Inhibitory effect of essential oils from local Thai medicinal plants against common human pathogens

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ABSTRACT: This study aimed to extract essential oils from some local Thai medicinal plants, namely Citrus hystrix DC. (Kaffir lime), Cymbopogon citratus Stapf. (Lemon grass), Cymbopogon nardus Rendle (Citronella grass) and Ocimum basilicum L. (Sweet basil) by hydro-distillation method and investigate their antimicrobial efficiency against four common bacteria species. By hydro-distillation method, the highest percentage yield was found from the essential oil extracted from sweet basil (1.19%), whereas lemon grass gave the lowest percentage yield of the essential oil (0.28%). Agar-disc diffusion technique was used to test for the antimicrobial activity of these essential oils against Staphylococcus aureus, Escherichia coli, and Salmonella Group B, isolated from specimens of patients from Kalasin Hospital. The result showed differences in antimicrobial efficiency of each essential oil against the same human pathogen. Essential oil, extracted from lemon grass, showed higher tendency to inhibit all pathogens. Antimicrobial index (AI) was used to report the antimicrobial efficiency. Result showed that S. aureus and Salmonella Group B were the most sensitive to the essential oil extracted from lemon grass, showing their AI as 5.07 and 1.15, respectively. The highest AI against E. coli was found when testing with the essential oil from citronella grass, followed by lemon grass, their AI as 2.28 and 2.22, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of essential oil extracted from lemon grass against the indicator strains were in the range 62.5-500 ppm. This result suggested the promising antimicrobial property of the essential oil from lemon grass could be useful in pharmaceutical treatment.

Keywords: essential oil; local Thai medicinal plant; antimicrobial index

Introduction

Consuming of foods contained with pathogenic microorganisms, leading to foodborne illness, is one of the most concerned topics in public health. It has been reported that as many as 30% of the world population suffers from foodborne diseases each year (Sara, 2004). Considering to the course of being suffer from foodborne diseases, bacteria pathogens are responsible for about 90% of the cases. *Salmonella* sp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Escherichia coli*; for example, are very common bacteria related to food poisoning, coursing mild foodborne diseases such as diarrhea to severe deaths (Sokovic et al., 2012). Each year, a lot of budgets are spent on foodborne disease treatments; for instance, in Canada, about 500 million on health budget is used for dealing with foodborne illness each year (Todd, 1989). To minimize the economic losses from food borne bacteria, the idea of using natural substances with antibacterial activity has been drawing a lot of attentions from researches (Oussalah et al., 2007; Upadhyay et al., 2010; Kamazen et al., 2012).

Essential oil or volatile oil is a secondary metabolite, commonly found in many parts of plants such as flower, bud, seed, bark and leaf. It is aromatic oily liquid which plays vital roles in plants, including protections of plants as antibacterial and antiviral agent, along with

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attraction of insects to dispersal pollens and seeds. For human, the essential oil has been used with various purposes for ages. In the past, the essential oil was mainly used with the intention of healing by folk healers and flavoring foods by locals (Bakkali et al., 2008). Many studies have recently reported a wide variety of benefits from using essential oil such as being favoring agents in food and drink industries, using as a fragrant in perfumery industries as well as preserving stored food and crops. Without doubt, due to their distinct benefits, the need of the essential oil has been increasing over the world. It is estimated that 40,000-60,000 tons of essential oil have been produced annually with approximately 700 million US dollars of market values (Raut and Karuppayil, 2014).

Therefore, the aim of this study was to extract essential oil from local medicinal plants in Kalasin area, growing all year round in order to investigate their antimicroorganism efficiency against the common foodborne pathogenic bacteria coursing diarrhea and other foodborne illnesses in Kalasin Hospital.

Methodology

Preparation of essential oil

The essential oil used in this study was extracted from Citrus hystrix DC. (Kaffir lime), *Cymbopogon citratus* Stapf. (Lemon grass), *Cymbopogon nardus* Rendle (Citronella grass) and *Ocimum basulicum* L. (Sweet basil) by the hydro-distillation method. The recovered essential oil was dried over sodium sulfate anhydrous and stored at 4 ºC until use. Yield of essential oil extraction was expressed in the unit of percent yield (%, v/w), described in equation (1) (Laohakunjit et al., 2009).

$$Percent\ yield\ of\ essential\ oil\ (\%) = \frac{volume\ of\ essential\ oil\ (ml)}{weight\ of\ raw\ material\ (g)} \times 100 \qquad (1)$$

Test microorganisms

Staphylococcus aureus, *Escherichia coli*, *Salmonella* Group B and *Pseudomonas aeruginosa* were kindly isolated in the Microbiology Laboratory of Kalasin Hospital using aseptic technique. These pure bacteria cultures were maintained in 30% glycerol before use.

