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Antimicrobial Activity of Banana (*Musa paradisiaca* L.) Peels against Food Borne Pathogenic Microbes

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Banana (*Musa paradisiaca* L.) peels are usually considered as wastes and are discarded during the processing, which eventually contribute to polluting the environment. Hence, this study was aimed to evaluate the antimicrobial activity of two different varieties of *M. paradisiaca* peels, i.e., *Nangka* (*M. paradisiaca* variety *Nangka*) and *Tanduk* (*M. paradisiaca* variety *Tanduk*) with regard to generate safe and cheap antimicrobials as well as address pollution related issues due to such wastes. Antimicrobial study was carried out on the extracts using disc diffusion and broth micro-dilution methods. The best activity through disc diffusion method for bacteria and fungi was demonstrated by *Tanduk* peel's ethanol and dichloromethane extracts against *S. aureus* (30 mm) and *C. krusei* (10 mm), respectively. However, the least active bacteria and fungi were found to be *V. parahaemolyticus* and *C. albicans,* respectively. The minimum inhibitory concentration (MIC) values ranged from 6.25 to 100 mg/mL. *Tanduk* peel's ethanol extract exhibited the lowest MIC and minimum bactericidal concentration (MBC) values against *B. cepacia* (6.25 mg/mL) whereas for fungi, *Tanduk* peel's dichloromethane extract exhibited lowest MIC and minimum fungicidal concentration (MFC) values against *C. albicans* (25 mg/mL). The results of MBC or MFC showed that some extracts were bactericidal or fungicidal while others were bacteriostatic or fungistatic against certain microbes. Banana peel waste's extracts could be potential antimicrobial alternatives and may be effective to utilize as a natural source of antimicrobial agent in pharmaceutical industries.

Key words: *Musa paradisiaca* L., *Nangka*, *Tanduk,* Antimicrobial activity, MIC, MBC, MFC.

Antibiotic resistance in microbes is an alarming and most debated issue of human health. In recent years, the bizarre ability of microbes to develop resistance to various antibiotics has become an increasing threat for successfully treating the infectious diseases due to pathogenic microbes. Consequently, researchers worldwide have been focusing on the herbal products, as a way to develop better drugs against multi drug resistant microbe strain[1].

For thousands of years, man is known to have been exploited plants as a source of medicinal drugs. Medicinal plants are possible sources of new drugs and possess boundless values for developing pharmaceutical products, phytomedicines, and dietary supplements[2]. Even

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though, the antimicrobial properties have been tested on many plant species, the majorities have not been sufficiently evaluated yet[3]. Recent researches have uncovered that the fruit and vegetable peels are potential antimicrobial agents[4-5]. Interestingly, in some fruits, the seeds and peels are found to have even higher antimicrobial activity than the pulp[6]. However, information about the antimicrobial activities of the various non-edible and waste parts of such fruits are very limited. It is expected that the findings of this research could be useful as a base line study to conduct more advance research for the sustainable utilization of agro-waste in Malaysia and its neighbouring countries.

Banana (*Musa paradisiaca* L.) is a tropical fruit of the family *Musaceae.* It is originated from the tropical region of Southern Asia[7]. It contributes about 16% of the world's total fruit production as it is the second largest produced fruit after citrus. Being one of the most widely grown tropical fruits, it is cultivated in over 130 countries. The peel which represents about 40% of the total weight of fresh banana protects the fruit and after the fruit is eaten, the peel is discarded as waste in municipal landfills[8]. Moreover, in Malaysia's industrial processing, the amount of green and ripe banana especially the species used for *Pisang goreng* (fried banana) i.e., *Musa paradisiaca* variety *Nangka* and *Musa paradisiaca* variety *Tanduk* are responsible for enormous waste which is not conducive for the environment if they are not managed or recycled appropriately. The *Pisang goreng* is one of Malaysian's favourite tea-time snacks and it can be found being sold in the stalls all over the country. So far, these agro industrial wastes were only made use as feed and fertilizer. It is acknowledged that the by-products of some fruits and vegetables represent the chief source of sugars, organic acid, minerals, dietary fiber and phenolic compounds that have a wide range of action, which includes antibacterial, antiviral, cardioprotective, antimutagenic and antitumoral activities. Hence, the new traits concerning the use of the wastes therapeutically are very attractive. Chanda *et al.*[4] reported from their studies that fruits and vegetables wastes are actually very rich in bioactive compounds which are said to have beneficial effects on health.

The idea of utilizing them therapeutically is still new and gradually getting some attention since their products are natural, eco-friendly, high value and the recovery are also economically good. Utilizing the peels as antimicrobial agents can help to recover the existing environmental waste problems and at the same time offer enormous benefits to mankind[4]. Based on the above perspectives, this study has been orchestrated for the sustainable utilization of agro-waste in Malaysia and its neighbouring countries. Several studies have shown that banana pulp and peel contain antimicrobial activities[9-10] but most of the studies focused on popular cultivars. However, the antimicrobial activities in fruits vary among species and cultivars[6]. The present investigation was undertaken to evaluate the antimicrobial activities of two (2) different varieties of bananas, Variety 1 (*M. paradisiaca* variety *Tanduk*) and Variety 2 (*M. paradisiaca* variety *Nangka*) peels extracts against several food borne pathogenic bacteria and fungi using disc diffusion and broth-micro dilution methods.

