MANGIFERA ODORATA (KUINI) FRUIT EXTRACTS AS POTENTIAL ANTI-LISTERIOSIS AGENTS

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ABSTRACT

Listeriosis or foodborne illness by pathogenic bacteria of Listeria sp. can caused death and seriously affect susceptible groups such as pregnant women, elderly and immunocompromised individuals. The most virulence strain, Listeria monocytogenes were able to inject human food chain and caused listeriosis in both human and animal hosts. The occurrence of L. monocytogenes due to antibiotic resistance has driven the use of natural products from plant-derived compounds as potential antimicrobial control. Thus, the activity of wild mango such as Mangifera odorata (kuini) extracts were studied against two Listeria species for the anti-listeria activity. The objective was to identify the anti-listeria activity from the peel, flesh and kernel seed of M. odorata fruit extracts against the L. monocytogenes and L. innocua using bioassay-guided method. The anti-listeria activity was indicated as a bacterial clear zone and expressed as percentage of inhibition. Results indicated that methanol extracts from the kernel seed (KCM and KTM) were potent (66.3 – 95.8 % inhibition) towards both bacteria. The peel extracts from ethyl acetate (PTE) and hexane (PTH and PCH) were very potent (> 100 % inhibition) against L. monocytogenes, whereas the peel extract from ethyl acetate (PCE) was very potent (80.8 %) to L. innocua. The anti-listeria activity of M. odorata extracts were probably contributed by the phenolics content, antioxidant (FRAP) value and scavenging (DPPH) activity of the extracts as reported in the earlier study. In conclusion, this study indicated that both control (C) and treated (T) extracts of M. odorata consist of anti-listeria activity against the L. monocytogenes and L. innocua. The potent anti-listeria activity showed by the M. odorata peel and kernel seed extracts indicated that the fruit contains prophylactic property that value-added to health benefit. Thus, the M. odorata peel and kernel seed can be developed into potential products with anti-listeria activity while the compounds in the potent extracts can be further identified in near future.

Key words: Listeria spp., Mangifera odorata, flesh, seed kernel and peel

INTRODUCTION

Foodborne illness such as listeria infection or listeriosis is due to consumption of food spoiled by pathogenic bacteria particularly in dairy, meat, poultry and ready-to-eat products. The listeriosis can be very serious and lead to septicemia or meningitis and death. The Listeria monocytogenes is one of the great public concern of human foodborne pathogens and the most frequent causes of death with high fatality rate due to food poisoning (Jemmi & Stephan, 2006). The L. monocytogenes is ubiquitous in the environment and primarily transmitted through the ingestion of contaminated food products and caused systemic infections. The frequent symptoms of illness were fever, chills, muscle aches, nausea and diarrhoea between 3 days to 10 weeks. The highest incidence and mortality rates of the L. monocytogenes infection were from food industrial sector and retail raw food (Wai et al., 2020). The L. monocytogenes is able to infect human food chain directly or through animal farms. The bacterium capable of multiplying at a range of 1-45°C and remains moderately inactivated at below 0°C (Swaminathan et al., 2007), thus gave particular concern on retail food with long shelf life.

The random use of antimicrobials in community and farms has led to increasing cases of antimicrobial resistant L. monocytogenes in the environments (Castanon, 2007). In the last three decades the L. monocytogenes has developed resistance and tolerance susceptibility towards certain antibiotics (Letchumanan et al., 2018; Srinivasan et al., 2005 & Pesavento et al., 2010). Tetracyclines, ampicillin, penicillin G, imipenem, amoxicillin, sulphonamides, aminoglycosides, macrolides, chloramphenicol and
glycopeptides were among antibiotics used as antibiotic therapy for listeriosis (Dortet et al., 2009). The *L. monocytogenes* able to resist an antibiotic concentration and developed resistance as response to adaptation or genetic change (Calderon & Sabundrayo, 2007). Thus, the infection caused by the antibiotic resistance of *L. monocytogenes* with varying virulence and pathogenicity is a major concerned of public health. Thus, the use of antibiotic in food chain should be control and managed in order to reduce the emergence of antibiotic resistant strain of *L. monocytogenes*. The other bacterium studied, the *L. innocua* is considered a nonpathogenic *Listeria* species. The *L. innocua* resembles a close relative to *L. monocytogenes*, but harmless to other organisms (Buchrieser et al., 2003). Atypical hemolytic *L. innocua* isolates have been characterised recently as virulent albeit less than *L. monocytogenes* (Moura et al., 2019). The septicemia and meningitis infections due to *L. innocua* in ruminants (Rocha et al., 2013) and human (Favaro et al., 2014) have been reported and suggested the presence of virulent atypical hemolytic *L. innocua* isolates as listeriosis agent is spreading. The exposure of virulence *L. innocua* in human fatality is rare, yet the prevalence is important for food safety and public health.

