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The efficiency and the correlation between testing methods on antimicrobial and antioxidant activities of selected medicinal essential oils

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Abstract

Eight selected medicinal essential oils—calamus, clove, betel, lime, Chinese cinnamon, lemon grass, vetiver and pine were determined for their antimicrobial and antioxidant activities. The inhibitory effect against pathogenic microorganisms using agar disc and agar well-diffusion methods demonstrated that *Candida albican* was extremely sensitive to Chinese cinnamon oil with an inhibition zone diameter of 2.4 and 1.5 cm for agar disc and agar well-diffusion tests, respectively. This microbe was also the most susceptible with MIC and MFC values of 0.075 $\mu L \cdot mL^{-1}$. The results from the MIC test showed that Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) were more resistant to Chinese cinnamon oil than Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*). The antioxidant activity tested using DPPH, ABTS and FRAP methods revealed that clove and betel oils were the strongest antioxidant agents with non-significant differences in comparison to the control. According to the Pearson's correlation coefficient, for the antimicrobial test, *C. albicans* had the highest significant correlation between the agar disc and agar well-diffusion methods (r = 0.927). Gram-negative bacteria also demonstrated a stronger and significant correlation than Gram-positive bacteria. The antioxidant testing methods indicated that DPPH, ABTS and FRAP showed strong positive correlations with each other with coefficients ranging from 0.808 to 0.989. In contrast, H_2O_2 scavenging assay showed no correlations. These results suggested that Chinese cinnamon and clove oils are the most effective natural agents for antimicrobial and antioxidant activities, respectively. Moreover, a positive correlation was found between the testing methods in both antimicrobial and antioxidant activity tests.

Introduction

In recent years, aromatic and medicinal plant essential oils have been evaluated for their effectiveness for food preservation and safety applications (Ait-Ouazzou *et al*., 2011; Wu *et al*., 2014; He and Xiao, 2016). Some volatile compounds from essential oils have a great potential of application as antimicrobial additives (Martucci *et al.*, 2015). Furthermore, the antioxidant properties of essential oils have been effective in retarding the process of the oxidative degradation of lipids in foods because of the hydroxyl groups presented in their chemical structure (Kim *et al.*, 2010). In general, food spoilage and pathogenic bacteria are controlled by chemicals, but the use of synthetic chemicals leads to undesirable aspects such as allergy and carcinogenicity. Chemical food additives also induce the emergence of multi-drugs resistant pathogens (Rath and Padhy, 2013). Thus, naturally occurring antimicrobial, particularly essential oils, are being of interest to be used as food additives. Herbs and spices commonly used as food ingredients and food seasonings are sources that provide essential oils. Essential oils are typically complex and composes of abundant biologically active compounds, especially monoterpenes (C10), sesquiterpenes (C15), a variety of a low molecular weight aliphatic hydrocarbon, alcohols, acids, aldehydes and phenolic compounds (Rota *et al*., 2004; Prabuseenivasan *et al*., 2006). Several reports have shown that essential oils extracted from herbs and spices possess antibacterial, antifungal and antioxidant properties (Adrar *et al.*, 2016; Jamkhande *et al*., 2016). The action mechanisms of essential oils against a wide range of food borne pathogens may relate to the hydrophobic property that leads

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to the ability of essential oil to penetrate microbial cell membrane resulting in disruption of membrane permeability, interference of energy generation system and cell death (Friedly *et al*., 2009).

In general, a variety of those antimicrobial and antioxidant screening methods are not based on the same reaction mechanism, thus they often give different results. Agar diffusion methods are the most well-known and basic methods for antimicrobial assay which are employing two different types of reservoirs consisting of a filter paper disc impregnated with compound–test and wells in dishes (Valgas *et al.*, 2007). For antioxidant activity, several methods have been widely used including 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation decolonization assay, ferric ion (Fe^{3+}) reducing antioxidant power (FRAP) assay and hydrogen peroxide (H_2O_2) scavenging activity assay (Cao and Prior, 1998; Pérez *et al*., 2000; Ou *et al*., 2002). To decide whether their properties could be predicted from one assay to another, a correlation analysis should be carried out (Terpinc *et al.*, 2012). Therefore, this study aimed to investigate the antimicrobial and antioxidant activities of some medicinal plant essential oils by using different testing methods and to evaluate the correlations among these testing methods.

