ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF SELECTED VARIETIES OF THAI MANGO SEED EXTRACT

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ABSTRACT

This study reports the antioxidant and antibacterial activities of four fresh mango seed extracts from Thai varieties. Total phenol contents determined by the Folin-ciocalteu method revealed the highest values to be in MKE, Chok-a-nan variety (399.8 mgGAE/g extract) and MSE of Nam-dok-mai variety (377.2 mgGAE/g extract). Both extracts showed potent $ABTS^{\cdot+}$ radical and $DPPH^{\cdot}$ radical scavenging activities with the lower half inhibition concentration (IC_{50}) values than those of the reference compounds; vitamin C, trolox and BHA, respectively. Their antioxidant property of MSE and MKE is strongly correlated with the total phenol contents (r = 0.98 and 0.98, respectively). When combined the MSE and MKE of the Fah-lun variety showed the strongest antioxidant activity. All mango seed extracts showed interesting antibacterial activity against both gram positive and gram negative bacteria as determined by disc diffusion method. The most sensitive pathogenic strain inhibited by all extracts (especially Kaew variety) was *Pseudomonas aeruginosa* ATCC 27853. This work suggests potential applications for practical uses of mango seed extracts from Thai varieties, as sources of antioxidant and antibacterial agents.

Keywords: Antibacterial; antioxidant; mango seed extract; phenol content; *Pseudomonas aeruginosa.*

INTRODUCTION

Free radicals are generated in our body during the normal metabolic processes and during exposure to adverse patho-physiological conditions (Van Langendonckt *et al.,* 2002). They are unstable species that are able to induce cellular damage in several ways. The most deleterious effects of free radicals is damage to DNA (Piconi *et al.,* 2003), which is associated with the process of carcinogenesis. Phytochemicals such as phenolics, carotenoids and dietary fibers are gaining increased attention because of their antioxidant, anticarcinogenic, antimutagenic, and other health promoting properties (Block and Langseth, 1994). Mango (*Mangifera indica* L.), a fruit consumed worldwide, belongs to the family Anacardiaceae. In Thailand, there are several mango varieties and the fruits of some varieties are available throughout the year. Apart from the fruit, mango pulp has been reported to have antilithiatic and free radical scavenging properties, which reduce lipid peroxidation and enhance antioxidant enzymes (superoxide dismutase and catalase) against isoproterenol (Bafna and Balaraman, 2005). Mango pulp contains vitamins, organic acids, carbohydrates, amino acids, polyphenols and certain volatile compounds (Pino *et al.,* 2005). Several studies have reported polyphenolic compounds in mango flesh and peel, including various ascorbic acids, dehydroascorbic acids, flavonoids, xanthones, phenolic acids, and gallotannins (Ribeiro and Schieber, 2010; Berardini *et al.,* 2005).

Mango peel extracts have been reported to show strong antioxidant activity (Ajila *et al.,* 2009). There are also reports about the antioxidant activity of mango flesh and seed (Ribeiro *et al.,* 2008; Soong and Barlow, 2004). Recently, an antioxidant study on mango flesh has been reported by Patthamakanokporn *et al.* (2008), antioxidant and hepatoprotective activities in mango seed kernels by Nithitanakool *et al.* (2009), and anti-hemorrhagic and anti-dermonecrotic activities of MSKE against snake venoms (Leanpolchareanchai *et al.,* 2009; Pithayanukul *et al.,* 2009). Apart from the antioxidant activity, mango seed extract has also exhibited potent antibacterial activity (Abdalla *et al.,* 2007; Kabuki *et al.,* 2000) and has been used as an immunomodulating agent in animals (Sahu *et al.,* 2007). Due to the beneficial uses of mango extracts from several parts of the fruit, which have been shown to have many potential applications, Thai varieties of mango should be investigated for feasibility of use. The aim of this research is to investigate the antioxidant activity and antibacterial activity of selected mango seeds from Thai varieties in order to gain a new source of bioactive compounds, add value and reduce solid waste.