MATERIALS AND METHODS

Sample collection

Two (2) types of bananas that are commonly used to make *pisang goreng* (fried banana) i.e., *Musa paradisiaca* variety *Tanduk* and *Musa paradisiaca* variety *Nangka* were purchased from Pahang Agricultural Park (Taman Pertanian Sultan Haji Ahmad Shah) and stored at room temperature in dark before being extracted in different solvents. Both varieties of banana were identified and authenticated by Dr. Norazian M. Hassan, PhD., (Taxonomist), Faculty of Pharmacy, Malaysia. The voucher specimens of both varieties (NMPC-Q31 & NMPC-Q32) have been deposited in the Herbarium, Faculty of Pharmacy, IIUM, Kuantan, Pahang DM, Malaysia for future references.

Extract preparation

The banana fruits were washed with distilled water and air dried at room temperature. After that, the peels of the banana were removed and weighed. Then, the peels were dried in an oven at 40°C for 5 days. When the peels were fully dried, it was pulverized using a Fritsch Universal Cutting Mill, Germany. The ground material (600 g each)

was extracted independently with 700 mL of ethanol (EtOH), dichloromethane (DCM) and distilled water at room temperature. Extraction was carried out for 7 days. The resulting extracts were filtered using filter paper (Whatman No. 1) and each filtrate was concentrated with a rotary evaporator. After that, all the extracts were stored at 4°C until further use[6].

Media preparation

Antimicrobial assays were performed using Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), Tryptic Soy Agar (TSA) and Tryptic Soy Broth (TSB) for bacteria whereby Saboraud Dextrose Agar (SDA) and Saboraud Dextrose Broth (SDB) were used for fungi. MHA, MHB, TSA, TSB, SDA and SDB were dissolved in distilled water and autoclaved for 15 minutes at 121°C before use.

Test organisms

For antibacterial activity determination, the following eight major food borne disease causing bacteria were used which were directly purchased either from the Fish Disease Laboratory (FDL), University Malaysia Terengganu (UMT), Malaysia or from the American Type Culture Collection (ATCC), USA, respectively (*Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 20922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (IMR S 1386/07A)). Besides, bacteria which are pure isolate from orange mud crab *Sylla olivacea* purchased from fish disease lab, UMT were also used; *Burkholderia cepacia* (FDL-UMT-12), *Vibrio alginolyticus* (FDL-UMT-13) and *Vibrio parahaemolyticus* (FDL-UMT-11). Some of these bacteria are known as harmful pathogenic bacteria for fish spoilage and diseases also. For antifungal testing, the following fungal strains were used; *Candida albicans* (IMR C 523/11A), *Candida tropicalis* (IMR C 480/08A) and *Candida krusei* (IMR C 434/07A). All fungal strains were directly purchased from the Institute for Medical Research (IMR), Malaysia.

Antimicrobial assay

Antimicrobial activity of all extracts was initially determined by paper disc diffusion method[6]. Sterilized filter paper disc (Whatman No. 1) with the diameter of 6 mm were impregnated with 20 µL of each of the extract (500 mg/mL) to give a final concentration of 5 mg/mL/disc, 10 mg/mL/disc and 20 mg/mL/disc and was left to dry under laminar flow cabinet. Disc injected with 20 µL of sterilized water used as the negative controls. For all the bacterial strains, the concentration of the overnight cultures grown in broth were determined using a spectrophotometer where the optical density should be between 0.08 and 0.1 at 625 nm for 0.5 McFarland standards whereby for fungi, the optical density should be between 0.11 and 0.14 at 530 nm[11-14].

The broth cultures were swabbed evenly on the surface of sterile MHA, TSA (bacteria) or SDA (fungi) plates using a sterile cotton swab. Each extract was assayed in triplicate. The plates were incubated at 37°C for 24 hours. Antimicrobial activity was evaluated by measuring the zones of inhibition in millimeter. Commercially available tetracycline and chloramphenicol discs (30 µg/disc) were used as positive control for bacteria while nystatin discs (20 µg/disc) were used for fungi[6].

Determination of MIC

The minimum inhibitory concentration (MIC) considered as the lowest concentration of the sample which inhibits the visible growth of a microbe. It was determined by broth micro-dilution method. A sterile 96 well plate was used. 100 µL of inoculum was added to all the wells. A volume of 100 µL of test material in 1% (V/V) dimethyl sulfoxide (DMSO), butanol or sterile water was pipetted into the first row of the plate. Serial dilutions were performed using a micropipette to obtain dilutions ranging from 100 to 3.125 mg/mL[15-17]. 1% DMSO and 1% butanol was used as the negative controls. The positive control used for bacteria was tetracycline and for fungi was nystatin. The culture tubes were then incubated aerobically at 37°C for 24 hours[6]. After incubation, 20 µL of 0.2 mg/mL aqueous solution of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well and further incubated for 30 minutes at room temperature. The presence of blue color indicates the presence of bacteria. MIC was defined as the lowest concentration in which no transformation of MTT was observed[18-20].

Determination of MBC or MFC

To determine the minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC), for each set of well in the MIC determination, a loopful of broth was collected

from those plates well, which did not show any visible sign of growth and inoculated on sterile MHA, TSA or SDA by streaking. Then, the plates were incubated at 37°C for 24 hours. After incubation, the concentration at which no visible growth of bacteria or fungi was seen and noted as the minimum bactericidal or fungicidal concentration (MBC/MFC)[21-22].

Statistical analysis

All values represent the mean from three independent experiments. Three replicates of each sample were used for statistical analysis. Analysis of the data was performed on the original data by one-way analysis of variance (ANOVA) by using SPSS software version 21 (Chicago, IL, USA).

