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In vitro Antibacterial Activity of *Averrhoa bilimbi* L. Leaves and Fruits Extracts

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Abstract: The present study was carried out to determine the *in vitro* antibacterial activity of aqueous and chloroform extracts of the leaves and fruits of *Averrhoa bilimbi* L. using the standard disc diffusion test. The air-dried leaves and fruits of *A. bilimbi* were separately soaked in distilled water or chloroform (1:10; wv^{-1}) for 24 h and the resultant supernatants were filtered. The aqueous extract was freeze-dried while the chloroform extract was evaporated to dryness under reduce pressure to obtained the crude dried extracts, which were prepared in the concentration of 25, 50 and 100 mg mL^{-1} prior to the antibacterial assay. The results obtained demonstrated the potential of *A. bilimbi* extracts against some of the Gram-positive and-negative bacteria. The 100 mg mL^{-1} aqueous extract of *A. bilimbi*'s leaves and fruits showed positive antibacterial activity against the Gram-positive *S. aureus, S. epidermis B. cereus, C. diphteriae* with the latter also gave positive effect against *K. rhizophila*. Both extracts also exhibited positive antibacterial activity against the Gram-negative *S. typhi, C. fuendii* and *A. hydrophila*. On the other hand, the 100 mg mL^{-1} chloroform extracts of *A. bilimbi*'s leaves and fruits demonstrated antibacterial activity against the Gram-positive *S. aureus, S. epidermis, B. cereus, K. rhizophila* and *C. diphteriae* and Gram-negative *S. typhi, C. fuendii, A. hydrophila* and *P. vulgaris*. In conclusion, the *A. bilimbi* leaves and fruits possess potential antibacterial activity that requires thorough study.

Key words: *Averrhoa bilimbi*, antibacterial activity, aqueous extract, chloroform extract

INTRODUCTION

Since antibiotics first became widely used in the World War II era, they have blunted serious complications of many feared diseases and infections. However, after more than 50 years of uses, most of the antibiotics have lost their effect due to the ability of microorganisms to develop tolerance towards their effects (Choy *et al*., 1999). Widespread uses of antibiotics have been thought to spur evolutionary changes in bacteria that allow them to survive and tolerate these powerful drugs effects. Diseases like tuberculosis, gonorrhea and malaria, for examples, are now difficult to treat than they were decades ago (Choy, 1999). While antibiotic resistance benefits the microbes, it presents humans with two major problems; firstly, resistance makes it more difficult to purge infections from the body and secondly, it heightens the risk of acquiring infections in the hospitals (Jorgenson *et al*., 1999). Other than that, several types of antibiotics also exhibited unwanted side effects to their users, which include stomach upset, diarrhea, vomiting and skin rash and to greater extent, breathing difficulties, hives and dizziness (Mark and Trattler, 1999).

Due to increase in the resistant of microbes against antibiotics and the development of unwanted side effects associated with the latter uses, studies on plant-animal- or microbe-derived natural products must be encourage to go on in order to discover novel and potential antibacterial and antifungal drugs. Herbal medicines have been the basis of treatment and cure for various types of diseases or physiological conditions in the Malays folklore medicines (Somchit *et al*., 2003). What is interesting, however, is that, quite often, a particular plant may be used for different diseases (Perumal, 1998).

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Averrhoa bilimbi L., locally known as '*Belimbing buluh*' to the Malays, belongs to the family *Oxalidaceae* (Morton, 1987). In Malaysia, particularly, *A. bilimbi* fruits were famously used as pickles or flavour-enhancer in traditional Malays dishes (Abdul Rahman, 2003). Medicinally, *A. bilimbi* leaves are use in the treatment of venereal disease (Abdul Rahman, 2003). Furthermore, the leaves' infusion or decoction are taken as a remedy for coughs or to relieve rectal inflammation and, respectively and sometimes taken as tonic after childbirth (Morton, 1987). On the other hand, *A. bilimbi* fruits are used to treat pimples, hypertension and diabetes. Other than that, the leaves, fruits and flowers of *A. bilimbi* are also use to treat coughs (Abdul Rahman, 2003). In Philippines, the leaves are applied as paste or poultice to treat itches, swellings due to rheumatism while in Java, Indonesia the fruits are use in the treatment of fever, coughs, inflammation and to stop rectal bleeding and to alleviate internal hemorrhoids (Morton, 1987). The present study was carried out to determine the *in vitro* antibacterial activity of the aqueous and chloroform extracts of the leaves and fruits of *A. bilimbi* against selected bacteria.

MATERIALS AND METHODS

Plant materials: The leaves and fruits of *A. bilimbi* were collected in January-February 2005 from its natural habitat in Kuala Lumpur, Malaysia and identified by Mr. Shamsul Khamis, a botanist from the Institute of Bioscience, UPM, Malaysia. A voucher specimen was deposited at the Herbarium of the Laboratory of Natural Products, Institute of Bioscience, UPM, Serdang, Selangor, Malaysia.

