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FULL PAPER

Effect of heat processing on antibacterial activity in fruit peel and seed of kuini (*Mangifera odorata*) extracts

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Abstract

Mangifera odorata or known locally as kuini is one of the underutilized fruits in Malaysia which belongs to the mango family (Anacardiaceae). It has been interestingly found to contain many nutrients. Studies have shown that kuini has high antioxidant properties including in its peel and seed. However, no studies have been conducted on the antimicrobial activity of kuini peel and seed. This study was conducted to determine the antibacterial activities of peel and seed of kuini in an effort to develop a functional bio-ingredient. Matured kuini fruits were boiled for 10 mins and steamed for 5, 10 and 15 mins, separately. Peel and seed of the fresh and heat-processed kuini were separated, dried and ground into powder and extracted using 70% methanol as solvent. The extracts were tested against pathogens *Escherichia coli* ATCC 48888, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 25923, *Salmonella enterica* serovar Typhimurium ATCC 14028 and *Enterobacter aerogenes* ATCC 13048 using the disc diffusion method at a concentration of 10 mg/mL. Results showed that fresh and heat-processed peel and seed extracts exhibited various inhibition zones against the tested pathogens. The mean for inhibition zones ranged between 11 to 29.33 mm where fresh peel showed the biggest inhibition zone. Penicillin 10 µg that was used as a positive control inhibited only two pathogens. The heat processing technique was found to affect the antibacterial activity of some of the peel and seed extracts. The antibacterial activity of peel that was subjected to heat was found to decrease for all the pathogens tested. Seed extract showed an increase in antibacterial activity against *B. cereus* when seeds were given heat treatments. Despite being subjected to heat treatment, the peel and seed of kuini still exhibited promising antibacterial activities. Hence, could be further utilized as a natural antibacterial agent or potentially be developed into a functional bio-ingredient.

1. Introduction

Many cases of food poisoning and food-borne diseases have been reported that are linked to food pathogens. Among the food pathogens that often cause food poisoning are *Salmonella enterica* serovar Typhimurium which can be found in eggs and poultry, *Listeria monocytogenes* in red meat, *Vibrio* spp. in seafood, *Bacillus cereus* in legumes, *Staphylococcus aureus* and *Escherichia coli* in most foods (Centre for Disease Control and Prevention, 2019). These pathogens when entering the human body will cause adverse implications. In recent years, bacterial resistance to antibiotics has become an important worldwide issue that needs serious attention. There have been cases of bacterial resistance to synthetic antibiotics which are very worrying and complicate the medical treatment of diseases caused by bacteria. The emergence of these antibiotic-resistant bacteria increases the need for the development of new antibiotics (Tollefson and Miller, 2000). Demand for antibiotics and natural food preservatives has also increased due to consumer awareness of the side effects of using synthetic substances on health. This has opened up the efforts of researchers, physicians and the pharmaceutical industry to explore antibiotics and preservatives alternatives from natural resources. Emphasis is also given by the World Health Organization (WHO) the need to include traditional medicine based on plants and natural

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ingredients in the national medicine policy because it is the best source for various types of medicine (Mushore and Matuvhunye, 2013).

