



Research Article

www.ijrap.net

(ISSN Online:2229-3566, ISSN Print:2277-4343)



EVALUATION OF ANTIMICROBIAL ACTIVITY AGAINST *PSEUDOMONAS AERUGINOSA*: A COMPARATIVE ANALYSIS BETWEEN THE HYDRO-ALCOHOLIC EXTRACT OF RIPE AND UNRIPE BANANA PEELS

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Received on: 18/09/23 Accepted on: 26/10/23

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DOI: 10.7897/2277-4343.15119

ABSTRACT

Background: Sustainable utilisation of agro-food wastes is rich in phytonutrients exhibiting a wide range of bioactivities and is the focus of recent research. Banana peels are waste products traditionally used for ailments like cough, burns, and inflammation. The present study evaluated the antimicrobial action of the ripe and unripe peel extract against *Pseudomonas aeruginosa*, which is responsible for most nosocomial infections and makes it challenging to achieve therapeutic compliance. Methods: The ripe and unripe banana peels were collected and authenticated, and hydro-alcoholic extracts were prepared using maceration. Phytochemical analysis, antioxidant and antimicrobial studies were performed with both the extracts and compared. Results: Both the peels were identified as *Musa paradisiaca* L., and hydro-alcoholic extracts showed the presence of flavonoid and phenolic compounds in significant amounts. The *in-vitro* antioxidant study revealed the ripe peels to be more potent, having an IC₅₀ value of 35.71 µg/ml in DPPH free radical scavenging action. It significantly inhibited gram-negative bacteria *Pseudomonas aeruginosa*, comparable with the Amoxicillin as standard. Both the extracts had MIC as 300 mg/ml, but the zone of inhibition produced by the ripe peels (1.63 cm) was more potent than the unripe peels (1 cm). Conclusion: The present study data indicated that the hydro-alcoholic extract of ripe banana (*Musa paradisiaca* L) peel was promising in antioxidant and antibacterial action primarily due to its significant phenolic contents. Further research is needed for a detailed evaluation of its phytopharmacological action, which can be beneficial for developing a cost-effective natural source of antimicrobial agents in pharmaceutical industries along with an eco-friendly environment.

Keywords: Banana peel, Ripe, Phenolic compound, Antimicrobial, *Pseudomonas aeruginosa*.

INTRODUCTION

Multi-drug resistance (MDR) has significantly developed in recent years and is now acknowledged as a major global problem. The most significant difficulty for doctors is providing adequate care for infections caused by gram-negative organisms because of the rise in antibiotic resistance in the healthcare environment. *Pseudomonas aeruginosa* has been leading in all infections brought on by gram-negative bacteria.¹ In severely ill and immunocompromised individuals, *Pseudomonas aeruginosa* infections have become a severe nosocomial (hospital-acquired infections) problem. The emergence of drug-resistant strains is the main issue contributing to high mortality.²

The World Health Organization (WHO) placed *Pseudomonas aeruginosa* in the category of highest priority in 2017 on the global priority list of diseases. *P. aeruginosa* has emerged as a significant concern in healthcare settings, leading to detrimental effects such as higher death rates, illness rates, and expenses for healthcare. Managing these infections continues to be difficult and necessitates implementing antimicrobial stewardship programs.³ It became necessary to investigate newer medications with reduced resistance. Medications derived from natural sources are crucial in preventing and treating human illnesses. In many of the developing countries, traditional medicine serves as a fundamental healthcare system.⁴ Recently, researchers have shown increased interest in exploring the advantages of agricultural refuse. Among the natural sources obtained, bananas are important edible fruits, extensively grown in tropical and

subtropical regions, possessing potential nutritional and health benefits with natural antioxidants. However, banana peels are a major issue as an overlooked waste.⁵

