ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF THAI HERBAL TEA EXTRACTS

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ABSTRACT

In this study, eight species of Thai herbal teas, including fruits of *Aegle marmelos* Correa, and leaves of *Annona squamosa* Linn., *Camella sinensis*, *Centella asiatica* Linn. Urban, *Morus alba*, *Pandanus amaryllifolius* Roxb, *Phyllanthus acidus* Skeels, and *Piper betle* Linn. were extracted using ethanol as a solvent, and tested for their antimicrobial activity against 9 species of bacteria and 6 species of yeasts using agar diffusion method as preliminary screening. Of these, five ethanolic extracts of plants, including *A. marmelos*, *C. sinensis*, *C. asiatica* , *P. amaryllifolius*, and *P. betle* showed great antimicrobial effect on microbial strains tested, and were selected to determine the minimum inhibitory concentration (MIC) using microbroth dilution test. Crude ethanolic extracts of *C. sinensis* and *A. marmelos* showed the highest antimicrobial activity, followed by *P. betle*, *P. amaryllifolius*, *C. asiatica*, *M. alba*, *A. squamosa*, and *P. acidus*. The most susceptible pathogenic bacteria to *C. sinensis* and *A. marmelos* extracts were *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus* (the MIC of 10.4-41.7 mg/ml). The most sensitive food spoilage bacteria to those extracts was *Leuconostoc mesenteroides* (the MIC of 10.4-20.8 mg/ml). The most vulnerable yeasts to *C. sinensis* extract were *Hanseniaspora uvarum* and *Rhodotorula glutinis* (the MIC of 41.7 mg/ml), while *Candida lipolytica* and *Pichia membranaefaciens* were the most sensitive yeast strains to *A. marmelos* extract (the MIC of 83.3 mg/ml).

Antioxidant activity of eight herbal tea extracts was studied. *P. betle* extract had the highest antioxidant activity, followed by *C. sinensis*, *A. squamosa*, *M. alba*, *C. asiatica*, *A. marmelos*, *P. acidus*, and *P. amaryllifolius* extracts. The EC_{50} values of the extracts were in the range of 699.29-13,886.94 µg extract/mg DPPH. Total phenolic contents of these extracts were also analyzed. The extract of *C. sinensis* had the highest phenolic content, followed by those of *P. betle*, *P. amaryllifolius*, *A. marmelos*, *A. squamosa*, *P. acidus*, *C. asiatica*, and *M. alba*. The total phenolic contents of these extracts were in the range of 6.0-546.0 µg gallic acid/ mg dry extract.

KEYWORDS: herbal tea, antimicrobial activity, antioxidant activity, phenolics

1. INTRODUCTION

Several studies have revealed that foods containing phytochemicals, such as fruits, vegetables, spices, teas, and other medicinal plants possessed antimicrobial and antioxidant activities. A recent report indicated that green tea (*Camella sinensis*) extract was found to inhibit growth of *Staphylococcus aureus* and *Bacillus cereus*. Some active compounds in tea extracts, such as polyphenols in green tea were reported to responsible for antioxidant and antimicrobial actions [1]. The active compounds, kuraridin and sophoraisoflavanone A in *Morus alba* were also found to have inhibitory effect against fungal and bacterial species [2]. Leaves of *Piper betle* contained eugenol and hydroxychavicol inhibiting fungi and bacteria [3]. Pandanin, a protein from *Pandanus amaryllifolius* plant which is commonly used for colouring and imparting fragrance to food was shown to have antiviral activity against virus infected in human [4].

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In addition to the antimicrobial property, many plants were found to possess antioxidative property [5-6]. Antioxidants are any substances that significantly delays or inhibits oxidation of substrates *in vivo* or in foods by inhibiting generation of reactive oxygen species, or by directly scavenging free radicals. Their physical role is to prevent damage of cellular components arising as a consequence of chemical reactions involving free radicals which are major contributors to aging and degenerative diseases such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction [7]. Dietary antioxidants may be effective in protection from oxidative damage. A number of synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been developed, and their uses have been restricted due to their toxicity [8]. As a result, there is considerable interest in development of natural antioxidants from botanical sources for use in food and in preventive medicine.