Materials and Methods

Plant material

Eight medicinal plant species were purchased from Tung Charoen O-Sod shop at a local market in Kamphaengsaen district, Nakorn Pathom province, Thailand–calamus (*Acorus calamus* Linn.), clove (*Eugenia caryophyllata* Thunb.), betel (*Piper betle* Linn.), lime (*Citrus aurantifolia* Swingle), Chinese cinnamon (*Cinnamomum cassia* Nees ex Blume), lemongrass (*Cymbopogon citratus* (DC.) Stapf), vetiver grass (*Vetiveria zizanioides* (L.) Nash.), and pine (*Pinus sylvestris* Linn.).

Essential oils extraction

The plant material was cut into small pieces and 100 g were hydro-distilled for 3 h using a Clevenger-Adams type apparatus. The essential oils were collected and dried over anhydrous sodium sulfate. The essential oils were stored in seal glass vials and kept at -18°C until use.

Microbial strain use in vitro antimicrobial assay

Escherichia coli and *Pseudomonas aeruginosa* were provided by the Department of Microbiology, Kasetsart University Kamphaeng Saen campus, Thailand. An enterotoxin A producing *Staphylococcus* aureus TISTR 029 was purchased from the culture collection of the Thailand Institute of Scientific and Technological Research (TISTR). *Staphylococcus epidermidis* ATCC 1228 and *Candida albicans* ATCC 10231 were purchased from the Department of Medical Sciences (DMSC), Ministry of Health, Thailand.

The bacteria and yeast strains were grown on Nutrient agar (NA) except for *S. epidermidis* which was cultured in Brain Heart Infusion (BHI) agar (Oxoid, England). Bacteria and yeast were activated on agar plate at 37°C for 24 h before used. All tested microorganisms were maintained on agar slant at 4°C. Stock cultures are kept at -70°C.

Agar disc diffusion method

Agar disc diffusion method was carried out according to Dobre *et al.* (2011). Microbial cell concentration was approximately adjusted to 1.5 x 10^8 CFU·mL^{-1} (0.5 McFarland) in sterile 0.85% sodium chloride (NaCl) (Ajax Finechem, Australia). The cell suspensions were spread uniformly with a sterile cotton swab on the surface of NA except for *S. epidermidis*, which was spread onto BHI agar. The essential oil was dissolved in dimethylsulfoxide (DMSO) (Merck, Germany) to obtain a 5.00% v/v solution. The essential oil/DMSO solution (5 µL) was deposited on sterile filter paper discs (6 mm in diameter) which were subsequently placed to the center of each plate. DMSO was used as the negative control. Antibiotics—ampicillin sodium (Chemical industries, Japan) (30 µg·$disc^{-1}$), tetracycline hydrochloride (Chemical industries, Japan) (30 µg·$disc^{-1}$) and amphotericin B (Chemical industries, Japan) (20 µg·$disc^{-1}$)—were used as the positive control. After 24h of incubation at 37°C, the diameter of growth inhibition zone was measured. All tests were done in triplicate.

Agar well diffusion method

Agar well diffusion method used in this study was modified from the procedure of Gupta *et al.* (2008). Microbial cell concentration was adjusted to 1.5 x 10^8 CFU·mL^{-1} (0.5 McFarland) in sterile 0.85% NaCl (Ajax Finechem, Australia). Twenty microliters of 5.00 % v/v of essential oils were introduced into the agar wells (6 mm in diameter). Positive and negative controls were performed as described above. After 24h of incubation at 37°C, the diameter of growth inhibition zones was measured. All tests were done in triplicate.