In recent times, in addition to the principles of 'Green Chemistry', the sustainable utilisation of agro-food waste, which contains high levels of phytonutrients or nutraceuticals, demonstrates a wide range of bioactivities.^{6,7} Research evidence has indicated that the rinds and seeds of fruits, such as apples, grapes, citrus fruits, jackfruit, mango, etc., contain approximately 15% more polyphenolic and flavonoid compounds than the pulp. The antioxidant, immunomodulatory, and anti-inflammatory properties of these by-products derived from vegetable and fruit waste can potentially prevent or be used as a treatment for numerous communicable and non-communicable diseases. Similarly, the peel of bananas, which makes up about 35% of the total weight of the fruit, has traditionally been used as a remedy for common ailments such as coughs, burns, and inflammation but is discarded as waste.⁸ It has been proven to be a good source of dietary fibre, polyphenolic compounds, and essential amino acids, even more so than the fruit itself. This bioactive compound has been reported to have pharmacological effects, particularly as an antioxidant, antidiabetic, anti-inflammatory, and antibiotic.⁹⁻¹¹

However, only a few studies have confirmed their pharmacological properties. The present study aims to compare ripe and unripe banana peels, focusing on their phytochemical, antioxidant, and antimicrobial activity against *P. aeruginosa*.

MATERIALS AND METHODS

Collection of Plant material and Identification

The unripe and ripe banana peels were collected and authenticated from the Botanical Survey of India, Howrah, West Bengal (CNH/Tech.II/2023/06 dated 24.02.2023) as *Musa paradisiaca* L. (Family- Musaceae).

Extraction of leaves of Plant material

The unripe and ripe banana peels were cleaned, shade-dried and crushed to powder by the grinder. They were macerated with 70% ethanol for 5 days. Thereafter, the extracts were collected after filtration and solvent evaporation. The dried samples were refrigerated for further experiments.

Phytochemical screening

Qualitative standard chemical methods were used to identify different phytoconstituents, like reducing sugar (Fehling's test), carbohydrate, alkaloids (Wagner, Hager, Mayer, Dragendorff's test), glycosides (Killer-Killani test), tannins, saponins, phenols (ferric chloride test), flavonoids, phytosterols, terpenoids (Salkowski test) present in the hydro-ethanolic extract of banana peels.

Total phenolic content

A 1 mg/ml stock solution of both the ripe and unripe peel extract was prepared in ethanol and centrifuged at 1000 rpm for 10 minutes, and supernatant was collected. A test sample of 0.2 mL was combined with 0.6 mL of water and 0.2 mL of Folin-Ciocalteu's phenol reagent in a 1:1 ratio. After 5 minutes, 1 mL of saturated sodium carbonate solution (8% w/v in water) was added, and the final volume was adjusted to 3 mL with distilled water. The reaction mixture was then kept in the dark for 30 minutes, and the absorbance was measured at 765 nm.¹² The total phenolic contents of extracts were measured using the Gallic acid standard curve by using the following formula:

$$\text{TPC} = c \text{ (V/m)}$$

where c = concentration of gallic acid obtained in mg/ml (calculated from the gallic acid standard curve)
V = volume of extract in ml, m = mass of extract in gm

Total Flavonoid content

The amount of total flavonoids present in the crude extract was assessed using the aluminium chloride colourimetric method.¹² The extract solutions for the ripe and unripe banana peel were prepared at a concentration of 1 mg/ml, and 0.15 ml of 5% sodium nitrite solution was added. After 5 minutes, 0.3 ml of 10% aluminium chloride solution was added to the mixture. After 6 minutes, 1 ml of 1M NaOH was added to the mixture. Then, the mixture was diluted by adding 1.55 ml of distilled water and mixed thoroughly. Then, the mixture was kept in the dark for 30 minutes, and the absorbance was determined at 510 nm compared with the control. Quercetin was used as a standard for the calibration curve. The following formula estimated the total flavonoid content (TFC) of the extract:

$$\text{TFC} = c \text{ (V/m)}$$

where c = concentration of quercetin obtained in mg/ml (calculated from the quercetin standard curve)
V = volume of extract in ml, m = mass of extract in gm

Anthocyanin content

The anthocyanin content of both the ripe and unripe peel extract was measured. 0.025M potassium chloride buffer with pH 1.0 and 0.4M sodium acetate buffer with pH 4.5 were prepared. 1 ml of the extract was mixed with 9 ml of potassium chloride buffer, and another part of 1 ml of extract was mixed with 9 ml of sodium acetate buffer. This process was carried out for both ripe and unripe peel extracts in a triplicate manner. The control contains all the chemicals with ethanol except the sample. The test tubes were then incubated at room temperature for 1 hour, and absorbance was measured at 520 and 700 nm, respectively, against the control.¹² Total anthocyanins were estimated using the following formula:

$$\text{Anthocyanin pigment (mg/L)} = (A \times \text{MW} \times \text{DF} \times 1000) / \epsilon \times l$$

where A = [(A_{520 nm} - A_{700 nm}) pH1.0 - (A_{520 nm} - A_{700 nm}) pH4.5]; MW (molecular weight) = 449.2 g/mol of cyanidin-3-glucoside; DF = dilution factor; l = path length in cm and ϵ = 26,900 molar extinction coefficient.