Several plants have been reported to have the antioxidant activity [6]. *Centella asiatica*, a plant commonly used for wound healing, memory improvement, and treating mental fatigue was found to have antioxidative activities [9]. Leaf of *Morus alba*, like green tea leaf, is one of medicinal plants commonly consumed as tea beverage. *M. alba* extract has been shown to have antioxidant activity [10]. The antioxidant activity of these plants has been attributed to the presence of polyphenolic compounds. However, little information is available on antimicrobial and antioxidant activities of plants, including *Aegle marmelos*, *Annona squamosa*, *Pandanus amaryllifolius*, *Phyllanthus acidus* and *Piper betle*. Therefore, the aim of this work was to determine antimicrobial and antioxidant activity, and total phenolic contents of eight plants commonly comsumed as herbal tea in Thailand, including fruit of *A. marmelos*, and leaves of *A. squamosa*, *C. sinensis*, *Centella asiatica*, *M. alba*, *P. amaryllifolius*, *P. acidus*, and *P. betle* to investigest whether they have potential to be biopreservative and bioantioxidant.

2. MATERIALS AND METHODS

2.1 Microorganisms

Fifteen microbial strains (5 species of pathogenic bacteria, 4 species of food spoilage bacteria, and 6 species of food spoilage yeasts) were used in this study. *Bacillus cereus* DMST 5040, *Escherichia coli* DMST 4212, *Listeria monocytogenes* DMST 11256, and *Salmonella* Enteritidis DMST 10633 were obtained from the culture collection of the Department of Medical Sciences, Ministry of Public Health, Thailand. *Staphylococcus aureus* TISIR 118, *Pseudomonas fluorescens* TISTR 358, *Lactobacillus plantarum* TISTR 050, *Leuconostoc mesenteroides* TISTR 053, *Candida lipolytica* TISTR 5655, *Hanseniaspora uvarum* TISTR 5153, *Pichia membranaefaciens* TISTR 5093, *Rhodotorula glutinis* TISTR 5159, *Schizosaccharomyces pombe* TISTR 5205, and *Zygosaccharomyces rouxii* TISTR 5044 were obtained from the Microbiological Resources Centre for Southeast Asian Region (Bangkok MIRCEN). *Lactobacillus fermentum* BCC 4398 was obtained from the National Centre for Genetic Engineering and Biotechnology, Thailand. Bacterial and yeast cultures were maintained on Nutrient Agar (NA, pH 6.8 ± 0.2, Difco) and Saboraud Dextrose Agar (SDA, pH 5.6 ± 0.2, Difco) slopes, respectively. They were subcultured monthly from slants and subsequently stored at 4°C.

2.2 Culture preparation

The microbial cultures were transferred individually to 5 ml of deMan Rogosa Sharpe Medium (MRS broth, pH 6.5 ± 0.2) for lactic acid bacteria, Nutrient Broth (NB, pH 6.8 ± 0.2, Difco) for other bacteria, and Saboraud Dextrose broth (SDB, pH 5.6 ± 0.2, Difco) for yeasts. After 24-hour incubation at 37°C for bacteria and at 30°C for yeast, microbial cells were collected by centrifugation at 3000 rpm for 15 min, washed twice and resuspended in 0.1% peptone water. Turbidity was adjusted to match that of 0.5 (for lactic acid bacteria) and 5 (for other bacteria) McFarland standard to obtain an inoculum concentration of 10^7 CFU/ml.

