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The *in vitro* Antibacterial Activity of *Muntingia calabura* Extracts

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Abstract: The present study was carried out to investigate on the possible antibacterial activity of aqueous (AEMC), methanol (MEMC) and chloroform (CEMC) extracts of *Muntingia calabura* using the *in vitro* disc diffusion methods. The sterilized blank discs (6 mm diameter) was impregnated with 20 μ L of the respective extract (in the concentration of 10,000, 40,000, 70,000 and 100,000 ppm) and tested against *Corneibacterium diphtheria*, *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus*, *Proteus vulgaris*, *Staphylococcus epidermidis*, *Kosuria rhizophila*, *Shigella flexneri*, *Escherichia coli* (O 157), *Aeromonas hydrophila* and *Salmonella typhi*. At all concentrations tested, the AEMC was effective against *S. aureus* and *K. rhizophila* while the MEMC was effective against *S. flexneri*, *B. cereus*, *S. aureus*, *P. vulgaris*, *A. hydrophila*, *K. rhizophila*. This activity was not observed with the CEMC. At the concentration of 40000 ppm and above, the AEMC exhibited significant antibacterial activity against *C. diphtheriae*, *P. vulgaris*, *S. epidermidis* and *A. hydrophila*; the MEMC was effective against *C. diphtheriae* and *L. monocytogenes*, and the CEMC was effective against *S. aureus*. Finally, we concluded that *M. calabura* possesses a potential antibacterial property that is comparable to the standard antibiotics used. The results also suggest the presence of more potent polar antibacterial compound.

Key words: *Muntingia calabura*, antibacterial activity, aqueous extract, methanol extract, chloroform extract, disc diffusion method

INTRODUCTION

Muntingia calabura L. (Kerukup siam), also known locally as Jamaica cherry, is a plant of the family Elaeocarpaceae (Morton, 1987). It is native to the American continent and is widely cultivated in warm areas of Asian region, including Malaysia (Chin, 1989). Its leaves, barks and flowers are believed to possess medicinal value as reported in Peru folklore medicinal uses (Morton, 1987). Scientifically, this plant has been proven to possess anti-tumour properties (Kaneda *et al.*, 1991; Su *et al.*, 2003; Chen *et al.*, 2005) and our recent study has also shown that the plant extract possesses antinociceptive activity, which is mediated, at least in part, through the opioid receptor (Zakaria *et al.*, 2004). Further studies have also demonstrated that the aqueous extract of *M. calabura* leaves has peripheral antinociception

which involved, at least in part, activation of μ -opioid, β -adrenergic and muscarinic receptors (data not published). In addition, we have also demonstrated that the *M. calabura* leaves extract also possessed anti-inflammatory and anti-pyretic properties (data not published).

It is generally accepted that plants still continue to be among the source of drugs for the majority of the world's population (Maisenbacher *et al.*, 1995; Meral and Karabay, 2002). Herbal remedies are widely known to be used in the treatment of many infectious diseases throughout the history of mankind. Plant materials continue to provide a major source of natural therapeutic remedies and play an important role in health care in many developing countries (Czygan, 1993; Ody, 1993). The fact that some of the available antibiotic also produce side effects and have limited efficacy (Gupta *et al.*, 1998;

Corazo *et al.*, 1999) has triggered researchers to discover for new safer and more effective antibiotics from natural products, especially from plants (Nitta *et al.*, 2002; Souza *et al.*, 2003). The basis for carrying the present study is attributed to several reports on plants that possessed pain or inflammation relieving properties as well as also showed antibacterial activity (Lin *et al.*, 1999) and the reason mentioned earlier. In this study the aqueous, methanol and chloroform extracts of *M. calabura* is screened against selected Gram positive and Gram negative bacteria for the presence of antibacterial activity using the disc diffusion method.

MATERIALS AND METHODS

Materials: *M. calabura* leaves were collected from Shah Alam, Selangor, Malaysia in January-February 2005 and a voucher specimen (SK 964/04) was deposited at the Herbarium of Institute of Bioscience, Universiti Putra Malaysia (UPM), Selangor, Malaysia.

