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***In vitro* Antimicrobial Evaluation of *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* Extracts as Natural Food Preservatives**

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Abstract: In the present study, antimicrobial activity of various extracts of *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* were screened against the common food borne bacteria such as *Escherichia coli*, *Salmonella enteritidis*, *Clostridium perfringens*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Bacillus cereus* and fungi such as *Saccharomyces cerevisiae*, *Hansenula anomala*, *Mucor mucedo*, *Candida albicans* using disc diffusion method. All the extracts showed significant antibacterial and antifungal properties. The methanol extracts ($100 \mu\text{g mL}^{-1}$) revealed maximum zone of inhibition ($p < 0.001$). *Zingiber officinale* and *Curcuma longa* possessed considerably greater activity than *Alpinia galanga*. These findings established the potential of the selected rhizomes of Zingiberaceae family as effective natural food preservatives.

Key words: Food preservatives, ayurveda, antibacterial, antifungal, disc diffusion

INTRODUCTION

Food is the most important basic need of man and hence preserving them is of utmost importance and is one of the oldest technologies. Food borne illness consequential from the consumption of contaminated food has been of great concern for consumers despite the use of various preservatives (Shan *et al.*, 2007). Safety researchers and regulatory bodies in food industries are constantly bothered with increase in the number of food poisoning and spoilage caused by pathogenic food borne microorganisms (Meng *et al.*, 1998). The emergence of antibiotic resistant pathogens that can amplify food borne infections is also another concern (Perrenten *et al.*, 1998; Stermitz *et al.*, 2000). The demand for food with extended shelf-life, lower level of harmful chemical preservatives and absence of risk causing food borne infections have made food processors to focus on exploring naturally occurring preservatives.

Herbs have been used in foods since ancient times, not only as folk medicine, but also as flavoring agents and food preservatives (Dillon, 1994; Cutler, 1995; Charalambous, 1994) due to their antimicrobial activity against certain pathogens (Tepe *et al.*, 2004; Erasto *et al.*, 2004; Fukai *et al.*, 2002; Puupponen *et al.*, 2001; Salie *et al.*, 1996; Xu *et al.*, 1998; Rauha *et al.*, 2000; Ahmad and Beg, 2001), antioxidative property (Beuchat and Golden, 1989; Shobana and Naidu, 2000) and wide array of medicinal values (Wood *et al.*, 2001). Herbs and

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spices are traditionally used as one of the safest and effective remedies in curing various ailments and even long-term consumption is not known to have produced any side effects (Nielsen and Rios, 2000). They do not exhibit toxicity at levels consumed and are considered as GRAS (Generally Recognized As Safe) substance (Souza *et al.*, 2005; Pandit and Shelf, 1994).

In Ayurveda, *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* belonging to the family Zingiberaceae are most commonly used rhizomes for their medicinal value. All these plants are distributed in warm tropical climates, predominantly found in Southeastern Asia and widely cultivated in India, China, Africa, Jamaica, Mexico and Hawaii (Evans, 1996).

Zingiber officinale is an aromatic plant and the rhizomes are scaly structures consisting of aromatic components, essential oil and oleoresins due to which it is used commonly in food products and beverages. *Curcuma longa* has been extensively utilized as a common household remedy and as a spice. It contains essential oil, yellow pigments, starch and oleoresin (Leung and Foster, 2003). Rhizomes of *C. longa* are utilized as carminative, anti-inflammatory and anthelmintic agents. Traditionally, the pastes from its rhizomes are applied as a remedy for wounds, bruises, inflamed joints and sprains (Eigner and Scholz, 1999). *Alpinia galanga* is also a traditionally used spice which has been comprehensively used as flavoring agent, carminative, treating diarrhea and for stomachache. For its distinctive aroma as well as strong flavor, the rhizomes of *A. galanga* are extensively used in foods of Thailand and Malaysia (Yang and Eilerman, 1999). Essential oils obtained from the rhizomes of *A. galanga* have been claimed to possess antimicrobial activities against bacteria, fungus, yeast and parasites (Farnsworth and Bunyapraphatson, 1992). Instead of these substantial findings showing wide range of potential therapeutic actions, these spices are still branded almost purely as flavoring agents.

