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In *vitro* Antimicrobial Activities of Aqueous and Ethanolic Aromatic Herb Extracts against Pathogenic Microorganisms

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Abstract. Selected aromatic herbs were extracted using water and ethanol, and were tested for their inhibitory activity against ten pathogenic microorganisms using Disc Diffusion Assay. The aqueous *E.elatior* extracts demonstrating the highest antibacterial activity against Gram-positive and Gram-negative bacteria tested at 1.25 and 2.5 mg/disc concentration. The Minimum Inhibitory Concentration (MIC) values ranged from 12.5-75 mg/mL, while Minimum Bactericidal Concentration (MBC) / Minimum Fungicidal Concentration (MFC) values are 50 mg/mL.

Keywords: Aromatic herbs, antimicrobial, MIC, MBC, MFC

1. Introduction

Food infection and intoxication are considered as the most common causes of foodborne diseases and still a concern for both consumers and the food industry. The growing number of foodborne diseases caused by some pathogenic and spoilage microorganisms in foods. The uses of plants in the indigenous cultures of developing countries are numerous and diverse. Various plants and their constituents have been recognized by their medicinal value for the purpose of food preservations. Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs. Their bioactive compounds referred as phytochemicals are gaining lot of interest due to their functional properties. Plants are rich in a variety of secondary metabolite such as tannins, terpenoids, alkaloids, flavonoids and phenols [1]. The majority of the active compound is phenolic compounds such as flavonoids possessed antioxidant properties and wide range of biological activities like antimicrobial and anti-allergic [2,3].

To our knowledge, most of the previous work focused mainly on the antioxidant activity of Malaysian herbs as compared to the antimicrobial activity. Hence, this study was designed to assess the effectiveness of different solvent extracts of three different Malaysian edible herbs in toward the growth inhibition of gram-positive, gram-negative bacteria and fungus. The analyses include the determination of minimum inhibitory concentration and minimum bactericidal/fungicidal concentration.

2. Materials and Methods

2.1 Plant Materials

Three aromatic Malaysian herbs used in this study were *Citrus hystrix* DC, *Etlingera elatior* (Jack) R.M. Sm. and *Cymbopogon citratus* Stapf. were collected from Kuala Selangor, Selangor, Malaysia. The herb was authenticated by a botanist from Unit of Biodiversity, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The vouncher specimens for each herb were preserved under the reference number fromSK 2032/12 to SK 2034/12 at the herbarium of Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

2.2 Extraction of Plant Materials

The fresh samples were cleaned, dried using cabinet dryer (Vission Scientific) until constant weight and ground into fine powder using ultra centrifugal mill (Restch, zm 200) to a uniform size of 0.5 mm. All herb samples were extracted using water and ethanol as solvents. The samples were weighed and boiled using distilled water in a ratio of 1:30 (herb:water) for aqueous extraction. However, for ethanolic extraction, the samples were soaked and stirred in 95% ethanol in a ratio of 1:20 (herb:ethanol). Then, the samples were filtered using Whatman No. 41 paper for both extractions. The samples were evaporated using a rotary evaporator (BUCHI) at 60 ^{o}C for aqueous extraction and 50 ^{o}C for ethanolic extraction. The viscous samples were dried using freeze drier (Christ Martin, alpha 1-4 LD plus). Both crude extracts were stored at -20 ^{o}C for further analysis.

2.3 Test Microorganisms

Ten pathogenic and spoilage microorganisms strains used in this study including *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 43300), *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus species* (ATCC 700405), *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 14028), *Proteus vulgaris* (ATCC 6380), *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404). All of the test microorganisms were obtained from the Department of Microbiology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia. Stock cultures were maintained at 4 ^{o}C on nutrient agar/potato dextrose agar.

2.4 Antimicrobial Activity (Disc Diffusion Assay)

Disc diffusion assay was used to determine antimicrobial activity of herbal extracts against ten microorganisms according to Mackeen *et al.* [4]. The antimicrobial activities were evaluated by measuring the diameter of inhibition zone of the tested bacteria in milimeters. Three replicates were prepared for each extract in this study.

2.5 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The minimum inhibitory concentration (MIC) determination of the extracts was performed by a serial broth dilution technique [3]. The lowest concentration of herb extracts that will inhibit the visible growth of microorganisms after incubation is known as MIC. To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto new sterile nutrient agar or potato dextrose agar by streaking. Nutrient agar or potato dextrose agar plates only were also streaked with the respective test organisms to serve as controls. All the plates were then incubated at 37±2 ^{o}C for 24 h (bacteria) or 30±2 ^{o}C for 72 h (fungi). After incubation the concentration at which no visible growth was seen was noted as the Minimum Bactericidal/Fungicidal Concentration (MBC/MFC).

