fig. 1.

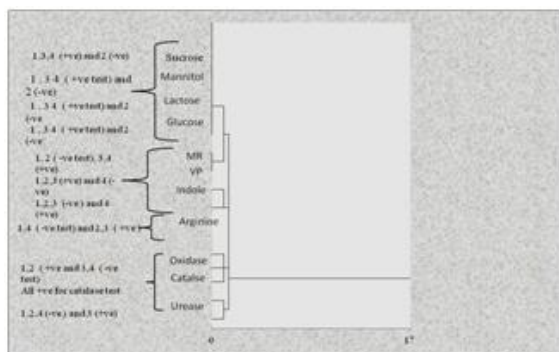


Figure 1: Dendrogram showing biochemical test of isolates based on rescaled distance

ABIS identification based on morphological and biochemical tests revealed isolate 1 as *Bacillus* and isolates 2, 3 and 4 as *Pseudomonas*, *Staphylococcus* and *E.coli* respectively.

Antibiotic Sensitivity Test

Agar well diffusion method: During agar well diffusion method an inhibition zone of 26mm was observed as highest when *Bacillus* producing bacitracin was screened against *Pseudomonas* followed by *Staphylococcus* (24mm) and *E.coli* (22mm), respectively (Fig. 2 and 3).



Figure 2: Petriplate showing inhibition of *Bacillus* (bacitracin) against pathogenic isolates

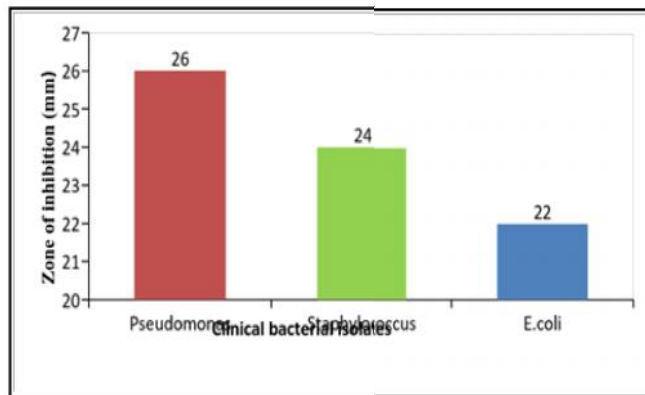


Figure 3: Zone of inhibition (mm) of clinical isolates

Disc diffusion method: During disc diffusion method an inhibition zone of 28 mm was observed as highest when *Bacillus* producing bacitracin was screened against *Pseudomonas* followed by *Staphylococcus* (26mm) and *E.coli* (24mm), respectively (Fig. 4).

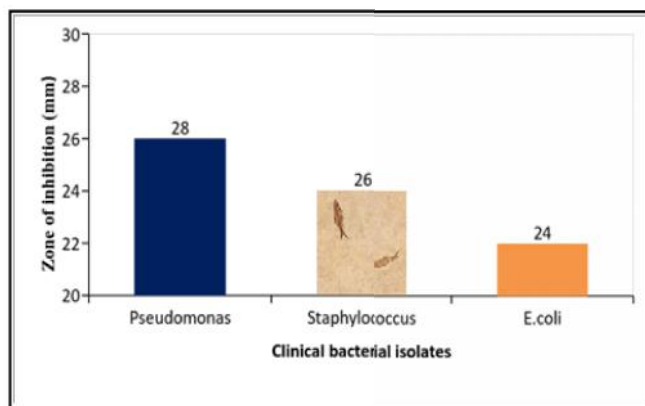


Figure 4: Zone of inhibition (mm) of clinical isolate

5. DISCUSSION

We explore rhizosphere as it has very dynamic environment and harbors a variety of microorganisms. In the present investigation soil samples were collected from the rhizosphere of medicinal plants from botanical garden of Abhilashi University (H.P.). Soil samples (3 in no.) were collected and isolated on nutrient media as well as on selective media. Phenotypic characterization was done by colony morphology and biochemical testing. We isolate *Bacillus* from rhizospheric part of *Aloe vera*, *Mentha* and *Salvia*. Lawrence *et al.*,2009 [12] isolated, purified and evaluated antibacterial agents from *Aloe vera*. Arora *et al.*, 2013 [13] isolated and characterized antibiotic producing microbes present in rhizospheric soil. Organism was preferred for isolation as it one of the useful microorganism produce a large number of antibiotics [6and14]. Sensitivity test was done on Muller Hinton agar Media [15] and then effect of antibiotic produced by microorganism was examined against pathogenic microorganisms including *S. aureus*, *E. coli* and *Pseudomonas*. It was concluded that *Bacillus* effects the growth of microbes and formed a zone of inhibition by both Agar Well diffusion method as well as Disc diffusion method. Antibiotics are affective only

against bacteria because they attack the unique peptidoglycan cell wall or smaller ribosomal unit of the bacteria [16]. Thus, in present paper the organism for bacitracin production was tested against *E. coli*, *Staphylococcus*, *Pseudomonas* spp. Results of antibiotics

activity expressed in terms of the diameter of the inhibition zone. Maximum inhibition caused by *Bacillus* producing bacitracin was screened against *Pseudomonas* (28mm) followed by *Staphylococcus* (26mm) and *E.coli* (24mm), respectively in disc diffusion method.

