Antibacterial activity of extracts of pericarp of *Garcinia mangostana*

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Received on: 08/11/06
Accepted on: 05/12/07

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 0.005 g/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 serotonin. 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 (10⁶ cfu/ml) of tested bacteria separately. Whatman 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.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Methanolic extract (0.5 g/ml)</th>
<th>Streptomycin sulphate (0.005 g/ml)</th>
<th>Penicillin (0.005 g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>20.60±0.75</td>
<td>31.50±0.29</td>
<td>19.75±0.48</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>16.62±0.93</td>
<td>19.50±0.50</td>
<td>19.00±0.41</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>17.11±0.51</td>
<td>29.50±1.32</td>
<td>17.50±0.29</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>no zone of inhibition</td>
<td>33.50±0.29</td>
<td>27.50±0.41</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>no zone of inhibition</td>
<td>31.50±0.87</td>
<td>29.75±0.75</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>no zone of inhibition</td>
<td>no colony growth</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Concentration of the extract (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (Control)</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>+</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>+</td>
</tr>
</tbody>
</table>

+ 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 ferricyanide-FeC\(_{6}\) 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\(_3\)COOH 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\(_3\)COOH, 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$_3$COOH, 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

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Ether fraction</th>
<th>Ethyl acetate fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>15.00±0.41</td>
<td>15.60±0.26</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>15.00±0.41</td>
<td>16.17±0.66</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>15.50±1.04</td>
<td>16.67±0.67</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Concentration of the extract (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td></td>
</tr>
</tbody>
</table>
Antibacterial activity of *Garcinia mangostana*

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.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Concentration of the fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em></td>
<td>+</td>
</tr>
</tbody>
</table>

+ 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).

<table>
<thead>
<tr>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45°C</td>
</tr>
<tr>
<td>After 24 h</td>
<td>-</td>
</tr>
<tr>
<td>After 48 h</td>
<td>-</td>
</tr>
</tbody>
</table>

- 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 (Chairungsriplerd et al. 1996).

To isolate the chemical constituents that are present in methanolic extract of pericarp of *G. mangostana*, 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 al. (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 ℃) 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. mangostana* against *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis* suggests that the compounds may be of useful pharmaceutical value.

5. References

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