The processing industry of juice, nectar, pulp, puree, snack and jam from fruits produces by-products or waste such as fruit peels and seeds. Around 15-20% and 20-60% of the weight of mangoes are contributed by the peels and seeds, respectively. Disposal of these peels and seeds will lead to the dumping of fruit waste (Tun Norbrillinda *et al*., 2020). Fruit peel and seed were found to contain significant amounts of phytochemicals. For example, *Mangifera indica* (mango) peel contains various functional compounds such as phytochemicals, polyphenols, carotenoids, vitamins E and C that contribute to antioxidant activity (Ajila *et al*., 2007). It is also a source of fibre, cellulose, hemicellulose, lipids, enzymes and pectin (Sogi *et al*., 2013). These components can be beneficial to human health when taken. Meanwhile, the seed is rich in phenolic acids such as gallic acid, ellagic acid, ferulic acid, cinnamic acid, tannin, vanillin, coumarin and mangiferin (Soong and Barlow, 2006). There have been several reports on the antimicrobial property of fruit peel and seed. Vaghasiya *et al*. (2011) reported that the methanol extract of *M. indica* seed showed potent antibacterial activity against 61 bacterial strains. Another report also agreed that there was an antimicrobial activity of ethanolic and methanolic extracts of *M. indica* seed against twenty-five bacterial and fungi strains (Awad El-Gied *et al*., 2012). Fruit peel of *M. indica* also exhibited antimicrobial activity as reported by Priyanka *et al*. (2016) where acetone extract peel powder showed the highest activity against *E. coli, S. enterica* ser. Typhimurium*, Shigella* spp. and *Enterobacter* spp., while ethanol and aqueous extract showed the highest antifungal activity against *Aspergillus brasiliensis* strain. This is supported by Kalpna *et al*. (2013) reported that the acetone extract of *M. indica* ripe peel showed the best antimicrobial and antioxidant activity. However, there is no information regarding the antibacterial activity of the seed and peel of *M. odorata*.

Mangifera odorata or known as kuini is a popular underutilized fruit in Malaysia, belonging to the same family as *M. indica*. Its shape is like mango but has a stronger fragrance and rubber under its skin. Several studies have reported the antioxidant and polyphenolic activities of kuini. Nur Amalina *et al*. (2019) described that *M. odorata* seed had the highest total phenolic content followed by peel and flesh, while total flavonoid content was highest in peel followed by seed and flesh. The same report also stated that *M. odorata* seed had higher scavenging activity as compared to ascorbic acid. However, there have been no published studies on the antibacterial activity of *M. odorata* peel and seed. Thus, the objective of this present study was to determine the antibacterial activity of *M. odorata* peel and seed against food pathogens and the effect of heat treatment on its antibacterial activity.

2. Materials and methods

2.1 Heat treatments and preparation of extracts

Mature and good quality kuini fruits were washed and subjected to heat processed by boiling at 100°C for 10 mins and steamed for 5, 10 and 15 mins separately. The peel and seed of unheated and heat-processed fruits were then separated and air-dried before grinding using a high-power industrial blender. A total of 100 mg of peel and seed powder were weighed and placed in a conical flask containing 100 mL 70% methanol (v/v) respectively. The mixture was shaken for 24 hrs at a speed of 200 rpm using an orbital shaker at room temperature before being filtered using Whatman No.1 filter paper. The filtered product was collected into a round flask and concentrated at 60°C using a rotary evaporator (BUCHI Rotavapor R-200). The extracts were allowed to air dry at room temperature and weighed. The percentage yield of extracts was calculated following the formula: % yield = (weight of extract/ weight of ground peel or seed samples used for extraction) × 100. The obtained crude extracts were prepared at a concentration of 10 mg/mL by dissolving them in sterile distilled water for further analysis.

2.2 Preparation of bacterial inoculums and growth media

The food pathogens used in this analysis consisted of Gram-positive bacteria which were *Bacillus cereus* ATCC 10876 and *Staphylococcus aureus* ATCC 25923. Gram-negative bacteria were *Escherichia coli* ATCC 48888, *Enterobacter aerogenes* ATCC 13048 and *Salmonella enterica* serovar Typhimurium ATCC 14028. All the bacterial cultures were obtained from Food Science and Technology Research Centre, MARDI. An aliquot of 100 µL of each bacterial inoculum was cultivated overnight in 90 mL of sterile nutrient broth at 37°C before use. The activated bacterial inoculum was diluted into a saline solution and set to a concentration of 1×10^8 CFU/mL, equivalent to 0.5 McFarland standard turbidity. The growth medium used in this analysis was Mueller-Hinton agar (MHA) and Nutrient Broth (NB). The prepared mediums were sterilized, and MHA was poured into sterile Petri dishes and left to solidify at room temperature before use.