In-vitro antioxidant studies

DPPH radical scavenging activity

The antioxidant studies were evaluated with the 70% ethanolic extract of ripe and unripe banana peels with various concentrations prepared by ethanol. The plant extract was assessed for the radical scavenging effect using stable 1,1-diphenyl-2-picrylhydrazyl (DPPH).¹² DPPH solution (0.002% w/v) was prepared in methanol. 1 ml of various concentrations of extracts (5 µg/ml - 1000 µg/ml dissolved in distilled water) was mixed with 1 ml DPPH solution and colour factors for each concentration were also prepared without DPPH. The reaction mixtures were kept in the dark at room temperature for 30 minutes of incubation. Subsequently, the absorbance of the samples was measured at 517 nm. All the measurements were repeated for at least three times. The percentage inhibition was calculated for each concentration of banana peel extract and compared with the control, and the IC₅₀ value was determined.

$$\text{Percentage inhibition was calculated as} = \left[\frac{(\text{absorbance of control} - (\text{absorbance of sample} - \text{colour factor}))}{(\text{absorbance of control})} \right] \times 100.$$

Reducing Power Assay

Various concentrations (100-500 µg/mL) of the hydro-alcoholic extract of *Musa paradisiaca* peel and its different fractions were added to a mixture containing 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution. The reaction mixture was thoroughly mixed using a vortex mixer and then incubated at 50 °C for 20 minutes. After the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture, and then centrifuged at 3,000 rpm for 10 minutes. The supernatant (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride. The absorbance of the coloured solution was measured at 700 nm against a blank using a UV Spectrophotometer.¹³ Ascorbic acid was used as a reference standard, and the reducing power of the samples was comparable to the reference standard.

Antimicrobial study with agar well diffusion method

The agar plates were prepared using clean, sterile Petri dishes, and after the media was solidified, 100 µl of *Pseudomonas aeruginosa* was spread over the agar plates. After that, sterilised borers were placed at the centre of the agar plates to make the

well, and 100 µl of the ripe and unripe peel extracts in different concentrations were applied in each well. Finally, they were incubated in the dark for 24 hours, and then the zone of inhibition of each plate was measured.¹⁴ Amoxicillin was used as a standard in this method; one plate was made as a negative control with distilled water and one positive control plate was prepared by adding only *Pseudomonas aeruginosa* to the media. To assess the effectiveness of the extracts against bacterial strains, the

researchers measured the minimum inhibitory concentration (MIC) and compared it with the standard drug Amoxicillin.

RESULTS

The phytochemical screening with the ripe and unripe peel extract of *Musa paradisiaca* revealed the presence of phytoconstituents like alkaloids, glycoside, flavonoids, terpenoids, phytosterols, carbohydrates and saponins in both extracts.

Table 1: Phytochemical screening of the hydro-alcoholic extract of *Musa paradisiaca* peels both ripe and unripe

Phytochemical analysis	Ripe sample	Unripe sample
1. Flavonoids	Positive	Positive
2. Carbohydrates	Positive	Positive
3. Reducing sugar (Fehling's B)	Negative	Positive
4. Tannin	Negative	Positive
5. Saponin	Positive	Positive
6. Alkaloids: Mayer's reagent Dragendorff's reagent Wagner's reagent Hager's reagent	Positive Negative Positive Positive	Negative Negative Positive Positive
7. Glycosides	Positive	Positive
8. Phenols	Negative	Positive
9. Terpenoids	Positive	Positive
10. Phytosterols	Positive	Positive

Reducing sugar and tannins were negative in the ripe peel extract but were present in the unripe peels.

Total Phenolic Content

The study showed both the samples had significant phenolic compounds present. The data were interpreted and compared to the Gallic acid standard curve. The ripe and unripe peel extracts comprise 40.246 mg/GAE g and 37.589 mg/GAE g total phenolic content, respectively. The ripe peel extract contains more phenolic content than the unripe peels.

