

# Detection of antimicrobial activity of banana peel (*Musa paradisiaca* L.) on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*: An *in vitro* study

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## Abstract

**Introduction and Aim:** Banana is used widely because of its nutritional values. In past, there are studies that show banana plant parts, and their fruits can be used to treat the human diseases. Banana peel is a part of banana fruit that also has the antibacterial activity against microorganisms but has not been studied extensively. Since, there are no studies that relate the antibacterial activity of banana peel against periodontal pathogens. Hence, the aim of this study is to determine the antimicrobial activity of banana peel extract on *Porphyromonas gingivalis* (*P. gingivalis*) and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*). **Material and Methods:** Standard strains of *P. gingivalis* and *A. actinomycetemcomitans* were used in this study which was obtained from the in-house bacterial bank of Department of Molecular Biology and Immunology at Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre. The banana peel extract was prepared, and the antibacterial activity was assessed using well agar diffusion method and minimum inhibitory concentration was assessed using serial broth dilution method. **Results:** In the current study, both the tested microorganisms showed antibacterial activity. In well diffusion method, *P. gingivalis* and *A. actinomycetemcomitans* showed 15 mm and 12 mm inhibition zone against an alcoholic extract of banana peel, respectively. In serial broth dilution method *P. gingivalis* and *A. actinomycetemcomitans* were sensitive until 31.25 µg/ml dilutions. **Conclusion:** From results of the study, it is suggested that an alcoholic extract of banana peel has antimicrobial activity against *P. gingivalis* and *A. actinomycetemcomitans*.

**Keywords:** *Aggregatibacter actinomycetemcomitans*, banana, chronic periodontitis, isopropyl alcohol, *Porphyromonas gingivalis*

## Introduction

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.<sup>[1]</sup> Periodontitis is a disease with multifactorial etiology leads to loss of tooth supporting structures and tooth.<sup>[2]</sup> Gram-negative bacteria play a central role in development and progression of periodontal diseases, among them *Aggregatibacter actinomycetemcomitans*

(*A. actinomycetemcomitans*) and *Porphyromonas gingivalis* (*P. gingivalis*) are major putative periodontal pathogens that have been associated with chronic periodontitis, aggressive periodontitis, and failure of periodontal therapy.<sup>[3]</sup> Various traditional plants and plant products have shown antimicrobial activity against periodontal pathogens.<sup>[4]</sup>

Banana, tropical fruit belonging to *Musaceae* family, is grown in many countries all over the world.<sup>[5]</sup> All parts of banana plant such as flower, pulp, stem, and leaves have a medicinal application.<sup>[6]</sup> Banana peel is a waste product of banana,<sup>[5]</sup> and studies have shown that banana peel also has medicinal properties.<sup>[6,7]</sup> 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.<sup>[7]</sup>

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Researchers have done studies demonstrating the antimicrobial activity of banana peel against various Gram-positive and Gram-negative bacteria,<sup>[7]</sup> but none of them were done on the periodontal pathogens. Therefore, the aim of this study is to evaluate the presence of antibacterial activity and minimum inhibitory concentration of alcohol extract of banana peel (*Musa paradisiaca*. L) on *P. gingivalis* and *A. actinomycetemcomitans*.

## Materials and Methods

The microorganisms used in the study were standard strains of *P. gingivalis* (ATCC 33277) and *A. actinomycetemcomitans* (ATCC 43718). They were obtained from the in-house bacterial bank of Department of Molecular Biology and Immunology at Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre, Belgaum. Banana used for the study was authenticated by the Indian Council of Medical Research Centre, Belgaum (RMRC 1277). Seventy percentage isopropyl alcohol was used as the control solution.

### Extract preparation from banana peel

Banana peels were coarsely chopped and were kept in 70% isopropyl alcohol. Then, the entire mixture was homogenized in blender. This homogenized mixture or slurry was left at room temperature for about 48 h. As the reaction continued, the yellow transparent liquid turned to amber and later to an opaque black liquid that served as the indicator for completion of the reaction. After completion of the reaction, the entire slurry was filtered to get banana peel extract.<sup>[8]</sup>

### Well diffusion assay

Well diffusion assay was carried out to check the antibacterial activity of banana peel extract against *P. gingivalis* and *A. actinomycetemcomitans* strains. A loop or swab was used to transfer the colonies to the blood agar plates. The inoculum was adjusted to standard McFarland 0.5 turbidity. The sterile cotton swab was then dipped into the inoculum, and the surface of agar plate was swabbed three times and then the plates were rotated approximately 60°C between streaking to ensure even distribution. Five-millimeter diameter hollow tube was used to prepare the wells on each plate. 75 µl, 50 µl, 25 µl, 10 µl, and 5 µl of banana extract were added into the respective wells on each plate. The plates were then incubated for 18–24 h at 37°C in incubator. The size of inhibition zone was measured by holding the measuring device.<sup>[9]</sup>

