Antimicrobial Activity of Cinnamon, Clove and Galangal Essential Oils and Their Principal Constituents, and Possible Application in Active Packaging

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ABSTRACT

Essential oils of cinnamon (Cinnamomum iners), clove (Syzygium aromaticum Linn.) and galangal (Alpinia galangal) and their principal constituents including cinnamaldehyde, eugenol and geraniol have been investigated for their antimicrobial activity against ten pathogenic and spoilage bacteria including Aeromonas hydrophila, Bacillus cereus, Escherichia coli, Escherichia coli O1587:H7, Listeria monocytogenes, Micrococcus luteus, Pseudomonas aeroginosa, Salmonella enteridis, Staphylococcus aureus and Enterococcus faecalis and three strains of yeast including Candida albicans, Saccharomyces cerevisiae, and Zygosaccharomyces rouxii using an 5 mm in diameter agar well diffusion assay. The minimum inhibitory concentration (MIC) of these essential oils and their principal constituents were determined using an agar dilution method ranged from 0.78 to 200 μL/mL. At the concentration of 50 μL/mL, all essential oils and their principal constituents showed a zone of inhibition, ranging from 8.16 to 30.09 mm in diameter. The MICs of cinnamon, clove and galangal oils in a range of 6.25-200 μL/mL could inhibit the growth of all test microorganisms except P. aeroginosa, which was not sensitive to all essential oils at the highest concentration used (200 μL/mL) and E. coli and S. enteridis which were not sensitive to galangal oil. The MICs of cinnamaldehyde, eugenol and geraniol in a range of 0.78-100 μL/mL could inhibit the growth of all test microorganisms except S. enteridis, which was not sensitive to geraniol. The principal constituents showed a higher antimicrobial activity than those of essential oils. Cinnamaldehyde possessed the strongest antimicrobial activity whereas galangal oil did not show growth inhibition of most microorganisms. It has also been revealed that geraniol was an excellent antimicrobial against all tested yeasts. Possible application in active packaging as antimicrobial edible films has also been demonstrated.

Keywords: Antimicrobial activity; Cinnamon; Clove; Galangal; Antimicrobial edible film; Minimum inhibitory concentration (MIC)

INTRODUCTION

The appearance of foods is one of the major determinants of its appeal to consumers and consequently, sales of the product. Microbial contamination is one of the main factors that determine food quality loss and shelf-life reduction. Therefore, preventing microbial contamination is highly relevant to food processors. The growth of microorganisms in food products may cause spoilage or foodborne diseases which, in turn, contribute to the deterioration in safety, flavour, texture and colour of the products (Decker et al., 1995).

Prevention of pathogenic and spoilage microorganisms in foods is usually achieved by using chemical preservatives. These chemical preservatives act as antimicrobial compounds which inhibit the growth of undesirable microorganisms. However, the onset of increasing demand for minimally-processed, extended shelf-life foods and reports of chemical preservatives as having potential toxicity demand food manufacturers to find alternative means (Nychas, 1995). There is a currently strong debate about the safety aspects of chemical preservative since they are considered responsible for many carcinogen and teratogenic attributes as well as residual toxicity. For these reasons, consumers tend to be suspicious of chemical additives and thus the exploration of naturally occurring antimicrobials for food preservations receives increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives (Schuenzel and Harrison, 2002).

Essential oils are well known inhibitors of microorganisms (Burt, 2004). Cinnamon (*Cinnamomum iners*), is traditionally harvested in Asian countries. It is, perhaps, one of the oldest herbal medicines, having been mentioned in Chinese texts as long as 4000 years ago. Cinnamon oil has exhibited health beneficial properties, such as antimicrobial activity. Clove (*Syzygium aromaticum* Linn) is widely cultivated in Madagascar, Sri Lanka, Indonesia and the south of China. Clove bud oil has biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties. Galangal (*Alpinia galangal* Linn), one of rhizomes of some members of the Zingiberaceae family, has been extensively used as condiment for flavoring and local medicines. It is known to contain antimicrobial agents.

The objectives of this study are aimed at investigating for antimicrobial activity of cinnamon, clove and galangal essential oils and their principal constituents against several pathogenic and spoilage bacteria and yeasts and at assessing for its possible application in active packaging.