Listeria infection is a public health worry due to antimicrobial resistant problems that effects healthcare treatment worldwide. Thus, non-antibiotic approach of utilising the natural products in food source may allow the withdrawal of antibiotic resistance profile in bacteria strains and prevent the over use of antibiotics for listeriosis treatments. This study therefore focused on the investigation of plant extracts from *Mangifera odorata* or locally named ‘kuini’ as anti-listeriosis agent against *L. monocytogenes* and *L. innocua*. The *M. odorata* is one of the mango family and a good source of natural products with a long history used in folk medicine. However, study on antipathogenic activity of *M. odorata* is scarce and the anti-listeria activity of *M. odorata* has not been reported elsewhere yet. The *M. odorata* is one of wild *Mangifera* species and contains antioxidants and total phenolic contents (Salahuddin et al., 2016) suggested that the *M. odorata* fruit may contributed to the antibacterial activity. Hence, the objective of this study was to identify the anti-listeria activity of *M. odorata* active extracts against *L. monocytogenes* and *L. innocua*. In this study, the extracts of *M. odorata* were prepared from the flesh, peel and kernel seed. The anti-listeria activity was analysed using bioassay-guided fractionation method and measured as the percentage of bacterial inhibition from a mean of triplicate readings. The expected output would reveal the *M. odorata* anti-listeria potent extracts that contain active compounds and potent to *L. monocytogenes* and *L. innocua*. The potent compounds in the active extracts could be further isolated and identified as anti-listerioses agent in near study.

### MATERIALS AND METHODS

#### PREPARATION OF *M. ODORATA* SAMPLES

The *M. odorata* fruits at stage 5 of maturity index were washed and air dried. The fruit sample were divided into fresh sample named as control (C) while fruits given hot steam pre-treatment named as treated samples (T). The fruits were peeled off and the flesh was sliced then blended into puree. The seeds were removed from the kernel and cut into small pieces. The preparation of samples as such peel, flesh and kernel seed for treated samples were similar as mentioned for control. The flesh puree, peel and seed kernel for control and treated samples were dried separately in an oven dryer at 40°C until approximately 10 % (w/w) of moisture content. All dried samples were blended into fine powder and separately packed in an aluminium foil bag, sealed and labelled.

#### PREPARATION OF EXTRACTS

All dried samples of *Mangifera odorata* such as flesh, peel and kernel seed were extracted using bioassay-guided fractionation method. Each sample was extracted separately by different solvents in sequences such as n-hexane (H), ethyl acetate (E) and methanol (M) for an hour at 25 °C using a sonicator (DECON F5100b, UK). All eluates were filtered using a qualitative filter paper (125mm, Fisher UK) and concentrated under reduced pressure using a rotary evaporator (Buchi R-205, Switzerland). The extracts were dried under a nitrogen flow to remove excessive solvent. All pure extracts were stored at − 20 °C prior to antibacterial analysis.

#### ANTIBACTERIAL ACTIVITY

Sample for each extract was prepared at 1 mg/mL in dimethyl sulfoxide (DMSO). The antibiotic tetracycline (1 mg/mL) was used as a positive control whereas the DMSO was used as a negative control. The bioassay for *Listeria monocytogenes* (ATTC® 51772) and *Listeria innocua* (ATTC® 33090) were tested on each sample using a disc diffusion assay method as mentioned by Adnan et al. (2017). The term anti-listeria was referred to antibacterial activity as shown by the presence of clear zones surrounding the disc and measured as diameter (Ø, mm) of inhibition. The clear zone indicated that the bacteria was susceptible to the sample that implies to the size of clear zone. The anti-listeria activity (% inhibition) was expressed as a percentage of growth inhibition compared with positive control. The anti-listeria activity was classified into active (≥ 50 %), moderate (≥ 70 %) and potent (≥ 90 %) inhibition, while inactive extracts were expressed as those with less than 50 % inhibition.