2.3 Extractions of herbal teas

Eight types of plants including fruit of *Aegle marmelos*, and leaves of *Annona squamosa*, *Camella sinensis* (Ootong tea, a partially fermented tea), *Centella asiatica* Linn. Urban, *Morus alba*, *Pandanus amaryllifolius* Roxb, *Phyllanthus acidus* Skeels, and *Piper betle* Linn. were purchased at retail in Bangkok, Thail and. These plants were extracted using ethanol as a solvent.

First of all, the plant materials were cut into small pieces; 20 g of each were soaked in 100 ml of 95% ethanol, and shaked at 150 rpm for 4 days at ambient temperature. The mixtures were then

filtered. The filtrates were evaporated using vacuum rotary evaporator (BÜCHI Rotavapor R-200/205, Model R205V800), and air dried at 40°C. Stock solutions of crude ethanolic extracts were prepared by diluting dried extracts with 10% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 400 mg/ml.

2.4 Screening of herbal tea extracts using disk diffusion test

The disk diffusion test was performed using the standard procedure as described by Jorgensen et al. [11]. The inoculum suspension of each microbial strain was swabbed on the entire surface of Mueller-Hinton agar (MHA, pH 7.3 ± 0.1, Difco) for bacteria, and SDA for yeast. Sterile 6-mm filter paper discs (Schleicher & Schuell) were aseptically placed on MHA and SDA surfaces. Crude ethanolic extracts were immediately added to discs in volumes of 20 µl. A 20-µl aliquot of 10% DMSO was also added to a sterile paper disc as a negative control. The plates were left at ambient temperature for 15 min to allow excess prediffusion of extracts prior to incubation at 37°C for 24 h for bacteria and at 30°C for 72 h for yeast. Diameters of inhibition zones were measured. The experiment was done in triplicate.

2.5 Determination of the minimum inhibitory concentration using microbroth dilution test

The dilution test was performed to determine minimum inhibitory concentrations (MICs) using the standard procedure as described by Jorgensen et al. [11]. One hundred microliter of Mueller-Hinton broth (MHB, for bacteria) or SDB (for yeast) were added in each well of a microtiter plate. The 100-µl aliquot of stock solution of crude ethanolic extract (400 mg/ml) was added, and subsequently two-fold serially diluted with MHB or SDB. The inoculum suspenion (20 µl) of each strain was then added in each well. The final concentrations of the extract were 166.7, 83.3, 41.7, 20.8, 10.4, 5.2, and 2.6 mg/ml. The negative control was also performed using 10% DMSO. Penicillin G (the concentration of 5,000, 500, 50, 5, 0.5, 0.05, and 0.005 unit/ml) and amphotericin B (the concentration of 4.17, 2.08, 1.04, 0.52, 0.26, 0.13, and 0.07 mg/ml) were used as positive controls. Duplicate wells were run for each concentration of extracts. The plates were incubated at 37°C for 24 h for bacteria, and at 30°C for 72 h for yeasts, and the turbidity was measured at 620 nm using the microplate reader (iEMS Reader MF, Labsystems). The lowest concentration that inhibited visible growth of the tested organisms was recorded as the minimum inhibitory concentration (MIC).

2.6 Determination of free radical scavenging activity using DPPH method

The free radical scavenging activity of eight herbal tea extracts was measured according to the procedure of Brand-Williams [12]. Each stock solution of extracts and positive control, α–tocopherol (10,000 µg/ml) were prepared and diluted to obtain final concentrations of 1,000, 500, 100, 50, 10, and 1µg/ml in methanol. Seventy five microliter of each diluted extract at six different concentrations were added to 2.925 ml of a 0.025 g/L DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution in methanol. The reaction mixtures were then incubated in the dark for 30 min, and the absorbance at 515 nm was measured at different time intervals using a UV-Visible spectrophotometer (Shimadzu, UV 1601). All tests were performed in triplicate. The remaining $DPPH^{\cdot}$ concentration in the reaction mixture was calculated from the DPPH standard curve, and the percentage of the remaining $DPPH^{\cdot}$ was calculated using the following equation.