Bacteria cultures: Microorganisms tested in this study were Staphylococcus aureus, Staphylococcus epidermis, Bacillus cereus, Corneybacterium diphtheria, Escherichia coli, Proteus vulgaris, Listeria monocytogenes, Salmonella typhi, Salmonella enteriditis, Aeromonas hydrophila, Citrobacter fuendii and Kochuria rhizophila.

Preparation of *A. bilimbi* aqueous and chloroform extracts: Fresh leaves and fruits of *A. bilimbi* were air-dried for 72 h at room temperature, ground into small pieces and then separately soaked with distilled water (dH_2O) or chloroform for 24 h in the ratio of 1:10 (wv^{-1}). The aqueous extract obtained was kept at -80°C for 48 h and then freeze-dried for 72 h while the resultant extraction of chloroform was completely evaporated using rotary evaporator. The obtained crude dried extracts of aqueous and chloroform extracts were prepared in the concentrations of 25, 50 and 100 mg mL^{-1} by dissolving the former in dH_2O and the latter in Dimethyl Sulfoxide (DMSO) and used in the antibacterial studies.

Antibacterial study: The inoculation and incubation of bacteria culture followed by the disc diffusion test were carried out according to the method described by Somchit *et al.* (2003). Briefly, 20 μL of the respective extract were loaded into empty sterilized blank discs (6 mm diameter, Oxoid, UK) and dried under room temperature. The discs were then positioned on the solid agar medium by pressing slightly. Petri dishes were placed in incubator according to their respective growth temperature and condition for 18-24 h. At the end of the period, inhibition zones formed was measured in mm. The study was performed in triplicate and the formation of the inhibition zones were compared with those of commercial antibiotic (ampicillin and kanamycin) discs.

RESULTS AND DISCUSSION

The antibacterial activity of aqueous and chloroform extracts of the leaves and fruits of *A. bilimbi* against Gram positive bacteria is illustrated in Table 1. The aqueous extract of *A. bilimbi* fruits was effective than its leaves counterpart indicated by the former ability to inhibit the growth of four bacteria (*S. aureus, S. epidermis, B. cereus* and *K. rhizophila*) at the concentration of 50 mg mL^{-1} compared to two bacteria (*S. aureus* and *C. diphteriae*) as seen with the latter. On the other hand, the chloroform extract of leaves was effective than its fruits counterpart indicated by the former ability to exhibit antibacterial activity at the lowest concentration (25 mg mL^{-1}) used. At all concentrations used, the chloroform extract of *A. bilimbi* leaves were effective against *S. aureus, B. cereus* and *C. diphteriae*. At the concentrations of 50 and 100 mg mL^{-1}, the aqueous extracts of the leaves and fruits and the chloroform extract of the fruits were effective against *S. aureus*, *C. diphteriae*, *K. rhizophila*, *S. epidermis* and *B. cereus*. At 100 mg mL^{-1} concentration, the chloroform extracts of the leaves and fruits and the aqueous extract of the leaves were effective against *K. rhizophila*, *S. epidermis*, *B. cereus* and *C. diphteriae*.

The antibacterial activity of the aqueous and chloroform extracts of the leaves and fruits of *A. bilimbi* against Gram negative bacteria is illustrated in Table 2. The chloroform extract of *A. bilimbi* leaves were effective, at all concentrations used, against *A. hydrophila* and *P. vulgaris*. At 100 mg mL^{-1}, the aqueous and chloroform extracts of *A. bilimbi* leaves and fruits were effective against *C. fuendii*, *S. typhi*, *A. hydrophila* and *P. vulgaris*. Based on the data obtained, *A. bilimbi* extracts were effective against the Gram-positive more than the Gram-negative bacteria. Unfortunately, bacteria like *L. monocytogenes*, *S. enteritidis* and *E. coli* were highly resistant against the effects of *A. bilimbi* aqueous and chloroform extracts.

Table 1: The antibacterial activity of aqueous and chloroform extracts of *A. bilimbi* against gram positive bacteria determined by disc diffusion method