MATERIALS AND METHOD

Materials

Cinnamon, clove and galangal oils were purchased from Thai-China Flavours and Fragrances Industry Co.,Ltd. (Bangkok, Thailand). Cinnamaldehyde, eugenol and geraniol were purchased from Sigma-Aldrich Sigma-Aldrich (Singapore). Dimethyl sulfoxide (DMSO), sodium dihydrogen orthophosphate (NaH_2PO_4. $2H_2O$), di-Sodium hydrogen orthophosphate (Na_2HPO_4), and sodium chloride (NaCl) were purchased from Fluka Chemie (Buchs, Switzerland). Glycerol was purchased from Sigma-Aldrich (Singapore). Ethanol was supplied by Liquor Distillery Organization, Excise Department, Ministry of Finance (Chachoengsao, Thailand). The media used in the present studies were nutrient broth, yeast malt broth and bacteriological agar were obtained from Hi-media (India). The count plates used in the experiments were 3M Petrifilm™ aerobic count plates and 3M Petrifilm™ yeast and mould count plates. All these count plates were supplied by 3M Microbiology Products, USA.

Microbial strains and inoculum preparation

The microorganisms used in this study were obtained from the Department of Medical Sciences (DMSC) and the culture collection at Thailand Institute of Scientific and Technological Research (TISTR), Thailand. Stock cultures were stored at frozen temperature in 40 % (v/v) glycerol-either nutrient or yeast malt broth. Working bacterial culture and yeast culture were grown at 37 °C for 24 h on nutrient agar and at 30 °C for 48 h in yeast malt agar, respectively. To obtain cells in the stationary growth phase, bacterial culture and yeast culture were subcultured twice at 37 °C for 24 h on nutrient broth and at 30 °C for 48 h in yeast malt broth, respectively. Cells were harvested by centrifugation at 6,000 × g for 2 min and washed once with a 5 mM NaCl solution. The supernatant was discarded and the cells were washed again. Bacterial cells and yeast cells were re-harvested and suspended in fresh nutrient broth and yeast malt broth, respectively. Cell densities of approximately 1×10^6 CFU mL^{-1} were calculated and prepared from cultures by dilution with 0.1 M sodium phosphate buffer (pH 7.0). Cell densities were also confirmed by 3M Petrifilm™ aerobic count plates for bacteria and 3M Petrifilm™ yeast and mould count plates for yeasts.

Determination of antimicrobial activity of essential oils and their principal constituents

The antimicrobial activity was investigated using a modified agar well diffusion technique (Chung et al., 1990). Petri dishes contained 15 ml of nutrient agar for bacteria or yeast malt agar for yeasts, supplemented by test strains at a density of 1×10^6 CFU mL^{-1}. Four wells (diameter 5 mm) were made in each agar plate using a sterile cork borer. Fourty microlitres of dilution of betel oil in DMSO to give final concentration was added in each well and DMSO blank was used as control. The plates of bacteria and yeasts were incubated at 37 °C for 24 h and at 30 °C for 48 h, respectively. The zone of inhibition surrounding the tested sample well was measured as diameter (mm) using Vernier calipers.

Determination of minimum inhibitory concentrations of essential oils and their principal constituents

The agar dilution method of the European Society of Clinical Microbiology and Infectious Diseases (2000) was adopted for determination of MICs. Petri dishes contained 15 ml of nutrient agar for bacteria or yeast malt agar for yeasts, supplemented by test strains at a density of 1×10^6 CFU mL^{-1}. Four wells (diameter 5 mm) were made in each agar plate using a sterile cork borer. Betel oil was dissolved in DMSO in two-fold serial dilutions from 0.5 to 200 μL mL^{-1}. Fourty microlitres of betel oil dilutions was individually added in wells and DMSO blank was used as control. The plates of bacteria and yeasts were incubated at 37 °C for 24 h and at 30 °C for 48 h, respectively. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of betel oil that resulted in a zone of inhibition.