Total Flavonoid Content

The flavonoid contents of the ripe and unripe banana peel extracts were evaluated from the Quercetin standard curve. The flavonoid contents in the ripe and unripe peel extracts were 22.075 mg/QE g and 21.97 mg/QE, respectively.

Anthocyanin Content

The ripe and unripe banana peel extracts possess anthocyanin content to some extent, compared with the cyanidine-o-3 glycoside standard curve. The ripe peel extract contains anthocyanin content of 1.56 mg / 100g, which is better than that of the unripe peels, i.e. 0.89 mg / 100 g.

In-vitro antioxidant study

DPPH free radical scavenging activity

The hydro-ethanolic extracts of *M. paradisiaca* peels, both ripe and unripe extract, significantly inhibited the DPPH radical in a dose-dependent manner (Figure 1).

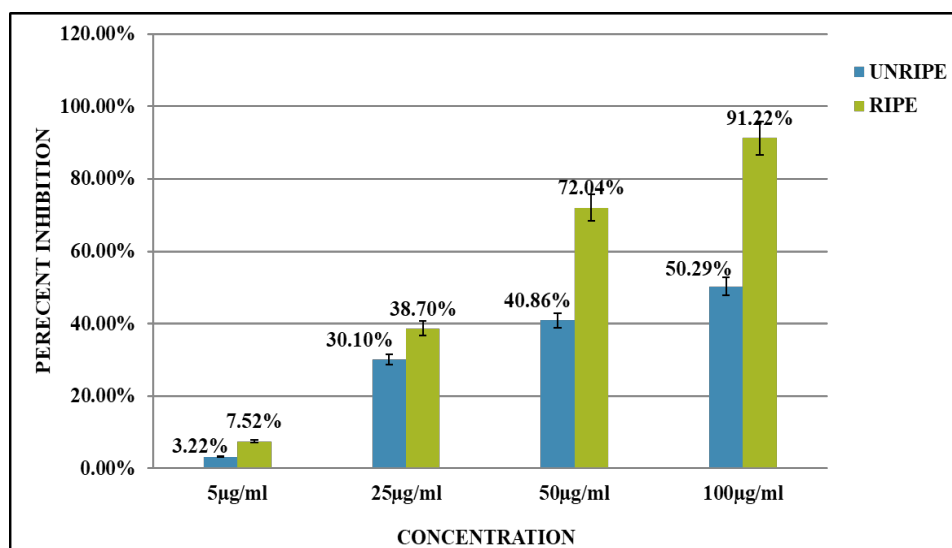
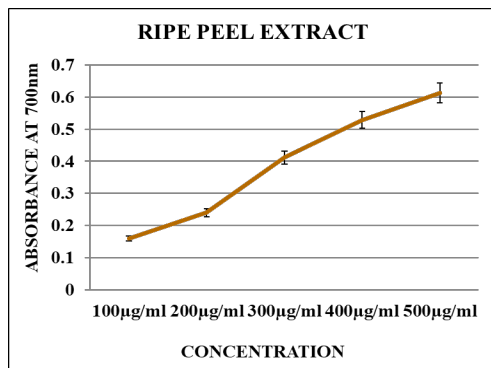


Figure 1: DPPH scavenging activity of hydro-alcoholic extract of *Musa paradisiaca* L.
Data are mean \pm S.E.M (n=4).

The ripe peel extract revealed a more significant antioxidant potency than the unripe one. The IC_{50} value of unripe peel was around 100 μ g/ml, whereas it was very significant for ripe peel extract at about 34.7 μ g/ml. The results indicate that the banana peel extract processes potent DPPH scavenging activities, and the ripe peels were found to be more beneficial.



Reducing Power Assay

The reducing power action of the hydro-alcoholic extracts of both the ripe and unripe *Musa paradisiaca* L. peels were analysed and compared with the ascorbic acid as a standard.