To inoculate bacteria culture (indicator strain), bacteria were grown on nutrient agar (NA) overnight at 37°C to obtain at single colony. Then, one single colony of pure culture was inoculated in nutrient broth (NB) and grown overnight at 37°C. The concentration of bacteria used was adjusted with normal saline to obtain 10^8 CFU/ml.

Antimicrobial Property

Agar-disc diffusion technique was used to test for the antimicrobial property of essential oil against the indicators strain. Briefly, sterile blank discs with 6 mm diameter were individually placed on NA plate, covered with 100 µL of the indicator strain. These sterile discs allowed 10 µL of essential oil to penetrate into the indicator strains. The plates were incubated at 37°C for 24 hours. The determination of antimicrobial property was done triplicate and evaluated by measuring diameter of inhibition zone of each essential oil against each indicator strains in millimetre (mm.). The antimicrobial efficiency was reported as the ratio of Antimicrobial index (AI), shown in equation (2). The "*Db*" and "*Da*" were the diameter of inhibition zone of essential oil and paper disc

against indicator strain, respectively (Villasenor, 2004).

$$Antimicrobial\ index\ (AI) = \frac{Db - Da}{Da} \qquad (2)$$

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of each essential oil were determined by using Resazurin Microtiter Assay Plate method with a slight modification (Rahman et al. 2004). One hundred microliter of NB was pipetted into each well in 96-well plate. Two-fold dilution was used to prepare the essential oil in different concentrations. One hundred microliter of the indicator strains was added into the mixture of essential oil. In this study, the concentration used for essential oil from *Cymbopogon citratus* Stapf. and *Cymbopogon nardus* Rendle was in the range of, 0.2-500 ppm, but the concentration used for essential oil from Citrus hystrix DC. and *Ocimum basulicum* L. was in the range of 0.6-1250 ppm. The 96-well plates were incubated at 37 C for 24 hours to obtain MIC. MIC is defined as the lowest concentration of the essential oil at which the indicator strain does not show the visible growth. After that, 30 microliter of 0.02 resazurin was added. The plates were subsequently incubated at 37 C for 24 hours to obtain MBC. MBC is defined as the lowest concentration of essential oil which inoculated bacteria were completely killed.

Results and Discussion

Percent yield of essential oil extraction

Hydro-distillation produced different yields of essential oils from four plant materials. The percent yields of essential oil ranged from 0.28-1.19 (**Table 1**). The highest and lowest percent yield were found from *Ocimum basulicum* L. (1.19%) and *Cymbopogon citratus* Stapf. (0.28%), respectively. Percent yields of essential oil extraction from Citrus hystrix DC. and *Cymbopogon nardus* Rendle were 0.87 and 1.00%, respectively. The percent yield of essential oil extraction from Citrus hystrix DC. in this study was not in agreement of the study from Laohakunjit et al. (2009), showing higher percent yield of Citrus hystrix DC. essential oil as about 2.875% when using simultaneous distillation-extraction method. The difference in percent yield of essential oil extraction may be affected by several factors such as extraction method, type and part of plant materials, cultivation site, climate, season, harvest method and post-harvest process (Laohakunjit et al., 2009). However, all the essential oil possessed strong characteristic and is distinct aromatic fragrances.