#### STATISTICAL ANALYSIS

The statistical data were analysed using Minitab® 16.1.1 Statistical Analyses (U.K). All data values were expressed as mean ± standard deviation (SD) and statistically analysed one-way ANOVA with significance difference at P values of ≤ 0.05 between treatments. The Turkey method was used to compare all possible pairwise difference of means at the same time.
RESULTS AND DISCUSSION

ANTIBACTERIAL ACTIVITY OF M. ODORATA FLESH EXTRACTS

The activities of untreated (FC) and treated (FT) of M. odorata flesh extracts against the L. monocytogenes (LM) and L. innocua (LI) were investigated (Figure 1). Results indicated that (FCH) extract was the most active against the L. monocytogenes. The (FCH) extract had exhibited significantly (P ≤ 0.05) high antibacterial activity (62-76 % inhibition) compared with other extracts. All untreated flesh extracts such as (FCH), (FCE) and (FCM) were active (≥ 50 % inhibition) against L. monocytogenes, whereas only (FTH) extract from the treated flesh (FT) was active. The antibacterial activities of these extracts however were insignificantly (P ≥ 0.05) difference. The antibacterial activity against L. innocua indicated that both untreated extracts of (FCH) and (FCE) were effective against the L. innocua, but the antibacterial activities between both extracts were not significantly (P ≤ 0.05) difference. All treated flesh extracts such as (FTH), (FTE) and (FTM) including (FCM) extract from untreated extracts were found inactive (< 50 % inhibition) against the L. innocua.

The antibacterial activity of flesh extracts against the L. monocytogenes showed that untreated flesh extracts were more active (> 50 % inhibition) than treated flesh extracts. Similar antibacterial activities against the L. innocua were showed by the extracts. Results on antibacterial activity against both bacteria showed that the untreated active extracts exhibited between 50-76 % inhibition, while the treated active extracts were much lower between 52-62 % inhibition. This finding revealed that untreated flesh (FC) extracts had explicit strong inhibition activity against the L. monocytogenes and L. innocua bacteria. The anti-listeria activity of flesh extracts as determined by the inhibition of L. monocytogenes and L. innocua bacteria may be supported by the total phenols, total flavonoids contents and antioxidant properties in the flesh (Ribeiro et al., 2008) that responsible to antimicrobial activity. The fruit flesh poses a lower content of phenolic compounds compared with the peel and seeds (Vega-Vega et al., 2013), thus exposure of fruits with hot steam during the pre-treatment possibly had denatured certain phenolic compounds in the flesh and affect the susceptible of extracts towards the bacteria thus reduced the antibacterial activity.

Amongst all extracts, the L. monocytogenes and L. innocua bacteria were more susceptible to hexane and ethyl acetate extracts indicated that the M. odorata flesh contains active compounds with anti-listeria activity were contributed from the nonpolar and medium-polar type of compounds. This finding was the first report to reveal the susceptibility of L. monocytogenes, a well-known pathogenic bacteria strain (Drevets & Bronze, 2008) towards the hexane and ethyl acetate extracts of M. odorata flesh. This finding revealed that nonpolar compounds in the untreated hexane (FCH) extract and medium-polar compounds in the ethyl acetate (FCE) extract of M. odorata flesh were significantly (P ≤ 0.05) affective against both L. monocytogenes and L. innocua. The nonpolar compounds in the treated hexane (FTH) extract however only significantly (P ≤ 0.05) affective against the L. monocytogenes.

