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Antimicrobial Activity of Some Medicinal Plants from Malaysia

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Abstract: Problem statement: About 32 extracts from eight selected medicinal plants, namely *Pereskia bleo*, *Pereskia grandifolia*, *Curcuma aeruginosa Roxb.*, *Curcuma zedoria*, *Curcuma mangga*, *Curcuma inodora aff. Blatter*, *Zingiber officinale var. officinale* (jahe gajah) and *Zingiber officinale var. rubrum* (jahe emprit) used by Malaysia traditional health care systems were screened for their antimicrobial activity against both Gram-positive bacteria and Gram-negative bacteria using agar disc diffusion assay. **Approach:** The efficacy of the extracts was compared to the commercially prepared antibiotic diffusion discs. **Results:** No inhibition was observed with the water fractions. **Conclusion/Recommendations:** None of the plants tested showed inhibition against *Escherichia coli*. *Curcuma mangga* showed some remarked inhibition against the bacteria used in this study.

Key words: Antimicrobial activity, agar disc diffusion assay, Malaysia medicinal plants

INTRODUCTION

Natural products perform various functions, and many of them have interesting and useful biological activities[3]. There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose. In Peninsular Malaysia, 1,200 species of higher plants and 2,000 species in Sabah and Sarawak are reported to have medicinal value and have been used for generations in various traditional health care systems[5]. Researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against cancer, as well as viral and microbial infections[3,4,15]

This study reports a screening programme of 32 methanolic extracts and from eight medicinal plants in Malaysia for their antimicrobial properties against two Gram-positive bacteria and two Gram-negative bacteria. These plants included two families namely Cactaceae (*Pereskia bleo* and *Pereskia grandifolia*) and Zingiberaceae (*Curcuma aeruginosa Roxb.*, *Curcuma zedoria*, *Curcuma mangga*, *Curcuma inodora aff. Blatter*, *Zingiber officinale* var. *officinale* and *Zingiber officinale* var. *rubrum*). There were no previous reports of antimicrobial study on *Pereskia bleo, Pereskia grandifolia, Curcuma mangga* and *Curcuma inodora aff. Blatter.*

There were numerous antimicrobial studies conducted on both essential oils and extracts of common ginger (*Zingiber officinale* var. *officinale*). However, there is no report on the antimicrobial activity of the variants of *Zingiber officinale* such as jahe emprit (*Zingiber officinale* var. *rubrum*) and jahe gajah (*Zingiber officinale* var. *officinale*). Sofia *et al.*[14] reported that the ginger extract showed insignificant antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*. In 2005, Lopez *et al.*[7]. reported that essential oil of ginger showed weakest inhibition against selected bacteria and fungi whilst Rath *et al.*[13]. reported that essential oil of ginger did not show any inhibition on the tested pathogens in their study.

Extracts of *Curcuma aeruginosa* obtained from supercritical fluid extraction have shown negligible inhibition activity against Gram negative bacteria *Escherichia coli* and yeast *Malassezia furfur*[8,9]. from Vietnam, however, isolated sesquiterpene constituents from the petroleum ether extract of *Curcuma aeruginosa* and found that these compounds have a broad spectrum of antimicrobial activity. There are a number of papers reported the antimicrobial activity of the essential oil of *Curcuma zedoaria* against Gram positive and negative pathogenic microorganism[2,6,12,16] reported that petroleum ether, hexane, chloroform, acetone and ethanolic extracts of *Curcuma zedoaria*

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exhibited antibacterial and antifungal activity whilst Phan *et al*.[8] isolated sesquiterpene constituents from the petroleum ether extract of *Curcuma zedoaria* which showed active inhibition against *Candida albicans*.

