

Antioxidant and antimicrobial activities of crude extracts from mangosteen (*Garcinia mangostana* L.) parts and some essential oils

^{1,*}Palakawong, C., ¹Sophanodora, P., ²Pisuchpen, S. and ³Phongpaichit, S.

¹Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.

²Department of Material Product Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.

³Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

Abstract: The antioxidant and antimicrobial activities of the extracts from peel, leaves, and bark of mangosteen (*Garcinia mangostana* L.), and some essential oils such as cinnamon and citrus were investigated. The antioxidant activities (IC₅₀) of peel, leaves, and bark extracted, which were evaluated by DPPH method, were 5.94, 9.44, and 6.46 µg/ml, respectively. Either cinnamon or citrus essential oil showed no antioxidant activities with DPPH. A broth dilution method was employed to evaluate the antimicrobial activity against some Gram-positive bacteria (*L. monocytogenes* and *S. aureus*) and Gram-negative bacteria (*E. coli* and *Salmonella* sp.). The minimum inhibitory concentration (MIC) values of peel, leaves, and bark extracted against Gram-positive bacteria were ranged from 0.025-0.78 mg/ml. While the minimum bactericidal concentration (MBC) values were between 0.05-0.39 mg/ml. MIC and MBC values of cinnamon against *S. aureus*, *E. coli* and *Salmonella* sp. were 3.13 and 6.25 mg/ml, respectively. Citrus oil showed effect on only *S. aureus* with MIC and MBC values of 6.25 and 12.50 mg/ml, respectively.

Keywords: Mangosteen, *Garcinia mangostana*, antioxidant, antimicrobial, MIC, DPPH

Introduction

Plants extracted have been added to many kinds of food to improve their flavor and organoleptic properties for many years. Especially, the extracts from herbs and spices are known as antimicrobial and antioxidation potential. The extracted plants were classified as natural compounds, which were the secondary metabolites. These metabolites have demonstrated biological activities and received particular attention as potential natural agents for food preservation. According to the current consumers, more natural and fresh-like foods with fewer synthetic additives but increase safety and shelf-life are needed (Negi *et al.*, 2008). Resulting from those demands, plants have emerged as popular ingredients and have a tendency of replacing synthetic antimicrobial and antioxidant agents (Mayachiew and Devahastin, 2008). The use of natural products as antimicrobial and antioxidant compounds seem to be an interesting way to support this trend in order to

reduce these health hazards and to extend the shelf-life of processed food.

Mangosteen (*Garcinia mangostana* L.) is one of the most famous fruits in Thailand. Previous studies have shown that the extracts from various parts contain varieties of secondary metabolites such as prenylated and oxygenated xanthenes. Xanthenes or xanthen-9H-ones is a secondary metabolite found in some higher plant that involves mangosteen (Peres *et al.*, 2000). Xanthenes could be isolated from peel, whole fruit, bark, and leaves of mangosteen. Several studies have shown that obtained xanthenes from mangosteen have remarkable biological activities such as antioxidant, antitumoral, anti-inflammatory, antiallergy, antibacterial, antifungal, and antiviral activities (Suksamrarn *et al.*, 2006; Pedraza-Chaverri *et al.*, 2008).

Other secondary metabolites extracted from plants that play an important role in biological activities are essential oils. Essential oils from aromatic plants, spices, and herbs have been used historically in

*Corresponding author.

Email: choothaweep@hotmail.com

Tel: +66-74-286330 ; Fax: +66-74-212889

the pharmaceutical, food, and perfume industries because of their antibacterial, culinary and fragrant properties (Salehi *et al.*, 2005). In addition, essential oils have been used for preventing food spoilage and deterioration, and also for extending shelf-life of food since ancient time. Cinnamon and citrus essential oils have been shown the possession of antimicrobial activities and could serve as a source of antimicrobial agents against food pathogen. Several references on the antimicrobial efficiency are available in the literature reviews such as cinnamon (Lopez *et al.*, 2005; Tzortzakis, 2009) and citrus (O'Bryan *et al.*, 2008; Fisher and Phillips, 2006)

Because of consumer awareness, food processors have desired to reduce the use of synthetic chemicals in food products. Then common culinary extracted plant such as ethanolic extract from mangosteen and essential oils extracted from cinnamon or citrus could be the sources of natural alternatives.