RESULTS

In the present study, eleven (11) food borne pathogens were tested for their sensitivity to different extracts of *Musa paradisiaca* variety *Tanduk* and *Musa paradisiaca* variety *Nangka* peels using disc diffusion and broth micro-dilution methods. All six extracts (ethanol extract of *M. paradisiaca* variety Nangka (EN), dichloromethane extract of *M. paradisiaca* variety Nangka (DN), aqueous extract of *M. paradisiaca* variety Nangka (HN), ethanol extract of *M. paradisiaca* variety Tanduk (ET), dichloromethane extract of *M. paradisiaca* variety Tanduk (DT) and aqueous extract of *M. paradisiaca* variety Tanduk (HT)) exhibited potent antibacterial activity against certain bacteria especially at 2000 mg/mL. ET inhibited all of the bacteria tested with zone diameters ranging from 7 to 26 mm while HT managed to inhibit 7 bacteria studied except *B. cepacia* from 7 to 12 mm. DT inhibited only 2 bacteria tested which were *S. aureus* and *V. alginolyticus* with zone diameters ranging from 8 mm to 10 mm. With respect to different solvents used in this assay, ET showed better antibacterial activity followed by HT and finally DT. On the other hand, for *M. paradisiaca* variety Nangka, EN inhibited five bacteria studied except *E. faecalis*, *V. parahaemolyticus* and *V. alginolyticus* with zone diameters ranging from 8 to 24 mm while DN and HN inhibited only one bacterium which was *S. aureus* from 9 to 15 mm (Table 1, Figures 1-6).

Moreover, in this study, apart from bacteria, 3 types of pathogenic fungi (*C. albicans*, *C. tropicalis* and *C. krusei*) were also tested for their sensitivity to different extracts of *M. paradisiaca* variety Tanduk and *M. paradisiaca* variety Nangka peels using disc diffusion and broth micro-dilution methods. Among the extracts, only ET and DT exhibited antifungal activity against the fungal strains. ET inhibited *C. tropicalis* (Figure 7) and *C. krusei* with diameters ranging from 8 to 10 mm whereby DT inhibited only *C. krusei* with zone of inhibition of 10 mm. Other extracts especially the one from *M. paradisiaca* variety Nangka did not show any antifungal activity (Table 1). The standard antibiotics and antifungal used, tetracycline, chloramphenicol and nystatin showed a zone of inhibition ranged from 8 to 35 mm against all test organisms while the negative controls did not show any antibacterial or antifungal activity (Table 1).

MIC of the tested banana peel extracts ranged from 3.125 to 100 mg/mL for the tested bacteria (Table 2). The positive control (tetracycline) had exhibited activity against all the tested bacteria. While the negative control (distilled water and 1% butanol) exhibited no antibacterial activity. The results indicate that all six extracts exhibited good antibacterial activity. ET was most effective against *B. cepacia* at 6.25 mg/mL whereby for other bacteria the MIC values ranged from 12.5 to 100 mg/ mL. Thus, the most susceptible bacteria for ET is *B. cepacia*. Furthermore, the results of MIC test for DT revealed that *S. aureus* and *B. cepacia* were inhibited at the concentration of 25 mg/mL. However, other bacteria were inhibited at a higher concentration from 50 to 100 mg/mL. As for HT, all the bacteria were inhibited at 25 mg/mL. On the other hand, EN was most effective against *S. aureus* and *B. cepacia* at 12.5 mg/mL followed by *E. faecalis*, *E. coli*, *V. alginolyticus* and *P. aeruginosa* at the concentration of 25 mg/mL. In the meantime, other bacteria were inhibited at 50 mg/ mL. For DN, the lowest MIC value was obtained for *B. cepacia* at 25 mg/mL whereby all other bacteria except *P. aeruginosa* were inhibited at 50 mg/mL and *P. aeruginosa* was inhibited at 100 mg/mL. HN had successfully inhibited *V. alginolyticus* at 25 mg/mL and other bacteria were only inhibited from 50 to 100 mg/mL. From the MBC assay, it was found that ET had good bactericidal effect against *B. cepacia* since it could

Table 1. Average diameters of zones of inhibition (mm) of ethanol, dichloromethane and aqueous extracts of banana (*M. paradisiaca* variety *Nangka* and *M. paradisiaca* variety *Tanduk*) peels against microbial strains.

