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Antimicrobial Activity and Minimum Inhibition Concentration of Banana Peel

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Abstract: Antimicrobial activity of banana plays a vital role in pharma industries because of its bioactive compounds. Previous research shows that banana plant parts and their fruits can be used to treat the human diseases. Banana peels have antibacterial activity against microorganisms but has not been studied extensively. Banana peels has lots of micro and macro biomolecules, and it is used to resist microbial growth. Plate diffusion method has to identify the zone of inhibition (ZOI), and it shows the capability of banana peel to resist microbes. The aim of this study is to determine the antimicrobial activity of banana peel. Methanolic extract against *Serratiamarscens*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Shigella flexneri*, *Vibrio parahaemolyticus*, and fungal species like *Aspergillus flavus*, *Trichoderma viride*, *Candida albicans*, *Penicillium griesiferum*. Zone of inhibition was higher in *Bacillus subtilis*. This study shows antimicrobial activity of banana peel. Least MIC was 15.6 µg/ml against *Shigella flexneri*, and highest MIC was 1000 µg/ml against *Serratia marscens*.

Keywords - Banana peels, Antimicrobial activity, Bioactive compounds, MIC (Minimum inhibition concentration), Microorganisms

I. INTRODUCTION

The healing powers of plants have been known to man for generations. A discussion of human life on this planet would not be complete without a look at the role of plants (Connie Veilleux *et al.*, 1993). Antimicrobials used earlier were derived from higher plants. But, discovery and subsequent extraction of effective antimicrobial compounds from other cheaper sources resulted in a shift of antimicrobial research from plants to laboratories and then, synthetic compounds. Recently, into interest in native plants, research has increased dramatically for a wide-range of reasons including an inability of many rural people and some governments to afford pharmaceutical care, revitalization of indigenous knowledge and "traditional" health systems, a greater appreciation of local and indigenous knowledge, international concerns for the conservation of biodiversity, and income-generating potential (LizFajber *et al.*, 1997). Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones (Momin *et al.*, 1987). In the traditional medicinal systems of India, all the parts of *Musa* spp. (family Musaceae) are used for the treatment of various diseases (Gurumaa *et al.*, 2008). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for potential antimicrobial activity. They have a long evolution of resistance against microbial agents, which has led to alternative directions in drug development. Extracts of plants are getting more importance as they have the great potential sources for microbial and viral inhibitors. Plant parts used for this purposes are bulb, gel, leaves, roots, barks, peels etc (Kinghorn *et al.*, 2010). There are around 70 species of *Musa* with a broad variety of uses. The common banana was scientifically known as *Musa sapientum* (Fereidoon *et al.*, 2004). Banana skin has many constituents like enzymes such as polyphenoloxidase, pectin as gelling agents. The banana peel extract is used alone or in combination with a cream or ointment, medicinal benefits of the extract include relief of pain, swelling and itching (Wuyts *et al.*, 2006; Daniells *et al.*, 2000). Banana a tropical fruit belonging to Musaceae family, is grown in many countries all over the world; (Shadma *et al.*, 2014). All parts of the banana plant such as flower, pulp, stem, and leaves have medicinal application (Imam *et al.*, 2011). Banana peel is a waste product of banana (Shadma *et al.* 2014) and studies have shown that banana peel also has medicinal properties (Chabuck *et al.*, 2013; Imam *et al.*, 2011). Bioactive compound such as flavonoids, tannins, phlobatannins, alkaloids, glycosides, and terpenoids are present in banana peel. This bioactive compound is reported to exert pharmacological effect, especially as an antioxidant, antidiabetic, anti-inflammatory, and antibiotic (Chabuck *et al.*, 2013). Additionally, flavonoids, tannins, phlobatannins, alkaloids, glycosides and terpenoids were found to be present in the peels of genus *Musa*. These plants have medicinal properties (Ighodaroro *et al.*, 2009). Researchers have done studies demonstrating the antimicrobial activity of banana peel against various Gram-positive and Gram-negative bacteria (Chabuck *et al.*, 2013). The current practice of medicine today has changed a lot from its practice in medieval times. However in India, we still use traditional practice for treatment of various diseases since Vedic period (Surathu *et al.*, 2011). Antibacterial and antibiotic principles are found in banana peel. Many studies are going on in pharmaceutical industries. All the parts of banana plant have medicinal applications (Amit and Shailandra., 2006). Bananas are

rich in vitamin B and they help to prevent nervous disorders (Singh and Bhat., 2003). This research study is aimed to investigate the antimicrobial activity of banana peel against some microorganisms (gram negative and gram positive bacteria).