Although the extracts from mangosteen and essential oils from cinnamon and citrus contain potent antimicrobials and antioxidation activities, such extracts have not been sufficiently tested for their activities. Even if extracted plants are considered to be safe (GRAS), however their uses are often limited by organoleptic criteria. For this reason, it will be necessary to determine the minimum concentration to inhibit the growth of pathogenic bacteria without affecting the sensory quality of the food. The objectives of the present study were to investigate the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) against four food-borne pathogens (Gram-positive and Gram-negative) and the antioxidant activity of mangosteen extracted from peel, leaves, and bark, and some essential oils such as cinnamon and citrus.

Materials and Methods

Chemicals

Ascorbic acid was purchased from Riedel-de-Hae (Seelze, Germany). DPPH, resazurin salt and ethanol were purchased from Sigma-Aldrich (St. Louis, MO, USA), while cinnamon and citrus essential oils (0.05%) were purchased from Flavor-Focus (Bangkok, Thailand). Gelatine powder was purchased from Ajax Finechem (Auckland, New Zealand). Cyprofloxacin and vancomycin were obtained from BJC Healthcare (Bangkok, Thailand). For antimicrobial tests, tryptic soy broth (TSB) and Mueller-Hinton broth (MHB) were purchased from HiMedia Laboratories (Mumbai, India).

Plant material and extraction method

The whole peel (outer and inner peels), leaves, and bark of *G. mangostana* were harvested from Songkhla province in April, 2009. The samples were first cleaned to remove any residual compost and washed thoroughly to remove impurities. After washing, the samples were chopped into small pieces ($0.5 \times 1.0 \text{ cm}^2$) and dried overnight in a tray dryer at 45°C . Then chopped samples were ground with a grinder to make powder (around 18 meshes).

All ground samples were placed in 70°C distilled water for 15 min at the ratio of sample powder:water of 1:4. The mixtures were boiled 4 times or until no content of tannin was found by dropping with 2% gelatin solution in the mixtures (Weecharangsan *et al.*, 2006). The mixtures were filtrated, the residues were then dried at $40\text{-}45^\circ\text{C}$ in the hot air oven. The dried powder was macerated at room temperature for 7 days with 50% ethanol. In order to know the exact weight, the crude extracts were filtered and evaporated to obtain the dried crude extracts. The obtained extracts were stored in a desiccator containing dry silica gel prior using in each experiment.

Microbial cultures

Escherichia coli DMST 15537, *Salmonella* sp. DMST 4464, *Listeria monocytogenes* DMST 17303, and *Staphylococcus aureus* DMST 6512 were obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. The microorganisms were maintained in TSA at 5°C . Stock culture of microbial was grown in MHB (*E. coli*, *S. aureus* and *Salmonella* spp.) and TSB (*L. monocytogenes*) at $37 \pm 2^\circ\text{C}$ in a shaker incubator for 3.5 h at 110 rpm (cells in early stationary phase). The bacterial suspension was subsequently adjusted to 10^6 CFU/ml using MHB and TSB.

Antioxidant activity

The scavenging of DPPH free radicals was used for measuring the antioxidant activity of the extracts according to the method of Weecharangsan *et al.* (2006) with slightly modification. Briefly, stock solutions of the crude extracts were prepared by dissolving 0.1 g of dry extracts in 50 ml 50% ethanolic solution. The stock solution was diluted with 50% ethanolic solution to obtain sample solutions at the concentrations of 1, 10, 50, and 100 $\mu\text{g/ml}$. The sample solutions were thoroughly mixed with freshly prepared 0.05% DPPH ethanolic solutions at the ratio of 1:1, and kept for 30 min in the dark at room temperature. The amount of the reaction mixture was determined by UV-VIS spectrophotometer at 517 nm. Neutralisation of DPPH radical was calculated

using the equation: DPPH inhibition (%) = $100 \times (A_0 - A_s)/A_0$, where A_0 is the absorbance of the control (containing all reagents except the test compound), and A_s is the absorbance of the tested sample.