$$\%DPPH^{\cdot}_{REM} = [DPPH^{\cdot}]_T / [DPPH^{\cdot}]_{T=0}$$

Where $[DPPH^{\cdot}]_T$ and $[DPPH^{\cdot}]_{T=0}$ were the concentration of $DPPH^{\cdot}$ at steady state and zero time, respectively. The percentages of the remaining $DPPH^{\cdot}$ in each reaction mixture of six different concentrations of all extracts were then plotted against µg of extract / mg of $DPPH^{\cdot}$ to obtain the amount of antioxidant or extract necessary to decrease the initial $DPPH^{\cdot}$ by 50% (EC_{50}). The EC_{50} values of all extracts were calculated by the following linear regression of plots, and the antiradical efficiency ($AE=1/EC_{50}$) values were also calculated.

$$[\%DPPH^{\cdot}_{REM}] = b\,[\mu g\ antioxidant/\ mg\ DPPH^{\cdot}] + a$$

2.7 Total phenolic assay

Total phenolic contents of plant extracts were determined by the procedure as described by Tepe et al. [13]. Each stock solution of the extracts was prepared to obtain the concentration of 1,000 µg/ml. One hundred microliter of each stock solution were transferred to a flask containing 46 ml

distilled water. One milliliter of Folin-Ciocalteu reagent was then added. The mixture was shaken thoroughly, and allowed to stand for 3 min. Three milliliter of 2% Na_2CO_3 were added to the mixture, and allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm using UV-Visible spectrophotometer (Shimadzu, UV 1601). Standard curve of gallic acid was prepared using the similar procedure. The results were expressed as μg GAE (gallic equivalents)/mg dry extract.

3. RESULTS AND DISCUSSION

3.1 Preliminary screening of herbal tea extracts

The results of the disk diffusion test (Table 1) indicated that the ethanolic extracts of *P. betle* showed the broadest antimicrobial activity by inhibiting growth of almost all microbial strains tested (14 strains) with the diameter of inhibition zone of 7.3-14.0 mm, followed by *C. sinensis* (11 strains), *A. marmelos* (10 strains), *M. alba* (8 strains), *P. amaryllifolius* (7 strains), *C. asiatica* (6 strains), *P. acidus* (4 strains), and *A. squamosa* (4 strains). *P. betle* extract had inhibitory effect on the growth of most bacterial and yeast strains tested, except for *L. plantarum*. *H. uvarum* was the most susceptible yeast strain to the extract of *P. betle* (14 mm). *C. sinensis* extract was more active against yeast and spoilage bacterial strains, especially *L. mesenteroides, P. membranaefaciens,* and *Z. rouxii* (20 mm), than pathogenic bacterial strains tested, whereas *A. marmelos* and *M. alba* extracts had higher antimicrobial activity against pathogenic bacterial strains than other two groups. *P. amaryllifolius* extract inhibited more species of spoilage bacteria than yeast and pathogenic bacterial strains tested. *C. asiatica*, *A. marmelos*, *M. alba*, *P. amaryllifolius*, and *A. squamosa* extracts showed the greatest antimicrobial activity against *L. fermentum, S.* Enteritidis, *B. cereus*, *P. fluorescens*, and *C. lipolytica*, respectively. However, extracts of *P. acidus* and *A. squamosa* had lower antimicrobial action, compared to other extracts.