### Minimum inhibitory concentration

Serial broth dilution method was carried out to check the minimum inhibitory concentrations of alcohol extract of banana peel.<sup>[9]</sup> Dilutions of alcoholic extract of banana peel have to be done with Thioglycollate broth for minimal inhibitory concentration (MIC). In the initial tube, 20 µl of alcohol extract of banana peel was added into the 380 µl of Thioglycollate broth. Then from the initial

tube 200 µl was transferred to the first tube containing 200 µl of Thioglycollate broth. By following this serial dilution, the concentrations of the alcohol extract of banana peel achieved were as the following – 500, 250, 125, 62.5, 31.25, 16, 8, 4, and 2 µl/ml, respectively. The tubes were then incubated for 48–72 h at 37°C. After the incubation, the MIC values were determined by visual inspection of the tubes. Turbidity in the MIC tube indicated growth of the bacteria implying that the bacteria were resistant to the alcoholic extract of banana peel. The Same procedure was carried out for 70% isopropyl alcohol.<sup>[10]</sup>

## Results

Both *P. gingivalis* and *A. actinomycetemcomitans* showed sensitivity against an alcoholic extract of banana peel.

### In well diffusion assay

*P. gingivalis* and *A. actinomycetemcomitans* showed 15 mm and 12 mm inhibition zone against an alcoholic extract of banana peel, respectively. In case of 70% isopropyl alcohol which is used as a negative control, *P. gingivalis* and *A. actinomycetemcomitans* showed 08 mm and 10 mm inhibition zone, respectively [Table 1]. Hence, this result suggested that alcoholic extract of banana peel showed antibacterial activity against *P. gingivalis* and *A. actinomycetemcomitans*.

### In serial broth dilution

*P. gingivalis* was sensitive until 31.25 µg/ml dilutions against both alcohol extract of banana peel and 70% isopropyl alcohol [Table 2], whereas in case of *A. actinomycetemcomitans* showed sensitivity up to 31.25 µg/ml and 250 µg/ml against alcoholic extract of banana peel and 70% isopropyl alcohol [Table 3], respectively. In this study, *A. actinomycetemcomitans* was found to be more sensitive than *P. gingivalis*.

## Discussion

The consumption of banana is good because of its nutritional value. It is used in anemia,<sup>[11]</sup> depression,<sup>[12]</sup> heartburn,<sup>[13]</sup> strokes,<sup>[11]</sup> stress,<sup>[12]</sup> etc., Banana peel which is an outer shell of banana also have been studied for the treatment of mosquito bites,<sup>[14]</sup> gastrointestinal disorders,<sup>[15]</sup> warts,<sup>[11]</sup> and nipple fissures caused by *Staphylococcus aureus*.<sup>[16]</sup>

There are several studies showing the antimicrobial activity of banana peel. Ighodaro<sup>[17]</sup> evaluated antibacterial activity of

**Table 1: Comparison of Inhibition zone of Banana peel extract and 70% Isopropyl alcohol against Pg and Aa in mm**

Strains	Banana peel extract	70% Isopropyl alcohol
<i>P. gingivalis</i>	15 mm	08 mm
<i>A. actinomycetemcomitans</i>	12 mm	10 mm

**Table 2: Minimum inhibitory concentration value of banana peel extract and 70% isopropyl alcohol against *Porphyromonas gingivalis* (Pg) in µg/ml**

	<i>P. gingivalis</i>									
	Concentration in µg/ml									
	500	250	125	62.5	31.25	16	8	4	2	1
Banana peel	S	S	S	S	S	R	R	R	R	R
70% Isopropyl alcohol	S	S	S	S	S	R	R	R	R	R

**Table 3: Minimum inhibitory concentration value of banana peel extract and 70% isopropyl alcohol against *Aggregatibacter actinomycetemcomitans* (Aa) in µg/ml**

	<i>A. Actinomycetemcomitans</i>									
	Concentration in µg/ml									
	500	250	125	62.5	31.25	16	8	4	2	1
Banana peel	S	S	S	S	S	R	R	R	R	R
70% Isopropyl alcohol	S	S	R	R	R	R	R	R	R	R

banana peel extract (*M. paradisiaca*) against human pathogenic bacteria and found that banana peel extract showed inhibition against *S. aureus*, *Escherichia coli*, and *Proteus mirabilis*. Chabuck *et al.*<sup>[7]</sup> studied antimicrobial activity on clinical isolates of two Gram-positive (*S. aureus* and *Streptococcus pyogenes*), four Gram-negative (*Enterobacter aerogenes*, *Klebsiella pneumoniae*, *E. coli*, and *Moraxella catarrhalis*), and one yeast (*Candida albicans*). Banana extract showed highest antibacterial activity against both *M. catarrhalis* and *S. aureus* followed by *S. pyogenes*, *E. aerogenes*, and *K. pneumoniae* and no effect against *E. coli* and *C. albicans*.