MATERIALS AND METHODS

Plant material: Eight traditional medicinal plants used in this study were *Pereskia bleo*, *Pereskia grandifolia*, *Curcuma aeruginosa Roxb.*, *Curcuma zedoria*, *Curcuma mangga*, *Curcuma inodora aff. Blatter*, *Zingiber officinale var. officinale* and *Zingiber officinale var. rubrum*. These medicinal plants were chosen based on their traditional medicinal use and reported biological activities. The fresh leaves of *Pereskia bleo* and *Pereskia grandifolia* were collected from Petaling Jaya, Selangor, Malaysia in May 2007. The rhizomes of *Curcuma aeruginosa*, *Curcuma mangga*, *Zingiber officinale var. officinale* and *Zingiber officinale var. rubrum* were obtained from Jogjakarta, Indonesia in 2006. Whereas both rhizomes of *Curcuma zedoaria* and *Curcuma inodora* were obtained from MARDI Kluang, Johor, Malaysia in January 2007. They were identified by Professor Dr. Halijah Ibrahim of Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia and voucher specimens were deposited at the herbarium of the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

Extraction of plant material: The fresh samples were washed, dried and ground to fine powders using a blender. The dried, ground samples were then soaked in methanol (1.5 L) for 3 days at room temperature. The solvent-containing extracts were then decanted and filtered. The extractions of the ground samples were further repeated (2x) with methanol (1.5 L each time). The filtrate from each extraction was combined and the excess solvent was evaporated under reduced pressure using a rotary evaporator to give crude methanol extracts. The methanol extracts were further extracted with hexane to give hexane-soluble fractions and hexane insoluble residues. The hexane-insoluble residues were further partitioned between ethyl acetate-water (ratio 1:1) to give ethyl acetate-soluble fractions. The water layers were freeze-dried to give water fractions. All the extracts and fractions were stored at 4°C for determination of antibacterial activity.

Test microorganisms and microbial culture: Four bacterial strains were used in this study: Gram negative bacteria; *Escherichia coli*, *Pseudomonas aeruginosa*, Gram positive bacteria; *Staphylococcus aureus*, *Bacillus subtilis*. The test microorganisms were obtained from the Microbiology Laboratory, Microbiology Division, Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia. Bacterial strains were cultivated at 37°C and maintained on nutrient agar (Difco, USA) slant at 4°C.

Antimicrobial activity assay: Antimicrobial activity was determined against four bacterial pathogens by the agar disc diffusion assay (NCCLS (National Committee for Clinical Laboratory Standards), 2005). The crude methanol and fractionated extracts were dissolved in Dimethyl Sulfoxide (DMSO) with the exception of the water fraction and then antimicrobial effect of crude methanol and fractionated extracts were tested using two different concentrations. Petri dishes (measuring 90 mm each side) containing 20 mL of mueller hinton agar (OXOID). At the same time, 6 mm diameter sterile Whatman Antibiotic disc were placed on the surface of the inoculated agar plates, and then appropriate concentration of the extracts in DMSO and water were applied onto the discs, 50 and 500 mg final concentrations were obtained for each discs. The plates were incubated at 37°C for 16-18 h. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs. Standard discs of the antibiotic gentamycin (10 μg) and ampicilin (10 μg) served as the positive antibacterial controls. Negative controls were done using paper discs loaded with 20 μL of DMSO and water. After that, the diameter of inhibition zone was measured in millimeters by Vernier Calipers. All tests were repeated three times to minimize test error. An inhibition zone of 14 mm or greater (including diameter of the disc) was considered as high antibacterial activity[11].

RESULTS AND DISCUSSION

This study reports the antimicrobial activity of 32 extracts from eight selected medicinal plants in Malaysia against two Gram positive bacteria and two Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. The results of the antimicrobial activity of the investigated extracts are shown in Table 1. None of the extracts showed activity against *Escherichia coli*. All the water fractions of the eight selected plants showed no inhibition against all the bacteria tested in this study. Generally, among the investigated extracts the ethyl acetate fractions exhibited the highest antibacterial effect followed by the methanol extracts.

