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**ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF ESSENTIAL  
OILS FROM BANANA (MUSA, ACUMINATE L, DWARF CAVENDISH  
BANANA) FRUITS PEEL: AN *IN VITRO* STUDY**

**RAMI E<sup>1\*</sup>, SWATI A<sup>1</sup> AND GANGAWANE AK<sup>2</sup>**

- 1: Department of Biotechnology, Parul Institute of Applied Science, Parul University, Vadodara,  
Gujarat, India
- 2: Department of Biochemistry, Parul Institute of Applied Science, Parul University, Vadodara,  
Gujarat, India

\*Corresponding Author: Dr. Esha Rami: E Mail: [esha.rami82036@paruluniversity.ac.in](mailto:esha.rami82036@paruluniversity.ac.in)

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**ABSTRACT**

Banana is used widely consumed because of its nutritional values. Banana peel is a part of banana fruit that also has the antioxidant and antibacterial activity against microorganisms but has not been studied extensively. There are few studies that relate the antioxidant status and antibacterial activity of essential oil from banana peel against common human pathogens. This study aimed to evaluate the antioxidant and antibacterial activity of peel of *Musa, acuminata* L, dwarf cavendish banana in an *in vitro* experimental set-up. The fruits of *M. acuminata* L (dwarf cavendish banana) were obtained from local market. Standard strains of *Escherichia coli* and *Staphylococcus aureus* were used in this study which were obtained from the National Centre For Cell Science, Pune, India. The antioxidant activities of the extracts were evaluated by using the Diphenyl-1- picrylhydrazyl (DPPH) free radical scavenging method. While, antimicrobial activities of the extracts and isolated components were evaluated using Minimum Inhibition Concentration (MIC). The essential oils obtained from hexane and chloroform extracts displayed high antimicrobial and antioxidant activity. Antioxidant activity of chloroform extracts was comparable to those of synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene. Among all isolated components  $\beta$ -sitosterol, malic acid, succinic acid, palmitic acid, 12-hydroxystearic acid,

glycoside, the d-malic and 12-hydroxystearic acid were the most active against both *Escherichia coli* and *Staphylococcus aureus* species tested. The MIC of d-malic and succinic acid was varying between 140-750 ppm, respectively. Essential oils extracted from peel extracts of *M. acuminata* L (dwarf cavendish banana) were able to scavenge free radicals, hence can be employed as an alternative source of medicine to mitigate the diseases associated with oxidative stress. Further, the essential oils extracted from peel extracts of *M. acuminata* L (dwarf cavendish banana) could be considered as a good antibacterial agent against both *Escherichia coli* and *Staphylococcus aureus* to replace the synthetic medicines in treatment of diseases caused by these bacteria.

**Keywords:** Banana, *Escherichia coli* and *Staphylococcus aureus*, Essential oils, antioxidant

## INTRODUCTION:

The Musaceae family of herbaceous plants, which includes the genus *Musa*, includes the edible fruit known as the banana. It is one of the most significant fruit crops in the world and is widely grown in tropical nations due to its useful applications in the food industry. Through the abundant production of antioxidant substances like gallic acid and dopamine, a banana plant defends itself against the oxidative stress brought on by intense sunlight and high temperatures [1]. A banana fruit should be regarded as a useful dietary source against cancer and heart disease and a rich source of natural antioxidants for foods. The isolation, characterization, and application of natural antioxidants have therefore received attention lately, with an increasing focus on essential oils as possible disease-preventing agents [2]. As these compounds are predominantly found in most of the fruit tissues, it would be worthwhile investigating the nature of

essentials that are present in the banana peel. Since banana fruits are widely available, have they been used as food without apparent toxic effects? The peel could be a potential source of antioxidant and antimicrobial activities [3]. The present investigation was undertaken to evaluate the antioxidant and antibacterial power of banana fruit peel and to identify the responsible compounds for those activities.

## MATERIAL AND METHODS:

**Plant material:** Banana *Musa acuminata* L (dwarf cavendish banana) fruits were purchased in Vadodara city at green stage without any ethylene treatment and stored at 20°C for 24hr before being extracted.

**Extraction procedures** [Hassan, 2010] [4]: The peel tissues of fresh banana fruit (300 g) at green with a trace of yellow stage were heated in 1L of distilled water for 2 min. The peel was homogenized with 70% acetone twice at room temperature in an electric

blender. The Peel paste obtained was dried in the oven at below 50°C. A fine powder was then obtained by grinding of dried peels at room temperature. Around 50 g of the powder was then subjected to Soxhlet extraction with analytical grade n-Hexane solvent and chloroform. Essential oil from both n-hexane and chloroform was obtained in solvent-to-solvent recovery. The antimicrobial and antioxidant activity of hexane and chloroform extracts were measured.