The present study was undertaken to determine and compare the potential of various extracts of *Z. officinale*, *C. longa* and *A. galanga* as an antimicrobial agent against food borne micro organisms such as *Escherichia coli*, *Salmonella enteritidis*, *Clostridium perfringens*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Bacillus cereus*, *Saccharomyces cerevisiae*, *Hansenula anomala*, *Mucor mucedo* and *Candida albicans* as natural food preservatives.

MATERIALS AND METHODS

Preparation of Extracts

Fresh rhizomes of *Z. officinale*, *C. longa* and *A. galanga* were purchased in the month of April 2008 from a local market in Cheras, Selangor, Malaysia and were authenticated by Dr. J. Anbu Jeba Sunilson, Pharmacognosist, Masterskill University College of Health Sciences, Malaysia. The rhizomes were washed under tap water to remove the dirt and soil and sliced into smaller pieces. They were dried separately in a vacuum drier at 25°C for 24 h and size reduced to coarse powder using a cutter mill. Powders of each rhizome (500 g) were extracted separately with petroleum ether, chloroform, methanol and water by Soxhlet extraction technique successively (Sunilson *et al.*, 2009). All the extracts were concentrated using rotary vacuum evaporator and kept in dessicator until further studies.

Selection of Microorganisms

Stock cultures of six different species of food-borne bacteria such as *Escherichia coli*, *Salmonella enteritidis*, *Clostridium perfringens*, *Staphylococcus aureus*, *Campylobacter jejuni* and *Bacillus cereus* and four different species of fungi such as *Saccharomyces cerevisiae*, *Hansenula anomala*, *Mucor mucedo* and *Candida albicans* were obtained from the microbiology laboratory, Masterskill University College of Health Sciences, Selangor,

Malaysia. The food-borne pathogens were maintained separately in different stock culture for bacteria (nutrient broth) and fungi (sabouraud dextrose broth). A loop full of each culture was inoculated individually into the respective agar broth and incubated at $37\pm 1^\circ\text{C}$ for 24 h for bacteria and $28\pm 1^\circ\text{C}$ for 48 h for fungi.

Antibacterial Assay by Disc Diffusion Method

All the extracts were concentrated and dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 10 mg mL^{-1} and sterilized by filtration through $0.45\ \mu\text{m}$ millipore filters. Screening of antimicrobial activity was carried out by disc diffusion method described by Murray *et al.* (2007) using $100\ \mu\text{L}$ of suspension containing 10^8 colony forming unit (cfu) mL^{-1} of bacteria, 10^4 spore mL^{-1} of fungi spread on nutrient agar and SDA medium, respectively. Discs of 6 mm in diameter were impregnated with $10\ \mu\text{L}$ of the extracts ($300\ \mu\text{g disc}^{-1}$) at a concentration of $100\ \mu\text{g mL}^{-1}$ and placed on the inoculated agar. Ofloxacin ($10\ \mu\text{g disc}^{-1}$) and fluconazole ($10\ \mu\text{g disc}^{-1}$) were used as reference standards for the antibacterial and anti fungal activities, respectively (Karaman *et al.*, 2003; Amerjothy *et al.*, 2007). All the inoculated plates were incubated at $37\pm 1^\circ\text{C}$ for 24 h for the bacteria and at $28\pm 1^\circ\text{C}$ for 72 h for fungus. The zones of inhibition were measured for determining the antimicrobial activity and the findings were tabulated.

Statistical Analysis

Data are expressed as Mean \pm SEM of triplicates. Students's t-test was used to compare the antimicrobial activity of the extracts against the standard antimicrobial agents. All statistical analysis were conducted with SPSS software (V. 12, SPSS, USA) at significant levels of 0.05, 0.01 and 0.001 (Sunilson *et al.*, 2008).

RESULTS

The antimicrobial activity of various extracts of *Z. officinale*, *C. longa* and *A. galanga* against the food-borne microorganisms examined in the present study were assessed by the diameter of zone inhibition (Table 1-3). On comparing various extracts, methanol extract of *Z. officinale*, *C. longa* and *A. galanga* exhibited moderate ($p<0.01$) to maximum ($p<0.001$) zone of inhibition against all food-borne pathogens whereas, petroleum extract exhibited lower ($p<0.05$) zone of inhibition against *E. coli*, *S. aureus*, *M. mucedo* and *C. albicans*. The chloroform and water extracts showed moderate zone of inhibition ($p<0.01$) against *S. aureus* and *H. anomala*, respectively.