Table 1: Results of the antimicrobial tests of the investigated plants in agar diffusion assay

			Inhibition zone (mm)[a] against			
Plant species	Extracts /fractions	Concentration (mg mL^{-1})	*E. c*	*P. a*	*S. a*	*B. s*
Pereskia bleo	Methanol	50	-	8.3	-	-
		500	-	9.8	-	-
	Hexane	50	-	-	-	-
		500	-	9.5	-	8.2
	Ethyl acetate	50	-	7.3	-	-
		500	-	8.5	-	7.8
	Water	50	-	-	-	-
		500	-	-	-	-
Pereskia grandifolia	Methanol	50	-	-	-	-
		500	-	-	-	-
	Hexane	50	-	-	-	-
		500	-	-	-	-
	Ethyl acetate	50	-	-	-	-
		500	-	8.0	9.2	8.5
	Water	50	-	-	-	-
		500	-	-	-	-
Curcuma aeruginosa Roxb	Methanol	50	-	-	-	-
		500	-	7.0	-	-
	Hexane	50	-	7.2	-	-
		500	-	7.5	7.5	-
	Ethyl acetate	50	-	-	-	7.0
		500	-	7.8	6.7	9.0
	Water	50	-	-	-	-
		500	-	-	-	-
Curcuma zedoaria	Methanol	50	-	-	-	-
		500	-	7.0	-	-
	Hexane	50	-	-	7.5	-
		500	-	7.7	8.5	8.5
	Ethyl acetate	50	-	-	-	-
		500	-	-	-	8.2
	Water	50	-	-	-	-
		500	-	-	-	-
Curcuma mangga	Methanol	50	-	7.2	7.5	9.3
		500	-	13.0	10.5	19.3
	Hexane	50	-	8.5	7.7	11.3
		500	-	15.0	9.5	13.5
	Ethyl acetate	50	-	-	7.0	8.7
		500	-	11.5	9.0	13.7
	Water	50	-	-	-	-
		500	-	-	-	-
Curcuma inodora aff. Blatter	Methanol	50	-	-	-	7.3
		500	-	7.8	8.3	8.0
	Hexane	50	-	-	-	-
		500	-	7.7	6.7	-
	Ethyl acetate	50	-	-	-	7.5
		500	-	7.8	10.0	9.0
	Water	50	-	-	-	-
		500	-	-	-	-
Zingiber officinale var. rubrum	Methanol	50	-	-	-	-
		500	-	-	-	-
	Hexane	50	-	-	-	-
		500	-	-	-	-
	Ethyl acetate	50	-	-	-	-
		500	-	-	-	7.5
	Water	50	-	-	-	-
		500	-	-	-	-
Zingiber officinale var. officinale	Methanol	50	-	-	-	-
		500	-	7.2	-	7.3
	Hexane	50	-	-	-	-
		500	-	-	-	-
	Ethyl acetate	50	-	-	-	-
		500	-	-	7.3	7.8
	Water	50	-	-	-	-
		500	-	-	-	-
Gentamycin, 10 µg/disc			20.7	18.0	22.0	19.0
Ampicilin, 10 µg/disc			NT	32.5	37.0	38.5

E.c: *Escherichia coli*; *P.a*: *Pseudomonas aeruginosa*; *S.a*: *Staphylococcus aureus*; *B.s*: *Bacillus subtilis*. -: no activity; NT: Not Tested, Negative controls did not show any activity, [a] Inhibition zones including the diameter of the paper disc (6 mm)

The most pronounced activity with inhibition zones of more than 14.0 mm was shown by methanol extract (inhibition zone 19.3 mm against *Bacillus subtilis* at concentration 500mg mL^{-1}) and hexane fraction (inhibition zone 15.0 mm against *Pseudomonas aeruginosa* at concentration 500mg mL^{-1}) of *Curcuma mangga*. In addition, the methanol extract of *Curcuma mangga* had a remarked sensitivity towards *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with inhibition zones 13.0 and 10.5 mm at concentration 500 mg mL^{-1} respectively. The hexane fraction of *Curcuma mangga* also showed significant antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* with inhibition zones 9.5 and 13.5 mm at concentration 500 mg mL^{-1}respectively whilst the ethyl acetate fraction showed inhibition against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* with inhibition zones 11.5, 9.0, 13.7 mm respectively at concentration 500 mg mL^{-1}. When the concentration of the extracts were decreased from 500-50 mg $mL^{-1,}$ slight decrease in inhibition zones were observed. A recent phytochemical study of *Curcuma mangga* revealed the presence of labdane-type diterpene compounds and these compounds are similar to those that have been reported to possess strong antimicrobial activity against Gram positive, Gram negative bacteria and pathogenic fungi[1,10]. It is likely that the presence of this type of compounds may have contributed to the antimicrobial activity of *Curcuma mangga*.