In the present *in vitro* study, we focused on determining the antibacterial activity of alcoholic extract of banana peel against standard stains of Gram-negative anaerobes like *P. gingivalis* and *A. actinomycetemcomitans* which are associated with periodontal diseases. *P. gingivalis* is a Gram-negative, anaerobic, nonmotile, saccharolytic rod that usually exhibits coccoid to short rod morphologies.<sup>[18]</sup> It is mainly associated with adult periodontitis, acute periodontal abscess, and failure of the regenerative procedure.<sup>[3]</sup> *A. actinomycetemcomitans* is a small, nonmotile, Gram-negative, saccharolytic, capnophilic, round-ended rod.<sup>[18]</sup> It is associated with adult periodontitis, aggressive periodontitis, refractory periodontitis,<sup>[3]</sup> and also associated with periodontitis lesion of Papillon-Lefèvre syndrome.<sup>[19]</sup> Both the bacterial strains showed sensitivity against an alcoholic extract of banana peel in the current study.

In our study, we carried out well diffusion assay and serial broth dilution method to detect the antibacterial activity of alcoholic extract of banana peel. In well diffusion assay, results have shown a 15 mm and 12 mm inhibition zone of *P. gingivalis* and *A. actinomycetemcomitans*, respectively, against the alcoholic extract of banana peel. Whereas, in serial broth dilution method both *P. gingivalis* and *A. actinomycetemcomitans*

have shown sensitivity until 31.25 µg/ml dilution against the same extract. In general, the presence of secondary metabolites in plants has been responsible for antibacterial activity.<sup>[20]</sup> Secondary metabolites such as flavonoids, tannins, phlobatannins, alkaloids, glycosides, and terpenoids were found in banana peel.<sup>[6]</sup> The presences of these phytochemicals/secondary metabolites might be responsible for the antibacterial activity of banana peel.

In the current study, banana peel extract was prepared using isopropyl alcohol, which is an organic solvent. Ehiowemwenguan *et al.*<sup>[21]</sup> studied on antibacterial activity of organic extract and aqueous extract of banana peel, and they concluded that organic extract had least MIC value compared to the aqueous extract. The author in the same study also carried out phytochemical screening of both organic and aqueous extract of banana peel and found that the organic extract of banana peel contains glycosides, alkaloids, flavonoids, and tannins. While, the water extract contains only glycosides and alkaloids. This result indicated that organic solvents dissolve more active compound than water. Ighodaro<sup>[17]</sup> also reported the same result, and he found that organic solvent had higher antibacterial activity than aqueous solution. Therefore, in this study isopropyl alcohol was used to dissolve more active compounds from the banana peel.

McDonnell and Russell<sup>[22]</sup> stated that alcohol has antimicrobial activity. Therefore, isopropyl alcohol was used as a negative control in this study. In well diffusion assay of this study, 70% isopropyl alcohol has shown 8 mm and 10 mm zones of inhibition with *P. gingivalis* and *A. actinomycetemcomitans*, respectively. Whereas, the alcohol extract of banana peel have shown 15 mm and 12 mm of inhibition zones with *P. gingivalis* and *A. actinomycetemcomitans*, respectively. In MIC, 70% isopropyl alcohol has shown least sensitivity

up to 31.25 µg/ml and 250 µg/ml against *P. gingivalis* and *A. actinomycetemcomitans*, respectively, whereas in alcoholic extract of banana peel showed sensitivity until 31.25 µg/ml against both strains. These results support the previous studies.<sup>[7,13,21]</sup> This also indicated that banana peel extract showed sensitivity against both strains, but it has no antibacterial activity against *P. gingivalis* at lower concentrations.

Singh et al. 2013<sup>[23]</sup> evaluated the antibacterial activity of three different color banana such as red, green, and yellow against ten clinical pathogens. The red banana showed a maximum zone of inhibition of 27 mm against *Planococcus citri* and 18 mm against *S. aureus*. The green banana peel showed inhibition zone of 19 mm against *Salmonella typhi* and *Aeromonas hydrophila*. The yellow banana peel exhibited 20 mm against *A. hydrophila* followed by 13 mm against *S. aureus*. This study enlightens a new line of approach for further study of different types of banana peels against different periodontal pathogens.

## Conclusion

The results suggest that banana peel has antibacterial activity against *P. gingivalis* and *A. actinomycetemcomitans*. This result opens new avenues of researches to be done on clinical isolates of all periodontopathogens with different varieties of banana and its extracts.

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## Conflicts of interest

There are no conflicts of interest.

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