**Diphenyl-1- picrylhydrazyl (DPPH) free radical scavenging method** [Ali *et al.*, 2017] [5]: For determination of antioxidant activity through DPPH scavenging potential, 1.0 mL of DPPH solution in methanol (0.13 mM) was mixed with equal volume of EOs in dimethyl sulfoxide (DMSO). Different concentrations of Eos were tested. The reaction mixture was kept at room temperature for 30 min in the dark place and, the absorbance of the mixture was measured by using a spectrophotometer at 517 nm. Antioxidant activity was measured as compared to control reaction without having a sample. The antioxidant potential of essential oil was calculated by the following equation:

Percentage of ABTS/DPPH scavenging activity =  $[(Abs_{\text{control}} - Abs_{\text{sample}}) / (Abs_{\text{control}})] \times 100$

Where,  $Abs_{\text{control}}$  is the absorbance of ABTS/DPPH radical with methanol and  $Abs_{\text{sample}}$  is the absorbance of ABTS/DPPH with EO solution.

**Determination of antioxidant activity using  $\beta$ -carotene linoleate model system:**

Antioxidant activity was measured using the methods of Jayaprakasha *et al.* [6] with slight modification. A 3.34 mg of  $\beta$ -carotene in chloroform solution (1 mL) and the chloroform were removed at 40°C under vacuum using a rotary evaporator. Thereafter, 40 mg of linoleic acid and 400 mg of Tween-20 were added and mixed well. The resulting mixture was immediately diluted with 5-10 mL of triple distilled water and was mixed well. The emulsion was further made up to 100 mL with 0.01M hydrogen peroxide ( $H_2O_2$ ). Aliquots (2 mL) of this emulsion were transferred into different test tubes containing 0.1 mL of test samples in methanol. In this experiment, BHA was used for comparative purposes. A control containing 0.2 mL of ethanol and 4 mL of the above emulsion was prepared. The tubes were placed in a water bath at 50°C. Absorbances of all the samples at 470 nm were taken at zero time and every 20 min

until the color of  $\beta$ -carotene disappeared in the control reaction. The blank was prepared as above mentioned but without  $\beta$ -carotene.

**An antioxidant assay using ferric thiocyanate methods:** A mixture of a 2 mL sample in 99.5% ethanol, 2.052 mL of 2.51% linoleic acid in 99.5% ethanol, 4 mL of 0.05M phosphate buffer (PH 7.0) and 1.948 mL of water was placed in a vial with a screw cap and placed in an oven at 60°C in the dark. To 0.1 mL of this sample solution, 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate was added. After the addition of 0.1 mL of  $2 \times 10^{-2}$  M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red color developed was measured in 3 min at 500 nm [7].

**Antimicrobial assay:** Fresh agar slants of the test strains were made from the obtained cultures. They were incubated for 24 hours at 30°C. A bacterial suspension was prepared by taking the bacterial colony from the agar slant into 6 ml of sterile water. Extracted essential oils from banana peel were dissolved in dimethyl sulphoxide (DMSO, 5.0 mg/mL) and diluted in neutral phosphate buffer. Dilution range used for the evaluation of antimicrobial activity: 1.0-0.1 mg/mL for *S.aureus* and 5.0-0.1 mg/mL for *E. coli*. Nutrient broth medium (composed of

peptone 0.5%, beef extract 0.15%, yeast extract 0.15%, sodium chloride 0.5% and pH adjusted to 7.2) was used for sub-culturing of selected bacteria and was incubated at 37°C for 24 h. For each experiment, 100  $\mu$ L of bacterial suspension was dispensed in a 96-well microplate (T941396, Tarson, India). 100  $\mu$ L of Standard antibiotics (Chloramphenicol for *S.aureus* and Ampicillin for *E.coli*) as positive control and dimethyl sulphoxide (DMSO) as a negative control, were mixed separately to it and, then the microplates were incubated at 37°C for 12 h. After incubation, the optical density of each well was measured by using a microplate reader at 600 nm (Radaelli *et al.*, 2016) [8].

**Determination of MIC:** The minimum inhibitory concentrations (MIC) were defined as the lowest concentration tested that showed no visible bacterial growth after incubation for 24 h (Radaelli *et al.*, 2016) [8].

## RESULTS:

**Antioxidant activities:** Banana peel essential oil extracts were tested for their antioxidant effects using hexane and chloroform, and the findings are shown in **Table 1** below. The extract from chloroform showed much higher antioxidant activity than the extract from hexane solvent.