Antimicrobial cellulose-based film preparation

Cellulose-based solution was prepared by dissolving 10 g of cellulose ether (Methocel Premium EP, Dow Chemical, USA) in a mixed solvent (300 mL) of ethanol (Sigma-Aldrich, USA) and distilled water. A 3 g of plasticizer (Cabrowax, Union Carbide Chemical and Plastic Inc., USA) was added to the above solution to prevent brittleness, and then the solution was heated on a hot plate and stirred to 65 °C. Essential oil or its principal constituent was added into the solution. This solution was degassed by keeping in an ultrasonic water bath (Model 275D, CREST Ultrasonics Corp, Trenton, NJ, USA) for 10 min. Antimicrobial film was cast by pouring film-forming solution (10 g) in a Petri dish cover. The covers were dried at room temperature for 24 h.

Determination of antimicrobial activity of the films

The films were tested for their inhibition against the target microorganisms. The antimicrobial activity was investigated using a vapour diffusion technique. Petri dishes containing 15 mL of nutrient agar for bacteria or yeast malt agar for yeasts, were inoculated with 0.1 mL of test strains suspension (5×10^2 CFU mL^{-1}), then spread in a circular motion until all the liquid was absorbed. Each film sample underneath a cover was sterilized with UV light for 2 min prior to being placed on a Petri dish, then tightly sealed with Parafilm™ stripe. The plates of bacteria and yeasts were incubated as described in previous section. The growth of bacteria or yeast colonies was measured as diameter (mm) using Vernier calipers, in comparison with the control. The

antimicrobial index was expressed as: (diameter of clear zone – diameter of well)/diameter of well. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Determination of antimicrobial activity of essential oils and their principal constituents

Cinnamon and clove oils showed antimicrobial activity against all test strains except *Ps. aeruginosa* (Table 1). At the concentration of 50 μL mL^{-1}, cinnamon and clove oils showed a zone of inhibition, ranging from 10.45 to 25.12 mm in diameter. They yielded the biggest zone of inhibition on *S. cerevisiae* and exhibited a wide spectrum of antimicrobial activity against 9 out of 10 bacteria including Gram-positive and Gram-negative and against all yeast strains used in this study. In addition, Escherichia coli O157:H7, a pathogen, was more sensitive to cinnamon, clove and galangal oils than its non-pathogenic strain. Cinnamaldehyde and eugenol revealed antimicrobial activity against all test strains, whereas geraniol exhibited a wide spectrum against 8 out of 10 test strains (Table 2). Eugenol and geraniol yielded the biggest clear zone on *S. cerevisiae*, whereas cinnamaldehyde yielded the biggest on *L. monocytogenes*. It has also been revealed that geraniol was an excellent antimicrobial against all tested yeasts.

Table 1. Antimicrobial activity of essential oils[a]

Microorganisms	Zone of inhibition /mm		
	Cinnamon	Clove	Galangal
Gram Positive Bacteria			
Bacillus cereus	12.22±0.17	11.51±0.35	7.94±0.37
Enterococcus faecalis	10.68±0.28	10.45±0.46	8.88±0.44
Listeria monocytogenes	12.24±0.19	11.18±0.72	9.15±0.24
Micrococcus luteus	21.89±1.18	21.46±1.54	ND[b]
Staphylococcus aureus	11.31±0.42	11.09±0.25	ND
Gram Negative Bacteria			
Aeromonas hydrophila	18.50±0.48	18.82±0.41	ND
Esherichia coli	10.98±0.28	10.61±0.15	ND
Escherichia coli O157: H7	12.11±0.32	11.59±0.45	8.95±0.72
Pseudomonas aeruginosa	ND	ND	ND
Salmonella enteridis	11.56±0.24	11.22±0.27	ND
Yeast			
Candida albicans	12.06±0.27	12.11±0.50	ND
Saccharomyces cerevisiae	25.12±1.77	24.84±0.86	ND
Zygosaccharomyces rouxii	15.36±0.22	14.44±0.81	ND

[a] Essential oils with a concentration of 50 μL mL^{-1}

[b] Not detected

Table 2. Antimicrobial activity of principal constituents of essential oils[a]