2.3 Disc diffusion test

The antibacterial activity of the extracts was

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determined by the disc diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) (1999) and Bauer (1966), with slight modifications. An amount of 100 µL of the standardized bacterial inoculum was pipetted on top of the solidified MHA in the petri dish and streaked evenly using a sterile cotton swab. Sterile 6 mm diameter blank paper discs were placed over the surface of the MHA using sterile forceps. An aliquot of 20 µL of each extract solution concentrated at 10 mg/mL was impregnated carefully on the paper disc and left to dry. The commercial antibiotic Penicillin disc 10 µg (Oxoid) was used as a positive control. All these Petri dishes were then incubated at 37° C for 24 hrs. Any formation of a clear zone around the paper disc was measured and recorded.

2.4 Determination of minimum inhibition concentration (MIC)

The minimum inhibition concentration (MIC) was determined using the broth dilution method according to a method by Mushore and Matuvhunye (2013) with slight modification. The extracts at 10 mg/mL were subjected to two-folded serial dilution, to obtain a range of concentrations of 10-0.312 mg/mL. An aliquot of 100 µL of previously prepared standardized bacterial inoculum was added to 9 mL of sterile Mueller Hinton broth before being mixed with 100 µL of each extract concentration. Tubes with broth only were set as a control. All tubes were incubated at 37°C for 24 hrs and were observed for the presence or absence of visible turbidity in the broth after the incubation period. The lowest concentration (highest dilution) that did not show any turbidity (growth) was considered and recorded as the MIC.

2.5 Statistical analysis

All treatments and analysis were carried out in triplicates and results were reported as mean with standard deviation. All data collected were subjected to analysis of variance (ANOVA) using statistical software IBM SPSS version 26. Means of treatments were separated using Duncan Multiple Range Test (DMRT) for statistical significance at a 95% confidence interval ($P<0.05$).

3. Results and discussion

The fresh peel and seed produced the highest yield percentage of extract compared to the peel and seed that were subjected to heat processing (Table 1). The methanolic extracts of the fresh and heat-processed peel and seed of kuini (*M. odorata*) were tested against five food pathogens using the disc diffusion method. Results showed that fresh (unheated) and heat-processed kuini peel and seed extracts had good antibacterial activity against all food pathogens tested (Table 2). This antibacterial activity was shown in the measurement of the diameter of the inhibitory zone obtained. The diameter of the inhibitory zone was of various sizes. Among the pathogens, the highest inhibition activity was found against *S. aureus* with a diameter of more than 20 mm in both peel and seed. The antibacterial activity of kuini peel and seed extracts was higher than the control (Penicillin 10 µg) against all pathogens except *S. aureus* and *S. enterica* ser. Typhimurium. Even though Penicillin showed the greatest inhibitory zones against *S. aureus* (38.5 mm) *S. enterica* ser. Typhimurium (21.5 mm), it showed no effect against other food pathogens. Most gram-negative bacteria are impermeable to the antibiotics Penicillin and Platensimysin (McDermott *et al*., 2002).

Table 1. Extraction yield of fresh and heat-processed kuini peel and seed extracts.

Part	Sample	Yield (%)
Peel	Fresh	5.12
	Steam 5 min	4.35
	Steam 10 min	2.94
	Steam 15 min	3.99
	Boil 10 min	3.78
Seed	Fresh	5.08
	Steam 5 min	2.06
	Steam 10 min	2.54
	Steam 15 min	2.45
	Boil 10 min	2.08

From the results (Table 2), it was found that the antibacterial activity of unheated peel was significantly ($P<0.05$) higher than that of boiled and steamed (heat-processed) peel for all the pathogens tested. This indicated that heat processing decreased the antibacterial activity in the peel. Contrary to peel extracts, the heat-processed seed extracts were found to increase the antibacterial activity against the pathogen *B. cereus*, while the rest of the seed extracts showed maintained activity. The growth inhibition diameter of *B. cereus* was found to increase from 13.33 mm in unheated seed to 17.50, 18.33, 19 and 20.67 mm when boiled for 10 mins, steamed for 5 mins, 10 mins and 15 mins respectively. Only seeds that were steamed for 5 and 15 mins were found to show no antibacterial activity against *E. coli*. From these findings, it can be suggested that the peel and seed of kuini exhibited promising antibacterial activity despite having undergone heat processing.