**Table 1** below displays the extracts' DPPH assay-measured capacity to scavenge free radicals. Chloroform extract had higher free radical scavenging capacity than hexane, which had low antioxidant capacity.

By using the DPPH free radical,  $\beta$ -carotene bleaching technique, and linoleic acid emulsion to assess antioxidant activity, banana peel essential oils showed significant levels of activity. As can be seen in **Figure 1** below, extracts significantly decreased the oxidative activity of linoleic acid.

The findings demonstrated that the synthetic antioxidant reagent BHA at 0.1 mg mL<sup>-1</sup> had a lower inhibitory action than CHCl<sub>3</sub> extracts of essential oils from banana peel using the thiocyanate technique. According to Jayaprakasha *et al.*, the absorbance of the control increased in the first six days and then declined because linoleic acid hydroperoxides are produced when linoleic acid is oxidised and they breakdown an excessive amount of secondary oxidation products.

**Figure 2** below depicts the oxidative activity of linoleic acid using the  $\beta$ -carotene bleaching method. Polar fraction of CHCl<sub>3</sub> extract considerably prevented linoleic acid oxidation in the linoleic acid system  $\beta$ -carotene bleaching procedure. The activity

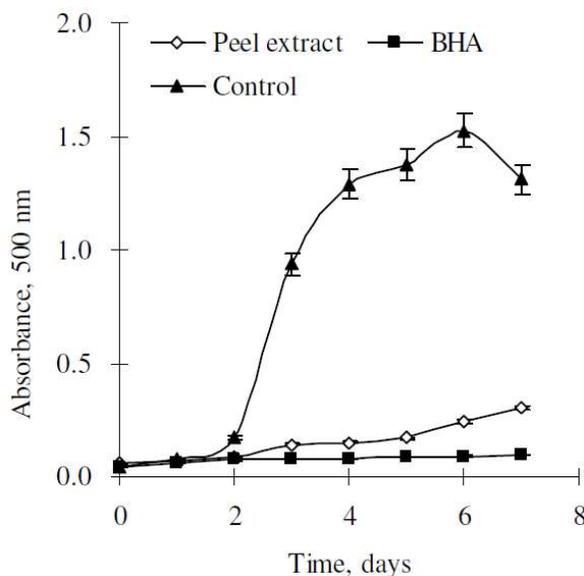
increased when the extract's polarity was increased, suggesting that the polyphenols, flavanones, and flavonoids in the essential oils may also be crucial players in the activities. These findings concurred with those of Baskar *et al.* In all instances, the sample inhibited the bleaching of  $\beta$ -carotene while the control without the addition of an antioxidant oxidised more quickly.

**Antimicrobial activities:** While CHCl<sub>3</sub> extract had minimal activity as evaluated by MIC techniques, hexane extract of the essential oils from banana peel had strong antibacterial properties (**Table 2**). After 48 hours of incubation at 36°C, the MIC was determined. The MIC of palmitic acid, succinic acid, malic acid, and  $\beta$ -sitosterol were examined against *S. aureus* and *E. coli*, as shown in **Table 2** below.  $\beta$ -sitosterol, malic acid, and succinic acid were effective against the studied bacterial species of *E. coli* and *S. aureus*, however palmitic acid had little effect. Malic acid, succinic acid, and  $\beta$ -sitosterol all have minimum inhibitory concentrations (MICs) that range between 140 and 750 ppm. Compared to  $\beta$ -sitosterol, succinic acid, and palmitic acid, these findings showed that malic acid had considerable antibacterial action.

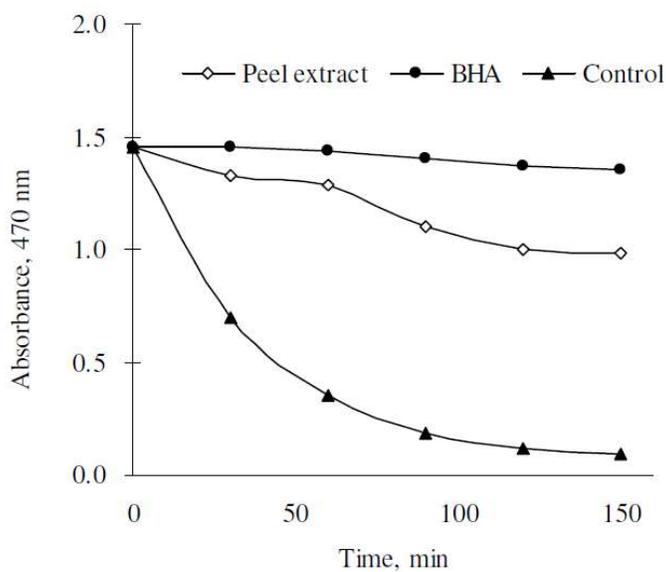
**Table 1: Antioxidant production and DPPH scavenging activity in banana peel tissues**