Table 1 Percent yield of essential oil extraction by hydro-distillation method

Material	Common name	Part of use	Percent yield (%, v/w)	Physical property of essential oil
Citrus hystrix DC.	kaffir lime	peel	0.87	clear solution with kaffir lime smell
Cymbopogon citratus Stapf.	lemon grass	stem	0.28	clear solution with lemon grass smell
Cymbopogon nardus Rendle	citronella grass	stem	1.00	dark yellow solution with citronella grass smell
Ocimum basulicum L.	sweet basil	leaf	1.19	light yellow solution with sweet basil smell

Antimicrobial property of essential oils

The antimicrobial property screening results from four essential oils against four common human bacteria strains, namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* Group B showed that these essential oil displayed varying degree of antimicrobial activity (**Table 2**). They could inhibit the growth of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* Group B, but their antimicrobial activity could not be observed on *Pseudomonas aeruginosa*. The *Cymbopogon citratus* Stapf. essential oil had the highest activity while the *Cymbopogon nardus* Rendle had moderate activity towards tested microorganisms. The essential oil of Citrus hystrix DC. and *Ocimum basulicum* L. showed mild inhibitory activity against microorganism tested.

Result from **Table 3** indicated that *Salmonella* Group B, gram negative bacteria, was the most sensitive bacteria as because it was successfully inhibited by all essential oils. On the other hand, *Ps. aeruginosa* was the most resistant bacteria since all essential oil showed no inhibitory effect towards it. These data were similar to the result found from the *Curcuma manga* oil which could be slightly inhibited *Ps. aeruginosa* (Kamazen et al., 2012).

To take the consideration on the antimicrobial efficiency of these four essential oil, the *Cymbopogon citratus* Stapf. essential oil showed broad range of antimicrobial activity (Fig 1). For this oil, gram-negative bacteria were more sensitive towards it than gram-positive bacteria, shown as a higher antimicrobial index (5.05, 2.22 and 1.15 for *Salmonella* Group B, *E. coli* and *S. aureus*, respectively). The essential oil from *Cymbopogon nardus* Rendle shared the similar inhibitory property, showing that it was more susceptible to gram-negative bacteria than gram-positive bacteria. Conversely, this results differed from the data from Suwanpugdee et al. (2012), indicating that the essential oil from lemon grass and citronella grass could successfully inhibit *S. aureus* (gram-positive bacteria), followed by *E. coli*. (gram-negative bacteria) Furthermore, the essential oil, obtained from Citrus hystrix DC. and *Ocimum basulicum* L. displayed the same level of inhibition on gram-positive and gram-negative bacteria.

MIC and MBC results (**Table 4**) showed MIC and MBC values of essential oil from *Cymbopogon citratus* Stapf. and *Cymbopogon nardus* Rendle against *S. aureus* were the same as 250 ppm. Likewise, MIC and MBC values of essential oil from Citrus hystrix DC. and *Cymbopogon nardus* Rendle against *Salmonella* Group B were

the same (625 and 250 ppm, respectively). The data indicated that these essential oil were inhibitory and bactericidal at a single concentration. Maximum activity of essential oil from *Cymbopogon citratus* Stapf. was observed against *Salmonella* Group B, which had the lowest MIC and MBC values of 62.5 and 125 ppm, respectively. However, its MBC value against *E. coli* was two-fold higher than MIC value (500 and 250 ppm, respectively). It implied that the tendency of susceptible microorganism for *Cymbopogon citratus* Stapf. essential oil was *Salmonella* Group B > *S. aureus* > *E. coli*. This susceptibility of indicator strain in the broth dilution assay was slightly different to that observed in the agar-disc diffusion assay. In agar-disc diffusion assay, the susceptibility of indicator strain for *Cymbopogon citratus* Stapf. essential oil was *Salmonella* Group B > *E. coli* > *S. aureus* (as shown in AI). The difference might be due to the variation in diffusibility of essential oil compounds in the agar-disc diffusion method. The inconsistency between agar-disc diffusion method and broth dilution method found in this study was similar to that reported by Rios et al. (1988), indicating that the MIC and MBC assay were more precise than the agar-disc diffusion assay to evaluate the strength of antimicrobial activity.

Comparison on the data obtained from this study with the previous results done by other research groups is a difficult as a result of many factors. Firstly, the major components, which were normally, were responsible for the antimicrobial activity of essential oils, differed and were varying due to geological areas, harvesting season, climate and extraction method (Oussalah et al., 2007). Not only the major components, but the minor components also played the important role in this activity. This data suggested that essential oils from different area might contain different chemical components, resulting in the diversity of biological property [6]. Secondly, test organisms, including the microbial growth, choice of bacteria as well as the resistance to antibiotic were also taken into the consideration. Using different type of tested microorganism may result differently. All of these factors contributed to the inhibitory effect of essential oils (Hammer et al., 1999).