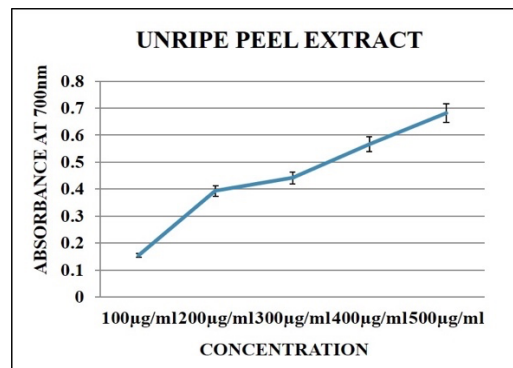


Figure 2: Reducing power analysis of hydro-alcoholic extract of *Musa paradisiaca* L. Data are mean \pm S.E.M (n=4).

Figure 2 denotes the reducing power action in a dose-dependent manner with both extracts. The ripe peel extract revealed better potential compared to the unripe one. Ascorbic acid was used as a standard to compare.

Antimicrobial study

The antimicrobial study used the agar well diffusion method to use the ripe and unripe peel banana extract (*Musa paradisiaca*) against the gram-negative bacteria *Pseudomonas aeruginosa*. Antimicrobial activity was observed in agar well diffusion assay with the extracts at 100 mg/ml, 200 mg/ml, 300 mg/ml and 500 mg/ml. The result revealed that both the extracts were suppressing the growth of the microorganisms with variable potency.

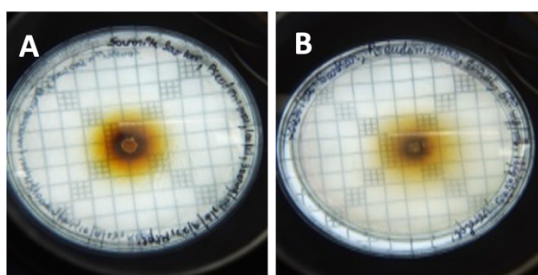


Figure 3: Antimicrobial study with ripe [A] and unripe peel [B] extract of banana (*Musa paradisiaca*) against *Pseudomonas aeruginosa* using agar well diffusion method

Table 2: The zone of inhibition study with the banana peel extracts against *Pseudomonas aeruginosa*

Concentration (mg/ml)	Zone of Inhibition by Ripe Peel	Zone of Inhibition by Unripe Peel
100 mg/ml	Nil	Nil
200 mg/ml	Nil	Nil
300 mg/ml	1.63 cm	1 cm
500 mg/ml	1.8 cm	1.23 cm

This table revealed that the ripe and unripe banana peel 70% hydro-alcoholic extract possesses antimicrobial activity against the microorganism *P. aeruginosa*. The activity was compared with the standard drug Amoxicillin 0.5 mg/ml at 1.68 cm. The MIC for both was found to be 300mg/ml.

DISCUSSION

Bananas are considered beneficial due to their nutritional value. Banana peels contain various compounds, including terpenoids, tannins, alkaloids, saponins, steroids, phenols, fixed oils, and fats. Among the extracts tested, the 80% ethanolic extract of banana peel exhibited the highest total phenolic content, total flavonoid content (TFC), and antioxidant activity.¹⁵ These secondary metabolites found in plants, such as flavonoids, tannins, phlorotannins, alkaloids, glycosides, and terpenoids, contribute to the antibacterial activity of banana peels and are enriched in them.¹⁰ Present study also revealed the 70% hydro-ethanolic extract of both the ripe and unripe peels of *Musa paradisiaca* contain phytoconstituents like alkaloids, glycoside, flavonoids, terpenoids, phytosterols, carbohydrates and saponins. A study by Ehiowemwenguan *et al.* found that the organic extract of banana peel contains glycosides, alkaloids, flavonoids, and tannins, whereas the water extract contains only glycosides and alkaloids. This observation suggests that organic solvents are more effective at dissolving the active compounds than water.¹⁶ Recently, Vu *et al.* also reviewed the phenolic compounds and their potential health benefits from banana peel.¹⁷ The major bioactive compounds in banana fruit, known for their antioxidant properties and health benefits, are phenolics. These include gallic acid, catechin, epicatechin, tannins, and anthocyanins, identified in various studies.¹⁸ Rebello *et al.* also demonstrated that the banana peel extract is a rich source of total phenolics, with a concentration of 29 mg/g as Gallic Acid Equivalents (GAE), contributing to its very high antioxidant activity.¹⁹ Some studies revealed significant bioactive components in the banana peels, particularly the phenolic compounds. The major phenolic compounds were classified as flavonols, hydroxycinnamic acids, flavan-3-ols, and catecholamines.²⁰ Similarly, the present study found that the ripe and unripe peel extracts are rich in phenolic contents. They consist of 40.246 mg/GAE g and 37.589 mg/GAE g total phenolic content, respectively.

