# BIOASSAY-GUIDED OF FRESH AND FERMENTED KUINI (*MANGIFERA ODORATA*) EXTRACTS AGAINST BACTERIAL ACTIVITY

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**ABSTRACT**

*Kuini (Mangifera odorata) is classified under the family of mango (Mangifera) and categories as one of underutilised mango species in Malaysia. Presently, kuini fruit is lack of market demands compared to commercial mango such as Mangifera indica. Interestingly, in term of remedy, kuini has been used in folk medicine since ages which indicated that the plant contain compounds with bioactivity. As part of an effort to explore the economic potential of kuini, the bioactivity of kuini flesh and peel were investigated. Thus the objective of this study was to identify the antibacterial activity of crude extracts from both fresh and fermented of kuini flesh and peel. Crude extracts from all four samples were tested against a selection of bacterial bioassay using disc diffusion method. The antibacterial activity was indicated as a clear zone around the disk and expressed as percentage of bacterial inhibition. Results indicated that extracts from fermented kuini flesh were more potent against Gram-positive bacteria but less susceptible to Gram-negative bacteria. Interestingly, extracts from fresh and fermented kuini peel were both effective against Salmonella typhimurium while only extracts from fermented kuini peel were effective against Pseudomonas aeruginosa PA14. Amongst all, fermented kuini extracts were able to exhibit a strong antibacterial activity up to 86 % whereas only 56 % was exhibited by fresh kuini extracts. The Gram-negative bacteria were more susceptible to non-polar extracts such as hexane-extracts, whereas medium to polar extracts such as ethyl acetate and methanol-extracts were more potent to Gram-positive bacteria. In conclusion, extracts from both fermented kuini flesh and peel had a strong antibacterial activity compared with their fresh counterparts. This study also revealed that fermentation was successfully transformed compounds in fresh kuini into active compounds with antibacterial activity in the fermented kuini. In future, the bioassay-guided isolation and purification of those bioactive extracts could be performed to identify the corresponding antibacterial compounds.*

Keywords: *Mangifera odorata*, flesh, peel, bioassay-guided, antibacterial

## INTRODUCTION

Plant consist a wide range of pharmacological properties and possessed a long history in folk medicine against many diseases. Bioassay guided method can be used to extract, fractionate and isolate compounds in plant with certain bioactivity. Plant natural products have diverse chemical, chirality and various functional groups that ideal as source for new antibacterial compounds

(Jiang *et al*. 2009). Plant-derived compounds with antibacterial property can be an ideal microbial control against certain bacteria and could provide templates for new antibacterial drug discovery.

Kuini (*Mangifera odorata*) fruit is known by its strong scent, sweet-sour taste, juicy and less fibrous flesh. The kuini tree is only planted as home garden or village orchard and lack of market demand. The unripe fruit is usually use as an appetiser (sambal), while the ripe fruit left rotten under the tree as less favour for fresh consumption. Kuini fruit however contains phytochemicals and bioactive compounds that potentially could attract consumer in preferences to nutritional quality and health benefit. At present, research on kuini is scarce (Mirfat *et al*. 2016; Brooke and Lau, 2013), thus this study attempt to raise awareness on the kuini bioactivity for health wellness and benefited to agricultural base industries.

Fermentation is a metabolic process that produces chemical changes in organic substrates using microorganisms as such yeasts and/or bacteria under certain conditions. Fermentation of kuini flesh and peel had created a favourable environment to the growth of microbial starter culture (Adnan *et al*. 2017) and assisted in the degradation of certain compounds into more beneficial compounds. Suitable microbial culture in fermentation could enhance the production of beneficial compounds (Adnan *et al*. 2018; Chen and Liu, 2000) in the fermented product as such phytochemicals and organic acids with antibacterial property (Yassine *et al*. 2016).