Minimum inhibitory concentration and minimal lethal concentration determination

The lowest concentration of essential oils required to inhibit the growth of the tested microorganisms was determined by the broth dilution method using a 96-well microplate (Stojkovic´*et al*., 2011). Microbial suspensions were prepared to obtain McFarland No. 0.5 standard turbidity. Two-fold serial dilutions of essential oils in 10% Tween-80 (Ajax Finechem, Australia) were performed in a 96-well microplate. Then 10 µL of microbial suspension and 90 µL of broth medium were added into each well which contained 100 µL of the diluted essential oil. One hundred and ninety microliters of nutrient broth and 10 µL of cell suspension were used as the negative control. Ampicillin Sodium (30 µg·mL^{-1}), Tetracycline Hydrochloride (30 µg·mL^{-1}) and Amphotericin B (20 µg·mL^{-1}) were used as the positive control. Triplicate wells were tested for each concentration of the essential oils. The plates were incubated at 37°C for 24h. The minimal lethal concentration (MLC) [minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC)] values were determined by sub-culturing the well with no growth apparent on agar plates. The least concentration showing no visible growth on agar plates was taken as the MLC (MBC or MFC) value.

2,2-Diphenyl-1-picrylhydrazyl free radical scavenging assay (DPPH assay)

The antioxidant activity of essential oils was measured in the form of hydrogen donating or scavenging activity according to the procedure of Dasgupta and De (2004). A solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany) in methanol (0.004 % w/v) and essential oil (100 mg·mL^{-1} in methanol) were prepared. The reaction was started by mixing of 3 mL of DPPH solution and 0.1 mL of essential oil. The mixture was incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. Ascorbic acid (Rankem, India), α-tocopherol (Sigma-Aldrich, Germany) and butylated hydroxytoluene (BHT) (Panreac Quimica SA, Spain) were used as the positive control. The capacity to scavenge the DPPH radical was calculated using the following formula:

$$\% \text{ DPPH radical scavenging} = \frac{(A-B)}{A \times 100}$$

Where, A is the absorbance of the negative control (DPPH and methanol) and B is the absorbance of the sample (DPPH, methanol and sample)

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation decolorization assay (ABTS assay)

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity using some modifications to the method of Dudonne *et al.* (2009) was determined. The pre-formed radical monocation of ABTS was prepared from reaction mixture of 7 mM ABTS (Sigma-Aldrich, Germany) in distilled water and 2.45 mM potassium persulfate ($K_2S_2O_8$) (Lobachemie, India). The ABTS solution was stored in the dark at room temperature for 12-16 h. The ABTS solution was diluted to an absorbance of 0.7±0.025 at 734 nm with methanol. Then, 0.2 mL of essential oils (100 mg·mL^{-1} methanol) was added to 2 mL of ABTS solution. The solution was well mixed and incubated in the dark at room temperature for 5 min and then an absorbance was measured at 734 nm. Ascorbic acid, α-tocopherol and BHT were used as the positive control. The capacity to scavenge the ABTS radical was calculated using the following formula:

$$\% \text{ ABTS radical scavenging} = \frac{(A-B)}{A \times 100}$$

Where, A is the absorbance of the negative control (ABTS and methanol) and B is the absorbance of the sample (ABTS, methanol and sample)

Ferric ion (Fe^{3+}) reducing antioxidant power assay (FRAP assay)

Ferric ion (Fe^{3+}) reducing antioxidant power assay was carried out using the method of Zhang *et al.* (2010). The reaction mixture containing 1 mL of essential oils (100 mg·mL^{-1} methanol) was mixed with 0.2 ml of 0.2 M phosphate buffer (pH 7.2) and 1 mL of 1% w/v potassium ferricyanide (Fisher scientific, India) in distilled water. The mixture was then incubated at 50°C for 20 min. Then, 0.2 mL of 10% w/v trichloroacetic acid (Merck, Germany) in distilled water was added to the mixture, which was then centrifuged for 10 min. The supernatant (2.0 mL) was mixed with 0.35 mL of 0.35% w/v ferric chloride (Sigma-Aldrich, Germany) in distilled water and the absorbance was measured at 700 nm. Ascorbic acid, α-tocopherol and BHT were used as the positive control.