Table 2 Antimicrobial activity of essential oils by hydro-distillation method

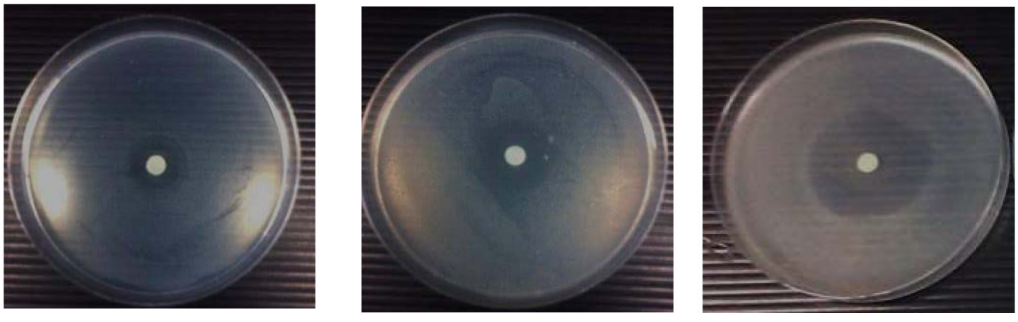
Detail of essential oil	Zone of inhibition (mm)			
	Gram-positive bacteria	Gram-negative bacteria		
Plants species	*S. aureus*	*E. coli*	*Salmonella* Group B	*Ps. aeruginosa*
Citrus hystrix DC.	11.42±0.24	11.60±0.65	11.69±0.53	-
Cymbopogon citratus Stapf.	12.88±0.46	19.35±0.23	36.28±0.20	-
Cymbopogon nardus Rendle	11.51±0.84	19.66±0.09	19.64±0.90	-
Ocimum basulicum L.	11.00±0.83	12.74±0.49	11.36±0.19	-
Distilled water	-	-	-	-
Ampicillin (50 g/disc)	20.53±0.56	19.67±0.37	20.94±0.17	18.65±0.12

Data expressed as mean of triplicate ± standard deviation (SD) including the disc diameter (6 mm). All values were rounded to two decimal places. "-" indicated no inhibition.

Table 3 Antimicrobial index of essential oils

Detail of essential oil	Antimicrobial index			
	Gram positive bacteria	Gram-negative bacteria		
Plants species	***S. aureus***	***E. coli***	***Salmonella* Group B**	***Ps. aeruginosa***
Citrus hystrix DC.	0.90	0.93	0.95	nd
Cymbopogon citratus Stapf.	1.15	2.23	5.05	nd
Cymbopogon nardus Rendle	0.92	2.28	2.27	nd
Ocimum basulicum L.	0.83	1.12	0.89	nd
Distilled water	nd	nd	nd	nd
Ampicillin (50 g/disc)	2.42	2.28	2.49	2.11

Data expressed in the ratio of antimicrobial index. All values were rounded to two decimal places. “nd” indicated not determination.



(a) (b) (c)

Figure 1 Inhibition zone of essential oil from *Cymbopogon citratus* Stapf. against common human pathogens; *S. aureus* (a, first figure), *E. coli* (b, second figure) and *Salmonella* Group B (c, third figure)

Table 4 Minimum Inhibitory concentration and Minimum bactericidal concentration of essential oils

Indicator strain	Source of essential oil							
	Citrus hystrix DC.		*Cymbopogon citratus* Stapf.		*Cymbopogon nardus* Rendle		*Ocimum basulicum* L.	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	625	1250	250	250	250	250	625	1250
E. coli	625	1250	250	500	250	500	625	1250
Salmonella Group B	625	625	62.5	125	250	250	625	1250

The unit of MIC and MBC values were expressed in ppm.

Conclusion

In summary, the *Cymbopogon citratus* Stapf. essential oil possessed the highest *in-vitro* antimicrobial activity against human pathogens. The data suggested that the essential oil of *Cymbopogon citratus* Stapf. showed a potent antimicrobial property to apply for many applications, such as pharmaceuticals, natural therapies and cosmetics. However, issues of safety and toxicity will need to be concerned and addressed

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