Microorganisms tested in this study were *Cornebacterium diphtheria*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus vulgaris*, *Staphylococcus epidermidis*, *Kosuria rhizophila*, *Shigella flexneri*, *Escherichia coli*, *Aeromonas hydrophila* and *Salmonella typhi*.

Methods: All of the procedures for the extraction of *M. calabura* leaves and preparation of microorganism culture to antibacterial assay were carried out in the Department of Food Technology, Faculty of Food Science, UPM, Selangor, Malaysia in March-May 2005. Fresh leaves of *M. calabura* were oven-dried for 24 h at 40°C according to the methods described by Somchit *et al.* (2003) but with slight modifications. It was then ground into small pieces under sterilized condition and extracted separately with aqueous, methanol or chloroform in the ratio of 1:20 (w/v) for 24 h by using Soxhlet apparatus. The aqueous extract obtained was kept at -80°C for 48 h and then freeze-dried for 72 h while the resultant extraction of methanol and chloroform was completely evaporated by using rotary evaporator machine (Somchit *et al.*, 2003). The obtained dried crude extracts of aqueous (AEMC), methanol (MEMC) and chloroform (CEMC) were then prepared into various concentrations (10,000 ppm, 40,000 ppm, 70,000 ppm and 100,000 ppm) by dissolving the dried AEMC in distilled water (DH₂O) and, the dried MEMC and CEMC in dimethyl sulfoxide (DMSO). Twenty microliter of the respective extract were then loaded into empty sterilized blank discs (6 mm diameter, Oxoid, UK). In addition, commercial antibiotic discs (Chloramphenicol; 30 µg µL⁻¹) were used for comparison.

Preparation of microorganism culture: The above-mentioned bacteria were incubated at 37°C±0.5 for 24 h after injection into nutrient broth. Mueller Hinton Agar (MHA) (Oxoid, UK), sterilized in a flask and cooled to 40-50°C, was poured (15 mL) into sterilized Petri dishes (diameter of 9 cm) and allowed to harden under room temperature. This is followed by homogenous distribution of 0.1 mL bacteria cultures (10⁶ bacteria per mL) onto medium in Petri dishes. Discs loaded with extracts were then positioned on the solid agar medium by pressing slightly (Sundar, 1996). Petri dishes were placed in incubator according to their respective growth temperature and condition for 18 to 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 antibiotic discs.

RESULTS AND DISCUSSION

As can be seen from the Table 1, the CEMC was less effective when compared to AEMC and MEMC, in terms of number of bacteria inhibited.

The AEMC was found to show inhibition ≥13.0 mm against *C. diphtheriae*, *S. aureus* and *S. epidermidis* with the 100,000 ppm concentration produced maximum inhibitory effect (≥20.0 mm) against *C. diphtheriae*. At the concentration of ≥40,000 ppm, the effect of AEMC was found to be linear (between 9.0-13.0 mm) throughout the concentration against *K. rhizophila* and *A. hydrophila*.

On the other hand, the MEMC was found to give inhibition ≥13.0 mm against *C. diphtheriae*, *S. aureus*, *P. vulgaris* and *K. rhizophila* and *S. flexneri*. Its 100,000 ppm concentration extract was found to produce maximum inhibitory effect (≥20.0 mm) against *S. aureus* and *S. flexneri*. At the concentration of ≥40,000 ppm, the effect of MEMC was found to be linear (between 13.0-16.0 mm) throughout the concentration against *P. vulgaris*. Interestingly, the MEMC was also found to produce inhibitory effect (between 9.0-13.0 mm) against *E. coli* at the concentration of ≥70,000 ppm but slight inhibition (≤9.0 mm) against *S. epidermidis*.

The present study carried out to screen for antibacterial activity in various solvent-based extracts of *M. calabura* leaves has demonstrated the potential use of *M. calabura*, especially its aqueous and methanol extracts, as an effective antibacterial agent against the infection of some of the bacteria used, particularly *C. diphtheriae*, *S. aureus* and *P. vulgaris*. The former and the latter were also effective in inhibiting the *S. epidermidis* and *S. flexneri*, respectively. This finding seems to indicate the presence of polar and hydrophilic type of antibacterial compound in *M. calabura*.