Hydrogen peroxide (H_2O_2) scavenging activity assay

The modified method of Ebrahimzadeh *et al.* (2010) was used to determine the hydrogen peroxide scavenging activity. The hydrogen peroxide (Ajax Finechem, Australia) solution (40 mM) was prepared in 0.1 M phosphate buffer (pH 7.4). Essential oils

Table 1. Antimicrobial activity of selected essential oils against various microorganisms using agar disc-diffusion method

Essential oil	Diameter of inhibition zone (cm) (mean±SD)				
	Gram-positive		Gram-negative		Yeast
	S. aureus	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*C. albicans*
Calamus	0.667±0.029 ^{d}	0.600±0.000 ^{c}	0.700±0.000 ^{de}	0.600±0.000 ^{c}	0.843±0.012 ^{d}
Clove	0.683±0.029 ^{d}	0.683±0.057 ^{c}	0.967±0.029 ^{b}	0.600±0.000 ^{c}	0.667±0.029 ^{ef}
Betel	0.667±0.029 ^{d}	0.600±0.000 ^{c}	0.817±0.126 ^{c}	0.600±0.000 ^{c}	0.700±0.000 ^{e}
Lime	0.800±0.000 ^{c}	0.600±0.000 ^{c}	0.667±0.029 ^{de}	0.600±0.000 ^{c}	0.667±0.029 ^{ef}
Chinese cinnamon	0.667±0.029 ^{d}	0.600±0.000 ^{c}	0.750±0.050 ^{cd}	0.783±0.289 ^{b}	2.400±0.000 ^{a}
Lemongrass	0.883±0.076 ^{c}	0.667±0.017 ^{c}	0.600±0.000 ^{e}	0.600±0.000 ^{c}	0.960±0.529 ^{c}
Vetiver	0.833±0.029 ^{c}	0.667±0.017 ^{c}	0.600±0.000 ^{e}	0.600±0.000 ^{c}	0.600±0.000 ^{f}
Pine	0.600±0.000 ^{d}	0.600±0.000 ^{c}	0.600±0.000 ^{e}	0.600±0.000 ^{c}	0.900±0.050 ^{cd}
Tetracycline	2.450±0.100 ^{a}	0.950±0.218 ^{b}	3.200±0.100 ^{a}	1.030±0.000 ^{a}	Nt
Ampicillin	1.033±0.115 ^{b}	2.050±0.132 ^{a}	3.233±0.058 ^{a}	0.600±0.000 ^{c}	Nt
Amphotericin B	Nt	Nt	Nt	Nt	1.967±0.115 ^{b}
Control (DMSO)	0.600±0.000 ^{d}	0.600±0.000 ^{c}	0.600±0.000 ^{e}	0.600±0.000 ^{c}	0.600±0.000 ^{f}

Diameter of inhibition zone including disk diameter of 0.6 cm.
The different letters within the same column indicate statistically significant difference at 0.05 probability level.
Nt = not tested.

(100 mg·mL^{-1} methanol) were added to 0.6 mL of H_2O_2 solution. Then, the solution was mixed and incubated at room temperature for 10 min and the absorbance was measured at 230 nm. Ascorbic acid, α-tocopherol and BHT were used as the positive control.

Statistical analysis

Data were recorded as the mean ± standard deviation of the results in triplicate. All statistical analysis was performed using one-way analysis of variance (ANOVA). Differences between means were considering using Duncan's New Multiple Range Test (DMRT) at the significance level of 0.05. The correlations of antimicrobial and antioxidant testing methods were calculated using the Pearson's correlation coefficient.

Results and Discussion

Antimicrobial activity

The antimicrobial activities of essential oils against five pathogenic microbes are summarized in Tables 1-2. The agar disc diffusion test demonstrated that the essential oils tended to be expressed as a low efficacy against Gram-positive bacteria (*S. aureus* and *S. epidermidis*) compared with the positive control (tetracycline and ampicillin) (Table 1). However, lemongrass, vetiver and lime oils showed inhibition of *S. aureus* with an inhibition zone diameter of 0.833, 0.833 and 0.800 cm, respectively, whereas, clove and Chinese cinnamon oil tend to inhibit Gram-negative bacteria (*E. coli* and *P. aeruginosa*) with an inhibition zone diameter of 0.967 and 0.783 cm, respectively. However, the inhibition zone was significantly lower than those of the positive control ($p<0.05$). Interestingly, Chinese cinnamon oil revealed a significant and strong efficacy against *C. albicans* with an inhibition zone diameter of 2.40 cm ($p<0.05$). These results suggested that all Gram-positive and Gram-negative bacteria were strongly affected by the tested essential oils, while the tested fungi were more sensitive to Chinese cinnamon oil. On the other hand, considering the agar well-diffusion method, this essential oil also showed the highest and significant antimicrobial activity against both Gram-positive and Gram-negative bacteria with its inhibition zone ranging from 1.10 to 2.67 cm ($p<0.05$). Moreover, it had a strong efficacy for the inhibition of *C. albicans* (Table 2). Generally, these two methods seem to be produce similar results. However, the agar well-diffusion method showed greater activity than the agar disc method. This might have resulted from the difference between the amounts of applied volume of essential oil onto the paper disc (5 µL) versus the agar well (20 µL). Thus, from those results, it can be concluded that the Chinese cinnamon oil proved to have greater microbial inhibitory efficiency than other essential oils which was supported by many previous reports (Devkatte *et al*., 2005; Matan *et al*., 2006; Gupta *et al*., 2008). Accordingly, with the highest efficacy against bacteria and yeast in both the