The antioxidant study of unripe banana peel revealed significant antioxidant properties and contained the highest total phenolic compound. The results showed a correlation between antioxidant activity and the total phenolic content.²¹ Based on these findings, it can be inferred that the unripe banana peel exhibited stronger antioxidant potency than the ripe peel.²² Some other studies also reported significant antioxidant studies using the banana peel

extracts.^{23,24} In the present study, the DPPH free radical scavenging method revealed the IC₅₀ value of unripe peel extract was 100 µg/ml and for ripe peel extract 34.7 µg/ml. This indicates that banana peels have significant antioxidant properties. The ripe banana peel extract processes more potent antioxidant action than the unripe peels. The reducing power assay also indicated the beneficial antioxidant property in the 70% hydro-alcoholic extract of both the ripe and unripe banana peels, comparable with that of the ascorbic acid. Studies conducted by researchers have shown that banana peel exhibits antimicrobial activity against a wide range of bacteria, including both gram-positive and gram-negative strains. Antibacterial activity of banana peel extract (*M. paradisiaca*) against human pathogenic bacteria showed inhibition against *S. aureus*, *Escherichia coli*, and *Proteus mirabilis*.²⁵ In another study, similar findings were reported, demonstrating the antimicrobial activity of banana peel against various clinical isolates, including two gram-positive bacteria (*S. aureus* and *Streptococcus pyogenes*), four gram-negative bacteria (*Enterobacter aerogenes*, *Klebsiella pneumoniae*, *E. coli*, and *Moraxella catarrhalis*), and one yeast (*Candida albicans*).¹¹ Ehiowemwenguan et al. investigated the antibacterial activity of organic and aqueous banana peel extracts. They concluded that the organic extract exhibited a lower minimum inhibitory concentration (MIC) value than the aqueous extract, indicating greater potency in inhibiting bacterial growth.¹⁶ But very few studies are against the pathogenic gram-negative bacterial strain *Pseudomonas aeruginosa*. Therefore, in the present study, the antibacterial potential of the banana peel extracts was evaluated against this pathogen using the agar well diffusion method. It was found that the hydro-alcoholic extract of both the ripe and unripe peel of *Musa paradisiaca* L (banana) revealed significant action against *Pseudomonas aeruginosa*, which is comparable with the standard drug Amoxicillin. The MIC for both was found to be 300 mg/ml. The ripe peels showed a zone of inhibition of 1.63 cm at 300 mg/ml dose, like the Amoxicillin at 0.5 mg/ml dose. In the case of 300 mg/ml unripe peels, the zone of inhibition was found to be 1 cm. Therefore, the present study has found that ripe peels had more significant antioxidant and antibacterial potential than unripe peels, possibly due to their rich phenolic content compared to unripe peels.

CONCLUSION

Recycling agricultural waste materials and by-products for utilisation as a commercial source of income is a very emerging field. Banana by-products are essential, having various medicinal values, and if these can be processed, they can benefit environmental waste management. In the present study, the hydro-alcoholic extract of the ripe and unripe banana peel was a rich phenolic content source. The ripe peels were more significant, with antioxidant and antimicrobial action against gram-negative *Pseudomonas aeruginosa*. Further research on the detailed phytopharmaceutical analysis of pharmacological action can lead to these waste materials as a sustainable source of pharmaceutical product development and management of an eco-friendly environment.

ACKNOWLEDGEMENT

The authors would like to acknowledge JIS University for providing assistance and support throughout the experiments and for academic tenure for the successful completion of the entire research work.

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Cite this article as:

Soumita Sarkar, Mayukh Bose, Moumita Ray. Evaluation of antimicrobial activity against *Pseudomonas aeruginosa*: A comparative analysis between the hydro-alcoholic extract of ripe and unripe banana peels. Int. J. Res. Ayurveda Pharm. 2024;15(1):88-93

DOI: <http://dx.doi.org/10.7897/2277-4343.15119>

Source of support: Nil, Conflict of interest: None Declared

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