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A Comparative Study on Secondary Metabolite Producing Microbes Isolated From Rhizospheric and Non Rhizospheric Region of Alove barbadensis

Chhavi Singh[1], Pratibha Teotia[2]

[1,2]Department of Biotechnology, Noida International University, Greater Noida, Gautam Buddha Nagar, Uttar Pradesh, India

Abstract: **The aim of the study was assess the antibacterial effect of plant extracts and their synergistic antibiotic and non antibiotic drugs against E.coli and S.aureus. The present study is carried out by the isolation and characterization of Rhizospheric and non-Rhizospheric region of Aloe Vera soil and comparative analysis of production of secondary metabolites against two bacterial pathogens (E. coli, S. aureus).The secondary metabolites will not involve in the growth of the cultures but they will be resultant of the primary metabolites. So, after isolation, the antibiogram analysis revealed the activity of isolates and further all were characterized through Bergey' manual. The study was started from isolation, Primary Screening, culture characterization, pure culturing, antibiogram test, secondary screening, secondary metabolite extraction, and at last antibiogram test of secondary metabolites. Thereby, our results indicate the possibility of using these extracts in the treatment of bacterial infections, and the results of this study was encouraging, despite the need for clinical studies to determine of the real effectiveness and potential toxic effects in vivo These results were revealed the importance of plant extracts when associated with antibiotic and Non-antibiotic drugs in control of bacteria.**

Keywords: **Rhizospheric, Non-Rhizospheric, Metabolites, Antibiogram.**

1. INTRODUCTION

The plants containing medicinal substances which can be used as antibacterial, antifungal, antipyretic, anti-cancerous are termed as medicinal plants .India has one of the richest plant medical cultures in the world. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all' plants as a potential source of medicinal substances.

Soil microorganism constitute world largest reservoir of biological diversity and are crucial to functioning of terrestrial ecosystems. The potential importance of microbial activity associated with root systems in plant nutrition and coined the term "rhizosphere" to describe zone of intense microbial activity. The region of soil surrounding and including the plant root is of crucial importance for plant health and nutrition. It has a high level of microbial activity, particularly because of nutrients secreted by plant roots in the form of soluble exudates as amino acids, organic acids and other photosynthtates.

2. 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.

Steps:

1: Prepare nutrient agar plate.

2: Spray 20 µl of selected pathogens (*Staphylococcus aureus, Escherichia coli*) onto the solidified nutrient agar plates.

3: Make three wells at appropriate distance onto the plate with the help of gel puncture.

4: Load 50 µl of the isolates' broth.

5: Incubate it at 37ºC for overnight

6: Observe the plates and take measurement of the zone of inhibition, if found.

Analysis of the Secondary Metabolite through Antibiogram Test

The Extracellular and Intracellular Secondary Metabolite obtained from the nutrient broth through centrifugation of the microbial sample of the Rhizospheric and Non-Rhzospheric soil samples are analysed for the Antimicrobial property.

The metabolites isolates are subjected to the Antibiogram test, as they are tested against the other pathogenic microbes for checking the resistivity and sensitivity of the microbes against the other pathogens.

Steps for the analysis:

1: Nutrient agar plate is prepared, and each plate is marked with the type of sample to be applied to the plate.

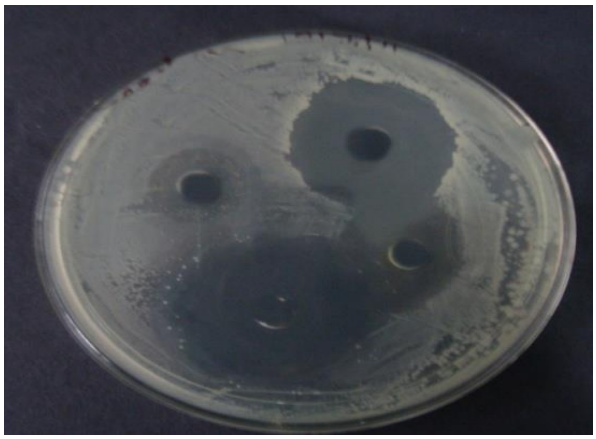
2: On solidifying, spary 20 µl of the selected pathogens (E. Coli and S. aureus) on the agar.

3: Make 4 wells on the agar at appropriate distance and with the help of gel puncture.

