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Antibacterial activity of extracts of pericarp of *Garcinia mangostana*

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Abstract

Pericarp of the fruit of *Garcinia mangostana* L is used for the treatment of skin infections, wounds and diarrhea in indigenous medicine of Thailand, India, Sri Lanka and Myanmar. In the present study, the inhibitory effect of extracts of pericarp of *Garcinia mangostana* on bacterial growth was investigated.

An inhibitory activity was exhibited by methanolic extract against Gram-positive bacteria tested *(Bacillus subtilis., Staphylococcus aureus.* and *Streptococcus faecalis)* whilst no inhibitory effect was observed against Gram -negative bacteria such as *Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa.*

Minimum inhibitory concentration (MIC) was determined using serially diluted extract with methanol on nutrient agar. After 24 hours of incubation at room temperature, the lowest concentration of the extract which inhibited the visible bacterial growth was recorded as MIC. The MIC of the extract was 0.0005 g/ml against *Bacillus subtilis* and *Staphylococcus aureus* while 0.005g/ml against *Streptococcus faecalis.*

Crude extract was fractionated with ether and ethyl acetate successively to obtain ether-soluble and ethyl acetate - soluble phenolics respectively. These two fractions were found to possess inhibitory effect on growth *of Bacillus subtilis., Staphylococcus aureus.* and *Streptococcus faecalis.* Chromatographic analysis of the two fractions showed the presence of several phenolic compounds.

Key words: *Garcinia,* extract, Gram-positive bacteria, inhibition zone

1. Introduction

Garcinia mangostana L. (Guttiferaceae) is a popular fruit tree, common in Thailand, India, Sri Lanka and Myanmar. In Thai indigenous medicine, pericarp of the fruit of *G. mangostana* is used for the treatment of skin infections, wounds and diarrhea. In Sri Lanka powder of pericarp has been reported to be used in treatment of wounds.

In recent years several workers have investigated the pharmaceutical value of extracts of *G. mangostana* and compounds isolated from it. Chairungrislerd *et al.* (1996) reported that crude methanolic extract of fruit hull of mangosteen inhibited the contractions of isolated thoracic rabbit aorta induced by histamine and seratonin. Chen *et al. (* 1996) have reported of a potent inhibitory activity of ethanolic extracts of mangosteen fruit peel against HIV-I protease. Extracts of *G. mangostana* fruit peels have also been reported to exhibit antibacterial activity against methicillin resistant *Staphylococcus aurens* (Linuma *et al.* 1996), inhibitory action on sarcoplasmic reticulum Ca(2+) pumping ATP-ase from rabbit skeletal muscle (Furukawa *et al.* 1996), inhibition of wheat embryo Ca^{-} dependent protein kinase and other kinases (Jinsart *et al.* 1992), antimicrobial activity (Sundaram *et al.* 1983), anti-inflammatory activity (Gopalakrishnan *et al.* 1980) and inhibition of oxidative modification of human LDL (Williams *et al.* 1995).

Present study was carried out to investigate the antibacterial activity of extract of pericarp of the *G.mangostana* fruit and to throw some light on the nature of active compounds in pericarp extracts against bacterial growth.

2. Material and Methods

Preparation of extracts

Pericarps of ripe fruits were collected and sun dried. Then they were ground into fine powder. Four grams of the powder was extracted with 100 ml of methanol and the extract was concentrated up to 8.0 ml by evaporating methanol in a water bath at 45°C. The concentrated crude extract was used to determine the antibacterial activity.

Fractionation of the crude extract

The crude extract was fractionated with ether and ethyl acetate. Two grams of powdered pericarp was extracted with 30 ml of methanol. Methanolic extract was shaken with 15 ml x 2 petroleum ether (bp 40-60 °C). Then the pet ether layer was removed and 10 ml of distilled water was added to aqueous phase and methanol was evaporated under reduced pressure. After evaporating methanol the precipitate was extracted with 1 ether (10 ml x 2) and 2 ethyl acetate (10 ml x 2) to obtain ether soluble phenols 1 and ethyl acetate soluble phenols and tannins.