No. Microbes	Samples	Concentration of extracts			Positive controls		Negative controls
		500 (mg/mL)	1000 (mg/mL)	2000 (mg/mL)	Tetracycline/ Nystatin 30 µg	Chloramp-henicol 30 µg	Water 20 µL/disc
1. *B. cereus*	N (E)	NA	10 ± 0	10 ± 1.4	16	13	NA
	N (D)	NA	NA	NA	16	17	NA
	N (H)	NA	NA	NA	17	14	NA
	T (E)	NA	15 ± 0	17 ± 0	17	15	NA
	T (D)	NA	NA	NA	16	12	NA
	T (H)	NA	9 ± 0.7	11± 0	17	14	NA
2. *B. cepacia*	N (E)	12± 0	9 ± 0.7	15 ± 1.4	28	30	NA
	N (D)	NA	NA	NA	30	30	NA
	N (H)	NA	NA	NA	26	28	NA
	T (E)	NA	11 ± 0.7	12 ± 0.7	28	28	NA
	T (D)	NA	NA	NA	29	29	NA
	T (H)	NA	NA	10 ± 0.7	27	30	NA
3. *E. feacalis*	N (E)	NA	NA	NA	8	24	NA
	N (D)	NA	NA	NA	9	22	NA
	N (H)	NA	NA	NA	9	23	NA
	T (E)	NA	9 ± 0.7	14 ± 0	8	24	NA
	T (D)	NA	NA	NA	8	23	NA
	T (H)	NA	7 ± 0	10 ± 0.7	8	22	NA
4. *E. coli*	N (E)	NA	10 ± 0	11 ± 1.4	17	11	NA
	N (D)	NA	NA	NA	16	14	NA
	N (H)	NA	NA	NA	16	11	NA
	T (E)	NA	14 ± 0.7	15 ± 0	15	11	NA
	T (D)	NA	NA	NA	16	12	NA
	T (H)	NA	9 ± 0.7	12 ± 0.7	17	11	NA
5.*P. aeruginosa*	N (E)	12 ± 0	8 ± 0.7	8 ± 0.7	12	10	NA
	N (D)	11± 0	NA	NA	9	12	NA
	N (H)	NA	NA	NA	8	14	NA
	T (E)	13 ± 0.7	8 ± 0.7	11 ± 1.4	9	10	NA
	T (D)	10 ± 1.4	NA	NA	11	11	NA
	T (H)	NA	10 ± 0.7	12 ± 0.7	8	12	NA
6. *S. aureus*	N (E)	14 ± 0	22 ± 0	24 ± 0	23	25	NA
	N (D)	9 ± 1.4	13 ± 2.1	11 ± 2.1	21	25	NA
	N (H)	11 ± 0	15 ± 0.7	13 ± 0.7	20	26	NA
	T (E)	20 ± 0.7	26 ± 0.7	30 ± 0.7	24	25	NA
	T (D)	8 ± 0	9 ± 0	10 ± 0.7	21	25	NA
	T (H)	10 ± 0	9 ± 0.7	11 ± 0.7	25	27	NA
7.*V. alginolyticus*	N (E)	NA	NA	NA	30	35	NA
	N (D)	NA	NA	NA	32	34	NA
	N (H)	NA	NA	NA	32	33	NA
	T (E)	NA	8 ± 0.7	9 ± 0.7	34	34	NA
	T (D)	NA	8 ± 0	10 ± 0.7	28	33	NA
	T (H)	NA	7 ± 0	10 ± 0	27	30	NA
8.*V. parahaemolyticus*	N (E)	NA	NA	NA	30	19	NA
	N (D)	NA	NA	NA	30	18	NA
	N (H)	NA	NA	NA	28	19	NA
	T (E)	NA	7 ± 0.7	8 ± 0	29	17	NA
	T (D)	NA	NA	NA	24	20	NA
	T (H)	NA	8 ± 0.7	12 ± 0.7	27	19	NA

9. *C. albicans*	N (E)	NA	NA	NA	17	-	NA
	N (D)	NA	NA	NA	17	-	NA
	N (H)	NA	NA	NA	17	-	NA
	T (E)	NA	NA	NA	15	-	NA
	T (D)	NA	NA	NA	15	-	NA
	T (H)	NA	NA	NA	17	-	NA
10.*C. tropicalis*	N (E)	NA	NA	NA	11	-	NA
	N (D)	NA	NA	NA	15	-	NA
	N (H)	NA	NA	NA	14	-	NA
	T (E)	NA	NA	8± 0.6	14	-	NA
	T (D)	NA	NA	NA	14	-	NA
	T (H)	NA	NA	NA	12	-	NA
11. *C. krusei*	N (E)	NA	NA	NA	14	-	NA
	N (D)	NA	NA	NA	15	-	NA
	N (H)	NA	NA	NA	12	-	NA
	T (E)	NA	9 ± 1.2	9 ± 1.5	13	-	NA
	T (D)	NA	10 ± 1.7	10 ± 1.2	15	-	NA
	T (H)	NA	NA	NA	14	-	NA

Note: Values are mean ± of triplicate tests with standard deviation. Diameter of inhibition zone (mm) include diameter of the disc which is 6 mm. N (E): *Musa paradisiaca* variety Nangka in ethanol extract, N (D): *Musa paradisiaca* variety Nangka in DCM extract, N (H): *Musa paradisiaca* variety Nangka in distilled water extract, T (E): *Musa paradisiaca* variety Tanduk in ethanol extract, T (D): *Musa paradisiaca* variety Tanduk in DCM extract, T (H): *Musa paradisiaca* variety Tanduk in distilled water extract. Positive control: Tetracycline, Chloramphenicol. NA: not active, no inhibition.

kill at 6.25 mg/mL which is also the MIC value. Other MBC values of ET for the rest of the bacteria ranged from 25 to 100 mg/mL. However, ET was found to be bacteriostatic for *E. coli*, *E. faecalis* and *V. alginolyticus.* Meanwhile, DT showed good bactericidal effect at 12.5 mg/mL against *B. cepacia* and *V. parahaemolyticus* and other bacteria at a higher concentration. But then, DT was bacteriostatic against *E. faecalis, B. cereus, S. aureus* and *V. alginolyticus*. As for HT, good bactericidal effect was observed for *E. coli, B. cepacia, V. parahaemolyticus* and *B. cereus* at 25 mg/ mL. On the other hand, *B. cepacia* was killed by EN at 12.5 mg/mL similar to its MIC value. However, it was bacteriostatic to *E. faecalis, E. coli, P.aeruginosa* and *V. alginolyticus*. As for DN, the MBC value was 25 mg/ mL for *B. cepacia* and *V. parahaemolyticus*. To *E. faecalis, B. cereus,* and *S. aureus*, DN was only bacteriostatic. Furthermore, HN could kill *E. coli* and *B. cepacia* at 50 mg/ mL and other bacteria at 100 mg/mL except for *E. faecalis* and *V. alginolyticus,* it was found to be bacteriostatic (Table 3). Although the antifungal activity of the banana peel extracts were only significant for certain extracts using disc diffusion method, all the extracts were still checked for MIC (Table 2, Figure 8).