Parts of plants	extracts	Concentration (mg mL^{-1})	Zone of inhibitions (mm) — Gram positive bacteria											
			S. aureus		*S. epidermis*		*L. monocytogens*		*B. cereus*		*K. rhizophila*		*C. diphtheriae*	
Antibiotics		30	a	b	a	b	a	b	a	b	a	b	a	b
			11	12	11	12	11	13	11	22	16	25	15	17
			12	12	12	12	11	13	11	22	16	25	15	17
			12	12	12	12	11	13	11	22	16	25	15	16
Leaves	Aqueous	25	-		-		-		-		-		-	
			-		-		-		-		-		-	
			-		-		-		-		-		-	
		50	7		-		-		-		-		7	
			7		-		-		-		-		7	
			7		-		-		-		-		7	
		100	8		7		-		13		-		7	
			8		7		-		13		-		8	
			8		7		-		13		-		7	
	Chloroform	25	10		-		-		12		-		10	
			10		-		-		12		-		10	
			10		-		-		12		-		10	
		50	10		-		-		15		-		11	
			11		-		-		15		-		11	
			11		-		-		15		-		12	
		100	11		-		-		16		11		12	
			11		-		-		16		11		12	
			11		-		-		16		11		12	
Fruits	Aqueous	25	-		-		-		-		-		-	
			-		-		-		-		-		-	
			-		-		-		-		-		-	
		50	9		8		-		13		11		-	
			9		7		-		11		10		-	
			8		7		-		10		11		-	
		100	10		8		-		13		12		-	
			10		8		-		13		13		-	
			10		8		-		13		13		-	
	Chloroform	25	-		-		-		-		-		-	
			-		-		-		-		-		-	
			-		-		-		-		-		-	
		50	-		-		-		-		16		-	
			-		-		-		-		15		-	
			-		-		-		-		15		-	
		100	10		9		-		-		20		10	
			10		9		-		-		20		10	
			10		9		-		-		20		10	

a = Amphicillin; b = Kanamycin; Data showed the diameter of inhibitory zone (mm) for each three replicates

The ability of both types of extracts to exhibit antibacterial activity against some of the bacteria suggested the presence of hydrophilic/polar and hydrophobic/non-polar antibacterial compounds. The extracts seem to show moderate antimicrobial activity indicated by their ability to affect some of the Gram-positive and -negative bacteria. However, the Gram-positive bacteria were more susceptible to the extracts when compared to the Gram-negative bacteria. This finding could be associated with the less complex and the lack of natural sieve effect, which is due to the presence of small pores in the cell envelope, in the Gram-positive bacteria's cell wall (Hawkey *et al*., 1998; Gould and Booker, 2000).

Generally, the mechanisms by which microorganisms survive the action of antimicrobial drugs/extracts are poorly understood and remain debatable (Okemo *et al*., 2001). Based on previous studies, the antibacterial activity of *A. bilimbi* could be associated with the presence of bioactive compounds of flavonoids type like luteolin and apigenin (Miean and Mohamed, 2001). In term of the aqueous extract, the antibacterial activity could be ascribed to the presence of thiocynate, nitrate, chloride and sulphates beside other water-soluble components naturally presence in most plant materials (Darout *et al*., 2000). Based on the data obtained, further studies involving isolation and identification of bioactive compounds from *A. bilimbi* extracts and antibacterial studies using these isolated constituents should be carried out as it will help us in facing the emergence and spread of antibacterial resistance. In conclusion, the *A. bilimbi* aqueous and chloroform extracts possess antibacterial activity against some bacteria that require further studies, possibly to the extent of isolating and identifying the responsible compounds.

Table 2: The antibacterial activity of aqueous and chloroform extracts of *A. bilimbi* against gram negative bacteria determined by disc diffusion method

Parts of plants	extracts	Concentration (mg mL^{-1})	Zone of inhibitions (mm) Gram positive bacteria *S. typhi* a	b	*C. fuendii* a	b	*S. enteritidis* a	b	*E. coli* a	b	*A. hydrophila* a	b	*P. vulgaris* a	b
Antibiotics		30												
Leaves	Aqueous	25	-		-		-		-		-		-	
			-		-		-		-		-		-	
			-		-		-		-		-		-	
		50	-		-		-		-		-		-	
			-		-		-		-		-		-	
			-		-		-		-		-		-	
		100	10		10		-		-		9		-	
			10		9		-		-		8		-	
			10		9		-		-		8		-	
	Chloroform	25	-		-		-		-		7		7	
			-		-		-		-		8		7	
			-		-		-		-		7		7	
		50	-		-		-		-		8		7	
			-		-		-		-		8		7	
			-		-		-		-		9		7	
		100	-		8		-		-		8		8	
			-		8		-		-		9		8	
			-		7		-		-		9		8	
Fruits	Aqueous	25	-		-		-		-		-		-	
			-		-		-		-		-		-	
			-		-		-		-		-		-	
		50	11		-		-		-		-		-	
			11		-		-		-		-		-	
			10		-		-		-		-		-	
		100	12		-		-		-		-		9	
			12		-		-		-		-		9	
			12		-		-		-		-		9	
	Chloroform	25	-		-		-		-		-		-	
			-		-		-		-		-		-	
			-		-		-		-		-		-	
		50	-		-		-		-		-		-	
			-		-		-		-		-		-	
			-		-		-		-		-		-	
		100	11		9		-		-		-		-	
			11		9		-		-		-		-	
			11		9		-		-		-		-	

a = Amphicillin; b = Kanamycin; Data showed the diameter of inhibitory zone (mm) for each three replicates

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