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The *in vitro* Antibacterial Activity of Methanol and Ethanol Extracts of *Carica papaya* Flowers and *Mangifera indica* Leaves

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Abstract: The present study was carried out to evaluate the possible antibacterial activity of methanol and ethanol extracts of *Mangifera indica* leaves and *Carica papaya* flowers using the *in vitro* disc diffusion methods. The sterilized blank discs (6 mm diameter) was impregnated with 20 μL of the respective extract in the concentrations of 12.5, 25, 50 and 100% and tested against *Corneybacterium diptheriae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*. The methanol and ethanol extracts of *M. indica* were effective against *C. diptheriae*, *S. aureus*, *S. typhi* and *P. aeruginosa*, with the latter producing slightly bigger inhibitory zone against some of the bacteria when compared to the former. The methanol and ethanol extracts of *C. papaya* were effective only against *S. aureus* and *S. pneumoniae*, with the latter also effective against *C. diptheriae*. As a conclusion, the present study demonstrated the potential of *M. indica* leaves and *C. papaya* flowers as antibacterial agents against some of the bacteria tested and thus may provide the basis for the isolation of bioactive compounds with antibacterial activity from the respective plant leaves or flowers.

Key words: *Mangifera indica*, *Carica papaya*, antibacterial activity, methanol extract, ethanol extract

Introduction

Plant materials have been a major source of natural therapeutic remedies and used to treat various infectious diseases in many developing countries (Czygan, 1993; Ody, 1993). Nowadays, natural products of plant sources have been the center of focus (Nitta *et al*., 2002; Souza *et al*., 2003) as the main source of new, safer and more effective bioactive compounds with antibacterial properties due to the raising problems of side effects and limited efficacy (Gupta *et al*., 1998; Corazo *et al*., 1999) of the available antibiotics.

Carica papaya, believed to be originated in Central America, is a plant that belongs to the family Caricaceaes. The fruits, leaves and latex of *C. papaya* have been used medicinally by peoples from various places to treat various ailments such as asthma, rheumatism, fever, diarrhea, boils and hypertension, to name a few. In Malaysia, *C. papaya*, locally known as betik, has been used to treat ailments in human colic, boils, vermifuge as well as to increase milk production.

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Mangifera indica, commonly called mango, is a plant belonging to the family Anacardiaceae. It is widely cultivated in Malaysia and locally known to the Malays as '*mangga*'. Besides being eaten as a ripe fruit, the green fruit is put in curries or made into brine pickles in many part of the world such as India and Malaysia. The Indians used the twigs and leaves to clean the teeth, while the bark is said to be useful for toothaches. The astringent stomachic bark is also used for internal hemorrhages, bronchitis and rheumatism. Furthermore, the resinous gum is used to treat cracked feet, scabies and ringworm while smoke from the burning leaves is believed to cure various throat disorders, from asthma to hiccups. In addition, the dried flowers are used to treat gleets while the green fruits are considered anticholeric, antidysmennorrheic, antiscorbutic, astringent and diaphoretic. The ripe fruits are considered as diuretic, laxative and ointment, the gum is used to treat scabies and the seeds are antihelmintic, antiasthmatic, antimenorrhagic, antidysenteric and unguent.

Since there were no scientific reports on the antibacterial properties of *C. papaya* flowers or *M. indica* leaves, the aims of the present study were to screen for their potential antibacterial activity against a selected group of bacteria available in our laboratory and to compare on their effectiveness against the standard antibiotic.

Materials and Methods

Materials

M. indica leaves and *C. papaya* flowers were collected from Gombak, Selangor, Malaysia in Oktober, 2004 and identified by Mr. Chan Yee Chong, a botanist at the Herbarium of the Forest Research Institute of Malaysia.

Microorganisms tested in this study were *Corneybacterium diptheriae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*.