MIC of the tested banana peel extracts ranged from 3.125 to 100 mg/mL for fungal strains. The positive control of fluconazole had exhibited activity against all three tested fungi. While the negative control (distilled water and 1% butanol) exhibited no antifungal activities. The results indicate that some extracts exhibited potential antifungal activity. From the results obtained, ET and EN had successfully inhibited the growth of all the fungal strains tested at 50 mg/ mL. Meanwhile, DT was effective against *C. albicans* at 25 mg/mL followed by *C. tropicalis* at 50 mg/ mL. As for HT, *C. albicans* could be inhibited at 50 mg/mL and *C. tropicalis* was only inhibited at 100 mg/ mL. On the other hand, DN was effective against *C. albicans* and *C. tropicalis* at 50 mg/ mL and HN could only inhibit *C. albicans* at 50 mg/ mL. Besides, *C. krusei* was found to be the most resistant strain since it was only inhibited by EN and ET extracts (Table 2). From the MFC assay, it was found that ET had good fungicidal effect against *C. albicans* and *C. tropicalis* at 50 mg/mL similar to the MIC value whereby *C. krusei* was killed at 100 mg/mL. Meanwhile, DT could kill *C. albicans* 25 mg/mL and *C. tropicalis* at 50 mg/mL. As for HT, it could kill only *C. tropicalis* at 100 mg/mL. The MFC values of EN against all three fungi were 100 mg/mL. For DN, *C. albicans* and *C. tropicalis* were destroyed at 50 mg/mL. However, HN showed only fungistatic effect to all the fungal strains (Table 3, Figs. 9-11).

DISCUSSION

In the present study, the antibacterial activity of ethanol, DCM and aqueous extracts of *M. paradisiaca* L. peels were evaluated against eleven food borne pathogens consisting three Gram-positive, five Gram-negative bacteria and

Table 2.MIC values of ethanol, dichloromethane and aqueous extracts of banana (*M. paradisiaca* variety *Nangka* and *M. paradisiaca* variety *Tanduk*) peels against microbial strains (mg/mL)

Microbial Strains	Samples					
	N (E)	N (D)	N (H)	T (E)	T (D)	T (H)
S. aureus	12.5	50	50	12.5	25	25
E. feacalis	25	50	50	100	50	25
B. cereus	50	50	50	50	50	25
E. coli	25	50	50	50	50	25
P. aeruginosa	25	100	50	50	100	25
B. cepacia	12.5	25	100	6.25	25	25
V. parahaemolyticus	50	50	100	25	12.5	25
V. alginolyticus	25	50	25	25	25	25
C. albicans	50	50	50	50	25	50
C. tropicalis	50	50	NA	50	50	100
C. krusei	50	NA	NA	50	NA	NA

Note: N (E): Ethanol extract of *M. paradisiaca* variety *Nangka*, N (D): DCM extract of *Musa paradisiaca* variety *Nangka*, N (H): Aqueous extract of *Musa paradisiaca* variety *Nangka*, T (E): Ethanol extract of *Musa paradisiaca* variety *Tanduk*, T (D): DCM extract of *Musa paradisiaca* variety *Tanduk*, T (H): Aqueous extract of *Musa paradisiaca* variety *Tanduk.* Positive control: Tetracycline (30µg) (bacteria) / Nyastatin (fungi) + test organisms, Negative control: Distilled water and 1% butanol+ test organisms: growth was observed. NA: not active, no inhibition.

Table 3. MBC and MFC values of of ethanol, dichloromethane and aqueous extracts of banana (*M. paradisiaca* variety Nangka and *M. paradisiaca* variety Tanduk) peels against microbial strains (mg/mL)

Microbial Strains	Samples					
	N (E)	N (D)	N (H)	T (E)	T (D)	T (H)
S. aureus	100	100	100	25	ND	100
E. feacalis	ND	ND	ND	ND	ND	ND
B. cereus	100	50	100	100	ND	25
E. coli	ND	50	50	ND	50	25
P. aeruginosa	ND	ND	100	50	100	ND
B. cepacia	12.5	25	50	6.25	12.5	25
V. parahaemolyticus	50	25	100	25	12.5	25
V. alginolyticus	ND	ND	ND	ND	ND	ND
C. albicans	100	50	ND	0	25	ND
C. tropicalis	100	50	ND	50	50	100
C. krusei	100	ND	ND	100	ND	ND

Note: N (E): Ethanol extract of *M. paradisiaca* variety *Nangka*, N (D): DCM extract of *Musa paradisiaca* variety *Nangka*, N (H): Aqueous extract of *Musa paradisiaca* variety *Nangka*, T (E): Ethanol extract of *Musa paradisiaca* variety *Tanduk*, T (D): DCM extract of *Musa paradisiaca* variety *Tanduk*, T (H): Aqueous extract of *Musa paradisiaca* variety *Tanduk.* Positive control: Tetracycline (30µg) (bacteria) / Nystatin (fungi) + test organisms, Negative control: Distilled water and 1% butanol+ test organisms: growth was observed. ND: not determined.

three fungal strains. The methods involved were disc diffusion to obtain average zones of inhibition and broth micro-dilution to find MIC.