The antioxidant activity of the crude extract was expressed as IC_{50} , defined as the concentration of the crude extract required to inhibit DPPH radicals by 50%, using the linear regression analysis. Ascorbic acid was used as a standard antioxidant.

Antimicrobial activity

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were performed by a broth dilution technique, using 96-well microtitre plates according to Kuete *et al.* (2008) and Saker *et al.* (2007). The bacterial inoculate applied contained approximately 1.0×10^5 cells in a final volume of 100 μ l/well.

The crude extracts were dissolved in 10% dimethylsulfoxide (DMSO) in sterile MHB to obtain a stock concentration of 12.50 mg/ml. Serial two-fold dilutions of each sample needed to be evaluated were made with MHB to yield volumes of 100 μ l/well with final concentrations ranging from 0.05-12.50 mg/ml. MHB was used as a negative control while ciprofloxacin and vancomycin were used as positive controls (0.5-2.0 μ g/ml). The cultured microplates were incubated for 24 h at 37 °C.

The MIC of samples was detected following the addition of 10 μ l resazurin (0.75%). Viable bacteria reduced the blue dye to a pink color. Microbial growth was determined by observing the change of color in the wells (blue when there is no growth and pink when there is growth). MIC was defined as the lowest sample concentration that had prevented this change. The minimum bactericidal concentration (MBC) was determined by subcultivation of 50 μ l of each blue well in plates containing Mueller-Hinton agar and then further incubation for 24 h at 37 °C. The lowest concentration with no visible growth was defined as the MBC.

Results and Discussion

Antioxidant activity

Extracted peel of *G. mangostana* expressed the strongest activity ($IC_{50} = 5.94$ μ g/ml) while bark and leaves extracted showed moderate activities ($IC_{50} = 6.46$ and 9.44 μ g/ml, respectively). Cinnamon oil showed no activity and the citrus oil did not reach 50% of DPPH-neutralisation at the highest concentration applied (Table 1).

In this experiment, the study found that the extracted peel showed IC_{50} value less than the

experiment of Weecharangsan *et al.* (2006). In those experiments, researchers used the same extraction method that was used in this study. However, the same methods were used, the different results were reported. It is possible that because the different in the maturity stage of raw material was used. This study used raw material in stage 3 of maturity but stage 5 or 6 by the experiment of Weecharangsan *et al.* (2006). From Table 1, this can be concluded that the extract from peel of mangosteen showed the best antioxidant activity among these extracts when compared to L-ascorbic acid.

Okonogi *et al.* (2007) reported that the antioxidant activity (IC_{50}) of the extract from peel of mangosteen was 0.023 μ g/ml, which was less than the IC_{50} found in this experiment (5.94 μ g/ml), for the reason of different extraction methods of this study. This implies that the different extraction methods could effect on the activity, agreed to the study of Liu *et al.* (2008).

Among these extracts from mangosteen parts, each part (peel, leaves, and bark) showed antioxidant activity with different IC_{50} values. Extracted peel exhibited lowest IC_{50} , followed by bark and leaves with IC_{50} 5.94, 6.46 and 9.44 μ g/ml, respectively. Zadernowski *et al.* (2009) reported that rind (inner peel) of mangosteen had composed more phenolic content than peel (outer peel) and aril parts. Tachakittirungrod *et al.* (2007) reported that the active compounds in higher plant were located in different parts with different contents. This is the answer why extracts of peel, leaves and bark showed the activity with different IC_{50} . In addition, Maisuthisakul *et al.* (2008), Mayachiew and Devahastin (2008), and Liu *et al.* (2008) reported that the antioxidant activity was correlated with phenolic content. Moreover, Mayachiew and Devahastin (2008) reported that crude extract with different chemical compositions can effect on the activity. These literatures may be concluded that extracted peel composes more phenolic content than those of bark and leaves, respectively.