Banana Peel tissue	Crude extract		
	Solvent	Yield (g)	Activity DPPH (%)
	CHCl <sub>3</sub>	1.2	52.1 ± 0.2
Hexane	0.73	26.2 ± 0.4	
BHA			82.3 ± 0.1
BHT			81.8 ± 0.3

Note: Values represent the mean of triplicate experiments for each Isolated compound tested Values are represented as mean, SD



**Figure 1: The thiocyanate technique was used to test the antioxidant properties of banana peel essential oil CHCl<sub>3</sub> extracts**



**Figure 2: Banana peel essential oil CHCl<sub>3</sub> extract antioxidant properties as determined by the β-carotene bleaching technique**

Table 2: Utilizing MIC techniques, molecules with antibacterial activity were identified from essential oils from banana peels.

Microorganisms	Isolated compounds															Positive Control		Negative Control
	$\beta$ -sitosterol	SD	SE	12-hydroxy stearic acid	SD	SE	Palmitic acid	SD	SE	d-Malic acid	SD	SE	Succinic acid	SD	SE	Chloramphenicol	Ampicillin	DMSO
	MIC (ppm)*																	
<i>Staphylococcus aureus</i>	330	84	32	200	28	11	>1000	217	98	180	19	12	620	114	63	5.5	2.7	0.0
<i>Escherichia coli</i>	300	77	24	250	31	17	>1000	194	86	420	36	21	750	137	71	5.5	6.2	0.0

\*Values of minimum inhibitory concentration (MIC) given as ppm for the isolated compounds and chloramphenicol antibiotics as a standard  
 Note: Values are represented as mean, SD and SE. Values represent the mean of triplicate experiments for each isolated compound tested  
 DMSO: Dimethyl sulfoxide

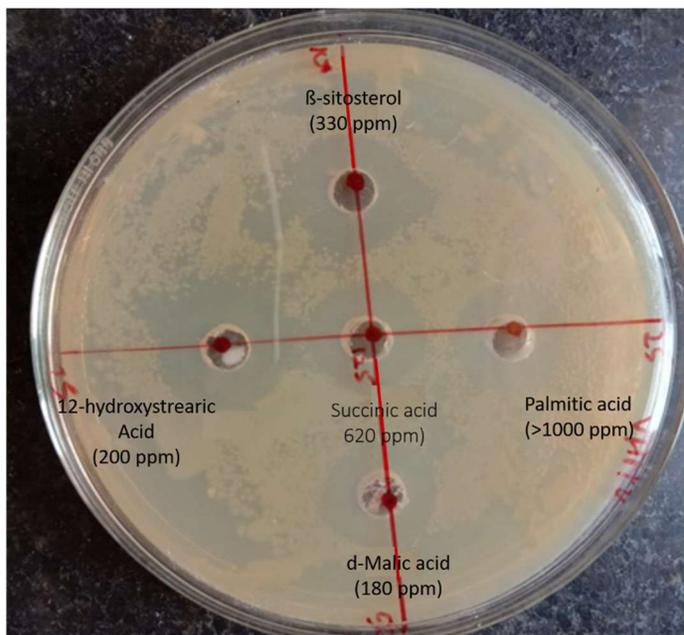


Figure 3a: Antimicrobial activity of isolated compounds on *Staphylococcus aureus*