Minimum inhibitory concentration (MIC) refers to the lowest concentration of the extract which is required for the inhibition of the visible growth of tested microorganisms (Table 3). Peel that was not subjected to

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Table 2. Diameter of inhibition zone (mm) of fresh and heat-processed kuini peel and seed extracts against tested pathogens

Part	Sample	Diameter of inhibition zone (mm)				
		E. coli	*B. cereus*	*S. aureus*	*S. enteritidis* serovar Typhimurium	*E. aerogenes*
Peel	Fresh	18.5±0.50^{h}	21.5±0.70^{f}	29.33±0.577^{b}	21.5±0.00^{e}	19±0.00^{g}
	Steam 5 min	13.5±0.70^{m}	14±0.00^{k}	24.5±0.707^{c}	16±0.00^{i}	14.33±0.57^{kl}
	Steam 10 min	13.5±0.70^{m}	11.5±0.57^{o}	22.67±0.577^{d}	14.5±0.57^{k}	13.5±0.70^{m}
	Steam 15 min	12.67±0.57^{n}	12.67±0.57^{n}	21.33±0.577^{e}	16±0.00^{i}	14±0.00^{l}
	Boil 10 min	14±0.00^{l}	13.5±0.70^{m}	22±0.00^{d}	15±0.00^{j}	14.5±0.70^{kl}
Seed	Fresh	16.67±1.15^{e}	13.33±0.57^{fg}	24±0.00^{c}	16.5±0.70^{i}	17±0.00^{e}
	Steam 5 min	0	19±0.00^{g}	23.33±0.57^{c}	19.33±1.15^{g}	15.33±0.57^{j}
	Steam 10 min	13.5±0.70^{m}	18.33±1.15^{h}	22.83±0.28^{cd}	14±0.00^{l}	14.33±0.57^{kl}
	Steam 15 min	0	20.67±2.51^{fg}	21±0.00^{e}	16.33±0.57^{i}	15.33±0.57^{j}
	Boil 10 min	15.33±0.57^{j}	17.5±0.70^{hi}	21.33±0.577^{e}	16.67±0.57^{i}	16±0.00^{i}
Positive control (Penicillin)		0	0	38.5±0.707^{a}	16±0.00^{i}	0

Values are presented as mean±standard deviation (n = 3). Values with different superscript within the same column are significantly different (P<0.05).

Table 3. Minimum inhibition concentration (MIC) of fresh and heat-processed kuini peel and seed extracts.

Part	Sample	MIC (mg/mL)
Peel	Fresh	0.31 – 1.25
	Steam 5 min	1.25
	Steam 10 min	1.25
	Steam 15 min	1.25
	Boil 10 min	1.25
Seed	Fresh	1.25
	Steam 5 min	1.25
	Steam 10 min	1.25
	Steam 15 min	1.25
	Boil 10 min	1.25

heat showed the lowest MIC against *S. aureus* (0.31 mg/mL), *B. cereus* and *S. enterica* ser. Typhimurium (0.63 mg/mL). The rest of the extracts showed a MIC of 1.25 mg/mL.