Determination of antibacterial activity of extracts

Antibacterial activity of crude methanolic extract as well as ether and ethyl acetate fraction was tested using filter-paper disc-agar diffusion technique as described by Cappucino and Sherman (1999). Nutrient agar plates were heavily inoculated with 20 µl of 24 h old broth culture (10^8 cfu/ml) of tested bacteria separately. Watman No. 1 sterile filter paper discs (diameter 6 mm) were impregnated with different

concentrations of extracts and then placed on the surface of an agar plate at well-spaced intervals that has been seeded with the bacterium to be tested. Streptomycin sulphate and penicillin were used as positive control and methanol was the negative control, (ether and ethyl acetate were used as controls when tested ether and ethyl acetate fractions) These plates were incubated at 37 ° C for 24 h. The degree of antibacterial activity against tested bacteria was assessed by measuring the diameter of zone of inhibition. Since tested Gram-positive bacteria showed the susceptibility they were selected for further investigations. Four replicates were used for each antibacterial agent

Determination of minimum inhibitory concentration

To determine the minimum inhibitory concentration (MIC) the extract was serially diluted with methanol and samples (1 ml) at each dilution were mixed with nutrient broth (9 ml) and 5 sets of tubes with different concentrations of methanol extract were prepared. The tubes were then inoculated with 20 µl of a broth culture of bacterium and incubated at 37 ° C for 24 h and examined for growth of bacteria by measuring the optical density. The lowest concentration of extract which inhibited the growth of the bacterium was recorded as the MIC. Nutrient broth containing appropriate amount of methanol was used as control. Five replicates were used for each concentration.

Effect of temperature on the antibacterial activity of methnolic extract

A set of nutrient broth tubes (10 ml) containing 1 ml of methnolic extract was prepared and it was kept in a water bath at a temperature range of 45 -75 ° C for 5 min. After keeping at particular temperature for 5 min, tubes were allowed to cool. These tubes were inoculated with 20 µl of over night broth culture of tested bacterium and incubated at 37 ° C for 24 h. After incubation tubes were examined for growth of bacteria by measuring the optical density. Four replicates were used for each temperature.

Chromatographic analysis

Crude extract, ether and ethyl acetate fractions of crude extract were examined separately by two dimensional paper chromatography for the presence of phenolic compounds, on Whatman No.1 paper using solvent system 2% acetic acid (v/v) followed by n-Butanol-acetic acid-water (4:1:2.2 v/v). Chromotograms were treated with potassium ferricyanide-ferric chloride reagent (Smith, 1969) for the detection of phenolic compounds.

3. Results

The methanolic extract of pericarp of *Garcinia mangostana* was found to have inhibitory activity against *Bacillus subtilis, Staphylococcus aureus,* and

Streptococcus faecalis, which are Gram - positive bacteria (Table 1). The extract gave a range of zone of inhibition, 16-21 mm at an extract concentration of 0.5 g/ml against those bacteria. The antibiotics; streptomycin sulphate and penicillin gave ranges of zones of inhibition, 17-32 mm and 17 -19 mm respectively, at a concentration of 0.005 g /ml against the same bacteria.

The extract of pericarp did not show any inhibitory activity against *Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa,* which are Gram-negative bacteria. These bacteria were found to grow even on the edges of the discs impregnated with the extracts as well as of those impregnated with ethanol after 24 hours of incubation. Antibiotics; streptomycin sulphate and penicillin showed inhibitory effect on all tested Gram-negative bacteria.

Table 1. Antibacterial activity of methanolic extract of *Garcinia mangostana* pericarp.

Bacterium	Diameter of the zone of inhibition (mm)		
	Methanolic extract (0.5 g/ml)	Streptomycin sulphate (0.005 g/ml)	Penicillin (0.005 g/ml)
Bacillus subtilis	20.60±0.75	31.50tO.29	19.75±0.48
Staphylococcus aureus.	16.62±0.93	19.50±0.50	19.00±0.41
Streptococcus faecalis	17.11±0.51	29.50±1.32	17.50±0.29
Escherichia coli	no zone of inhibition	33.50±0.29	27.50±0.41
Proteus mirabilis	no zone of inhibition	31.50±0.87	29.75±0.75
Pseudomonas aeruginosa	no zone of inhibition	no colony growth	no colony growth

Table 2. Determination of minimum inhibitory concentration (MIC) of extracts of *Garcinia mangostana* pericarp.