Table 1 showed not only cinnamon but also citrus essential oil that exhibited no activity with DPPH. This result agrees with Poloteo *et al.* (2006) who reported that cinnamon essential oil had had low activity with DPPH. Eyob *et al.* (2008) reported that the activity of essential oil from korarima was very low, for the reason that the OH group in aromatic ring of phenolic compound was replaced by some functional group as a result of their hydrogen donating ability. This may be used to support for the result of this study that why cinnamon and citrus essential oil used in this study had low activity.

Table 1. Antioxidant activity (IC₅₀, µg/ml) of *G. mangostana* extracted from peel, leaves, and bark and some essential oils (cinnamon and citrus).

	IC ₅₀
Peel	5.94±0.14
Leaves	9.44±0.39
Bark	6.46±0.36
Cinnamon	no activity
Citrus	no activity
L-Ascorbic acid	4.30±0.14

Data are expressed as means ± standard deviation of three trials.

Antimicrobial activity

The MIC and MBC of the tested extracts ranged from 0.05 to 6.25 mg/ml against the 4 tested microorganisms (Table 2). This experiment confirmed the strong antibacterial activity of extracted mangosteen on Gram-positive bacteria but no activity on Gram-negative bacteria. The lowest MIC value (0.025-0.05 mg/ml) was observed with extracts of peel and bark on *L. monocytogenes* and *S. aureus*. Cinnamon oil was moderately active against *E. coli*, *Salmonella* sp. and *S. aureus* (MIC 3.13 mg/ml, MBC 6.25 mg/ml) while citrus oil was active only against *S. aureus* (MIC 6.25 mg/ml, MBC 12.5 mg/ml).

Xanthone, an active compound found in all parts of mangosteen. More than 20 xanthones were found in mangosteen. Both of α - and β -mangostin were mainly found in this fruit (Furukawa *et al.*, 1996; Chen *et al.*, 2008), especially in peel of mangosteen. Xanthone can inhibit several microorganisms. Inuma *et al.* (1996) and Sakagami *et al.* (2005) reported that the MIC value of α -mangostin against *S. aureus* was lower than β -mangostin (6.25 and >100 µg/ml, respectively). From these literatures and results of this study, these can conclude that the efficiency of microbial inhibition might be resulted directly from α -mangostin. Because peel and bark were composed more of α -mangostin than leaves, so this study found that the extracts from peel and bark can inhibit both of *L. monocytogenes* and *S. aureus* with lower MIC than extracted leaves. In addition, the study also found that only Gram-positive was susceptibility to extracted mangosteen.

Canillac and Mourey (2001) reported that if the MBC/MIC ratio was found to be less than or equal to 4, the bacteria was considered to be susceptible; on the other hand, if this ratio was greater than 4, it was considered to be tolerant. From these results, MBC/MIC ratios of all extracts on Gram-positive bacteria were less than 4, so all of them were considered to be sensitive to these extracts. The reason that the

sensitivity of the Gram-positive bacteria was higher than Gram-negative bacteria could be attributed to their differences in cell membrane constituents and arrangement (Negi *et al.*, 2008). The Gram-positive bacteria contains an outer peptidoglycan layer, which is an ineffective permeability barrier (Scherrer and Gerhardt, 1971). The resistance of Gram-negative bacteria towards antibacterial substances may be due to outer phospholipidic membrane carrying the structural lipopolysaccharide components, which makes it impermeable to lipophilic solutes and porins constitute of a selective barrier to the hydrophilic solutes (Nikaido and Vaara, 1985).

Cinnamon and citrus essential oil showed that the effect against 4 microorganisms were different from those extracts of mangosteen. Generally, the study found that the essential oils had higher MIC or MBC than extracted mangosteen. Citrus can inhibit against only Gram-positive, especially *S. aureus*. Not only *S. aureus* but also all of Gram-negative were inhibited by cinnamon.