MATERIALS AND METHODS

Chemicals

Glacial acetic acid, HCl, sodium acetate, methanol, ethanol, sodium carbonate and Folin-ciocalteu reagent were purchased from BDH, England. Ascorbic acid (vitamin C) was obtained from Merck, Germany. Trichloroacetic acid (TCA) was purchased from Riedel-de Haën. 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonate), di-ammonium salt (ABTS), 1,1-diphe-nyl-2-

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picryl-hydracyl (DPPH), Butylated hydro-xylanisole (BHA) and 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid (trolox) were obtained from Sigma, Germany. Bio-Rad protein assay reagent was a product of Bio-Rad, USA. Gallic acid and kanamycin were purchased from Fluka, Switzerland.

Preparation of mango seed extracts

The seeds of four mangoes (*Mangifera indica* L.) Thai varieties including Chok-a-nan, Fah-lun, Kaew and Nam-dok-mai, were obtained from a local market near Mahasarakham University from June to December, 2008; they were washed and air dried. The kernels and kernel sheathes were removed manually from the seeds. Fresh kernel seeds and kernel sheaths were chopped and blended with distilled water at a ratio of sample/water of 1:3 (w/v). The residue was then removed by filtering through cheese cloth; the filtrate was then centrifuged at 8,100 x g, for 5 min. After centrifugation, the supernatant was lyophilized. The extraction yield of each sample was calculated and reported as a percentage (g dwt extract/100 g dwt sample).

Determination of the total phenol contents

The amounts of phenolic compounds in the extracts were determined according to the Folin-Ciocalteu colorimetric method based on the procedure described by Singleton and Rossi (1965) with some modifications, using Folin-Ciocalteu reagent. Gallic acid was used as the standard phenol compound. The extracted solution, in the appropriate dilution, (0.5 mL) was transferred to a test tube containing 0.5 mL of Folin-Ciocalteu reagent. After three minutes, 0.5 mL of 35% w/v sodium carbonate solution was added. The mixture was allowed to stand for 90 min at room temperature in the dark and then 3.5 mL distilled water was added to adjust the total volume to 5.0 mL. The sample was shaken well before being measured at 725 nm. The experiment was carried out in triplicate and the total phenol content was expressed as milligrams of Gallic acid equivalents (GAE) per gram of dried weight.

Determination of antioxidant activity

$ABTS^{\cdot+}$ radical scavenging activity

The $ABTS^{\cdot+}$ cation radical solution was prepared as perviously described (Khammuang and Sarnthima, 2008). Briefly, the reaction consisted of 0.1 mM ABTS and 0.2 U mL^{-1} crude laccase from *Lentinus polychrous* Lév. in distilled water, which was incubated at 32 ºC for 10 min. Laccase was removed from the ABTS radicals by centrifugation in a selection membrane with a molecular weight cut off of 10 kDa (viva spin, USA). The stock $ABTS^{+\bullet}$ radical solution was diluted in distilled water to give an initial absorbance (A_{734}) at 0.7 before use. An aliquot of samples at various concentrations (10 µL) was added to 990 µL of $ABTS^{+\bullet}$ radical solution. The absorbance was measured at 734 nm by a spectro-photometer after 30 min of incubation. Total antioxidant capacity was calculated relative to the reactivity of Trolox under a parallel experiment and the result was reported as µmol Trolox/g sample.

% $ABTS^{+\bullet}$ scavenging = A_c-A_t/A_c *100

Where A_c was the absorbance of the control and A_t was the absorbance of the mixture containing extracts.

DPPH radical scavenging activity

The stable free radical scavenging activity was determined by the $DPPH^{\bullet}$ method (Brand-Williams *et al.* 1995). The $DPPH^{\bullet}$ radical was prepared by dissolving in methanol (2.4 mg/mL). Before use, stock $DPPH^{\bullet}$ radical solution was diluted in methanol to give initial OD_{515} at 0.7. An aliquot of samples at various concentrations (10 µL) was added to 990 µl of $DPPH^{\bullet}$ radical solution. The absorbance was assayed by decreasing absorbance at its maximum absorption (λ_{max} 515 nm) for 30 min. Total antioxidant capacity was calculated relative to the reactivity of Trolox under the same conditions and the results were shown as µmol Trolox/g sample.

The ability to scavenge $DPPH^{\bullet}$ was calculated as percent $DPPH^{\bullet}$ scavenging using the following equation:

% $DPPH^{\bullet}$ scavenging = A_c-A_t/A_c *100

Where A_c was the absorbance of the control and A_t was the absorbance of the mixture containing extracts.