Based on the literature search, there have been no antimicrobial studies conducted on *M. odorata*. However, there were a few studies on the peel and seed of *M. indica* that can be used as a reference as *M. indica* and *M. odorata* are from the same family. According to Priyanka *et al*. (2016), mango peel acetone extract exhibited the highest antibacterial activity against *E. coli*, *S. enterica* ser. Typhimurium, *Shigella* spp. and *Enterobacter* spp., while ethanol and aqueous extracts exhibited antifungal activity against *Aspergillus niger*. Awad El-Gied *et al*. (2012) reported that methanol and ethanol extracts of *M. indica* seed showed growth inhibitions against 23 strains tested. On the other hand, Vaghasiya *et al*. (2011) found the antibacterial activity of *M. indica* seed extract against 58 out of 61 human pathogenic bacterial strains. In another study, Kalpna *et al*. (2013) determined the promising antimicrobial activity of peel and seed of mature and immature *M. indica* against seven Gram-positive bacteria and ten Gram-negative bacteria. The MIC for ripe peel was 1.25 mg/mL, while 0.15-1.25 mg/mL and 0.31-1.25 mg/mL were recorded for ripe and unripe seed, respectively.

The antimicrobial and antioxidant activity found in most fruits and plants is due to the presence of phytochemical compounds. Bioactive compounds in plants such as alkaloids, flavonoids, tannins, sterols, and triterpenes have been proven to exhibit antimicrobial activities (Saadabi *et. al*, 2006). Kuini was found to contain 73 phytochemical compounds with the main classes being monoterpene (45.4%), ester (33%) and α-terpineol (31.9%) as reported by Nur Amalina *et al*. (2019). The same author also reported that the total phenolic and flavonoid content of kuini was found to be higher in peel and seed extract, compared to the flesh. The presence of phenolic acids such as gallic acid, ferulic acid and ellagic acid contributes to the high phenolic content in the seeds of the genus *Mangifera* (Soong and Barlow, 2006). There are various mechanisms of phytochemicals that may contribute to antimicrobial activities. Phenolic and flavonoid compounds inhibit bacterial growth by interfering with the biological process in the cell. Flavonoids inhibit membrane-bound enzymes such as ATPase and phospholipase in addition to affecting the membrane permeability (Havsteen, 1983). Tannins are responsible for coagulating the protein wall (Li *et al*., 2003) while alkaloids cause cytotoxicity effect on the cell (Nobori *et al*., 1994).

Processing temperature can affect the functional activity of a phytochemical compound. Processing that involves the use of high heat can cause the decomposition of active phytochemical compounds in biological materials which leads to the removal,

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separation or release of certain phytochemical components which in turn alters biological activity (Negi, 2012). Ginovyan (2017) conducted a study on the effect of processing temperature on the antibacterial activity of four Armenian herbs where the results showed that methanol extract of *Agrimona eupatoria* lost its antibacterial activity against *S. aureus* after exposure at 60°C for 30 mins. Meanwhile, *Hypericum alpestre* and *Sanguisorba officinalis* extract still maintained their antibacterial activities even after exposure at a temperature of 121°C at the same time period. Chan *et al*. (2008) in their study reported that herbs boiled for 20 mins showed higher antibacterial activity compared to herbs that were not given a heat process. Chua and Aminah (2017) also reported a similar finding in which the antibacterial activity of nutmeg extract against *S. aureus* increased in tandem with the given temperature increase (50, 100, 121°C).

4. Conclusion

Kuini (*M. odorata*) peel and seed were found to have promising antibacterial activity against common food pathogens. The 10-min boiling and steaming for 5, 10 and 15 mins showed different antibacterial effects. The activity in peel was found to decrease for all the pathogens tested when the heat was given. Meanwhile, antibacterial activity in seed was increased specifically against *B. cereu*s when the seed was heated. From the findings, the peel and seed of kuini could be further utilized as a natural antibacterial agent or developed into functional bio-ingredients for nutraceutical or food products with the ability to combat food pathogen contamination. The development of potential products from the peel and seed can also reduce the problem of dumping agricultural waste.

Conflict of interest

The authors declare no conflict of interest.

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