II. MATERIALS AND METHODS

A. Sample collection and preparation

The bananas were purchased from a local super market in Chennai, Tamil Nadu. The banana peels were removed and air-dried for two weeks and ground into powder with a mechanical blender and sieved with a mesh of size less than 0.5 mm. The powdered samples were stored at room temperature for further studies.

B. Preparation of methanol extract

The banana peel powder was washed with distilled water to remove any adherent particles and Shade dried. 25g of banana peel sample was mixed with 300 ml of methanol by continuous hot percolation with the help of soxhlet apparatus for 10 hr. The extract was filtered and concentrated using a rotary evaporator in the temperature range of 50° C-60° C. The concentrated extract was stored in the refrigerator.

C. Source of microorganisms

Pure culture of pathogenic bacteria *serratia marscens*, *pseudomonas aeroginosa*, *bacillus subtilis*, *shigella flexneri*, *vibrio parahaemolyticus* were obtained from Life Tech Research Center, Chennai, Tamil Nadu, India. The organisms were subcultured in a nutrient broth and incubated at 37° C for 24 hr.

D. Antibacterial activity assay

Antibacterial activity of extracts was determined by agar disc diffusion method on Muller Hinton agar (MHA) (Nazrul *et. al.*, 1984). The organisms to be tested was inoculated in stock cultures, and maintained at 4° c on a nutrient broth. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tube containing nutrient broth , and were incubated for 24 hrs at 37° C. Muller Hinton agar (MHA) was poured in to a petriplate. After the agar solidified, the inocula were spread on the solid plates with sterile swab moistened with the bacterial suspension. The discs were placed in MHA plates and 20 micro litre of samples were added (concentrations: 1000 µg/ml , 750 µg/ml , 500 µg/ml) were placed in the disc. The plates were incubated at 37° C for 24 hr. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition. The inocula tube and the 0.5 McFarland standard were compared against a card with a white background and contrasting black lines. Optimally within 15 minutes of preparation, dilute the adjusted inoculum suspension was diluted in a broth. After inoculation, each tube contains approximately 5×10^5 CFU/ml. This can be accomplished by diluting the 0.5 McFarland suspension 1:150, resulting in a tube containing approximately 1×10^6 CFU/ml. The subsequent 1:2 dilution in step 3 brings the final inoculum to 5×10^5 CFU/ml. 1 mg of sample was taken and mixed with 1ml of DMSO obtaining a concentration of 1 mg/ml.

E. Minimum Inhibitory concentration (MIC) determination

Minimum inhibition concentration (MIC) is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The inoculums are prepared by making a direct broth suspension of isolated colonies selected from an 18 - to 24 -hour agar plate (use a nonselective medium, such as blood agar). The suspension was adjusted to achieve a turbidity equivalent of a 0.5 McFarland turbidity standard. This results in a suspension containing approximately 1 to 2×10^8 colony forming units (CFU)/mL for bacterial cultures viz., *Pseudomonas aeroginosa*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Staphylococcus aureus*. This assay consists the determination of chemical agent spectrum of action, according to resistance of studied microorganisms. 1 ml of sterile LB broth was distributed for every tube and was submitted to autoclave under constant pressure at a temperature of 121° C. After the broth reaches room temperature 1 ml of diluted sample is added in tube1. 1 ml was transferred from tube 1 to tube 2. The transfer was repeated successively until tube 8. 100 µl of bacterial cultures were added to all the tubes from 1 to 8. Incubation was done at 37°C for 24 hrs. After incubation, the turbidity was observed. MIC is the concentration of higher dilution tubes in which there was no bacterial growth.