The results obtained from this investigation revealed that all six extracts taken into consideration exhibited potent antimicrobial activity against certain microorganisms. The difference in activity was due to the type of solvents used to extract the plant material. Ncube *et al.*[23] stated that methanol, ethanol and water are the most frequently used solvents to study antimicrobial activity in plants and some also use

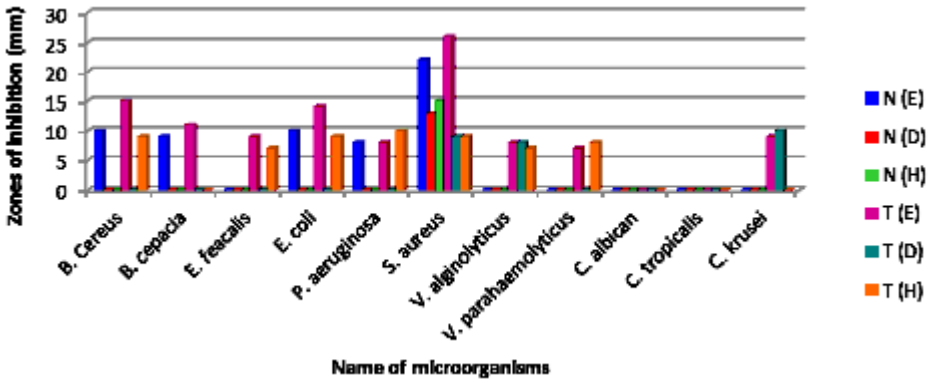

Fig. 1. Average diameters of zones of inhibition (mm) for different extracts of banana peels at 1000 mg/mL by disc diffusion assay against microbial strains

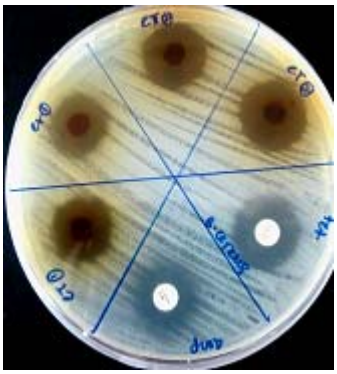

Fig. 2. Disc diffusion assay of ethanol extract of *Musa paradisiaca* variety Tanduk against *B. cereus.*

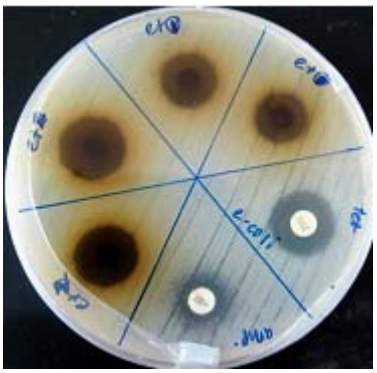

Fig. 3. Disc diffusion assay of ethanol extract of *Musa paradisiaca* variety Tanduk against *E. coli.*

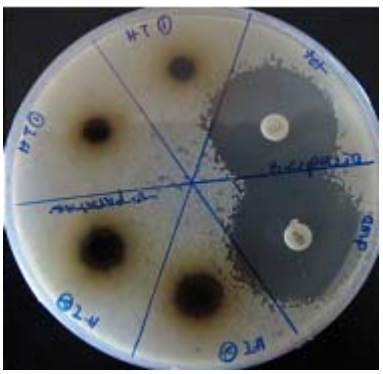

Fig. 4. Disc diffusion assay of ethanol extract of *Musa paradisiaca* variety Tanduk against *B. cepacia.*

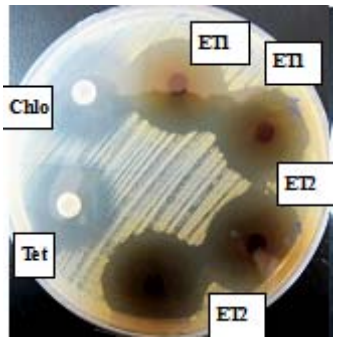

Fig. 5. Disc diffusion assay of ethanol extract of *Musa paradisiaca* variety Tanduk against *S. aureus.*

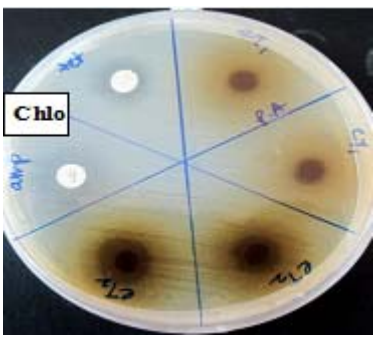

Fig. 6. Disc diffusion assay of ethanol extract of *Musa paradisiaca* variety Tanduk against *P. aeruginosa.*

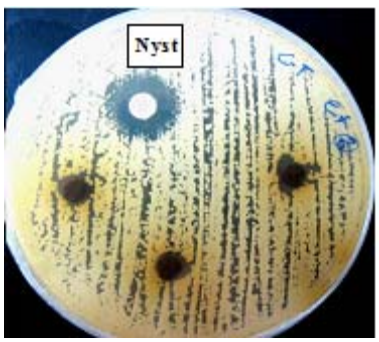

Fig. 7. Disc diffusion assay of ethanol extract of *Musa paradisiaca* variety Tanduk against *C. tropicalis.*