Table 1: The antibacterial activity of aqueous, methanol and chloroform extracts of *Muntingia calabura* determined by disc diffusion method

Bacteria	Concentration (ppm)											
	AEMC				MEMC				CEMC			
	10K	40K	70K	100K	10K	40K	70K	100K	10K	40K	70K	100K
<i>C. diphtheriae</i>	-	+++	++++	++++	-	++	+++	++++	-	-	-	+
<i>S. aureus</i>	++	++++	++++	++++	+	+++	++++	++++	-	+	+	+
<i>B. cereus</i>	-	-	+	+	+	+	++	++	-	-	+	+
<i>P. vulgaris</i>	-	++	+++	+++	+	+++	+++	+++	-	-	-	-
<i>S. epidermidis</i>	-	+++	++++	++++	-	-	-	+	-	-	-	+
<i>K. rhizophila</i>	+	++	++	++	+	++	+++	+++	-	-	-	-
<i>S. flexneri</i>	-	+	+	++	+	+++	++++	++++	-	-	-	-
<i>E. coli</i>	-	-	-	-	-	-	+	++	-	-	-	-
<i>A. hydrophila</i>	-	++	++	++	+	+	++	++	-	-	-	-
<i>L. monocytogenes</i>	-	+	+	++	-	-	-	+	-	-	-	-

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
+++++ 20.0 mm<IZ, Except for *P. vulgaris* (IZ = 9 mm), Chloramphenicol gave inhibition zone of ≤20 mm against all bacteria

Furthermore, the broad antimicrobial action of the aqueous extract of *M. calabura* leaves could be ascribed to the anionic components such as thiocyanate, nitrate, chloride and sulphates beside other water soluble components which are naturally occurring in most plant materials (Darout *et al.*, 2000). However, the CEMC was found to be less effective as antibacterial agents against the selected bacteria. This finding may be attributed to little diffusion properties of the extract in the agar or because fresh plants contain active substances which may be affected or may have disappeared in the steps of extraction methods (El Astal *et al.*, 2005). In comparison to standard chloramphenicol (30 µg µL⁻¹) the activities of those extracts were not so promising which seems to support the fact that the Peruvian, or even the Malays, did not use *M. calabura* traditionally to treat infectious diseases (Morton, 1987).

The findings that *S. aureus* is susceptible to both the aqueous and methanol extracts of the *M. calabura* are also similar to the susceptibility of that microbe to different plant extracts reported by several researchers (Arora and Kaur, 1999; Okemo *et al.*, 2001; Digraki *et al.*, 1999; Madamombe and Afolayan, 2003). However, from this study it is clear that Gram positive bacteria were found to be more susceptible than Gram negative bacteria. This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lack the natural sieve effect against large molecules due to the small pores in their cell envelope (Hawkey, 1998; Gould and Booker, 2000). *E. coli* showed no response to each of AEMC and CEMC but slightly inhibited by MEMC. The resistance of *E. coli* as observed in this study could be due to cell membrane permeability or other genetic factors.

The AEMC and MEMC showed slightly better killing action than the CEMC and thus should be used for further investigations to distinguish its components and their individual antimicrobial effect. Our findings have validated the use of *M. calabura* leaves for the treatment

of some microbial infections like urinary tract infection and bacterial food poisoning. Since this is a preliminary screening for the presence of antimicrobial properties in *M. calabura* leaves extract, at the moment, the identification of chemical constituents is not part of the objective of this study. In general, the mechanisms by which microorganisms survive the action of antimicrobial agents are poorly understood and remain debatable (Okemo *et al.*, 2001). However, based on previous studies using leaves of various types of plant, the antibacterial activity of *M. calabura* is suggested to be due to the presence of compounds, such as tannins and flavonoids (Diaz *et al.*, 1988; Ogunleye and Ibitoye, 2003), glycosides (Chukwurah and Ajali, 2000; Ogunleye and Ibitoye, 2003) and saponins (Pretorius *et al.*, 2003). It seems important to recommend that, further studies using isolated constituents instead of whole extract must be done in this field since it will also offer a great help in facing the emergence and spread of antimicrobial resistance. Thus, the result may provide a basis for the isolation of compounds of biological interest from *M. calabura*.

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