ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF GUAVA (PSIDIUM GUAJAVA L.) LEAVES

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ABSTRACT

Ethanolic extract of shed dried and powdered leaves of Psidium guajava L. collected from Murshidabad district exhibited very good antibacterial activity against Gram positive bacteria viz. Staphylococcus aureus, Bacillus subtilis, Listeria monocytogenes and Gram negative bacterium Escherichia coli when tested by disc diffusion method. It has slight inhibitory effect against Pseudomonas aeruginosa but was unable to produce zones of inhibition against Salmonella typhimurium during in vitro study. By counting the colony forming units, minimum inhibitory concentration (MIC) was decided as 4 mg/ml in case of Gram positive bacteria and 5 mg/ml in case of Gram negative bacteria. A remarkable decrease in the number of CFUs indicated cidal mode of action of the leaf extract against the Gram positive bacteria but in case of Gram negative bacteria, pattern of CFU decrease in treated set suggested the bacteriostatic mode of action of the leaf extract. The extract also had very strong antioxidant property with IC50 value 4.1 µg/ml.

KEYWORDS: Antibacterial activity, Minimum inhibitory concentration, Cidal mode of action, Static mode of action, Antioxidant property.

INTRODUCTION

Plant bioactive compounds are utilised in keeping human beings away from sufferings since antiquity. Most of these bioactive compounds are produced as their secondary metabolites.1 Medicinal plants which serve as natural sources of compounds can be used against many diseases today.2

Psidium guajava L. (Guava) is a very common fruit plant available all over the world, belonging to the family Myrtaceae. It is an important plant in therapeutic aspects. The leaves of this plant have a valuable medicinal importance. Various secondary metabolites obtained from the leaf extract of Psidium guajava L. are used to control bacterial and fungal infections. These leaves contain a number of beneficial substances, including antioxidants like vitamin C and flavonoids such as quercetin. Antioxidants are those compounds which protect the health from the damage by different free radicals. The guava leaves have been used in traditional medicine to control diarrhea, dysentery, malaria, wounds, gastroenteritis, vomiting, ulcers, sore throat, toothache, inflamed gums and a number of other conditions like hypertension, obesity and diabetes.3,4,5

As the constituent of the plants varies from place to place, the main objectives of the present study were to determine the antibacterial activity of Ethanolic extract of guava leaves grown in Murshidabad district against number of pathogenic bacteria and evaluated antioxidant property.

MATERIALS AND METHOD

Collection of plant material
Fresh and healthy leaves of Psidium guajava L. were collected from Murshidabad and was identified by Prof. S. Mondal, Angiosperm Taxonomist of the department of Botany, Visva-Bharati. A voucher specimen is also kept in the departmental Herbarium.

Preparation of crude extract
Collected leaf samples were washed properly in distilled water, shed dried, coarsely powdered and then subjected to ethanolic extraction. 10 g of the powdered leaf samples were extracted with 100 ml of ethanol for overnight under shaking condition. The concentrated ethanolic extract was then centrifuged at 10000 rpm for 10 minutes to discard the debris. The supernatant was taken and evaporated to dryness. The dried leaf extract was stored at 4° C for further work.
Antibacterial activity of ethanolic extract of guava leaves

Test micro-organisms
Three Gram positive bacteria viz. Staphylococcus aureus MTCC 96, Bacillus subtilis MTCC 121, Listeria monocytogenes MTCC 657 and three Gram negative bacteria viz. Escherichia coli MTCC 1667, Salmonella typhimurium MTCC 98 and Pseudomonas aeruginosa MTCC 741 were used in the present study. All the stains were collected from Institute of Microbial Technology, Chandigarh and were maintained on nutrient agar (NA) slants at 37 °C (for E. coli, S. aureus, P. aeruginosa, S. typhimurium) or at 28 °C (for B. subtilis, L. monocytogenes), with regular transfer.