3.2 Minimum inhibitory concentration

Five plant extracts of *A. marmelos*, *C. sinensis, C. asiatica, P. betle,* and *P. amaryllifolius* were selected to determine the minimum inhibitory concentration against 15 microbial strains. The results indicated that the extracts of *A. marmelos*, *C. sinensis*, and *P. betle* had higher antibacterial action, compared to *C. asiatica* and *P. amaryllifolius* extracts (Table 2). *A. marmelos* and *C. sinensis* extracts showed greater antimicrobial activity than *P. betle* extract. Of all pathogenic bacterial strains tested, *B. cereus, E. coli* and *S. aureus* were more sensitive to the extracts of *A. marmelos* and *C. sinensis* with the MIC of 10.4-41.7 mg/ml, compared to *L. monocytogenes* and *S.* Enteritidis (the MIC of 83.3 to >166.7 mg/ml). *S. aureus*, as well as *L. monocytogenes*, was also sensitive to Penicillin G (MIC of 0.005 unit/ml). For food spoilage bacteria, *L. mesenteroides* was the most susceptible strain to the extracts of *A. marmelos* and *C. sinensis* (MIC of 10.4-20.8 mg/ml), followed by *L. plantarum*, *P. fluorescens*, and *L. fermentum*. Of all yeast strains tested, *H. uvarum* and *R. glutinis* were the most sensitive strains to the extract of *C. sinensis* (MIC of 41.7 mg/ml), while *C. lipolytica* and *P. membranaefaciens* were the most susceptible strains to *A. marmelos* extract (MIC of 83.3 mg/ml). Penicillin G had high antibacterial activity to most of bacteria tested, except for *S.* Enteritidis and *L. plantarum*. Amphotericin B showed greater inhibitory action against yeast strains tested, compared to bacterial strains. The most sensitive yeast strain to amphotericin B was *S. pombe* (MIC of 0.07 mg/ml).

In this study, extracts of *P. betle*, *A. marmelos*, and *C. sinensis* strongly inhibited the growth of most strains tested. The antimicrobial activity of *P. betle* is attributed to its essential oil which contains several compounds, such as chavicol, chavibetol, eugenol, p-cymene, methyl ether, cadinene, and caryophyllene. Haeborne and Williams [14] found that *P. betle* possessed antibacterial, antiviral, anti-inflammatory, antioxidant and antiplatelet activities. *A. marmelos* also had antimicrobial action. Methanolic extract of *A. marmelos* unripe fruit was reported to reduce induction time of diarrhea in mice [15]. The unripe fruit of *A. marmelos* is useful for treatment of diarrhea as it contains tannin or mucilaginous substances. Moreover, essential oil of *A. marmelos* leaves strongly inhibited growth of several fungal species and spore germination [16].

In the present study, *C. sinensis* extract showed high inhibitory action against *B. cereus, S. aureus, L. plantarum, L. mesenteroides* and *P. fluorescens*. Chou et al. [17] also reported that *P. fluorescens* was sensitive to tea extract. Yam et al. [18] demonstrated that different types of tea extracts inhibited a wide range of pathogenic bacteria, including *S. aureus* and *Staphylococcus epidermidis*. The antimicrobial activity of green tea can be explained by its content of polyphenol compounds, including

catechin, theaflavins and thearubigins catechins, (-)-epigallocatechin gallate (EGCG). EGCG is the most abundant in most green, oolong, and black teas. Green tea has a higher content of catechins than oolong tea (semifermented tea) and fermented teas (red and black teas) [19]. Gallocatechins and their gallates are the main chemical compounds responsible for the antibacterial activity of green tea extracts. The antimicrobial activity of tea may be related to its catechin content [17].

P. amaryllifolius and *M. alba* extracts had relatively low inhibitory action. Leaf of *P. amaryllifolius* contains several volatile compounds, especially 2- acethyl-1-pyroline [20]. Alkaloids, such as pandanamine and pandanamerilactones was found in *Pandanus amaryllifolius* leaves [21]. Pandanin, an unglycosylate protein was found to inhibit herpes simplex virus type-1 (HSV-1) and influenza virus (H1N1) infected in human [4]. *M. alba* extract was also found to inhibit the growth of *S. aureus* and *B. subtilis* [22], since *M. alba* plant contains bioactive compounds, including asiatic acid, asiaticoside, madecassic acid, and madecassoside [23]. The present study showed that leaves of *A. squamosa* and *P. acidus* had lower antimicrobial activity, compared to other extracts. Hadi and Bremner [24] found that 23% out of 100 plant species predicted to have antimicrobial and antimalarial activities were tested positively for alkaloids, but leaves, barks and roots of *P. acidus* showed negative result for alkaloid testing.