2.6 Statistical Analysis

All analyses were run in triplicates. Data were analysed by the Windows SAS program (Version 9.0, 2009). Data were expressed as mean ± standard deviation using ANOVA. Differences were considered statistically significant if $P<0.05$.

3. Results and Discussion

3.1. Antimicrobial Activity

In the present study, ten food borne pathogens and spoilage microorganisms were tested for their sensitivity to different extracts of aromatic herbs. Antimicrobial activity (assessed in terms of inhibition zone) of the herbs extracts tested against selected microorganisms were shown in Table 1.

This study showed increasing concentration of herb extracts in discs, the growth inhibition has also been increased. In the present study, among 6 extracts of aromatic herbs tested for their bioactivity, 4 herb extracts inhibited the growth of selected pathogenic and spoilage microorganisms. Aqueous *E.elatior* extracts exhibited the highest antibacterial activity. It was the extract that showed high activity against four of Gram-positive and three of Gram-negative bacteria tested at 1.25 and 2.5 mg/disc concentration. The ethanolic

C.hystrix extracts exhibited activity against *B.subtilis* at all concentrations. *A.niger* showed sensitivity to ethanolic *C.citratus* extracts, while no zone of inhibition was observed for the other 2 extracts.

Table 1: Diameter of inhibition zone of aqueous and ethanolic aromatic herb extracts against the microorganisms based on disc diffusion method

Extracts	Samples	Conc. (mg/ disc)	Diameter of inhibition zone (mm)									
			Gram-positive bacteria				Gram-negative bacteria				Fungi	
			BS	*SA*	*SE*	*MS*	*EC*	*PA*	*ST*	*PV*	*CA*	*AN*
Aqueous	*C.hystrix*	2.5	-	-	-	-	-	-	-	-	-	-
		1.25	-	-	-	-	-	-	-	-	-	-
		0.625	-	-	-	-	-	-	-	-	-	-
	E.elatior	2.5	8.0 ±0.0	10.0 ±1.1	14.0 ±1.6	18.0 ±1.5	8.0 ±0.8	9.0 ±0.9	-	11.0 ±1.1	-	-
		1.25	-	8.0 ±0.0. 9	9.0 ±0.5	9.0 ±0.8	6.5 ±0.0	8.0 ±0.9	-	10.0 ±0.8	-	-
		0.625	-	-	-	-	-	-	-	-	-	-
	C.citratus	2.5	-	-	-	-	-	-	-	-	-	-
		1.25	-	-	-	-	-	-	-	-	-	-
		0.625	-	-	-	-	-	-	-	-	-	-
Ethanolic	*C.hystrix*	2.5	11.0 ±0.8	-	-	-	-	-	-	-	-	-
		1.25	11.0 ±1.0	-	-	-	-	-	-	-	-	-
		0.625	11.0 ±2.0	-	-	-	-	-	-	-	-	-
	E.elatior	2.5	11.7 ±2.1	-	10 ±1.2	11.0 ±1.3	-	-	-	-	-	-
		1.25	-	-	7.0 ±1.0	-	-	-	-	-	-	-
		0.625	-	-	-	-	-	-	-	-	-	-
	C.citratus	2.5	-	-	-	-	-	-	-	-	-	12 ±0.1
		1.25	-	-	-	-	-	-	-	-	-	-
		0.625	-	-	-	-	-	-	-	-	-	-
Standard	Gentamicin	0.03	25.7 ±0.6	19.0 ±3.1	31.0 ±1.5	22.3 ±2.1	20.0 ±2. 0	18.3 ±2.7	21.3 ±2.7	11.3 ±2.5	-	-
Standard	Nystatin	0.10	-	-	-	-	-	-	-	-	13.0 ±0. 5	12.0 ±1. 5
	Water	-	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-	-

Values are expressed as mean ± standard deviation (n=3). " – ", no inhibition zone. BS=*Bacillus subtilis* (ATCC 6633), SA=*Staphylococcus aureus* (ATCC 43300), SE=*Staphylococcus epidermidis* (ATCC 12228), MS=*Micrococcus species* (ATCC 700405), EC=*Escherichia coli* (ATCC 11229), PA=*Pseudomonas aeruginosa* (ATCC 27853), ST=*Salmonella typhimurium* (ATCC 14028), PV=*Proteus vulgaris* (ATCC 6380), CA=*Candida albicans* (ATCC 10231) and AN=*Aspergillus niger* (ATCC 16404).