Bacterial cultures

Microorganisms used in this study consisted of four strains of pathogenic bacteria, including *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 7058, *Salmonella typhi* DMST 5784 and *Pseudomonas aeruginosa* ATCC 27853. Bacteria were obtained as a kind gift from Dr. Sompong Thammasirirak, Department of Biochemistry, Khon Kaen University and from Dr. Rungruedee Thiewthong, Department of Biology, Mahasarakham University. The bacteria were grown and maintained on Luria-Bertani (LB) slants. The inoculated agar slants were incubated at 37ºC.

Disc diffusion assay

The antibacterial activity assay was based on the disc diffusion method (Nair *et al.*, 2007) using bacterial cell suspensions grown at 37 ºC in Luria-Bertani media (LB; HiMedia Laboratories, India). The exponentially growing bacteria (OD_{600} = 0.5, ~10^{12} cfu/mL) were mixed with melted warm LB agar and pour into the Petri dishes (100 mm diameter). Sterile paper discs (6 mm diameter) were placed on the agar. Then the solution of mango seed aqueous extract (348 µg dwt extract) was added onto each paper disc. Discs with distilled water and the solvent used for extraction, were used as negative controls and 1 mg kanamycin discs were used as positive controls. The plates were incubated at 37ºC for 16-20 h. After incubation, the zones of inhibition was measured. The experiment was performed in a triplicate and all results were expressed as means ± standard deviation.

STATISTICAL ANALYSIS

Results are presented as mean values ± standard deviation (three replicate experiments). Analysis of variance and significant differences among means were determined by one-way ANOVA using SPSS (Version 17, SPSS Inc., 2008, Chicago, USA.). Significant differences were declared at $P < 0.05$.

RESULTS

The extraction yields, total phenol contents, antioxidant and antibacterial activities of 4 fresh mango seed extracts, from the Thai varieties Chok-a-nan, Fah-lun, Kaew and Nam-dok-mai were investigated. The results (Table 1) showed that the highest extraction yields were found in sheath seed extract (MSE) of Fah-lun variety (12.35%), followed by seed kernel extract (MKE) of Nam-dok-mai variety (11.90%).

Total phenol contents determined by Folin-ciocalteu method revealed that MKE of Chok-a-nan variety showed the highest values (399.8 mgGAE/g extract), followed by MSE of Nam-dok-mai variety (377.2 mgGAE/g extract) (table 1).

Comparisons of antioxidant activity against $ABTS^{\cdot+}$ and $DPPH^{\cdot}$radicals were evaluated against reference compounds. It was found that MKE of Chok-a-nan variety showed the highest $ABTS^{\cdot+}$ scavenging activity (half inhibition concentration, IC_{50} 4.13 μg/mL) followed by MSE of Nam-dok-mai (IC_{50} 4.84 μg/mL) (table 1). The $ABTS^{\cdot+}$ scavenging activity of both extracts were correlated as both high vitamin C equivalent and trolox equivalent values (table 1).

The MSE of Nam-dok-mai and MKE of Chok-a-nan also showed the highest $DPPH^{\cdot}$ scavenging activity with the lowest IC_{50} equaled to 2.14 and 2.45 μg/mL respectively. These values are better than the reference compounds, BHA (3.25 μg/mL), Trolox (4.82 μg/mL) and vitamin C (8.85 μg/mL) respectively (fig. 1).

The antioxidant activity of most seed extracts had a positive correlation with their total phenol contents (fig. 2). This indicated that phenol compounds present in mango seed extracts resulted in antioxidant activity.