Phytochemical contents and antibacterial activity in fresh and fermented kuini is an interesting study. In this study extracts from two kuini parts, the flesh and peel were chosen to be analysed for their antibacterial potential. By-using bacterial assay, fresh and fermented extracts were tested against both Gram-negative and Gram-positive bacteria to identify the active antibacterial compounds. Thus the purpose of this study is to investigate the antibacterial activity of kuini extracts and concomitantly indicate extracts that active, susceptible or resistance against certain bacteria. Active kuini extracts are potential to be further isolate to purify and identify the compounds that potentially use as antibacterial drug template.

## MATERIALS AND METHODS

### PREPARATION OF KUINI SAMPLES

Kuini fruit at maturity stage was washed, air dried and peeled. The flesh was sliced and blend as puree. Both flesh (puree) and peel were dried separately in a dryer at 40ºC for 2 days with approximately 10 % moisture content. Dried samples of kuini flesh and peel were blend as fine powder and separately packed in a plastic bag and sealed. In this study fresh kuini was referred to dry powdered of kuini flesh or peel, whereas fermented kuini was referred to kuini flesh or peel obtained after fermentation process.

### FERMENTATION OF KUINI FLESH AND PEEL

Powdered kuini flesh and peel (3 and 5 %, w/v) were poured into a separate conical flask. A volume of distilled water was slowly added into the sample and stirred to form a mixture. Mixtures were pasteurised and once cool the mixtures were aseptically inoculated with starter culture of *Gluconacetobacter sp.* (1 and 2 %, w/v). The mouth of each conical flask was covered with sterilised cotton wool, tight using rubber bands and then incubated at 30°C. After 2 weeks of fermentation, the yield known as fermented kuini was filtered to separate the fermented liquid and solid.

### PREPARATION OF EXTRACTS

Fermented kuini liquid was dried using freeze dryer, whereas fermented kuini solid was dried in an oven dryer at 40ºC. After dried, both fermented samples were blended to fine powder. Each dried sample (powder) as such fermented flesh, fermented peel including dried fresh flesh and dried fresh peel were extracted by three different eluent as such *n*-hexane (-H), ethyl acetate (-E) and methanol (-M) for an hour at 25 ºC using a sonicator (DECON F5100b, UK), excluding fermented kuini liquid (-W). The eluate was filtered using a qualitative filter paper (125mm; Fisher UK). The extract obtained was concentrated under reduced pressure using a rotary evaporator (Buchi R-205, Switzerland) and dried under a nitrogen flow to remove excessive remaining eluent. All extracts were kept in glass containers, sealed and stored at – 20 °C. Prior to antibacterial analysis, 1 mg/mL working concentration of each extract was prepared using dimethyl sulfoxide (DMSO).

### ANTIBACTERIAL ACTIVITY

Bacteria cultures were obtained from culture collection of Mycology Laboratory, MARDI. The tested Gram-negative bacteria were *Salmonella enteritidis, Escherichia coli* strains 0157, 1370 and 303, *Pseudomonas aeruginosa* strain PA14 and *Salmonella typhimurium* whereas for Gram-positive bacteria were *Listeria monocytogenes, Listeria innocua, Bacillus cereus, Staphyloccus aureus* and *Staphyloccus epidermis*. The preparation of bioassay and antibacterial activity was measured using disc diffusion method as mentioned by Adnan *et al*. (2017). The antibacterial activity was shown by the presence of clear zones of inhibition (diameter, mm) surrounding the disc. The antibacterial activity was classified into low (< 50 %), moderate (≥ 50 %) and strong (≥ 60 %) inhibition. Active extracts are expressed as those exhibited antibacterial activity with more than 50 % inhibition.

## RESULTS AND DISCUSSION

### ANTIBACTERIAL ACTIVITY OF FRESH AND FERMENTED FLESH EXTRACTS

In this study, the antibacterial activity for all extracts of fresh kuini flesh (Figure 1a-b) and fermented kuini flesh (Figure 1c-d) were investigated. Results indicated that all flesh (fresh) extracts (KF-H/-E/-M) exhibited low antibacterial activity (< 50 %) thus ineffective against both Gram-negative (Figure 1a) and Gram-positive bacteria (Figure 1b). In comparison, fermented flesh extracts (KF3/5-A2-H) and (KF3/5-A2-E) were managed to produce a moderate antibacterial activity (50-52 %) against the Gram-negative bacteria (Figure 1c) while moderate to strong antibacterial activity (54-68 %) were obtained against the Gram-positive bacteria (Figure 1d), respectively.