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of aqueous and ethanolic aromatic herb extracts

Herbs	Extract	MIC/MBC/ MFC	MIC/MBC/MFC (mg/mL)									
			Gram-positive bacteria				Gram-negative bacteria				Fungi	
			BS	*SA*	*SE*	*MS*	*EC*	*PA*	*ST*	*PV*	*CA*	*AN*
C.hystrix	Aqueous	MIC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		MBC/MFC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Ethanol	MIC	12.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
		MBC/MFC	++	ND	ND	ND	ND	ND	ND	ND	ND	ND
E.elatior	Aqueous	MIC	75	50	50	50	50	50	ND	50	ND	ND
		MBC/MFC	++	++	++	++	++	++	ND	50	ND	ND
	Ethanol	MIC	75	ND	50	75	ND	ND	ND	ND	ND	ND
		MBC/MFC	++	ND	50	++	ND	ND	ND	ND	ND	ND
C.citratus	Aqueous	MIC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		MBC/MFC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Ethanol	MIC	ND	ND	ND	ND	ND	ND	ND	ND	ND	100
		MBC/MFC	ND	ND	ND	ND	ND	ND	ND	ND	ND	++

++= profuse growth, ND = not determined. BS=*Bacillus subtilis* (ATCC 6633), SA=*Staphylococcus aureus* (ATCC 43300), SE=*Staphylococcus epidermidis* (ATCC 12228), MS=*Micrococcus species* (ATCC 700405), EC=*Escherichia coli* (ATCC 11229), PA=*Pseudomonas aeruginosa* (ATCC 27853), ST=*Salmonella typhimurium* (ATCC 14028), PV=*Proteus vulgaris* (ATCC 6380), CA=*Candida albicans* (ATCC 10231) and AN=*Aspergilus niger* (ATCC 16404).

The results of MIC and MBC/MFC for both herb extracts against pathogenic and spoilage microorganisms are listed in Table 2 which were expressed in mg/mL. The MIC values were ranging from 12.5-100 mg/mL. The MIC value was maximum for *A.niger*, therefore this fungus is least effective. The lowest value was for *B.subtilis* which shows that the ethanolic *E.elatior* extracts were more effective against

B.subtilis. The MBC/MFC values were 50 mg/mL. The MIC value of the seven active herb extracts obtained in this study were lower than the MBC values suggesting that the plant extracts were bacteriostatic at lower concentration and bactericidal at higher concentration [5].

Based on the results obtained from disc diffusion, MIC and MBC/MFC, it can be suggested that in comparison with aqueous and ethanolic extracts, the organic extracts were more effective and consistent activity than aqueous extracts except for *E.elatior* extracts. This may be due to the better solubility of the active components in organic solvents and the active substances were present in aqueous extracts at concentrations at which bioactivity was not longer detectable [6]. According to Ahmad *et al.*, [7], alcoholic extracts was found to be a better solvent for extraction of antimicrobial active substances compared to water. In this finding, the extracts inhibiting the growth of the bacteria was better than inhibiting the growth of fungi. According to Oladunmoye [8], this might be due to ability of fungi to produce extracellular enzymes in which the enzymes are known to be degradative in nature. Besides, due to the fungi eukaryotic nature which more complex cell wall with rigidity and less permeability than the thin cell wall membrane of bacteria.

4. Conclusion

This work has revealed further potential of herb in the area of pharmacology as an antimicrobial agent. As a result of the high antimicrobial activity value, *Etlingera elatior* (Jack) R.M. Sm. extracts is considered a safe antimicrobial agent. Further studies should be conducted on the identification of bioactive compound to human cell line culture of antimicrobial effect.

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6. References

[1] F.O. Ekundayo, C.A. Adeboye and E.A. Ekundayo. Antimicrobial activities and phytochemical screening of pignut (*Jatrophas curcas* Linn.) on some pathogenic bacteria. *J. Med. Plants Res.* 2011, 5(7): 1261-1264.

[2] P. Hodek, P. Trefil and M. Stiborova. Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. *Chemico-Biological Interactions,* 2002, 139: 1-21.

[3] J.H. Doughari, A.M. Elmahmood and S. Manzara. Studies on the antibacterial activity of root extracts of *Carica papaya* L. *African J.Microb. Res.* 2007, 037-041.

[4] M.M. Mackeen, A.M. Ali, S.H. El-Sharkawy, K.M. Salleh, N.H. Lajis and K. Kawazu. Antimicrobial and cytotoxic properties of some Malaysian traditional vegetables. *Int. J. Pharmacognosy*, 1997, 35: 174-178.

[5] O.A. Kabir, O. Olukayode, E.O. Chidi, C.I. Christopher and A.F. Kehinde. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *BMC Complementary and Alternative Med*. 2005, 5(6).

[6] H.J. De Boer, A. Kool, A. Broberg, W.R Mziray, I. Hedberg and J.J. Levenfors. Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. *J. Ethnopharmacol.* 2005, 96: 461-469.

[7] I. Ahmad, Z. Mehmood and F. Mohammad. Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethnopharmacol.* 1998, 62: 183–193.

[8] M.K. Oladunmoye. Comparative evaluation of antimicrobial activities and phytochemical screening of two varieties of *Acalypha wilkesiana. Int. J. Tropical Med.* 2006, 1(3): 134-136.