Table 2. Antimicrobial activity of selected essential oils against various microorganisms using agar well-diffusion method

Essential oil	Diameter of inhibition zone (cm) (mean±SD)				
	Gram-positive		Gram-negative		Yeast
	S. aureus	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*C. albicans*
Calamus	0.717±0.029 ^{g}	0.600±0.000 ^{e}	0.923±0.251 ^{g}	0.600±0.000 ^{d}	0.600±0.000 ^{c}
Clove	0.767±0.058 ^{g}	0.850±0.050 ^{c}	1.500±0.173 ^{d}	0.600±0.000 ^{d}	0.600±0.000 ^{c}
Betel	0.717±0.029 ^{g}	0.750±0.050 ^{d}	1.133±0.058 ^{f}	0.600±0.000 ^{d}	0.600±0.000 ^{c}
Lime	0.900±0.050 ^{f}	0.600±0.000 ^{e}	1.017±0.029 ^{fg}	0.600±0.000 ^{d}	0.600±0.000 ^{c}
Chinese cinnamon	2.033±0.104 ^{b}	1.367±0.764 ^{b}	2.667±0.115 ^{c}	1.100±0.000 ^{b}	1.500±0.000 ^{b}
Lemongrass	1.583±0.076 ^{d}	0.867±0.289 ^{c}	1.333±0.058 ^{e}	0.600±0.000 ^{d}	0.600±0.000 ^{c}
Vetiver	1.100±0.000 ^{e}	0.900±0.500 ^{c}	0.600±0.000 ^{h}	0.600±0.000 ^{d}	0.600±0.000 ^{c}
Pine	1.525±0.090 ^{d}	0.600±0.000 ^{e}	0.600±0.000 ^{h}	0.867±0.058 ^{c}	0.600±0.000 ^{c}
Tetracycline	2.217±0.029 ^{a}	0.700±0.000 ^{d}	3.233±0.029 ^{a}	1.160±0.026 ^{a}	Nt
Ampicillin	1.693±0.101 ^{c}	2.083±0.289 ^{a}	3.067±0.029 ^{b}	0.600±0.000 ^{d}	Nt
Amphotericin B	Nt	Nt	Nt	Nt	2.033±0.029 ^{a}
Control (DMSO)	0.600±0.000 ^{h}	0.600±0.000 ^{e}	0.600±0.000 ^{h}	0.600±0.000 ^{d}	0.600±0.000 ^{c}

Diameter of inhibition zone including well diameter of 0.6 cm
The different letters within the same column indicate statistically significant difference at 0.05 probability level.
Nt = not tested.

Table 3. MICs, MBCs and MFC of Chinese cinnamon oil using 96-well micro plate dilution method