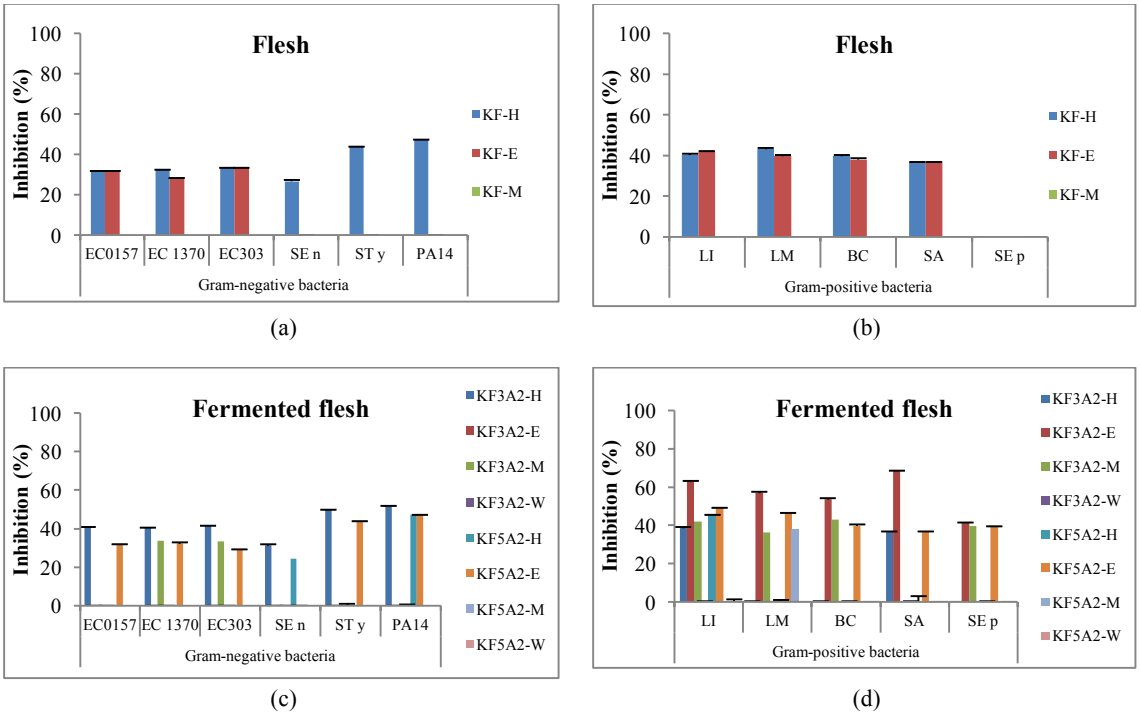
The antibacterial activity between flesh (Figure 1a) and fermented flesh (Figures 1c) against Gram-negative bacteria indicated that hexane-extract of fermented flesh (KF3A2-H) was more active than hexane-extract of flesh (KF-H). Results showed that fermented extract (KF3A2-H) exhibited moderate antibacterial activity (≥ 50%) against both *S. typhimurium* and *P. aeruginosa* PA14 (Figure 1c) but only low antibacterial activity (< 50%) managed to exhibit by the flesh extract (Figure 1a). This finding revealed that extracts from fermented flesh were more active than flesh (fresh) in inhibiting the Gram-negative bacteria.

The antibacterial activity between flesh (Figure 1b) and fermented flesh (Figures 1d) against Gram-positive bacteria also indicated that fermented flesh explicit high antibacterial activity compare to flesh (fresh) counterpart. All flesh extracts exhibited low antibacterial activity (< 50%) and failed to inhibit *S. epidermis* (Figure 1b). The fermented flesh however managed to exhibit high antibacterial activity (> 60%) against Gram-positive bacteria including inhibition of *S. epidermis* (Figure 1d) although at low antibacterial activity (< 50%).