Table 1: Antimicrobial activity of various extracts of *Zingiber officinale*

Test organisms	Diameter of zone of inhibition (mm)				Reference standard
	Extract concentration ($100\ \mu\text{g mL}^{-1}$)				
	PE	CE	ME	WE	
<i>E. coli</i>	8.7 \pm 1.2*	9.5 \pm 0.8*	13.6 \pm 0.4***	12.5 \pm 1.2**	16.2 \pm 2.3*** (O)
<i>S. enteriditis</i>	8.4 \pm 0.2*	8.9 \pm 1.3*	17.3 \pm 1.2***	10.3 \pm 1.8**	14.6 \pm 1.3*** (O)
<i>C. perfringens</i>	10.4 \pm 2.1**	9.2 \pm 1.1*	15.7 \pm 0.9***	8.0 \pm 0.1*	15.7 \pm 1.2*** (O)
<i>S. aureus</i>	8.2 \pm 0.3*	12.5 \pm 1.5**	13.8 \pm 1.6***	10.6 \pm 0.4**	17.3 \pm 0.8** (O)
<i>C. jejuni</i>	9.3 \pm 1.7*	8.9 \pm 2.2*	12.3 \pm 0.8**	11.4 \pm 0.6**	10.8 \pm 0.9*** (O)
<i>B. cereus</i>	8.6 \pm 0.7*	12.4 \pm 2.3**	16.8 \pm 0.7***	11.3 \pm 0.8**	17.3 \pm 1.8*** (O)
<i>S. cerevisiae</i>	10.6 \pm 1.6**	9.8 \pm 1.4**	15.4 \pm 1.7***	9.6 \pm 2.3**	18.6 \pm 2.1*** (F)
<i>H. anomala</i>	8.7 \pm 0.8*	11.6 \pm 0.9**	16.2 \pm 1.2***	8.9 \pm 0.7**	18.6 \pm 1.9*** (F)
<i>M. mucedo</i>	8.2 \pm 1.2*	10.2 \pm 1.2**	14.9 \pm 1.7***	8.4 \pm 0.9*	16.9 \pm 0.7*** (F)
<i>C. albicans</i>	9.7 \pm 1.3*	8.1 \pm 0.3*	15.3 \pm 0.8***	14.2 \pm 1.2**	13.8 \pm 1.2** (F)

PE: Petroleum ether extract, CE: Chloroform extract, ME: Methanol extract, WE: Water extract, O: Ofloxacin ($10\ \mu\text{g disc}^{-1}$), F: Fluconazole ($10\ \mu\text{g disc}^{-1}$). Values are means of three times \pm SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, zone of inhibition of extracts against bacteria and fungus vs. the normal diameter of disc

Table 2: Antimicrobial activity of various extracts of *Curcuma longa*

Test organisms	Diameter of zone of inhibition (mm)				Reference standard
	Extract concentration (100 µg mL ⁻¹)				
	PE	CE	ME	WE	
<i>E. coli</i>	9.1±2.3*	11.3±0.6**	18.7±1.2***	10.3±3.7**	16.2±2.3*** (O)
<i>S. enteritidis</i>	10.4±1.5**	13.6±0.9**	21.2±2.3***	9.5±2.1*	14.6±1.3*** (O)
<i>C. perfringens</i>	9.8±0.3*	12.4±2.3**	12.5±0.3**	11.3±0.8*	15.7±1.2*** (O)
<i>S. aureus</i>	8.2±0.9*	10.5±1.2**	16.8±0.8***	15.3±1.3**	17.3±0.8*** (O)
<i>C. jejuni</i>	10.3±0.8**	8.7±1.6*	14.3±0.1***	9.3±1.2*	10.8±0.9** (O)
<i>B. cereus</i>	12.5±2.5**	9.8±2.3*	16.3±0.9***	13.8±1.3***	17.3±1.8*** (O)
<i>S. cerevisiae</i>	9.2±1.7*	8.8±0.9*	15.5±1.2***	16.8±3.5***	17.9±1.4*** (F)
<i>H. anomala</i>	13.4±2.6**	10.2±1.2**	16.9±2.0***	12.1±0.9**	18.1±2.2*** (F)
<i>M. mucedo</i>	8.5±0.8*	8.9±1.1*	15.2±2.4***	13.6±3.0**	19.3±2.8*** (F)
<i>C. albicans</i>	9.3±1.2*	14.8±2.3**	18.3±2.9***	12.8±2.9**	13.8±1.2** (F)