Methods

Fresh leaves of *M. indica* and flowers of *C.* papaya were oven-dried for 24 hours at 48°C according to the methods described by Zakaria *et al*. (2006a) but with slight modifications. The respective leaves and flowers of *M. indica* and *C. papaya* were then ground into small pieces under sterilized condition. Each sample (100 g) was then extracted separately with methanol or ethanol in the ratio of 1:10 (w/v) for 24 h by using the Soxhlet apparatus. The supernatants collected after the methanol or ethanol extraction of both samples were then completely evaporated at 40°C under reduced pressure using the rotary evaporator machine (Buchi, Germany). The obtained dried crude methanol (MEMI) and ethanol (EECP) extracts of *M. indica* and *C. papaya* were prepared in the concentrations of 12.5, 25, 50 and 100% by dissolving them in dimethyl sulfoxide (DMSO). Twenty microliter of the respective extract were then loaded into empty sterilized blank discs (6 mm diameter, Oxoid, UK) and left to dry at room temperature under sterilized condition prior to subjection to antibacterial assay. In addition, commercial antibiotic discs (Tetracycline; 30 μg μL^{-1}) were used for comparison.

Preparation of Microorganism Culture

The procedures for the preparation of microorganism culture were as described by Zakaria *et al*. (2006a, b).

Results and Discussion

As can be seen from the Table 1 MEMI was slightly more effective than the EEMI in term of the size of inhibitory zone of bacteria growth, particularly of the *C. diptheriae* and *P. aeruginosa*. The inhibitory zone of more than 13 mm was seen after pre-treatment with the 50 and 100% concentrations MEMI, but not EEMI that give the inhibitory zone of less than 13 mm. Generally, from the data obtained, the MEMI and EEMI were effective only against two of the Gram negative bacteria (*S. typhi* and *P. aeruginosa*).

On the other hand, Table 2 shows that the MECP and EECP were effective only against the selected Gram positive, but not the Gram negative, bacteria namely *C. diptheriae*, *S. aureus* and *S. pneumoniae*. Furthermore, the MECP was found to produce a more effective antibacterial activity against the said bacteria when compared to the EECP.

Although majority of the diameter of the inhibitory zone obtained was below 16 mm and the number was of bacteria affected by both plants was small, the present study did demonstrate the potential antibacterial properties of *M. indica* leaves and *C. papaya* flowers. Except for MEMI and EEMI that were effective against *S. typhi* and *P. aeruginosa*, the lower value obtained in term of the diameter of inhibitory zone could be due to the types of bacteria used, in which out of eight bacteria used, five of them are Gram negative bacteria. The presence of lipopolysaccharide (LPS) in the Gram negative bacteria (Levin *et al*., 1993; Whitfield, 1995; Maskell and Allen, 1997) could be used to support the lack of antibacterial activity of both the MECP and EECP. LPS has been associated with intracellular survival of certain Gram negative bacteria, particularly *Salmonella* spp. (Ernst *et al*., 1999) and has been thought to interact with antibacterial peptides, making the latter less effective in inhibiting the bacteria growth. However, further extensive researches need to be carried out to confirm on whether the same LPS interact with the antibacterial compounds present in those extracts that lead to the extract ineffectiveness. Furthermore, recent studies have demonstrated that the long chain LPS only plays a secondary role in invasiveness of Gram negative bacteria like *S. enteriditis*, (Martin *et al*., 2000). Several findings have also associated the differences in virulence with the capacity of LPS to activate complement through the alternate pathway (Grossman and Leive, 1984; Saxen *et al*., 1984; 1987).

Based on the recent observation, it is plausible to suggest that the *M. indica* extracts showed a broad spectrum activity similar to amphicilin, tetracycline and streptomycin since they were effective against all of the Gram positive and some of the Gram negative bacteria while those of *C. papaya* exhibited a narrow spectrum of activity as can be seen with clindamycin, gentamicin and penicillin G since they were effective only against the Gram positive bacteria (Cheesbrough, 1994). Other than the water-soluble compounds naturally present in most plants, the broad antimicrobial action of the MEMI and EEMI could also be due to the presence of anionic components such as thiocynate, sulphates, nitrate and chloride (Darout *et al*., 2000).

In comparison to the standard antibiotic chloramphenicol (30 μg μL^{-1}), the antibacterial activity of *M. indica* and *C. papaya* extracts were less promising and could be attributed to the fact that both plants' extracts used are crude extracts. Except for *P. aeruginosa* that gave an inhibitory zone of less than 13 mm, treatment of the other bacteria with chloramphenicol were found to produce the inhibitory zones that are greater than 20 mm.