3.3 Antioxidant activity

Free radical scavenging activity of eight herbal tea extracts determined by DPPH assay was expressed as an EC_{50} value which is the concentration of antioxidant needed to decrease by 50% of the initial substrate concentration. The lower the EC_{50}, the higher the antioxidant power [25]. The results indicated that among all extracts, the most active one was *P. betle*, with an EC_{50} of 699.29 μg extract/ mg DPPH (AE = 1.43×10^{-3}), followed by *C. sinensis*, *A. squamosa*, *M. alba*, *C. asiatica*, *A. marmelos*, *P. acidus*, and *P. amaryllifolius* (Table 3). The positive control, ∝-tocopherol had the highest antioxidant power with the EC_{50} of 314.47 μg extract/ mg DPPH (AE = 3.18×10^{-3}).

3.4 Total phenolic content

The results of total phenolic analysis are shown in Table 4. Total phenolic content of eight herbal tea extracts was in the range of 6.0-546.0 μg GAE/mg dry extract. The highest phenolic content in *C. sinensis* extract (546.0 μg GAE/ mg dry extract) was observed, followed by *P. betle*, *P. amaryllifolius*, *A. marmelos*, *A. squamosa*, *P. acidus*, *C. asiatica*, and *M. alba*.

In the present study, extracts with high antioxidant power (extracts of *P. betle* and *C. sinensis*) had high phenolic contents, but good correlations could not be found with the other extracts. These findings are in agreement with earlier studies. Atoui et al. [5] reported that Chinese green tea showed the highest antioxidant activity, with the EC_{50} of 0.15 mg extract /mg DPPH, as compared to other plant extracts. Sixty different phenolic compounds were detected in these nine teas and herbs. Catechin was present in green tea. Runnie et al. [26] also determined total phenolic content in *P. betle* extract, and found that it contained 67 GAE value (mg/g dry extract). The results of antimicrobial acitivity of *P. betle* and *C. sinensis* may be related to their high contents of phenolic compounds and high antioxidant power. Phenolics are thought to exert inhibitory action against microorganisms by membrane disruption [27]. Polyphenols which are strong antioxidant have biological activity, such as antibacterial, anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic, and immune-stimulating effects. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [10, 28].

M. alba leaf extract showed high antioxidant activity (1021.7 μg extract/mg DPPH). Ohsugiet al. [10] revealed that water extract of *M. alba* had strong scavenging activity against hydroxy radical. Leaves of *C. asiatica* had less antioxidant activity (1054.64 μg extract/mg DPPH) than *M. alba*, but contained low phenolic contents (8.03 μg GAE/ mg dry extract). Zainol et al. [9] reported that both leaf and root of *C. asiatica* had high antioxidative activity, and the total phenolic content varied from 3.23-11.7 g/100 g dry sample. Leaf of *C. asiatica* contained higher phenolic contents than its root. Similarly, *A. marmelos* fruit extract was found to have less antioxidant activity than *C. asiatica*, but higher total phenolic contents. Similar results were reported by Kamalakanan and Prince [29]. *A. marmelos* fruit extract showed antioxidant activity in mice. Lampronti et al. [30] showed that *A. marmelos* extract was able to inhibit the *in vitro* proliferation of human tumor cell lines. Three derivatives (butyl p-tolyl sulfide, 6-methyl-4-chromanone and butylated hydroxyanisole) in *A. marmelos* extracts were identified, and exhibited strong activity in inhibiting cell growth of human K562 cells. In this study, *P. amaryllifolius* and *P. acidus* leaf extracts showed low antioxidant activity. Abas et al. [31] also reported that methanolic extract of *P. amaryllifolius* had lower antioxidant activity than other vegetables tested including *Anacardium occidentale*, *Cosmos caudatus*, *Curcuma mangga*, *Melicope ptelefolia*, *Persicaria tenella* and *Portulaca oleracea*.