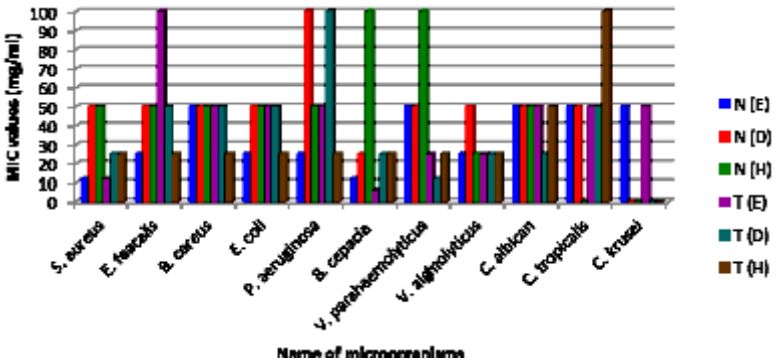

Fig. 8. MIC values (mg/mL) of different extracts of banana peels against microbial strains

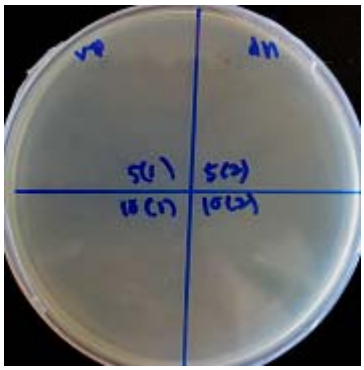

Fig. 9. MBC of DCM extract of *Musa paradisiaca* variety Nangka against *V. parahaemolyticus*

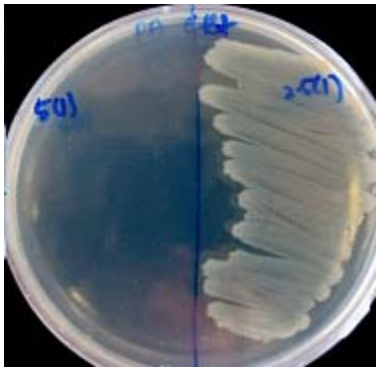

Fig. 10. MBC of ethanol extract of *Musa paradisiaca* variety Tanduk against *P. aeruginosa*

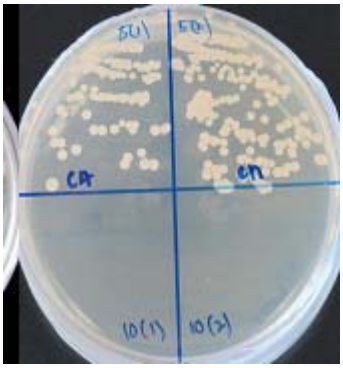

Fig. 11. MBC of ethanol extract of *Musa paradisiaca* variety Nangka against *C. albicans*

DCM. They also pointed out that the type of solvents used will determine the biologically active compounds present in the plant material. Therefore, the selection of solvent should depend on the targeted compounds from the extract. This study had used ethanol, DCM and water for the extraction of two varieties of *M. paradisiaca* L. peel.

Among the solvents, ethanol extracts from both types of banana (*Nangka* and *Tanduk*) showed a significant antibacterial activity against almost all the tested microorganisms with T (E) giving better activity than N (E). This finding is in agreement with the finding observed by Fagbemi *et al.*[10] where they investigated the potency of unripe banana (*Musa sapientum* L.) against pathogens and revealed that the ethanol and aqueous extracts of banana gave higher antibacterial activity against all tested organisms with zone diameters ranged from 8 to 31 mm. On the other hand, a study on green banana peel by Mokbel and Hashinaga[9] indicated that the ethyl acetate extract of green banana peel displayed high antibacterial activity while their aqueous extract did not show any activity. On the contrary, the aqueous extract in our study showed a good antibacterial activity where the inhibition zones ranged from 10 to 12 mm and the MIC value was 25 mg/mL against all the bacteria tested.

Since polyphenolic compounds such as flavonoids and most other bioactive compounds are said to be more soluble in polar solvents[23], the exceptional activity by ethanol extract of banana peels was believed to be due to the presence of various phenolic compounds in banana such as gallocatechin and anthocyanins like peonidin and malvidine in banana as stated by Yusoff[24]. Furthermore, preliminary phytochemical screening of the banana peels by Alisi *et al.*[25] revealed the presence of some glycosides, anthocyanine, tannins, flavonoids and carbohydrate. They also suggested the possible antimicrobial activity was due to the presence of tannins, flavonoids and

saponins in the peels. Besides, it was reported by Rather *et al.*[26] that fruit peel yields 1000 folds more phenolic compound than pulp. Also, from an antibacterial activity study carried out by Shan *et al.*[27] on 46 spices and herbs extracts from different regions discovered that there is a good linear relationship between antibacterial activity and total phenolic content.

The standard antibiotics and antifungal used, tetracycline, chloramphenicol and nystatin showed a zone of inhibition ranged from 8 to 35 mm against all test organisms while the negative controls did not show any antibacterial activity. When the inhibition zones of the extracts compared to the inhibition zones produced by the positive controls used, better results were still exhibited by tetracycline chloramphenicol and nystatin. This could be due to the fact that the conventional drugs were already an established one and in pure form while the nature of the extract is still in crude form. However, T (E) inhibited *S. aureus* with zone diameters of 30 mm while tetracycline inhibited *S. aureus* with zone diameters of only 24 mm and chloramphenicol was 25 mm. This indicates that T (E) has a better antibacterial activity than the conventional drugs and it could replace these drugs as an antibacterial agent in the future. It is interesting to note from this study that even crude extracts of the plants showed good activity against multidrug resistant strains where modern antibiotic therapy has failed[28].