	Concentration of the extract (g/ml)					
	0 (Control)	0.5	0.05	0.005	0.0005	0.00005
Bacillus subtilis	+	-	-	-	-	+
Staphylococcus aureus.	+	-	-	-	-	+
Streptococcus faecal is	+	-	-	-	+	+

+ There was a growth of the bacterium
- No growth of the bacterium

The minimum inhibitory concentration (MIC) of crude extract was 0.0005 g/ml against *Bacillus subtilis* and *Staphylococcus aureus* and 0.005 g/ml against *Streptococcus faecalis* (Table 2).

Paper chromatography of methanolic extract of pericarp of *G. mangostana* gave ten spots with ferrycynide-$FeCc_3$ reagents indicating the presence of phenolic compounds. Four of them very quite prominent (Figure 1). No attempt was made to identify them.

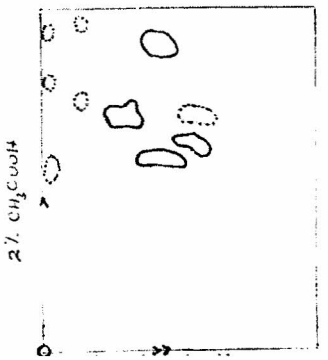

Figure 1:Chromatogram of phenolics in methanolic extract of pericarp of *G. **mangostana.*** Run first in 2 % CH_3COOH followed by n-Butanol: acetic acid : water (4:1: 2.2 v/v). Spots were located with $FeCl_3$ - $Fe(CN)_6$.

Ethyl acetate fraction gave five spots, all of which corresponded well with the spots detected in the crude extract (Figure 2). Ether soluble fraction gave three spots (Fig 3) with only one of them corresponding to crude extract.

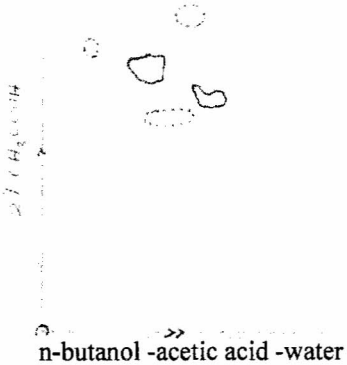

Figure 2: Chromatogram of phenolics in ethyl acetate fraction of methanolic extract of pericarp of *G. mangostana.* Run first in 2% CH_3COOH, followed by n-Butanol: acetic acid : water (4:1: 2.2 v/v). Spots were located with $FeCl_3$ - $Fe(CN)_6$.

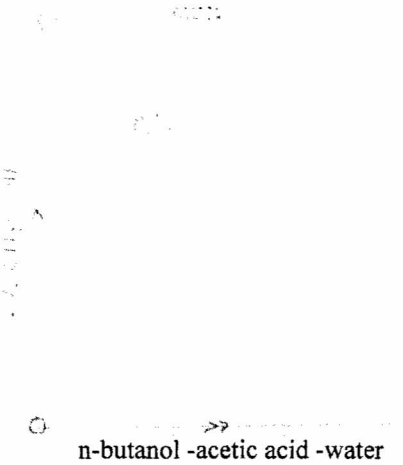

Figure 3: Chromatogram of phenolics in ether fraction of methanolic extract of pericarp of *G. mangostana*. Run first in 2% CH_3COOH, followed by n-Butanol: acetic acid : water (4:1: 2.2 *v/v)*. Spots were located with FeCh - $Fe(CN)_6$.

Table 3. Antibacterial activity of ether fraction and ethyl acetate fraction of *G.magostana*

Bacterium	Ether fraction	Ethyl acetate fraction
Bacillus subtilis	15.00±0.41	15.60±0.26
Staphylococcus aureus.	15.00±0.41	16.17±0.66
Streptococcus faecalis.	15.50±1.04	16.67±0.67

Both ether and ethyl acetate soluble fractions of the extract showed inhibitory activity against tested Gram-positive bacteria (Table 3). Inhibitory activity is more or less same in the two different fractions. Minimum inhibitory concentration of ether fraction and ethyl acetate fraction showed similar concentrations (Figures 4 and 5).

Table 4. Minimum inhibitory concentration of ether fraction of crude extract (0.2 g/ml) of *Garcinia mangostana* pericarp.