Table 1 Antimicrobial activity of crude ethanolic extracts of plants using disk diffusion test

Microbial species	Diameter of Inhibition Zone (mm)[a] ± SD							
	Aegle marmelos	*Annona squamosa*	*Camella sinensis*	*Centella asiatica*	*Pandanus amaryllifolius*	*Piper betle*	*Phyllanthus acidus*	*Morus alba*
Pathogenic bacteria								
Bacillus cereus	-	8.0±0.0	[b]	7.0±0.0	-	9.7±3.8		10.0±1.4
Escherichia coli	9.7±3.8		-		-	10.3±1.2		-
Listeria monocytogenes	9.7±1.2	-	8.5±0.7		8.5±0.7	9.5±0.7		9.5±2.1
Salmonella Enteritidis	10.0±1.4	8.5±0.7	7.7±1.2			7.3±0.6		8.5±2.1
Staphylococcus aureus	9.0±3.5		-	7.0±0.0	8.5±0.7	9.3±2.1		8.5±0.7
Food spoilage bacteria								
Pseudomonas fluorescens	8.0±1.4	-	12.7±4.4	-	10.0±4.0	8.0±1.4	-	-
Lactobacillus fermentum			12.0±5.3	20.0±0.0	8.5±2.1	10.0±1.4	-	
Lactobacillus plantarum	-	-	12.3±5.9	-	-		-	7.5±0.7
Leuconostoc mesenteroides	8.3±0.6	7.5±0.7	20.0±0.0	10.0±1.4	9.0±1.0	11.3±2.5	7.5±0.7	8.5±1.6
Food spoilage yeasts								
Candida lipolytica	9.3±1.5	9.0±0.0	10.0±4.2	7.0±0.0	9.0±1.0	8.3±0.6	7.5±0.7	9.3±2.3
Hanseniaspora uvarum		-	9.0±2.0	-	-	14.0±0.0	-	-
Pichia membranaefaciens	8.3±0.6	-	20.0±0.0	-	-	9.3±0.6		-
Rhodotorula glutinis	8.5±2.1	-	7.5±0.7		8.3±1.2	8.0±1.4	7.5±0.7	7.7±1.2
Schizosaccharomyces pombe	-	-	-	-	-	11.7±1.5	-	-
Zygosaccharomyces rouxii	7.3±0.6	-	20.0±0.0	7.7±0.6	-	10.7±0.6	7.5±0.7	-

[a]Data are mean of three replications.
[b]No inhibition was observed.

Table 2 Minimum inhibitory concentrations of crude ethanolic extracts of plants

Microbial species	Minimum inhibitory concentrations (mg/ml) / (units/ml)*						
	Aegle marmelos	*Camella sinensis*	*Centella asiatica*	*Pandanus amaryllifolius*	*Piper betle*	Penicillin G	Amphotericin B
Pathogenic bacteria							
Bacillus cereus	41.7	10.4	>166.7	>166.7	>166.7	5.0	2.08
Escherichia coli	41.7	83.3	166.7	166.7	83.3	5.0	4.17
Listeria monocytogenes	83.3	166.7	>166.7	>166.7	>166.7	0.005	2.08
Salmonella Enteritidis	83.3	>166.7	>166.7	166.7	83.3	5,000	>4.17
Staphylococcus aureus	41.7	20.8	>166.7	>166.7	>166.7	0.005	>4.17
Food spoilage bacteria							
Pseudomonas fluorescens	83.3	41.7	>166.7	>166.7	>166.7	0.5	2.08
Lactobacillus fermentum	83.3	166.7	>166.7	>166.7	>166.7	5.0	4.17
Lactobacillus plantarum	83.3	20.8	>166.7	166.7	166.7	5,000	1.04
Leuconostoc mesenteroides	10.4	20.8	>166.7	>166.7	>166.7	0.5	4.17
Food spoilage yeasts							
Candida lipolytica	83.3	83.3	>166.7	166.7	>166.7	5,000	1.04
Hanseniaspora uvarum	>166.7	41.7	>166.7	>166.7	>166.7	50	1.04
Pichia membranaefaciens	83.3	166.7	>166.7	>166.7	166.7	>5,000	0.26
Rhodotorula glutinis	166.7	41.7	>166.7	>166.7	83.3	>5,000	0.52
Schizosaccharomyces pombe	>166.7	>166.7	>166.7	>166.7	>166.7	5,000	0.07
Zygosaccharomyces rouxii	166.7	>166.7	>166.7	>166.7	>166.7	>5,000	4.17