Antibacterial activity from the disc diffusion method of these mango seed extracts (sheath & kernel) against four strains of both gram positive and gram negative bacteria (table 2) showed that all extracts inhibit the growth of all tested bacteria. The most sensitive pathogenic strain,

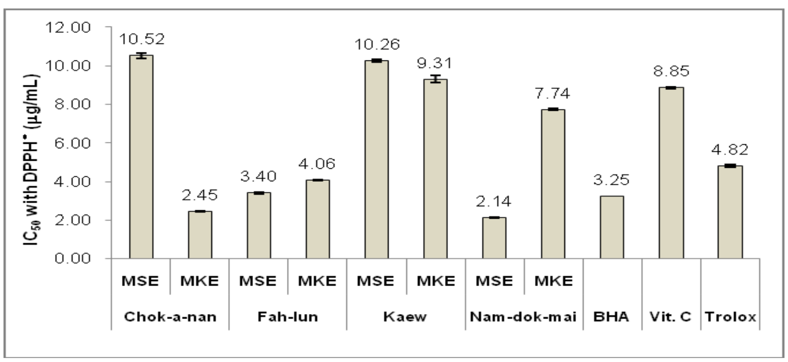

Fig. 1: Half maximum inhibition concentrations (IC_{50}) for $DPPH^{\cdot}$ radical scavenging activity of fresh *Mangifera indica* L. aqueous seed extracts and comparison with reference antioxidant compounds, BHA and vitamin C. Error bars indicate mean ± standard deviation (n = 3)

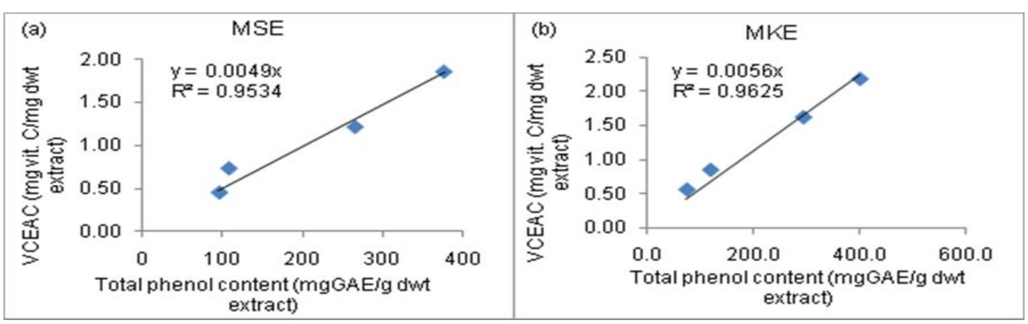

Fig. 2: Correlation of antioxidant activity and total phenol content of four *Mangifera indica* L. fresh seed sheath extracts (MSE) (a) and seed kernel extracts (MKE) (b). Data is average value from a triplicate experiment (n = 3).

inhibited by all seed extracts (especially Kaew variety), is *Ps. aeruginosa* ATCC 27853. All seed extracts showed a greater bacterial growth inhibition than the positive control, kanamycin. This phenomenon is also observed in the other gram negative strain, *S. typhi* DMST 5784.

DISCUSSION

The extraction yields of some fresh mango seed extracts, from the Thai varieties had been reported. Hot ethanol extraction of Thai mango, Fah-lun cultivar had been reported to yield 8.66% (w/w) (Nithitanakool *et al.,* 2009). Using a different extraction method, Fah-lun variety yielded a slightly larger amount than previously reported. Maisuthisakul and Gordon (2009) reported that Sun-dried mango seed kernel (SDMSK) and oven-dried mango seed kernel (ODMSK) of Chok-a-nan cultivar extracted with ethanol or water yielded about 2.01-3.83%. In that report ODMSK extraction with water gave a yield similar to that of Chok-a-nan fresh kernel extracted with water reported here (3.92%, table 1). According to the findings of Maisuthisakul and Gordon (2009) different experimental conditions of extraction gave yields of 3.31, 11.9 and 10.8% for shaking, reflux and acid hydrolysis, respectively.

Total phenol content of mango seed kernel (MSK) extract (~117 mgGAE/g) and the heating the mango seed kernel at 160°C (~160 mgGAE/g), reported by Soong and Barlow (2004) were less than the highest values from the present work except for Kaew variety (73.8 mgGAE/g). These differences might be due to mango varieties and geographical location (Yuri *et al.,* 2009). From Table 1, the total phenol contents from mango seed sheath extracts were also higher than the values from their report, except for Chok-a-nan and Kaew varieties. The content of phenol compounds in MKE of Chok-a-nan from our work was far higher than those found in extracts of MSK extracted with 95% ethanol and water by Maisuthisakul and Gordon (2009) which were expressed as tannic acid equivalents, TAE (varied from 44.5 to 89.92 mg/g). Their report showed that the extraction and hydrolysis procedure had a substantial effect on the content of phenol compounds present in MSK. Where MSK values extracted after acid hydrolysis were higher than the values for samples of MSK extracted using shaking and refluxing conditions (ranging from 90.0 to 286 mgTAE/g). Even then the highest value from acid hydrolysis (286 mgTAE/g), was still lower than the values presented here. This implies that water extraction of Thai mango varieties in the present work gives greater phenolic content.