Disc diffusion method
Antibacterial activities of guava leaves extract was checked by disc diffusion method against pathogenic bacteria. The dried ethanolic extract was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/ml and from this stock solution different concentrations (1-100 mg/ml) of the extract were prepared. Overnight grown cultures of pathogenic bacteria were spread over the nutrient agar plates with the help of sterilised cotton swab. Sterilised filter paper disc were soaked in different concentrations of guava leaves extract and placed on pathogenic bacterial lawn of NA plates. Only DMSO was used as negative control. All the plates were incubated at their respective growth temperatures for 24 h, inhibition zones were observed around the paper disc and the diameters were measured.

Determination of minimum inhibitory concentration (MIC)
Minimum inhibitory concentration (MIC) values of guava leaves extract was determined by counting the number of colony forming units (CFUs) of pathogenic bacteria after treatment with different concentrations (1 - 6 mg/ml) of guava leaves extract for 24 h. In control sets only equal volume of DMSO were added. 100 µl of bacterial cultures from untreated control and treated sets were taken and spread on the NA plates after serial dilution and incubated at respective growth temperatures. After overnight incubation the number of CFUs were counted and compared with control.

Study of mode of action
Mode of action of guava leaves extract was checked against one Gram positive bacterium S. aureus and one Gram negative bacterium E. coli. On actively growing cultures (mid log phase) of pathogenic bacteria, ethanolic extract of guava leaves were added at its MIC values and CFUs of pathogenic bacteria were counted at regular time intervals. In control set only DMSO was added. Mode of action was determined by observing the pattern of changes in CFU counts in treated set in comparison to untreated control.

Antioxidant activity of ethanolic extract of guava leaves

Antioxidant activity of ethanolic extract of guava leaves was checked by 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay as described by Lee et al.[7] Various concentrations (1-10 µg/ml) of leaf extract were prepared by dissolving the powdered leaf samples in methanol. 100 µl of different concentrations were taken and added to 2900 µl of DPPH solution, mixed properly and left for 30 minutes in dark condition. For control set, only DPPH solution was used and only methanol was taken as blank. The reduction in the free radical was determined by measuring the absorption at 517 nm (UV-visible spectrophotometer, Shimadzu). The percentage of free radical scavenged was calculated following this formula.

\[
\text{[(A control-A sample)/A control] ×100}
\]

Where, A control: Absorbance of control A sample
Absorbance of solution containing leaf extract.

IC50 value was calculated by plotting the values in the graph. Ascorbic acid was used as standard in this experiment.

RESULTS AND DISCUSSION
The dried leaf samples were subjected to ethanolic extraction to obtain the biologically active ingredients of the plant. The dried crude extract was dissolved in non toxic organic solvent DMSO for antimicrobial study. The ethanolic extract of guava leaves showed prominent zones of inhibition against all the three Gram positive bacteria tested and one Gram negative bacterium E. coli (Fig. 1). It had very minute inhibitory effect against Pseudomonas aeruginosa and no effect against Salmonella typhimurium. The zones of inhibition ranges from 14-15 mm in case of Gram positive bacteria whereas in Gram negative bacteria these ranges from 8-12 mm. During disc diffusion method the inhibition zones observed around the paper disc at 5 mg/ml concentration against all the Gram positive bacteria tested and Gram negative bacteria E. coli. The crude extract showed zones of inhibition against P. aeruginosa at 30 mg/ml concentration. The maximum zone of inhibition (15 mm ± 1.5) was observed against S. aureus which is responsible for several infections of skin and soft tissues. Whereas in case of endospore forming B. subtilis and food borne as well as food spoilage bacteria L. monocytogenes, the maximum inhibition zones was 14.1 mm ± 1 and 14 mm ± 1.5 respectively. The ethanolic extract produced maximum zones of inhibition against Gram negative bacteria E. coli and P. aeruginosa which was 12 mm ± 1 and 8 mm ± 1 respectively (Fig 2). Biswas et al.[8] have reported the antibacterial activity of guava leaves extract only against Gram positive bacteria whereas Vieira et al.[9] found the inhibitory effect of guava sprout extract against E. coli.
Figure 1: Zones of inhibition produced by ethanolic extract of guava leaves against pathogenic bacteria A: *Staphylococcus aureus*, B: *Escherichia coli*.

Figure 2: Antibacterial activities of the ethanolic extract of guava leaves against pathogenic bacteria.