Microorganisms	Zone of inhibition /mm		
	Cinnamaldehyde	Eugenol	Geraniol
Gram Positive Bacteria			
Bacillus cereus	22.95±0.26	11.44±0.77	11.09±0.33
Enterococcus faecalis	27.76±0.92	11.00±1.29	11.91±0.41
Listeria monocytogenes	30.09±0.80	11.40±0.55	11.05±0.43
Micrococcus luteus	22.35±1.39	14.96±2.35	17.01±0.54
Staphylococcus aureus	28.93±0.62	10.56±0.54	9.71±0.47
Gram Negative Bacteria			
Aeromonas hydrophila	22.27±0.95	12.79±0.63	12.05±0.46
Esherichia coli	23.45±1.30	11.42±0.42	9.81±0.46
Escherichia coli O157: H7	21.08±0.62	12.16±0.15	ND[b]
Pseudomonas aeruginosa	12.66±0.35	8.70±0.42	8.16±0.21
Salmonella enteridis	22.76±1.55	11.56±1.01	ND
Yeast			
Candida albicans	21.01±0.48	12.08±0.20	16.25±2.59
Saccharomyces cerevisiae	24.78±0.98	19.77±0.56	17.89±0.32
Zygosaccharomyces rouxii	24.18±0.79	14.08±0.42	14.03±0.35

[a] Principal constituent of essential oils with a concentration of 50 μL mL^{-1}

[b] Not detected

Determination of minimum inhibitory concentrations of essential oils and their principal constituents

The MICs of cinnamon and clove oils in a range of 6.25-25 μL mL^{-1} could inhibit the growth of all test microorganisms except *Ps. aeruginosa*, which was not sensitive to this oil at the highest concentration used (200 μL mL^{-1}) (Table 3). The MICs of galangal oil were in a range of 25-200 μL mL^{-1}. It is interesting to note that among the test microorganisms, *S. cerevisiae* was the most sensitive to cinnamon and clove oils whereas *Bacillus cereus* and *L. monocytogenes* were the most sensitive to galangal oil. Cinnamaldehyde showed the lowest MICs against all test strains, in comparison with eugenol and geraniol. *S. enteridis* was the most resistant to geraniol.

Cinnamon oil contains cinnamaldehyde, ethyl cinnamate, eugenol, β-caryophyllene, linalool and methyl chavicol. Clove oil contains eugenol, caryophyllene, furfurol, α-pinene and eugenyl acetate. Galangal oil contains geraniol, 1,8-cineole, β-bisabolene, α-pinene, β-caryophyllene, eugenol, camphor and methyl cinnamate. Cinnamaldehyde and eugenol were among the most active components against Gram-positive and Gram-negative bacteria (Dorman and Deans, 2000; Friedman et al., 2002). Mechanism of action of monoterpenes (e.g. geraniol, 1,8-cineole, pinene and limonene), sesquiterpene (e.g. caryophyllene and bisabolene), phenylpropanes (e.g. eugenol, methyl eugenol, and methyl chavicol) (Pauli, 2001) in cinnamon, clove and galangal oils should be similar to other terpenes and phenolic compounds as indicated an involvement in disruption of the cytoplasmic membrane and coagulation of cell content.

Cinnamon, clove and galangal oils could not inhibit the growth of *P. aeruginosa* (Gram-negative bacteria) due to a failure of outer membrane penetration. The resistance of this Gram-negative bacteria towards these oils is related to lipopolysaccharides in its outer membrane (Gao et al., 1999). Recently, Pasqua et al. (2006) have studied the changes in membrane fatty acids composition of microbial cells in the presence of a sublethal concentration of antimicrobial compound (e.g. thymol, carvacrol, limonene, cinnamaldehyde and eugenol) in response to a stress condition. It was found that *Pseudomonas* sp. did not show substantial changes in its fatty acid compositions. This is an indication of the high resistance of *Pseudomonas* sp. to the tested compounds.