Bacterium	Concentration of the extract (g/ml)					
	Control	Original	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Bacillus subtilis	+	-	-	-	+	+
Staphylococcus aureus.	+	-	-	-	+	+
Streptococcus faecalis	+	-	-	-	+	+

+ There was a growth of the bacterium
- No growth of the bacterium
* Original - ether fraction of 0.2 g/ml of crude extract

Table 5. Minimum inhibitory concentration of ethyl acetate fraction of crude extract (0.2 *g/ml) Garcinia mangostana* pericarp.

Bacterium	Concentration of the fraction					
	Control	Original	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Bacillus subtilis	+	-	-	-	+	+
Staphylococcus aureus.	+	-	-	-	+	+
Streptococcus faecalis	+	-	-	-	+	+

+ There was a growth of the bacterium
- No growth of the bacterium
* Original - ether fraction of 0.2 g/ml of crude extract

Table 6. Effect of temperature on antibacterial activity of extract (Response of *Streptococcus faecalis* to the extract).

Time	Temperature						
	45°C	50°C	55°C	60 °C	65 °C	70°C	75°C
After 24 h	-	-	-	-	-	-	-
After 48 h	-	-	-	-	-	-	-

- no growth of the bacterium

All heat-treated, tested samples of extracts inhibited the growth of tested bacterium when the culture tubes were incubated at 37 °C (Table 6).

4. Discussion

Methanolic extract of pericarp of *G.magostana* at the concentration of .0.5 g/ml had the highest inhibitory activity against *Bacillus subtilis* and the activity is similar to the antibiotic penicillin (0.005 g/ml) against *Bacillus subtilis and Staphylococcus aureus*. Similar results have been reported by Linuma *et al.* (1996). Extracts of peel of *G.magostana* showed inhibitory effects against methicillin-resistant *Staphylococcus aureus*.

Ethyl acetate-soluble phenols (ethyl acetate) showed a MIC of 0.005 g/ml of crude extract against *Bacillus subtilis, Staphylococcus aureus* and *Streptococcus faecalis.* Ether fraction of crude extract was also examined and the antibacterial activity against tested Gram-positive bacteria was similar to that of ethyl acetate fraction.

Linuma *et al.* (1996) too have reported the presence of one active isolate, alpha-mangostin, a xanthone derivative with a minimum inhibitory concentration (MIC) of 1.57 -1.5 micrograms mL^{-1}. The extract of the fruit hull has been fractionated by silica gel chromatography to monitor the pharmacological activity and active compounds (Chairungsrilerd *at el.* 1996).

To isolate the chemical constituents that are present in methanolic extract of pericarp of *G.magostana* , phytochemical screening was performed to fractionate the same into ether soluble and ethyl acetate soluble phenolics. The results of phytochemical screening of crude extract of the pericarp provide information on the nature of compounds, which may include some active compounds possessing antibacterial activity. It is reported that secondary plant metabolites such as alkaloids, flavonoids and steroids show antimicrobial activities (Bandaranayake,1995).

Extensive phytochemical studies have shown that *Garcinia* spp. are rich in a variety of oxygenated and prenylated xanthones (Bennet and Lee, 1989). Since xanthones have phenolic functional groups, they exhibit a wide range of biological activities including antimicrobial activities (Russian *et al.* 1982). Hence, results of this study is agreeable with the results reported by Hussian *et* a/.(1982). It was found that ether soluble compounds and ethyl acetate soluble phenols possess inhibitory activities on tested Gram-positive bacteria. Results revealed that active compounds of pericarp of *G. mangostana* could tolerate higher temperatures (up to 75 °C) without losing antibacterial properties.

Since crude methanolic extract was used in plate assay, there is a possibility that the mixture of several active compounds is responsible for the inhibition of bacterial growth instead of a single compound. Synergistic activity is evident when the sum of the effects of the active components used in combination is significantly greater than the sum of their effects when used individually. However, separation of constituents by chromatographic analysis could fractionate the active fractions. No inhibition of bacterial growth was observed in controls. Ether and ethyl acetate are volatile and would have got evaporated.

The strong *in vitro* antibacterial activity of crude methanolic extract and, ether and ethyl acetate fractions of *G.magostana* against *Bacillus subtilis, Staphylococcus aureus* and *Streptococcus faecalis* suggests that the compounds may be of useful pharmaceutical value

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