Results indicated that fermented flesh extracts had explicit strong activity against Gram-positive bacteria than Gram-negative bacteria (Figure 1c-d). Prominent antibacterial activity of fermented flesh was mainly exhibited by the ethyl acetate extracts (KF3A2-E) from 54-68% of inhibition (Figure 1d). Amongst all, *P. aeruginosa PA14* and *S. typhimurium* were more susceptible to a hexane-extract, whereas *S. enteritidis* and all strains of *E. coli* were resistant to all extracts (Figure 1a-b).

The *P. aeruginosa* PA14 are a well-known resistant bacteria strain (Lee *et al*. 2006) and this finding was the first report to reveal the susceptibility of *P. aeruginosa PA14* to hexane-extract from fermented kuini flesh. This finding indicated that ethyl acetate-extract of fermented kuini flesh (Figure 1d) were affective against Gram-positive bacteria as such *S. aureus, L. innocua, L. monocytogenes* and *B. cereus*. On the other hand, most Gram-negative bacteria were more resistant against fermented kuini flesh extracts except for *P. aeruginosa PA14* and *S. typhimurium.* Gram-positive bacteria were more susceptible to fermented flesh extracts than Gram-negative bacteria (Figure 1c-d) while all flesh extracts were less effective against all bacteria (Figure 1a-b).

Figure 1: Antibacterial activities of kuini flesh (KF, Figure 1a-b) and fermented kuini flesh (KF3/5, Figure 2c-d) extracts expressed as percentage of bacterial inhibition (%). Extracts with symbols H, E, M and W respectively referring to the *n*-hexane, ethyl acetate, methanol and water extracts. Error bars represents the mean ± SD of three replicates. The encoded bacteria are referring respectively to *E. coli* (EC), *S. enteritidis* (SEn), *S. typhimurium* (STy), *P. aeruginosa* (PA14), *L. innocua* (LI), *L. monocytogenes* (LM), *B. cereus* (BC), *S. aureus* (SA) and *S. epidermis* (SEp).




To date this study is the first reports revealed on antibacterial activity of fresh and fermented kuini flesh. In related study, antibacterial activities of mango (*M. indica*) extracts have been reported (Sahrawat *et al*. 2013; Stoilova *et al*. 2005) and the antibacterial activity was due to the phenolic compounds (Engels *et al*. 2011). Phenolic compounds such as gallic acid in kuini could influence the ionic strength and altered the permeability of bacteria thus lead to cell death (Borges *et al*. 2013) of *S. aureus*, *E.coli* and *P. aeruginosa* PA14. In addition, fermentation had produced fermented kuini flesh with active phytochemical (e.g. phenolic) and high antioxidant (e.g. scavenging activity) as exhibited by the fermented flesh extract which also contributed to the antibacterial activity (Adnan *et al*. 2018).

**ANTIBACTERIAL ACTIVITY OF FRESH AND FERMENTED PEEL EXTRACTS**

In this study, the antibacterial activity for all extracts of fresh kuini peel (Figure 2a-b) and fermented kuini peel (Figure 2c-d) were investigated. Results indicated that almost all fresh peel extracts (KP-H/-E/-M) exhibited low antibacterial activity (< 50 %) against both Gram-positive and Gram-negative bacteria, except *S. typhimurium* (Figure 2a). In comparison, fermented peel extracts (KP3/5-A2/1-H/-E/-M/-W) managed to exhibit moderate to strong antibacterial activity (50-86 %) against both Gram-negative (Figure 2c) and Gram-positive bacteria (Figure 2d). All fresh peel extracts were mostly ineffective against both Gram-positive and Gram-negative bacteria compared with fermented peel extracts.