* Units/ml for penicillin G only.

Table 3 DPPH radical-scavenging activity of plant extracts

Plant extracts	EC_{50} (μg extract/ mg DPPH)[a] ± SD	AE ($\times 10^{-3}$) ± SD
Aegle marmelos	1201.99 ± 90.44	0.83 ± 0.01
Annona squamosa	911.65 ± 21.04	1.10 ± 0.05
Camella sinensis	729.93 ± 40.01	1.37 ± 0.02
Centella asiatica	1054.64 ± 62.89	0.95 ± 0.01
Morus alba	1021.70 ± 60.57	0.98 ± 0.02
Pandanus amaryllifolius	13,886.94 ± 111.63	0.07 ± 0.009
Phyllanthus acidus	5926.82 ± 34.86	0.17 ± 0.03
Piper betle	699.29 ± 29.10	1.43 ± 0.03
α-tocopherol	314.47 ± 24.59	3.18 ± 0.04

[a]Data are mean of three replications.

Table 4 Total phenolic contents of plant extracts

Plant extracts	Total phenolic content[a] ± SD (μg Gallic Acid Equivalents (GAE) /mg dry extract)
Aegle marmelos	42.53 ± 3.48
Annona squamosa	26.03 ± 0.82
Camella sinensis	546 ± 0.00
Centella asiatica	8.03 ± 1.89
Morus alba	6.00 ± 0.00
Pandanus amaryllifolius	46 ± 2.00
Phyllanthus acidus	26 ± 0.00
Piper betle	59.7 ± 1.04

[a]Data are mean of three replications.

4. CONCLUSION

Crude ethanolic extracts of *C. sinensis* and *A. marmelos* had the highest antimicrobial activity, whereas *P. betle* showed the broadest antimicrobial action. *B. cereus*, *E. coli*, *S. aureus* were the most sensitive pathogenic bacteria to *C. sinensis* and *A. marmelos* extracts (the MIC of 10.4-41.7 mg/ml), while *L. mesenteroides* was the most sensitive food spoilage bacteria (the MIC of 10.4-20.8 mg/ml). The most vulnerable yeasts to *C. sinensis* extract were *H. uvarum* and *R. glutinis* (the MIC of 41.7 mg/ml), while *C. lipolytica* and *P. membranaefaciens* were the most sensitive yeast strains to *A. marmelos* (the MIC of 83.3 mg/ml). Therefore, there is the possibility to use these extracts as biopreservatives in foods.

Each plant extract had different antioxidant power, and contained different amount of total phenolic compounds. *P. betle* and *C. sinensis* had higher antioxidant activity and total phenolic

contents than the other extracts tested. According to their antimicrobial and antioxidant activities, *C. sinensis*, *P. betle*, *A. marmelos*, *A. squamosa*, *C. asiatica*, and *M. alba* are potential sources of natural preservatives and antioxidants. Isolation and identification of active compounds attributed to antimicrobial and antioxidant activities should be further studied to establish the connection between those activities and chemical composition of these plants.

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