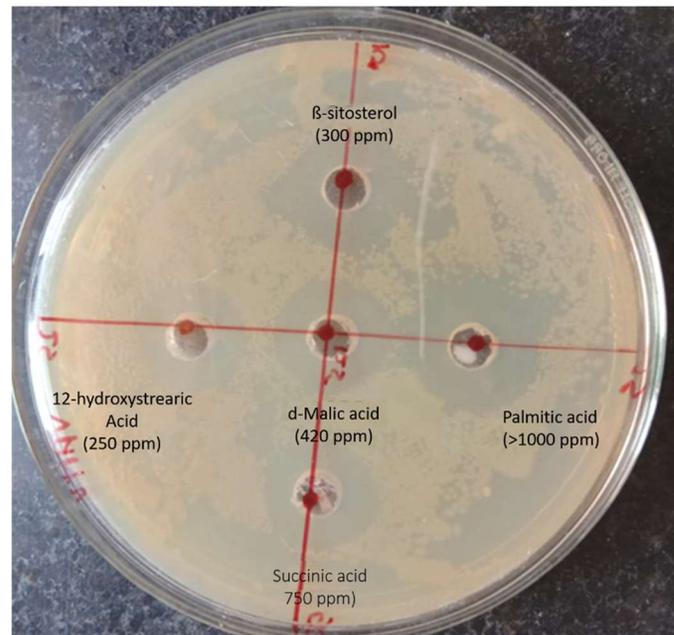


Figure 3b: Antimicrobial activity of isolated compounds on *Escherichia coli*

**DISCUSSION:**

Banana peel contains a lot of phytochemicals, primarily antioxidants. Alkaloids, flavonoids, tannins, and saponins are some of the secondary metabolites found in banana peel extract that are responsible for its antioxidant effect.<sup>9</sup> Strong antioxidants known as flavonoids can lower levels of free radicals while also producing flavonoid molecules. Free radicals are dangerous, highly reactive molecules that can injure organ tissue and lead to several diseases. Since antioxidants can both prevent free radicals and increase endurance, they are crucial for battling their effects on the body [9].

The essential oils found in banana peels include 0.90 to 3.0 g of phenolic compounds overall per 100 g of dry weight [10]. Gallic acid was discovered by Someya *et al.* at a concentration of 160 mg/100 g dry weight. Other substances, including catecholamines and the anthocyanins delphinidin and cyanidin, are also present in ripe banana peel essential oils [11]. Additionally, lutein equivalents of 300–400 g have been found in banana peel essential oils, along with sterols and triterpenes like -sitosterol, stigmasterol, campesterol, cycloeucaleanol, cycloartenol, and 24-methylene cycloartanol, as well as

carotenoids like -carotene, -carotene, and various xanthophyl [12].

Someya *et al.* examined the relationship between the gallic acid concentration of banana peels and their antioxidant activity as determined by their impact on lipid autoxidation [11]. In Coimbatore, India, Baskar *et al.* looked into the antioxidant potential of nine regional banana peel variants. According to the findings, banana peel extract significantly exhibited antioxidant activity. According to this study, disorders caused by free radicals can be effectively treated using the extract of this type of banana [13]. Banana peel (*Musa paradisiaca* L.) extract demonstrated the maximum antibacterial and antioxidant activity at 600 ppm, according to Aboul-Enein *et al.* Gallic acid, quercetin, and chrysin was the primary phenolic compounds found in the essential oils from the extract [14].

With an IC<sub>50</sub> value of 4.44 ppm, the raw banana peel extract (*Musa paradisiaca* forma typica) from Jaro Village in Tabalong Regency, South Kalimantan, exhibited exceptionally potent antioxidant activity. According to this study, Banana peel extract can be used as an effective therapeutic or preventative treatment to boost the body's antioxidant levels, which are crucial for the scavenging of free radicals [15]. The amount

of free radical scavenging activities increased due to the elevated phenolic and flavonoid content [16]. A total phenolic content of 20.47 mg gallic acid equivalents (GAE)/100 g was found in eight cultivars of Malaysian bananas [17]. Our research found that aqueous banana extracts had an inhibitory effect on *S. aureus* and *E. coli* bacteria.

Regarding the high susceptibility of *K. pneumoniae*, *E. aerogenes*, and resistance of *E. coli* to banana peel extract, the findings of Fapohunda *et al.* [18] and Bankar *et al.* [19], respectively, were in agreement. Banana peel gel was also found by Lino *et al.* to stop *Enterobacteria* and pyogenic bacteria from growing [20]. According to Aldean *et al.*, *Streptococcus* species and other Gram-positive and negative bacteria that cause gingivitis were resistant to banana skin extract in varying degrees [21, 22].

According to Sumathy's research, yellow banana fruit peel has antifungal and antibacterial characteristics that are efficient against a variety of Gram-positive and negative bacteria. Future studies on the use of plant extracts as therapeutic agents, particularly those pertaining to the control of bacteria resistant to antibiotics, are conceivable.

#### CONCLUSION:

A biological characteristic of particular interest is the antioxidant activity of essential oils, which may protect food from the harmful effects of oxidants. This study determined whether banana peel might be used for antibacterial and antioxidant properties. The results of the banana peels showed that the banana peels extracts encouraging potentials for biological activities as antioxidant and antimicrobial activities. we suggestions for future work on banana waste, especially on the biological activities. Further studies should investigate how to identify of biologically active compounds present in banana peel and other plant residues to which biological activity is due.

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