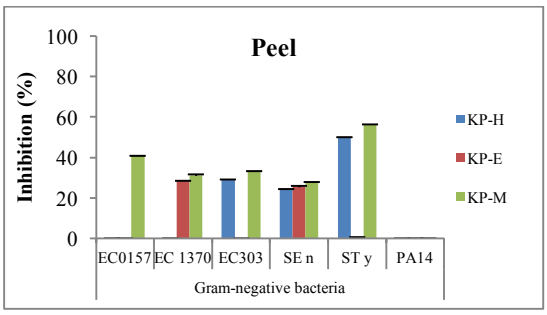
The antibacterial activity between fresh peel (Figure 2a) and fermented peel (Figures 2c) against Gram-negative bacteria were studied. Results indicated that hexane-extract (KP-H) and methanol-extract (KP-M) from fresh peel were moderately (50-56 %) active against *S. typhimurium* but failed to inhibit *P. aeruginosa* PA14 (Figure 2a). In comparison, the hexane-extract (KP5A1-H) and ethyl acetate-extract (KP5A1-E) of fermented peel were also moderately (50-54 %) active against *S. typhimurium* but exhibited strongly (70-86 %) against *P. aeruginosa* PA14 (Figure 2c) by the hexane-extracts of (KP5A1-H) and (KP5A2-H).

This finding was the first report revealed the susceptibility of *P. aeruginosa PA14* to hexane-extracts of fermented kuini peel (KP5-A2/1-H) and susceptibility of *S. typhimurium* to methanol-extract of kuini peel (KP-M) and hexane-ethyl acetate-extracts of fermented kuini peel (KP5-A2/1-H/-E) . This finding also revealed a weak antibacterial activity (< 50% inhibition) of fermented peel extracts towards *S. enteritidis* and all *E. coli* strains (Figure 2a-b) which indicate the resistance of *S. enteritidis* (Jaradat *et al*. 2014) and *E. coli* (Mizielinska *et al*. 2017).

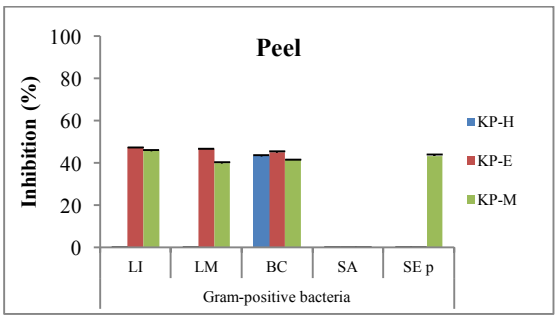
Antibacterial activity against Gram-positive showed that fresh peel extracts exhibited weak antibacterial activity but failed to inhibit *S. aureus* (Figure 2b), whereas fermented peel extracts namely (KP3A2-M) and (KP5A2-E) managed to exhibit strong antibacterial activities including against *S. aureus* (Figure 2d). The *B. cereus* was susceptible to ethyl acetate- (KP5A2-E) and methanol-extracts (KP3A2-M) of fermented peel whereas *S. epidermis* was susceptible to hexane- (KP5A2-H) and ethyl acetate-extracts (KP5A2-E) as showed in Figure 2d. High antibacterial activity towards the same bacteria was exhibited by fermented peel extracts. The antibacterial activity against *S. typhimurium* indicated that extracts (KP5A1-H) from fermented peel exhibited 54 % inhibition whereas extract (KP-H) from peel only managed to exhibit 50 % of inhibition. This finding indicated that fermented peel extracts were more active than fresh peel extracts and same extracts could active against both Gram-negative and Gram-positive bacteria.

The fermented peel of hexane-extract (KP5A2-H) was found highly effective against *P. aeruginosa* PA14 while fresh peel of methanol-extract (KP-M) was found effective against *S. typhimurium*. Similar extracts however were not effective on other Gram-negative bacteria (Figure 2a-c). The fermented peel of ethyl acetate- (KP5A2-E) and methanol-extracts (KP3A2-M) were both strongly effective against *B. cereus,* while hexane- (KP5A2-H) and ethyl acetate-extracts (KP5A2-E) were both moderately effective against *S. epidermis.* On the other hand, fermented flesh of ethyl acetate-extract (KF3A2-E) was moderately effective against *L. monocytogenes* and *B. cereus,* and strongly effective to both *S. aureus* and *L. innocua*. Similar extracts could effective on other Gram-positive bacteria but at different efficacy (Figure 2b-d).