Microorganism	MIC (μL·mL^{-1})	MBC/MFC (μL·mL^{-1})
Gram-positive bacteria		***MBC***
Staphylococcus aureus	1.250	2.500
Staphylococcus epidermidis	2.500	5.000
Gram-negative bacteria		***MBC***
Escherichia coli	0.600	0.600
Pseudomonas aeruginosa	0.600	1.250
Yeast		***MFC***
Candida albicans	0.075	0.075

agar disc diffusion and agar well-diffusion methods, it was selected for evaluation of MIC, MBC and MFC using the broth dilution method in 96-well microplates. The results showed that *C. albicans* was the most susceptible microorganism with MIC and MFC values of 0.075 μL·mL^{-1}. Gram-negative bacteria (*E. coli* and *P. aeruginosa*) seem to be more resistant to Chinese cinnamon oil than Gram-positive bacteria (*S. aureus* and *S. epidermidis*) (Table 3). This result was in contrast to many previous reports which indicated that it was normally more effective in inhibiting Gram-positive than Gram-negative bacteria (Marino *et al*., 1999; Ruberto *et al*., 2000; Delaquis *et al*., 2002; Harpaz *et al*., 2003). Nevertheless, not all studies on the antimicrobial activity of essential oils have revealed that Gram-negative bacteria are more resistant (Wilkinson *et al*., 2003; Baljeet *et al*., 2015). Additionally, the main compound of cinnamon oil was identified as cinnamaldehyde (52.4%) (Prabuseenivasan *et al*., 2006) which belongs to the group of aromatic terpene aldehydes. Its carbonyl group was found to be an effective inhibitor of amino acid decarboxylase (Wendakoon and Sakaguchi, 1995). In addition, the lipophilic property of the essential oils makes it easy for the oil to penetrate the microbe membrane, disturbing the structures and leaking the ions and other cell contents (Burt, 2004).

Antioxidant activity

The *in vitro* antioxidant activities of eight essential oils were determined through the comparison of the standards (ascorbic acid, α-tocopherol and BHT) using DPPH, ABTS, FRAP and H_2O_2 assays and the results are presented in Table 4. With respect to

Table 4. Antioxidant activities of selected essential oils using different testing methods

Essential oil	Antioxidant activity			
	DPPH (100 mg·mL^{-1})	ABTS (100 mg·mL^{-1})	FRAP (100 mg·mL^{-1})	H_2O_2 (25 mg·mL^{-1})
Calamus	33.86±0.21 ^{f}	34.69±0.74 ^{c}	0.85±0.02 ^{e}	40.44±0.42 ^{c}
Clove	94.18±0.11 ^{ab}	99.47±0.09 ^{a}	2.07±0.23 ^{a}	21.14±1.65 ^{g}
Betel	93.48±0.17 ^{b}	99.23±0.00 ^{a}	1.47±0.04 ^{b}	14.24±0.54 ^{i}
Lime	49.26±0.30 ^{e}	70.24±0.37 ^{b}	0.89±0.05 ^{de}	38.39±1.61 ^{d}
Chinese cinnamon	21.66±0.62 ^{g}	26.94±1.27 ^{d}	0.37±0.01 ^{g}	28.28±0.31 ^{f}
Lemongrass	17.55±0.59 ^{i}	18.76±0.46 ^{e}	0.55±0.03 ^{f}	66.49±0.81 ^{b}
Vetiver	91.24±0.23 ^{d}	98.93±0.19 ^{a}	0.95±0.01 ^{d}	21.50±0.37 ^{h}
Pine	18.74±1.44 ^{h}	19.50±0.40 ^{e}	0.61±0.02 ^{f}	16.32±0.66 ^{i}
Ascorbic acid	94.66±0.07 ^{a}	99.28±0.00 ^{a}	1.10±0.07 ^{c}	82.08±0.38 ^{a}
α-Tocopheral	92.14±0.12 ^{c}	99.23±0.09 ^{a}	1.42±0.00 ^{b}	30.54±0.16 ^{e}
BHT	92.16±0.07 ^{c}	99.52±0.09 ^{a}	1.46±0.03 ^{b}	17.41±0.41 ^{i}

The different letters within the same column indicate statistically significant difference at 0.05 probability level.

the DPPH test, clove oil revealed a remarkable free radical scavenging effect with an inhibition value of 94.18%. Its efficacy was homogenous with ascorbic acid and slightly better than that of α-tocopherol and BHT. Similarly, betel oil showed significantly higher activity than α-tocopherol and BHT ($p<0.05$). The result of the ABTS test also indicated that clove, betel and vetiver had strong ABTS radical scavenging activity with an inhibition value of 99.47, 99.23 and 98.93%, respectively. Moreover, these plant oils had no significant difference in efficiency when compared with those of the standards; α-tocopherol, BHT and ascorbic acid ($p>0.05$).