The $ABTS^{\cdot+}$ scavenging activity of both extracts were correlated as both high vitamin C equivalent and trolox equivalent values. For trolox equivalent antioxidant capacity (TEAC) of MKE, Chok-a-nan variety had a higher value than those reported by Maisuthisakul and Gordon (2009). Soong and Barlow (2004) had reported antioxidant activity of fresh mango kernel extract in terms of ascorbic acid equivalent (AEAC) ~762 µmol/g and ~2,568 µmol/g for MSK product after heating at 160 °C. In later work with improved extraction methods giving better gallic acid recovery; AEAC values were lower than 1,500 µmol/g (Soong and Barlow, 2006). The present work showed AEAC values (or VCEAC as presented here) of MKE form Thai varieties in the range of 0.56 to 2.18 mg vitamin C equivalent/mg (or 3,179 to 12,377 µmol/g calculated according to FW 176.13 of ascorbic acid). These results showed levels far higher than those reported elsewhere indicating that mango seed kernel extracts of Thai varieties might be good sources as antioxidants.

The $DPPH^{\cdot}$ scavenging activity of MKE, Chok-a-nan variety showed similar results to that of sun-dried MSK extracted with water as reported by Maisuthisakul and Gordon (2009), where the IC_{50} was 2.61 µg/mL (2.45 µg/mL in present study). However, the ethanolic extract of MSK showed more than four times the $DPPH^{\cdot}$ scavenging activity (IC_{50}=0.56 µg/mL) when compared to these results (Maisuthisakul and Gordon, 2009). The IC_{50} of aqueous mango seed extracts reported in the present work is lower than those reported by Vaghasiya and Chanda (2010) which showed maximum antioxidant activity by acetone extract (IC_{50} = 11 µg mL^{-1}) followed by methanol extract (IC_{50}=12 µg mL^{-1}). When combining both MSE and MKE, Fah-lun variety showed the strongest antioxidant activity.

Most seed extracts antioxidant activity had a positive correlation with their total phenol contents indicated that phenol compounds present in mango seed extracts resulted in antioxidant activity. A positive correlation of antioxidant activity and total phenol content has also been observed in other reports (Khammuang and Sarnthima, 2008; Soong and Barlow, 2004).

All extracts showed antibacterial activity in all tested bacteria. The antimicrobial properties of ethanol extract of mango seed kernel (MKE) were also reported by Kabuki *et al.* (2000). They reported that the MKE had a broad antimicrobial spectrum, and mostly was more active against gram-positive than gram-negative bacteria (Kabuki *et al.,* 2000). Antibacterial activity of MKE and MSE of Thai varieties of mango could inhibit both gram positive and gram negative bacteria differently, depending on the strain and mango variety. Our results showed that kanamycin seemed to be more active against gram positive bacteria than gram negative ones, whereas most mango seed extracts showed the opposite. This might suggest a different action mode of bacterial growth inhibition between kanamycin and compounds presence in mango seed extract. However, screening with more strains or species would probably be able to summarize

Table 1: Extraction yields and total phenol contents of fresh Thai *Mangifera indica* L. aqueous seed extracts and $ABTS^{\cdot+}$ radical scavenging activity compared with the reference antioxidant compounds, vitamin C and trolox