PE: Petroleum ether extract, CE: Chloroform extract, ME: Methanol extract, WE: Water extract, *O: Ofloxacin (10 µg disc⁻¹), F: Fluconazole (10 µg disc⁻¹). Values are means of three times ±SEM. *p<0.05, **p<0.01, ***p<0.001, zone of inhibition of extracts against bacteria and fungus vs. the normal diameter of disc

Table 3: Antimicrobial activity of various extracts of *Alpina galanga*

Test organisms	Diameter of zone of inhibition (mm)				Reference standard
	Extract concentration (100 µg mL ⁻¹)				
	PE	CE	ME	WE	
<i>E. coli</i>	8.3±0.3*	11.25±1.33**	15.5±1.32***	8.4±0.3*	16.2±2.3*** (O)
<i>S. enteritidis</i>	12.3±1.2**	8.30±2.4*	10.4±0.5**	8.1±0.1*	14.6±1.3*** (O)
<i>C. perfringens</i>	8.6±1.9*	10.50±0.5**	12.1±0.7**	13.4±0.2**	15.7±1.2*** (O)
<i>S. aureus</i>	8.3±0.1*	10.30±0.8**	13.4±1.5***	14.3±1.6***	17.3±0.8*** (O)
<i>C. jejuni</i>	8.8±1.0*	11.20±0.7**	12.8±1.5**	10.3±1.8**	10.8±0.9** (O)
<i>B. cereus</i>	10.8±1.3**	9.80±0.8*	11.8±2.8**	12.6±1.3**	17.3±1.8*** (O)
<i>S. cerevisiae</i>	9.6±0.8*	8.90±1.2*	13.6±3.8**	10.2±0.7**	18.2±2.3*** (F)
<i>H. anomala</i>	10.1±1.3*	10.50±1.8**	15.2±1.4***	12.1±2.6**	17.1±1.9*** (F)
<i>M. mucedo</i>	8.5±0.6*	10.20±2.1*	12.3±2.7**	10.5±2.8**	15.6±1.7*** (F)
<i>C. albicans</i>	8.8±0.6*	8.60±0.6*	13.7±1.3**	8.7±0.9*	16.3±1.2*** (F)

PE: Petroleum ether extract, CE: Chloroform extract, ME: Methanol extract, WE: Water extract, *O: Ofloxacin (10 µg disc⁻¹), F: Fluconazole (10 µg disc⁻¹). Values are means of three times ±SEM. *p<0.05, **p<0.01, ***p<0.001, zone of inhibition of extracts against bacteria and fungus vs. the normal diameter of disc

The results revealed that all the extracts had significant antibacterial activity, among which methanol extracts (100 µg mL⁻¹) exhibited maximum effect. When comparing the antimicrobial activity among different rhizomes, *Z. officinale* and *C. longa* possessed significant activity than *A. galanga* which was well comparable with reference standards ofloxacin and fluconazole.

DISCUSSION

The use of naturally occurring preservatives has gained momentum in the pharmaceutical and food industries due to their ability in prolonging the storage life of foods by preventing rancidity through their antioxidant or bacteriostatic activity (Beuchat and Golden, 1989).

Many medicinal plant extracts have been known to possess antimicrobial activity and are used for the purpose of food preservation (Shelef, 1983; Cowan, 1999; Zaika, 1988; Aktug and Karapinar, 1986). In this study, the methanol extracts of *Z. officinale*, *C. longa* and *A. galanga* displayed effective antimicrobial activity against food spoilage and food-borne

pathogens. The zone of inhibition against sensitive microorganisms was in the range of 10-21 mm. These results are in parallel with the findings of previously reported studies that methanol is a better solvent for consistent extraction of antimicrobial components from medicinal plants compared to other solvents (Eloff, 1998).