MIC values were determined at 4 mg/ml in case of Gram positive bacteria and 5 mg/ml in case of Gram negative bacteria by counting the number of CFUs of pathogenic bacteria (Table 1). The more effectiveness of the leaf extract against Gram positive bacteria was probably due to the structural difference of bacterial cell wall. The Gram negative bacteria contain an outer lipopolysaccharide layer in addition to peptidoglycan layer. On the other hand Gram positive bacteria possess only peptidoglycan layer which make it more susceptible to plant extracts.

Table 1: Effect of ethanolic extract of guava leaves on CFUs of pathogenic bacteria.

<table>
<thead>
<tr>
<th>Concentration of leaves extract (mg/ml)</th>
<th>S. aureus</th>
<th>B. subtilis</th>
<th>L. monocytogenes</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$5.24 \times 10^{11}$</td>
<td>$5.71 \times 10^{8}$</td>
<td>$3.77 \times 10^{7}$</td>
<td>$3.98 \times 10^{6}$</td>
</tr>
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<td>1</td>
<td>$6.60 \times 10^{8}$</td>
<td>$2.53 \times 10^{6}$</td>
<td>$3.82 \times 10^{5}$</td>
<td>$5.18 \times 10^{4}$</td>
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<td>2</td>
<td>$2.67 \times 10^{7}$</td>
<td>$5.86 \times 10^{5}$</td>
<td>$2.29 \times 10^{4}$</td>
<td>$4.85 \times 10^{3}$</td>
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<td>$1.12 \times 10^{6}$</td>
<td>$3.79 \times 10^{4}$</td>
<td>$3.65 \times 10^{3}$</td>
<td>$6.6 \times 10^{2}$</td>
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<td>$2.43 \times 10^{2}$</td>
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</tr>
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<td>$2.064 \times 10^{1}$</td>
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<td>$6.85 \times 10^{-1}$</td>
</tr>
<tr>
<td>6</td>
<td>$1.65 \times 10^{4}$</td>
<td>$1.55 \times 10^{2}$</td>
<td>$2.21 \times 10^{0}$</td>
<td>$3.44 \times 10^{-1}$</td>
</tr>
</tbody>
</table>

During mode of action study, ethanolic extract of guava leaves were added at its MIC to the actively growing cultures of *S. aureus* and *E. coli*. A sharp decrease in the number of CFUs in case of *S. aureus* indicated cidal mode of action of the leaf extract against that Gram positive bacteria (Fig. 3A). On the other hand in case of *E. coli* pattern of CFU decrease in treated set suggested the bacteriostatic mode of action of the leaf extract (Fig.3B). In most of the cases the cidal effect is found when cell walls are disrupted as it was found in pathogenic bacteria treated with triterpenoid compounds obtained from *Limnophila indica*.[11] Such cidal mode of action against Gram positive bacteria by the metabolites of the guava leaves was not reported earlier.
The crude ethanolic extracts of guava leaves tested for the antioxidant activity using the DPPH free radical scavenging method and compared with ascorbic acid (standard). The color changes from pink to yellow indicate the antioxidant property. The ethanolic extract of guava leaves showed very strong antioxidant activity, with IC_{50} value of 4.1 µg/ml whereas IC_{50} value of ascorbic acid was 20.23 (Fig. 4). Tachakittirungrod et al.\textsuperscript{[12]} also reported similar type of antioxidant activity of guava leaves extract grown in Thiland.

CONCLUSION
The crude ethanolic extract of guava leaves was able to kill which are surface infecting, endospore forming and food spoilage microorganisms like \textit{S. aureus}, \textit{B. subtilis}, \textit{L. monocytogenes} respectively. It also exhibited its inhibitory activity against \textit{E. coli} and mild activity against \textit{P. aeruginosa} but was unable to kill \textit{S. typhimurium}. The results indicate that the guava leaves extract contain some antimicrobial compounds which can be very useful to control different Gram positive pathogenic bacteria with cidal mode of action. The guava leaf extract also showed very good antioxidant activity. Further research is needed to identification and purification of bio active compounds of the leaf extract.

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REFERENCES