Many studies reported that Gram-positive bacteria are more sensitive as compared to Gram-negative bacteria because of the differences in their cell wall structures[29-30]. However, there was no clear trend observed for Gram-positive and Gram-negative bacteria in this study which was similar with the findings observed by Nurmahani *et al.*[31] where no clear trend was also found on the different types of bacteria when the antibacterial activity of dragon fruit peel extracts was studied. Based on the results, the MIC values for most of the extracts were lower than their MBC values, suggesting that these extracts inhibited the growth of the test microorganisms while being bactericidal at higher concentrations. Pankey and Sabath[32] defined bacteriostatic as the agent that prevents the growth of bacteria by keeping them in the stationary phase of growth and bactericidal was defined as the agent that kills bacteria. They also stated that the *in vitro* microbiological determination of whether an antibacterial agent is bactericidal or bacteriostatic may be influenced by growth conditions, bacterial density, test duration, and extent of reduction in bacterial numbers.

Khan *et al.*[33] proposed that the mechanism of the antimicrobial effects covers the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and lastly ion leakage from the cells. In this study, among the eight bacteria tested by disc diffusion method, *S. aureus* was the most sensitive to the extracts because it was successfully inhibited by all the banana peel extracts with highest mean inhibition zone of 30 mm, while *V. parahaemolyticus* was the most resistant since it was least inhibited by the extracts with a lowest zone of 7 mm. The highest sensitivity of *S. aureus* may be due to its cell wall structure and outer membrane[27]. Likewise, in a study conducted by Jain *et al.*[6], the antibacterial activity of various extracts of peel, pulp and seed portions of local seeded banana fruit was tested. The results obtained showed that *S. aureus* was also the most susceptible bacteria to ethyl acetate extract of banana pulp with 24 mm of clear inhibition zone. Among the fungal strains tested, *C. krusei* was found to be the most resistant strain based on broth micro-dilution method since it could be inhibited by only N (E) and T (E) whereas *C. albicans* was identified as the most susceptible strain.

During this investigation, most of the extracts gave antimicrobial activity only at a higher concentration which ranged from 25 to 100 mg/mL. This may be due to the method of extraction, solvents used in extraction and season at which samples were collected[29]. Moreover, the difference in the phytochemical composition or difference in the genotypes of the plant used could also contribute to this low activity of some extracts[15]. Though, this extract is still in crude form and further isolation and purification are believed to give better results. Besides, it can be deduced from the results that the antimicrobial activity of the extracts declined with decreased concentrations in the majority of the cases. However, there were exceptions. For example, when N (E) was tested against *P. aeruginosa*, the inhibition zone diameter achieved with the highest concentration (2000 mg/mL) was 8 mm but at lowest concentration (500

mg/mL), the inhibition zone diameter was 12 mm. Similar observations were noticed against *B. cepacia*. The reasons for these results are unknown but similar results were obtained by a study done by Nweze and Onyishi[34] when they evaluated the *in vitro* antimicrobial activity of ethanol and methanol fruit extracts of *Xylopia aethiopica* against few pathogenic bacteria.

Throughout this investigation, it was found that most of the results obtained from disc diffusion and broth micro-dilution methods were not similar. For instance, most of the extracts did not give good antibacterial activity at low concentration in disc diffusion test but gave better activity in broth micro-dilution test. Kumar *et al.*[15] stated that MIC by broth dilution method showed good results compared to disc diffusion method because in disc diffusion method, there may be a problem with the diffusion of the biological component into the agar and the hydrocarbon components either remain on the surface of the medium or evaporate. Besides, broth micro-dilution method also has the advantages where lower workloads are required for a larger number of replicates, increased sensitivity for small quantities of extract, ability to distinguish between bacteriostatic and bactericidal effects, only small volumes of the test substance growth medium will be needed and it presents reproducible results[23,35]. Hence, the results obtained from broth micro-dilution method can be considered as more reliable than the results from disc diffusion method.

Similarly, the antimicrobial activity studies by broth micro-dilution test also revealed that the antimicrobial activity of the *M. paradisiaca* variety *Tanduk* peel extracts was generally higher than the *M. paradisiaca* variety *Nangka* peel extracts since the *M. paradisiaca* variety *Tanduk* peel extracts could inhibit at a lower concentration compared to *M. paradisiaca* variety *Nangka*. The most potent antimicrobial extract that has high potential and could be commercialized in the future was found to be T (E). This is because it successfully inhibited all the microorganisms with the range of 7 to 30 mm by disc diffusion method and the MIC values ranged from 6.25 to 100 mg/mL. This high activity could be due to the presence of particular secondary metabolites in this extract that could inhibit broad spectrum bacteria. Therefore, isolation and identification of bioactive compounds are required in order to obtain pure and effective antimicrobial agents which can efficiently inhibit the growth of different ranges of microorganisms.

CONCLUSION

The present study revealed that all the pathogenic microorganisms tested were sensitive to different extracts of *M. paradisiaca* variety *Tanduk* and *M. paradisiaca* variety *Nangka* peels. Among the different solvent extracts used, ethanol extract of both the varieties of *M. paradisiaca* L. peels showed highest antimicrobial activity with *M. paradisiaca* variety *Tanduk* peel being better than *M. paradisiaca* variety *Nangka* peel. With respect to both extracts, ethanol extract of *M. paradisiaca* variety *Tanduk* peel (T (E)) can be categorized as a good source of potent natural antimicrobial agent for both Gram-positive and Gram-negative bacteria. The demonstration of broad spectrum antimicrobial activity by *M. paradisiaca* L. peels may help to discover new types of antibiotic like substances that could serve as selective and low cost source of natural antimicrobial agents and may also help to conserve our environment.

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