From literature, main components in citrus oil were limonene, linalool, and citral. Among these components, limonene showed the most abundant content, followed by linalool and citral, respectively. Fisher and Phillips (2006) reported that limonene had showed lowest effect against microorganisms. Inhibition effect against microorganisms was resulted from linalool rather than citral or limonene. Gram-negative was tolerant with citrus because of the lipopolysaccharide present in outer membrane which provided protection against different agents (Mahboubi and Haghi, 2008; Oussalah *et al.* 2007).

Cinnamaldehyde and eugenol were found to be a main chemical composition in cinnamon essential oil. Oussalah *et al.* (2007) reported that cinnamaldehyde was found mainly in bark or branch of cinnamon tree while eugenol was found in leaves. Besides this, eugenol was lower activity against microorganism than cinnamaldehyde. Hussain *et al.* (2008) reported

Table 2. Minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) of *G. mangostana* extracted from peel, leaves, and bark and some essential oils (cinnamon and citrus).

Bacteria	MIC/ MBC	Mangosteen parts (mg/ml)			Cinna- mon (mg/ ml)	Citrus (mg/ml)	Cipro- floxacin (µg/ml)	Vanco- mycin (µg/ml)
		Peel	Leaves	Bark				
<i>L. monocytogenes</i>	MIC	0.05	0.78	0.025	>12.5	>12.5	0.78	0.78
	MBC	0.10	1.56	0.05	-	-	0.78	0.78
<i>S. aureus</i>	MIC	0.025	0.20	0.05	3.13	6.25	<.05	0.78
	MBC	0.05	0.39	0.10	6.25	12.50	-	0.78
<i>E. coli</i>	MIC	>3.13	>3.13	>3.13	3.13	>12.5	<.05	ND
	MBC	-	-	-	6.25	-	-	ND
<i>Salmonella</i> sp.	MIC	>3.13	>3.13	>3.13	3.13	>12.5	<.05	ND
	MBC	-	-	-	6.25	-	-	ND

ND = Not Detected

that the different chemical composition in an essential oil could result in different biological activity. Lopez *et al.* (2005) and Inouye *et al.* (2001) found that cinnamon with eugenol had showed higher activity against Gram-negative than Gram-positive. Cinnamon with cinnamaldehyde showed higher activity against Gram-positive than Gram-negative. From the result of this study, cinnamon can inhibit both of Gram-positive and Gram-negative. This result agreed with Oussalah *et al.* (2007) who reported that both of them were inhibited by this essential oil. It is possible that cinnamon in the present study composes of both of eugenol and cinnamaldehyde.

These differences in the susceptibility of the test organisms to essential oil could bring to the conclusion of the variation in the rate of essential oil constituent's penetration through the cell wall and cell membrane structures. The ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control are the most likely reasons for its lethal action (Cox *et al.*, 2000). Nedorostova *et al.* (2009) reported that the leakage of intracellular metabolites due to their activity on cell membranes seemed to be the main mechanism or mode of action. On the other hand, essential oils can interact with intracellular sites and cause death of the cell e.g., by alteration of protein structures after penetration into the cells.

The MBC/MIC ratio of citrus on *S. aureus* and the MBC/MIC ratio of cinnamon on *E. coli*, *Salmonella* sp. and *S. aureus* were less than 4 according to Table

2. Therefore, *S. aureus* was considered to be sensitive to both of essential oils while *E. coli* and *Salmonella* sp. were considered to be sensitive to cinnamon essential oil.

Conclusions

Thesesampledatareceivedshowtheinhibition potency on extracts of peel, cinnamon, and the citrus oil that can be considered as preservative agents for both antibacterial and antioxidant activities. This study provides the important baseline information for the use of extracted peel from *G. mangostana* as well as the essential oils as the preservative agents for some types of food. Then *in vivo* experiment should be conducted for further study. The antibacterial and antioxidant studies of these peel and essential oils are being considered as the future objective for the planning of using these potential preservation agents in fresh-cut mangosteen.

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