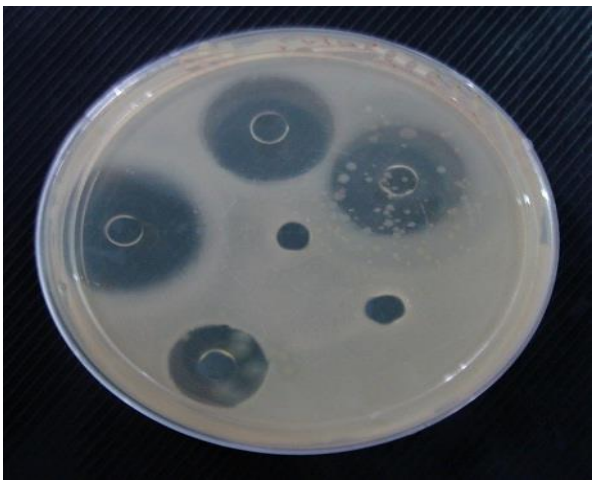
4: Load 50 µl of the metabolite isolates of the different samples.

5: Incubate it at 37ºC for overnight.

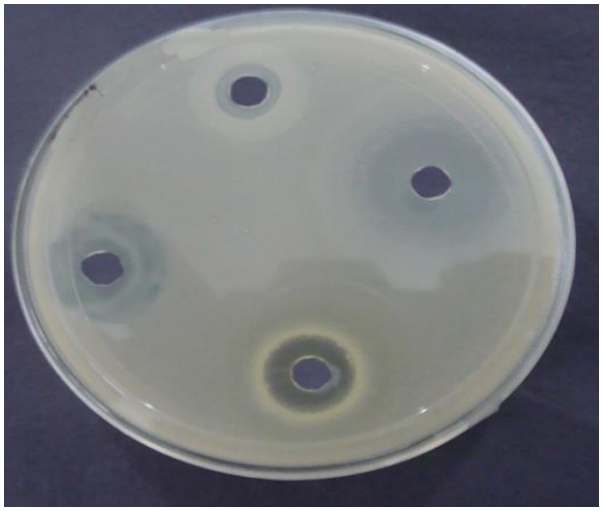
6: Observe the plates and take measurement of the zone of inhibition, and record it.



Zone of Inhibition for the Intracellular Metabolite isolate on E.coli



Zone of inhibition for the Intracellular Metabolite on S.aureus



Zone of Inhibition for the Extracellular Metabolite on E.coli

Zone of Inhibition In mm

S no.	E. Coil (Intracellular metabolites)	S. Aureus (Intracellular metabolites)	E. coli (Extracellular Metabolites)
AL1 R	10±2	6±1	2±2
AL2 R	8±1	7±1	5±1
AL3 NR	3±1	9±1	4±1
Al4 R	12±2	5±1	5±2

Zone of Inhibition observed for the Isolates in millimetre.

The "±" denotes the precision error in measuring the zone.

3. RESULT AND DISCUSSION

The soil samples collected for the Aloevera were screened and the bacteria were isolated and purified and characterized. The bacterial culture were obtained by the serial dilution and then spread plate technique, and characterized by streak plate method. The total 9 cultures were isolated out of which only 4 were characterized.

All these 4 samples were subjected to the further processes from which the secondary metabolites were obtained.

The extracellular and intracellular metabolites obtained were tested for the antimicrobial property against the two selected pathogens (E. Coli and S. aureus). From the results we can analyze that the zone of inhibition is best given by the intracellular metabolites compared to extracellular metabolites. Two potent isolates were found that showed the best activity against E. coli is AL1 and AL4 and against S. aureus is AL2 and AL3.

4. CONCLUSION

The 9 cultures were obtained from the Rhizospheric and Non-rhizospheric region of the Aloe barbadensis.

Out of this 9 only 4 were potent isolates. The activity of the intracellular and extracellular secondary metabolites was observed and after various comparisons it was concluded that only 4 isolates were potent and had shown best activity against the two pathogens.

The activity of the **Intracellular Secondary Metabolite** isolated from **the Rhizospheric soil sample** has shown the best effect against the pathogens **E. coli** as compared to the **Extracellular Secondary Metabolite** isolated from the **Rhizospheric soil sample**.

The activity of the **Intracellular Secondary Metabolite** isolated from **the Non-rhizospheric soil sample** has given the best activity against **S.aureus** as compared to **Rhizosphric soil sample.**

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