The methanol extract of *Z. officinale* showed significant ($p < 0.001$) zone of inhibition against all the microorganisms such as *E. coli*, *S. aureus*, (Archana *et al.*, 2009) *S. enteritidis*, *C. perfringens*, *B. cereus*, *S. cerevisiae*, *H. anomala*, *M. mucedo* and *C. albicans* and moderate inhibition ($p < 0.01$) against *C. jejuni*. Water extracts showed moderate inhibition ($p < 0.01$) against *E. coli*, *S. enteritidis*, *S. aureus*, *C. jejuni*, *B. cereus*, *S. cerevisiae*, *H. anomala* and *C. albicans* and less inhibition ($p < 0.05$) against *C. perfringens* and *M. mucedo*. Chloroform extract exhibited moderate ($p < 0.01$) zone of inhibition against *S. aureus*, *B. cereus*, *S. cerevisiae*, *H. anomala* and *M. mucedo* whereas petroleum ether extract exhibited lower ($p < 0.05$) zone of inhibition against all food-pathogens except *C. perfringens* and *S. cerevisiae*. *Z. officinale* is known to contain resins and volatile oils such as borneol, camphene, citral, eucalyptol, linalool, phenllandrene, zingiberine and zingiberol phenols (Ahmad *et al.*, 2008; Hirasu and Takemasa, 1998) which may be responsible for its potent antimicrobial activities.

Methanol extract of *C. longa* showed significant ($p < 0.001$) zone of inhibition against all strain of tested microorganisms except *C. perfringens* which was moderately ($p < 0.01$) inhibited, followed by water extract that revealed significant inhibition ($p < 0.001$) against *S. aureus*, *B. cereus* and *S. cerevisiae* and moderate ($p < 0.01$) zone of inhibition against *E. coli*, *C. perfringens*, *C. albicans*, *H. anomala* and *M. mucedo*. The chloroform extract of *C. longa* revealed moderate ($p < 0.01$) inhibition against all micro organisms except *C. jejuni*, *B. cereus*, *S. cerevisiae* and *M. mucedo* which displayed lower susceptibility. Petroleum ether extract exhibited moderate ($p < 0.001$) zone of inhibition against *S. enteritidis*, *C. jejuni*, *B. cereus* and *H. anomala*. Also, previous scientific studies on *C. longa* reported that ethanol and chloroform extract possess potent antifungal activity (Misra and Sahu, 1971). The antibacterial activity of various extracts of *C. longa* may be attributed to the presence of active ingredients p-tolymethyl-carbinol, curcumin (Lutomoski *et al.*, 1974; Ramprasad and Sirsi, 1956; Huhtanen, 1980) and essential oils (Banerjee and Nigam, 1978).

All the extracts of *A. galanga* displayed moderate ($p < 0.01$) to lower zone ($p < 0.05$) of inhibition except methanol extract which showed significant ($p < 0.001$) inhibition against *E. coli*, *C. perfringens*, *S. aureus* and *H. anomala*. *Alpinia galanga* contains volatile oils, resins, galangol, kaempferid, galangin, alpinin and starch. The major constituents of its essential oils are β -farnesene, myrcene and 1, 8 cineole, β -bisabolene, β -caryophyllene and β -selinene (Kubota *et al.*, 1999) which may be responsible for its antibacterial and antifungal activities.

Generally, the high concentrations of phenolic compounds in these natural products accounts for their antioxidant property (Burt, 2004; Lin *et al.*, 2005; Delaquis *et al.*, 2002; Nevas *et al.*, 2004). Addition to the enormous scientific studies reported by several researchers on the antimicrobial activities of these herbs, there is also enough documented data suggesting the high positive correlation between antimicrobial activity, total phenolic content and antioxidant property (Shan *et al.*, 2005; Wu *et al.*, 2006; Kudo *et al.*, 2004). Several studies have reported on the antioxidant property of *Z. officinale* (Dhuley, 1999; Shobana and Naidu, 2000; Halvorsen *et al.*, 2002), *C. longa* (Jay, 2006; Cai *et al.*, 2004) and *A. galanga* (Poh and Hasim, 2000; Juntachote and Berghofer, 2005).

CONCLUSION

The results of the present study reveals that all the selected species of Zingiberaceae possess potent antimicrobial activity against selected food borne pathogens which might be due to the presence of phenolic compounds and is well substantiated with the evidence of previously documented study. Hence, due to its strong antimicrobial activity imparting extended shelf-life with less harmful effects, they can be used as natural preservatives with considerable market prospects in the food industry.

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