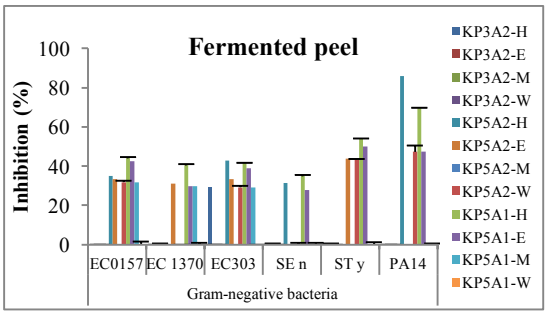
Figure 2: Antibacterial activity measured as bacterial inhibition (%) by kuini peel for both fresh (KP, Fig. 2a, and c) and fermented (KP3/5, Fig. 2b, d) extracts. Extracts with symbols of H, E, M and W respectively referring to the *n*-hexane, ethyl acetate, methanol and water extracts. Error bars represents the mean ± SD of three replicates. The codes are referring to bacteria *E. coli* (EC), *S. enteritidis* (SEn), *S. typhimurium* (STy), *P. aeruginosa* (PA14), *L. innocua* (LI), *L. monocytogenes* (LM), *B. cereus* (BC), *S. aureus* (SA) and *S. epidermis* (SEp).



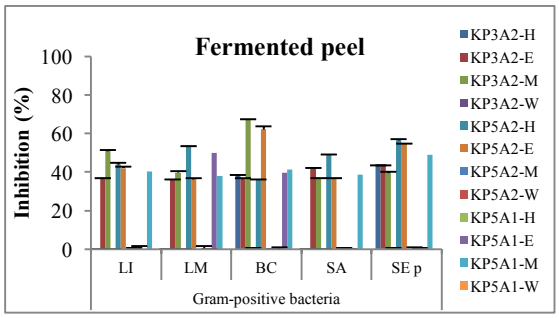

(a)




(c)




(b)




(d)

Results indicated that bacteria exhibited different resistance efficacy to extract. The susceptibility of bacteria could be due to the presence of anionic groups in their membrane at which the magnitude of the charge varies from species to species and can be influenced by ionic strength and pH (Borges *et al*. 2013). Compounds such as ethyl gallate and penta-O-galloyl-glucoside reported in mango peel had potent ability to scavenge hydroxyl radical and superoxide anion (Jiang *et al*. 2010) including other polyphenols in mango peel such as quercetin, kaempferol, gallic acid and mangiferin (Barreto *et al*. 2008). The antibacterial activity in this study could possibly resulted by the effect of similar phenolic compounds present in fresh and fermented kuini peel extracts that disturb the bacteria cell membrane plasma causing protein denaturation and death due to disruption of bacteria cell.

## CONCLUSION

Our study highlighted the potential extracts of fresh and fermented kuini for both flesh and peel to inhibit bacteria that poses a dangerous threat to public health. The hexane-extracts of fermented kuini peel were potent against *P. aeruginosa* PA14 while the methanol-extract was potent against *B.cereus*. The ethyl acetate-extract of fermented kuini flesh was potent against *S. aureus* and *L. innocua*. In comparison, fermented kuini extracts able to inhibit many bacteria compare to fresh kuini extract with prominent antibacterial activities were shown by fermented peel extracts. To the best of our knowledge, this is the first report concerning such fermentation activity of kuini flesh and peel and their antibacterial properties. In near future the active extracts with high antibacterial activity could be further isolate and purify to identify the bioactive compounds. Limitation of this research is that scientific study on kuini is presently new and information reported on kuini is scarce. Thus these findings gave value added to the beneficial of kuini and probably could increase the market demand. As such chokanan and harumanis varieties, hopefully kuini could also be one of the Malaysian commercial mangoes in the future.

## CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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