Figure 1: Antibacterial activity of M. odorata flesh for control (FC) and treated sample (FT) against Listeria monocytogenes (LM) and Listeria innocua (LI). The anti-listeria activity was expressed as percentage of bacterial inhibition (%). Error bars represents the mean ± SD of three replicates. Samples with symbols H, E and M were respectively referring to the n-hexane, ethyl acetate and methanol extracts. Sample that do not share a letter are significantly different at 5 % level (P ≤ 0.05).
ANTIBACTERIAL ACTIVITY OF M. ODORATA KERNEL SEED EXTRACTS

Figure 2 shows the activity of M. odorata kernel seed extracts from untreated (KC) and treated (KT) against L. monocytogenes (LM) and L. innocua (LI). Results indicated that the methanol extract of untreated (KCM) kernel seed was potent against both L. monocytogenes and L. innocua bacteria at 96 and 84 % inhibition, respectively. The treated kernel seed extract of (KTM) were also showed inhibitory affect against both L. monocytogenes and L. innocua but slightly lower between 65-66 % inhibition. Another treated kernel seed extract, (KTE) was active against the L. innocua but failed to inhibit the L. monocytogenes. The inhibitory effect on L. monocytogenes indicated that the untreated methanol extracts of (KCM) were significantly (P < 0.05) potent compared to the treated methanol extracts of (KTM). Similarly, a significantly (P < 0.05) inhibitory effect on L. innocua was shown by the (KCM) and (KTM) extracts. The ethyl acetate extracts of untreated (KCE) and treated (KTE) kernel seed were active (> 50 % inhibition) against the L. innocua, but inactive towards the L. monocytogenes. The inhibitory effect of both (KCE) and (KTE) extracts against the L. innocua however was insignificantly (P ≤ 0.05) difference.

The antibacterial profiles indicated that the untreated methanol kernel seed of (KCM) extracts were the most potent (> 87 % inhibition) followed by the moderate activity from the treated methanol kernel seed of (KTM) extracts (> 65 % inhibition). The untreated and treated ethyl acetate extracts, the (KCE) and (KTE) were active (56-65 % inhibition) against L. innocua, whilst all hexane extracts were inactive towards both bacteria. All methanol extracts (KCM and KTM) were potent against the L. monocytogenes and L. innocua indicating that the extracts contain strong anti-listeria compounds. The potent inhibitory effect of kernel seed methanol-extracts on the L. monocytogenes and L. innocua were probably due to the damaging effects on the cell membrane from the phenolic compounds that led to the alteration in cell morphology and interference with bacterial division (Jiamboonsri et al., 2011). The kernel seed contains relatively high phenolic contents such as pentagalloglucopyranose and relatively small amounts of methyl gallate and gallic acid (Nithitanakool et al., 2009). The ethyl acetate extracts of (KCE) and (KTE) were only active against the L. innocua indicating that the mixtures of nonpolar and polar compounds in the extracts were more susceptible on L. innocua.

The activity of extracts reflects from the preparation of sample. The pre-treatment gave a significant (P ≤ 0.05) inhibitory effect with slightly lower antibacterial activity of treated kernel seed of (KTM) compared to the untreated (KCM) extract. The possible reason could be due to denature of certain active compounds during the high temperature treatment that reduced the compounds bioactivity (Rawel et al., 2005). Compounds such as phenolics are thermostable and could denature at high temperature at short time period or at moderate temperature but increasing exposure time. This study revealed that methanol extracts of M. odorata kernel seed were affective against the L. monocytogenes and L. innocua, while L. innocua were susceptible to most extracts. This finding was the first report to reveal the susceptibility of L. monocytogenes to potent methanol extracts of M. odorata kernel seed. This finding also indicated that the untreated of M. odorata kernel seed were more effective than the treated extract and exhibited strong anti-listeria activity against both the L. monocytogenes and L. innocua.

Figure 2: Antimicrobial activity of M. odorata kernel seed for control (KC) and treated sample (KT) against Listeria monocytogenes (LM) and Listeria innocua (LI). The anti-listeria activity was expressed as percentage of bacterial inhibition (%). Error bars represents the mean ± SD of three replicates. Samples with symbols H, E and M were respectively referring to the n-hexane, ethyl acetate and methanol extracts. Sample that do not share a letter are significantly different at 5 % level (P < 0.05).