At concentration 500 mg mL^{-1}, the methanol, hexane and ethyl acetate extracts of *Curcuma inodora* showed inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The methanol and ethyl acetate extracts of *Curcuma inodora* also showed modest inhibition against *Bacillus subtilis* at both concentrations of 500 and 50 mg mL^{-1}.

The methanol, hexane and ethyl acetate extracts of *Pereskia bleo*, at the concentration of 500 mg mL^{-1}, exhibited modest inhibition against *Pseudomonas aeruginosa* at 9.8, 9.5 and 8.5 mm, respectively. When the concentrations of these three extracts are lowered to 50 mg mL^{-1}, a slight decline in the inhibition zone were shown by the methanol and ethyl acetate extracts whilst the hexane extract showed no inhibition at all (Table 1). The hexane and ethyl acetate extracts of *Pereskia bleo*, at the concentration of 500 mg mL^{-1}, also showed modest inhibition against *Bacillus subtilis* at 8.2 and 7.8 mm, respectively. However, only the ethyl acetate extract of *Pereskia grandifolia* showed some antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* at concentration of 500 mg mL^{-1}.

Antimicrobial activity of jahe gajah (*Zingiber officinale* var. *officinale*) showed no inhibition against all the bacteria used in this study except a small inhibition zone of 7.5 mm against *Bacillus subtilis* at concentration 500 mg mL^{-1}. The methanol extract of jahe emprit (*Zingiber officinale* var. *rubrum*) showed inhibition against *Pseudomonas aeruginosa* and *Bacillus subtilis* whilst its ethyl acetate extract inhibited the growth of *Staphylococcus aureus* and *Bacillus subtilis* at concentration of 500 mg mL^{-1} (Table 1).

At concentration of 500 mg mL^{-1}, the methanolic extract of *Curcuma zedoaria* exhibited antimicrobial activity against *Pseudomonas aeruginosa* whilst the ethyl acetate extract of *Curcuma zedoaria* showed antimicrobial activity against *Bacillus subtilis*. The hexane extract of *Curcuma zedoaria*, however, is observed to inhibit the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* at concentration of 500 mg mL^{-1}. There is a slight decrease in inhibition of *Staphylococcus aureus* by the hexane extract of *Curcuma zedoaria* concentration of 50 mg mL^{-1}.

The ethyl acetate extract of *Curcuma aeruginosa* showed inhibition against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* at concentration of 500 mg mL^{-1} while at 50 mg mL^{-1}, its ethyl acetate extract showed inhibition against *Bacillus subtilis*. Both hexane and methanolic extract of *Curcuma aeruginosa* showed antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with inhibition zones of 7.5 mm each, at concentration of 500 mg mL^{-1}. The hexane extract of *Curcuma aeruginosa* also showed antimicrobial activity against *Pseudomonas aeruginosa* with inhibition zone of 7.2 mm at concentration of 50 mg mL^{-1}.

CONCLUSION

Curcuma mangga exhibit some degree of antibacterial activity towards *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. Thus, it shows that some of the medicinal plants used in traditional medicine are potentially effective antimicrobial agents. None of the plants tested in this study inhibited the growth of *Escherichia coli*. Investigation of the antimicrobial compounds in *Curcuma mangga* is now underway. The resulting information will contribute to a better understanding of the antimicrobial activity of the plant.

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