Table 2: Table showed physiological characteristics of selected isolates with respective staining techniques

S.No.	Sample	Gram staining	Media	Colony Morphology
1.	Sample No. 1	Gram positive rods	Nutrient agar	White, dry, flat, irregular and circular colonies with green colored endospore and purple colored capsule.
	Sample No.2	Gram negative bacilli	Nutrient agar	Colonies are grey, smooth, translucent, irregularly around and emit a characteristics fruity odour.
3.	Sample No.3	Gram positive cocci	Nutrient agar	White, smooth, convex with entire and shiny margins
4.	Sample No.4	Gram negative cocci	Mac Conkey agar	Colonies are bright pink circular and convex.

4. Conclusion

From the extensive literature review, it is evident that soil is an important part of the earth and can also serve as a good medium for growth of microorganisms. These microorganisms have the property of producing antibiotics and therefore useful from medicinal point of view. The pathogenicity of undesired microorganisms and medicinal property of useful microorganisms were reviewed in this study and hence one of the aims of this study was to assess the antibiotic producing microorganisms. There is often one source of bacteria such as *Bacillus* which showed a strong antimicrobial activity against the pathogenic microorganisms. *Bacillus* isolated from soil has antimicrobial activities, hence used as an antimicrobial agent against isolated pathogenic microbes.

5. References

- [1] JA De Mondena, SAJ Gutierrez, RAJ Falchini, L Gallazo, DE Hughes, JE Bailey and JF Martin. Intracellular expression of *Vitreoscilla* haemoglobin improves cephalosporin C production by *Acremonium chrysogenum*. *Biotechnology*, 1996, 11: 926-929
- [2] N Parmar and M Rawat. Medicinal plants used as antimicrobial agents: a review. *International Research Journal of Pharmacy*, 2012, 3: 31-40.
- [3] M Choudhary, V Kumar, H Malhotra and S. Singh. Medicinal plants with potential anti-arthritis activity. *Journal of Interculture Ethnopharmacology*, 2015, 4: 147-179.
- [4] AK Singhal, V Naithani, and OP Bangar. Medicinal plants with a potential to treat Alzheimer and associated symptoms. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, 2012 2: 84-91.
- [5] C Nirmala, M Sridevi and AK Kushwah. Screening and isolation of bacteria from Kanjamalai hill for antimicrobial activity. *International Journal of Pharmaceutical Science and Research*, 2016, 7: 221-227.
- [6] S Tamilarasi, K Nanthakumar, K Karthikeyan and P Lakshmana perumalsamy. Diversity of root associated microorganisms of selected medicinal plants and influence of rhizomicroorganisms on the antimicrobial property of *Coriandrum sativum*. *Journal Environmental Biology*, 2008, 29: 127-134.
- [7] J Olmos, and J Paniagua-Michel. *Bacillus subtilis*: a potential probiotic bacterium to formulate functional feeds for aquaculture. *Microbial Biochemistry and Journal Technology*, 2014, 6: 361-365.
- [8] D Dhanasekara, G Rajakumar, P Sivamani, S Selvamani, A Panneerselvam and N Thajuddin. Screening of salt pans Actinomycetes for antibacterial agents. *The Internet Journal of Microbiology*, 2005, 1: 1 - 6.
- [9] S Magaldi, S Mata-Essayag, C Hartung de Capriles. Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases*, 2004, 8: 39-45.
- [10] C Valgas, SM De Souza, EFA Smânia. Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, 2007, 38: 369-380.
- [11] NG Heatley. A method for the assay of Penicillin. *Journal of Biochemistry*, 1944, 38: 61-65.
- [12] R Lawrence, P Tripathi and E Jeyakumar . Isolation, purification and evaluation of antibacterial agents from *Aloe vera*. *Brazilian Journal of Microbiology*, 2009, 40: 906-915.
- [13] S Arora, D Nandi, N Prasad, S Rawat, and A Pandeya. Isolation and characterization of antibiotic producing microbes present in rhizospheric soil. *International Journal of Scientific & Engineering Research*, 2013, 4: 1157-1166.
- [14] A Pandey and Malviya. Production of antibiotics isolated from soil bacteria from rhizospheric and non-rhizospheric region of medicinal plants. *Indian Journal of Applied Research*, 2014, 4: 25-32.
- [15] WM Kirby, AW Bauer, JC Sherris and M Turck. Antibiotic susceptibility testing by a standardized

single disk method. *American Journal of Clinical Pathology*, 1966, 45: 493–496.

- [16] MS Butler and AD Buss. Natural products-the future scaffolds for novel antibiotics *Biochemistry Pharmacology*, 1944, 71.