Table 3. Minimum inhibitory concentration of essential oils and their principal constituents against test microoraganisms

Microorganisms	MIC /μL mL^{-1}					
	Cinnamon	Cinnamaldehyde	Clove	Eugenol	Galangal	Geraniol
Gram Positive Bacteria						
Bacillus cereus	12.5	3.12	12.5	12.5	25	6.25
Enterococcus faecalis	12.5	0.78	12.5	12.5	50	3.12
Listeria monocytogenes	12.5	6.25	12.5	12.5	25	6.25
Micrococcus luteus	25	6.25	25	25	200	12.5
Staphylococcus aureus	25	1.56	25	25	200	50
Gram Negative Bacteria						
Aeromonas hydrophila	12.5	0.78	12.5	12.5	200	25
Esherichia coli	25	12.5	12.5	50	> 200	50
Escherichia coli O157: H7	25	6.25	25	25	50	100
Pseudomonas aeruginosa	> 200	12.5	> 200	50	> 200	50
Salmonella enteridis	12.5	6.25	12.5	25	> 200	> 200
Yeast						
Candida albicans	25	6.25	25	25	100	3.12
Saccharomyces cerevisiae	6.25	1.56	6.25	12.5	100	3.12
Zygosaccharomyces rouxii	25	1.56	25	12.5	100	3.12

Determination of antimicrobial activity of the films

Cellulose ether films containing cinnamaldehyde were used to assess for its possible application in active packaging as antimicrobial film. It showed positive antimicrobial activity against all test strains (including *L. monocytogenes*, *S. aureus*, *E. coli* and *S. enteridis*) in a vapour diffusion test (Table 4 and Figure 1). Cinnamaldehyde-cellulose ether film was found to be strong against *L. monocytogenes* (antimicrobial index = 0.56). *S. aureus*, *E. coli* and *S. enteridis* were also prone to growth inhibition with the index of 0.45, 0.34 and 0.19, respectively. Cinnamaldehyde is known to have a broad spectrum of antimicrobial activity against a variety of microorganisms. It is noted that cellulose ether film containing cinnamamldehyde inhibited the activity of Gram positive bacteria better than that of Gram negative bacteria.

Table 4. Antimicrobial activity of cellulose ether film containing cinnamaldehyde

Microorganisms	Diameter of colony /mm		Antimicrobial index
	Control film	Film with 1 % (w/w) cinnamaldehyde	
Gram Positive Bacteria			
Listeria monocytogenes	3.54±0.17	1.57±0.16	0.56
Staphylococcus aureus	4.30±0.18	2.35±0.15	0.45
Gram Negative Bacteria			
Esherichia coli	5.52±0.44	3.64±0.23	0.34
Salmonella enteridis	3.56±0.21	2.89±0.33	0.19

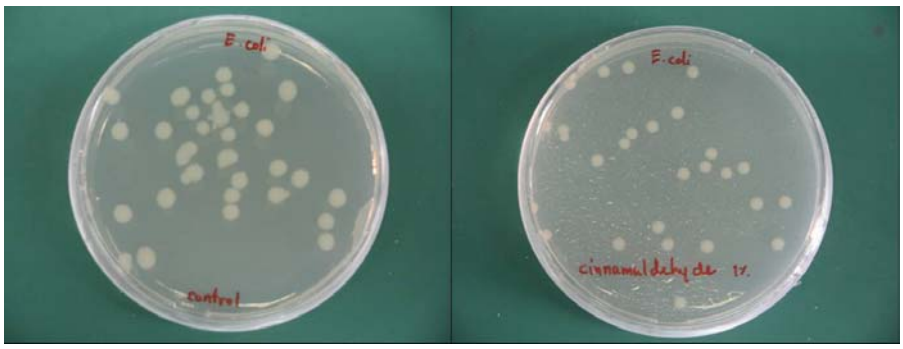

Figure 1. Growth inhibition of *Escherichia coli* by cellulose ether film containing cinnamaldehyde

CONCLUSIONS

This study revealed that cinnamon and clove oils could inhibit a wide spectrum of food pathogenic and spoilage microorganisms, whereas galangal oil showed slightly inhibitory effect against test strains. Cinnamaldehyde showed the lowest MICs against all test strains, in comparison with eugenol and geraniol. Cellulose ether films containing cinnamaldehyde demonstrated a possible application in active packaging as antimicrobial film. It showed positive antimicrobial activity against all test strains. However, further research is necessary to assess the antimicrobial activity of the film against other food pathogenic and spoilage microorganisms and the types of food that can benefit from this antimicrobial packaging material.

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