The leaves of *M. indica* have been reported to contain glucoside and mangiferin while its sap was reported to contain mangiferin, resinous acid, mangiferic acid, resinol and mangiferol. Mangiferin, for examples, has been demonstrated to possess antiviral activity against herpes simplex virus type 2

Table 1: The antibacterial activity of methanol and ethanol extracts of *Mangifera indica* determined by disc diffusion method

	Concentration (%)							
	MEMI				EEMI			
Bacteria	12.5	25	50	100	12.5	25	50	100
C. diptheriae	++	++	+++	+++	+	+	++	++
S. aureus	+	+	++	++	+	+	++	++
S. pneumoniae	-	-	-	+	-	-	-	+
S. typhi	++	+++	+++	+++	++	+++	+++	+++
P. aeruginosa	++	++	+++	+++	++	++	++	+++
E. coli	-	-	-	-	-	-	-	-
K. pneumoniae	-	-	-	-	-	-	-	-
P. vulgaris	-	-	-	-	-	-	-	-

IZ = Inhibition zone (mm), -No inhibition zone, +IZ ≤ 9.0 mm, ++ 9.0 mm<IZ≤13.0 mm, +++13.0 mm <IZ≤16.0 mm, ++++16.0 mm <IZ≤20.0 mm. Except for *P. aeruginosa* (IZ = 10 mm), Chloramphenicol gave inhibition zone of ≤20 mm against all bacteria

Table 2: The antibacterial activity of methanol and ethanol extracts of *Carica papaya* flowers determined by disc diffusion method

	Concentration (%)							
	MECP				EECP			
Bacteria	12.5	25	50	100	12.5	25	50	100
C. diptheriae	-	+	+	++	-	-	-	+
S. aureus	++	++	+++	+++	+	++	+++	+++
S. pneumoniae	+++	+++	+++	++++	++	++	+++	+++
S. typhi	-	-	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-
K. pneumoniae	-	-	-	-	-	-	-	-
P. vulgaris	-	-	-	-	-	-	-	-

IZ = Inhibition zone (mm), -No inhibition zone, +IZ≤9.0 mm, ++ 9.0 mm <IZ≤13.0 mm, +++13.0 mm <IZ≤16.0 mm,++++ 16.0 mm <IZ≤20.0 mm. Except for *P. aeruginosa* (IZ = 10 mm), Chloramphenicol gave inhibition zone of ≤20 mm against all bacteria.

(Zhu *et al*., 1993), hypoglycemic activity (Aderibigbe *et al*., 2001) and, antihyperlipidemic, anti diabetic and antiartherogenic activities (Muruganandan *et al*., 2005). In addition, formulation comprising of *C. papaya* roots, *M. indica* leaves, *Citrus limon* fruit and *C. citratus* leaves has also been reported to possess antibacterial activity against *S. typhi, S. paratyphi* and *S. typhimurium* (Nkuo-Akenji *et al*., 2001). Although various types of compounds have been identified from the leaves, bark, fruits, barks, roots, seeds and latex of *C. papaya* (Bennett *et al*., 1997; MacLeod and Pieris, 1983; Pousset *et al*., 1981; Sandhya and Veerannah, 1996; Schwab and Schreier, 1988; Sheu and Shyu, 1996; Tang 1979; Winterhalter *et al*., 1986), there has been no report on the chemical constituents of its flowers. *C. papaya* contains various types of biologically active compounds with the two important compounds are chymopapain and papain (Brocklehurst and Salih, 1985), which are thought to aid in digestion. Furthermore, papain also has been used in the treatment of arthritis. Among the various types of bioactive compounds isolated from *C. papaya*, alkaloids, carpaine, dehydrocarpaines, flavonols, tannins and benzyglucosinolate have been reported to be presence in the leaves while linalool, cis- and trans-linalool oxide, α-linolenic acid, α-phellandrene, α- and γ-terpinenes, 4-terpineol, terpinolene have been reported to be presence in the fruits as mentioned earlier. It is plausible to suggest the involvement of mangiferin, at least in part, in the antibacterial activity of *M. indica* based

on the previous reports mentioned earlier. However, it was not possible to link any of the compounds isolated from various parts of *C. papaya* as described earlier with the observed antibacterial activity since there was no report on the chemical constituents of its leaves. However, their present and possibility of occurrence in the flowers of *C. papaya* should not be excluded.

As a conclusion, the present study has proven that the respective *M. indica* and *C. papaya* leaves and flowers possessed antibacterial activity and thus provide the initial steps for future isolation and identification of antibacterial agents from those plants.

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