III. RESULTS AND DISCUSSION

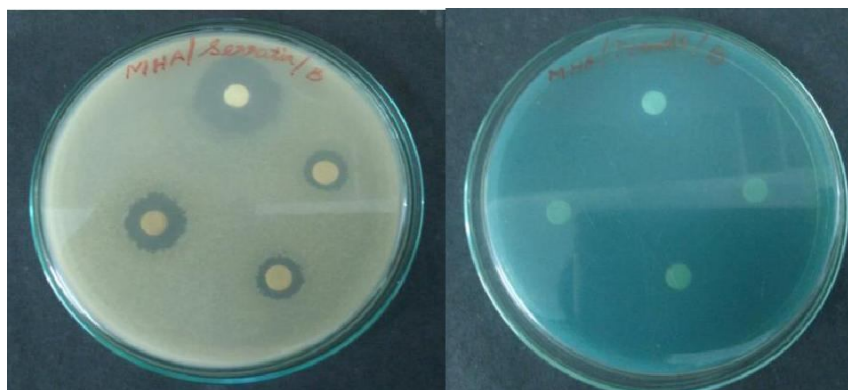
A. Antimicrobial activity

In the present study, the antibacterial activity of banana peel extract was studied *invitro* by disc diffusion method against five bacterial strains. The result of antibacterial activity of the methanol extracts of banana peel as shown in Table-1. The zone of inhibition of the growth of the isolates was obtained to be a function of the relative antibacterial potency of the extracts. The zone of inhibition decreased as the concentration of the extract decreased. The highest zone of inhibition was obtained at a concentration of 1000 µg/ml, against *bacillus subtilis* with a diameter of 27 mm. The antimicrobial activity was calculated by using the standard formula,

$$\text{Percentage Viability} = (\text{TOD} / \text{COD}) \times 100$$

TABLE 1
ZONE OF INHIBITION FOR AMPICILLIN

Name of microorganisms	Standard (Ampicillin) (mm)	1000 µg/ml (mm)	750 µg/ml (mm)	500 µg/ml (mm)
<i>Serratiamarscens</i>	23	15	11	10
<i>Pseudomonas aeruginosa</i>	23	09	07	06
<i>Bacillus subtilis</i>	42	27	19	21
<i>Shigella flexneri</i>	16	12	07	6.5
<i>vibrio parahaemolyticus</i>	25	14	11	12



Serratia Marscens

Pseudomonas Aeruginosa



Bacillus subtilis

Shigella flexneri



Vibrio Parahaemolyticus

Figure 1. Zone of inhibition of pathogens against banana peel extract

B. Minimum inhibition concentration

In the present study, antibacterial activity of banana peel extract was assayed *in vitro* by disc diffusion method against five bacterial strains. Some research studies show that banana peel contains secondary metabolites such as glycosides, alkaloids, saponins, volatile oil, flavonoids, and tannins. In general secondary metabolites are widely present in plants and earlier this was reported by Rabe (2000) to be responsible for their therapeutic activity. Plant drugs are less toxic when compared to any other drugs and they also have medical properties (Momin *et. al.*, 1987). The increase in the failure of both chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial infectious agent has led to the screening of several medical plants for potential antimicrobial activity (Kinghorn *et. al.*, 2010). The results obtained in this study indicate the use of banana peel by traditional medical practitioners.

TABLE 2
MINIMUM INHIBITION VALUES OF MICROORGANISMS

Name of microorganism	Minimum inhibition concentration (MIC) µg/ml
<i>Serratia marcescens</i>	1000
<i>Pseudomonas aeruginosa</i>	62.5
<i>Bacillus subtilis</i>	62.5
<i>Shigella flexneri</i>	15.6
<i>Vibrio flexneri</i>	125

IV. CONCLUSION

The present study shows that zone of inhibition were higher in *Bacillus subtilis* at lower concentration, and minimum inhibition concentration activity was higher in *shigella flexneri* when compared to the other four microorganisms. Banana peels have antimicrobial activity against pathogenic microorganisms. This work also suggests that all parts of banana plant have medicinal applications. According to this study it was concluded that banana peels serve as potential natural source of bioactive compounds and can be effectively utilized against many microorganisms.

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