Extracts	Extraction yield (%)		Total phenol content (mgGAE/g dwt extract)		IC_{50} (µg/mL)		VCEAC[a]		TEAC[b]	
	sheath	kernel	sheath	kernel	sheath	kernel	sheath	kernel	sheath	kernel
Chok-a-nan	6.87	3.92	95.4±0.5	399.8±8.2	19.39±0.62	4.13±0.10	0.46±0.01	2.18±0.05	0.66±0.02	3.10±0.07
Fah-lun	12.35	5.36	265.7±13.6	293.1±22.5	7.57±0.16	5.34±0.37	1.19±0.03	1.69±0.12	1.69±0.04	2.40±0.17
Kaew	4.09	2.53	107.4±2.5	73.8±3.2	11.83±0.58	16.18±1.45	0.76±0.04	0.56±0.05	1.08±0.05	0.80±0.07
Nam-dok-mai	9.13	11.9	377.2±22.5	118.1±0.2	4.84±0.17	10.56±0.31	1.86±0.06	0.85±0.02	2.65±0.09	1.21±0.04

[a] vit. C equivalent antioxidant capacity (mg vit. C/mg dwt extract)

[b] Trolox equivalent antioxidant capacity (mmol trolox/g dwt extract)

Table 2: Antibacterial activity of fresh *Mangifera indica* L. aqueous seed kernel extracts (MKE) and seed sheath extracts (MSE) against certain gram positive and gram negative bacteria by disc diffusion method.

Microorganism	Kanamycin (1 mg)	Inhibition zone diameter[a] (mm)							
		MKE				MSE			
		Chok-a-nan	Fah-lun	Kaew	Nam-dok-mai	Chok-a-nan	Fah-lun	Kaew	Nam-dok-mai
Gram positive bacteria									
Bacillus cereus ATCC 11778	14.0±1.1 (4.9)[b]	7.63±0.3	6.83±0.3	4.33±1.2	5.13±0.3	3.80±0.0	5.00±0.0	6.50±0.0	6.67±0.3
Bacillus subtilis ATCC 7058	13.4±1.7 (4.7)	6.83±0.3	5.00±0.0	2.67±0.6[c]	3.17±0.3	4.00±0.0	3.83±1.2	4.67±0.6	4.97±0.3
Gram negative bacteria									
Pseudomonas aeruginosa ATCC 27853	6.1±0.8 (2.1)	7.00±0.0	5.67±0.3[c]	10.33±2.4	5.83±0.3	4.00±0.0	4.17±0.3	11.83±0.3	6.00±0.0
Salmonella typhi DMST 5784	9.1±1.6 (3.2)	5.83±0.3	5.67±0.6	3.83±0.6	4.00±0.0	8.83±0.3	4.17±1.9	5.17±0.3	5.33±0.6

[a] data shown is mean ± standard deviation of clear zone of tests, subtracted from a negative control (n = 3)
[b] value in parentheses is zone of inhibition produced by 348 µg kanamycin
[c] indicates significant differences ($p < 0.05$) between MKE of bacterial strains

the preference of action. Moreover, isolation and characterization of the bioactive compounds from the mango seed extracts would be very interesting and useful to gain more understanding and knowledge for further application.

Sahu and co-workers (2007) reported that mango kernel powder incorporated into the fish feed of *Labeo rohita* at 5 g kernel/kg dry diet showed the highest percentage survival (98%) indicating that mango kernel stimulates the immunity and makes *L. rohita* more resistant to *Aeromonas hydrophila* ATCC 49040 infection. Gallotannins extracted from mango kernels also showed antibacterial activity against several pathogenic bacteria as reported by Engels *et al.* (2009) supporting the conclusion that inhibitory effects of hydrolyzable tannins are due to their iron-complexing properties. A combination of mango and neem chewing stick extracts showed antibacterial activity against *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis* which were involved in the development of dental caries (Prashant *et al.,* 2007).

CONCLUSION

Mango seed extracts from certain Thai varieties showed strong antioxidant activities against both $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radicals, especially Chok-a-nan and Nam-dok-mai as well as Fah-lun varieties in which antiradical activity is stronger than vitamin C, trolox, as well as a synthetic antioxidant compound, BHA. Interestingly, all seed extracts inhibit growth of both gram negative and gram positive bacteria. The most sensitive strain is the opportunistic gram negative bacteria, *Ps. aeruginosa* (ATCC 27853) which was strongly inhibited by all mango seed extracts, but especially extracts of Kaew variety compared to the positive control, kanamycin . These results reveal potential applications of mango seed extracts from Thai varieties as good sources of antioxidant and antibacterial agents in foods and pharmaceuticals.

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