ANTIBACTERIAL ACTIVITY OF M. ODORATA PEEL EXTRACTS

The antibacterial activity of M. odorata peel extracts toward the L. monocytogenes (LM) and L. innocua (LI) showed different inhibition profiles as presented in Figure 3. All extracts except (PTH) were active against both L. monocytogenes and L. innocua. The untreated (PCH) and treated (PTH and PTE) of peel extracts were significantly (P < 0.05) potent against the L. monocytogenes, however moderately active against the L. innocua. The antibacterial activity of treated peel (PT) extracts were significantly (P ≤ 0.05) potent than the untreated peel (PC) extracts in inhibiting the L. monocytogenes, but slightly lower towards the L. innocua. These antibacterial activities as between treated and untreated of peel extracts were contrary as shown by the flesh and kernel seed extracts. Amongst all, the ethyl acetate (PTE) and hexane extracts (PTH and PCH) of M. odorata peel were the most potent extracts.
to the *L. monocytogenes* exceeding 100% inhibition in comparison to control antibiotic (tetracycline). Tremendous high anti-listeria activity on the *L. monocytogenes* indicated that treated peel extracts consists of valuable compounds such as polyphenols, carotenoids, enzymes and dietary fibre (Ajila et al., 2007) that possibly contributed to the potent anti-listeria activity.

Results indicated that pre-treatment affect the bioactivity of certain compounds in the peel of *M. odorata* fruits as the treated peel extracts exhibited stronger anti-listeria activity than the untreated peel extracts against both bacteria. The most potent extract against *L. monocytogenes* was the ethyl acetate extract of (PTE), while the (PCE) was the most potent extract against the *L. innocua*. This finding was the first report revealed the susceptibility of *L. monocytogenes* to (PTH), (PTE) and (PCH), indicated that the bacteria exhibited different resistance efficacy to the peel extracts. The active compounds in the peel extracts probably exhibited different efficacy and susceptible towards different bacteria species (Jiamboonsri et al., 2011). The susceptibility of *L. monocytogenes* and *L. innocua* 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). The 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 polyphenols such as quercetin, kaempferol, gallic acid and mangiferin (Barreto et al. 2008). The anti-listeria activity of *M. odorata* peel could possibly due to effect of phenolic compounds that disturb and disrupted the bacteria cell membrane plasma that caused protein denaturation and death.

The antibacterial activity was found in the *M. indica* (mango) extracts (Sahrawat et al. 2013; Stoilova et al. 2005) and suggested that the antibacterial activity was due to the phenolic compounds (Engels et al. 2011). Hence, the phenolic compounds such as gallic acid in *M. odorata* could influence the ionic strength and altered the permeability of bacteria thus lead to cell death (Borges et al. 2013) of *L. monocytogenes* and *L. innocua*. High phytochemical (e.g. phenolic) content and high antioxidant (e.g. scavenging activity) also contributed to the antibacterial activity in *M. odorata* (Adnan et al. 2018). High amount of total phenolic compounds (TPC), FRAP value and scavenging activity could possibly responsible to the potent anti-listeria activity in the flesh, peel and kernel seed of *M. odorata* fruit.

**CONCLUSION**

To date this is the first reports revealed on the anti-listeria activity of untreated and treated extracts of *M. odorata* flesh, peel and kernel seed against the *L. monocytogenes* and *L. innocua* bacteria. The pathogenic bacteria of *L. monocytogenes* caused disease called listeriosis with fatal cases while the *L. innocua* which is lacks of virulence locus contributed to lesser pathogenicity. This study was the first to reveal the bioactivity of *M. odorata* extracts with anti-listeriosis activity. The fractionation method based on solvent polarity successfully exhibited active extracts with anti-listeria activity from the *M. odorata*. The anti-listeria of flesh and peel were from the n-hexane and ethyl acetate extracts, whereas for the kernel seed were from the ethyl acetate and methanol extracts. This study also revealed that the activity of potent extracts was significantly high beyond the activity of antibiotic tetracycline used as positive control. Thus, suggested the *M. odorata* peel extracts as potent natural antibacterial agents. The study has highlighted the potential *M. odorata* extracts from the flesh, peel and kernel seed against two *Listeria* sp. bacteria causing listeriosis that poses a dangerous threat to public health. To the best of author knowledge, this is the first report concerning such activity of *M. odorata* fruit parts on their anti-listeria properties. Thus, these findings had value added the *M. odorata* fruit as potential anti-listeriosis agent that beneficial to health and food safety.
CONFLICT OF INTEREST STATEMENT

We declare that there is no conflict of interest regarding the publication of this paper.

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