Similar results were also found in the reducing power capacity by FRAP assay where clove and betel oils exhibited the significantly highest activity with no significant difference compared with the standards ($p>0.05$). In contrast to the results from DPPH, ABTS and FRAP, all essential oils at a concentration of 25 mg·mL^{-1} showed a significantly lower hydrogen peroxide radical scavenging activity than ascorbic acid ($p<0.05$). However, the lemongrass oil demonstrated 66% inhibition which was higher than for α-tocopherol and BHT. Thus, clove and betel are among the essential oils that possess strong antioxidant activity. The antioxidant activities of essential oils from aromatic plants are mainly attributed to their major constituents. The major compound that confers the antioxidant activity of clove and betel is eugenol, with 91.2% and 36.2% obtained from clove and betel, respectively (Politeo *et al.*, 2006; Row and Ho, 2009). Its antioxidant effect was due to the presence of a hydroxyl group in their chemical structure where the hydrogen donating ability occurred (Kulisic *et al*., 2004). Moreover, the presence of a benzene ring is responsible for electron delocalization (Pietta, 2000).

Pearson's correlation coefficient results among testing methods for antimicrobial and antioxidant activity

The Pearson's correlation coefficient values among the methods used to determine the antimicrobial and antioxidant activity were calculated and are presented in Table 5. For the antimicrobial test, the agar disc method had the highest, significant correlation with the agar well method on *C. albicans* with a correlation coefficient of 0.927 ($p<0.01$). Gram-negative bacteria also demonstrated stronger, significant correlation than Gram-positive bacteria with a correlation coefficient value of 0.786 and 0.788 for *E. coli* and *P. aeruginosa* ($p<0.01$), respectively. *S. epidermidis* also showed significant correlation between the testing methods with a correlation coefficient of 0.723 ($p<0.01$). However, no significant correlation was found with *S. aureus* ($p>0.01$). These results indicated that the antimicrobial results of agar well and agar disc diffusion methods are significantly correlated for most of the tested microbes except for *S. aureus.*

As indicated in Table 5, the correlation coefficients obtained from a comparison of the results of antioxidant activity using different methods (DPPH, ABTS, FRAP and H_2O_2 methods) revealed

Table 5. Pearson correlation coefficient among testing methods for antimicrobial (a) and antioxidant activity (b)

(a) Antimicrobial activity		(b) Antioxidant activity	
Testing microbe	**r**	**Testing methods**	**r**
S. aureus	0.583	DPPH assay with ABTS assay	0.989**
S. epidermidis	0.723*	DPPH assay with FRAP assay	0.827**
E. coli	0.786**	ABTS assay with FRAP assay	0.808**
P. aeruginosa	0.788**	DPPH assay with H_2O_2 assay	-0.148
C. albicans	0.927**	ABTS assay with H_2O_2 assay	-0.156
		FRAP assay with H_2O_2 assay	-0.297

* Correlation significant at 0.05 level.
** Correlation significant at 0.01 level.
r = Pearson correlation coefficient.

that the DPPH and ABTS assays had the highest, positive correlation coefficient of 0.989 followed by the DPPH and FRAP methods and the ABTS and FRAP methods with correlation coefficients of 0.827 and 0.808, respectively. In contrast, no testing methods had a significant correlation with the H_2O_2 assay. Generally, the different assays for measuring antioxidant capacity are based on different reaction mechanisms, thus different results are often revealed. However, from our results, it could be suggested that DPPH, ABTS and FRAP were strongly positively correlated with each other. This could be explained by their same reaction mechanisms which all belong to the electron transfer reaction method (Miguel, 2010).

Conclusion

This study clearly revealed that Chinese cinnamon oil had effective, antimicrobial activity against both fungi and pathogenic bacteria. Clove and betel oils demonstrated a highly significant antioxidant activity in different in vitro assays. The Pearson correlation coefficient among the methods for antimicrobial testing exhibited a significant correlation between the agar disc and agar well methods for *C. albicans*, *P. aeruginosa, E. coli* and *S. epidermidis*. The DPPH, ABTS and FRAP assays were also found to be significantly correlated in antioxidant activity testing.

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