The efficacy of guava (*Psidium guajava* L.) leaf extract on some bacterial isolates from milk

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**Abstract**

Pathogenic bacteria isolated from cow milk represent a major role in mastitis and are not easy to treat even with the use of a wide range of antibacterial agents. Finding alternative substances that may eliminate those bacteria with less side effects especially if they are derived from natural sources. Here, guava (*Psidium guajava* L.) leaf extracts (GLE) of six solvents; n-hexane (HGLE), methanol (MGLE), ethanol (EGLE), ethyl acetate (EtGLE), and chloroform (CGLE) were used to evaluate their Mueller-Hinton-II based inhibition activities against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Salmonella* spp. (isolated from milk of local cows in the current work). The results showed that the CGLE was highly effective in decreasing the growth of the selected bacteria, followed by, EtGLE, MGLE, and EGLE when compared with the ciprofloxacin control. The findings revealed that HGLE did not show any activity in inhibiting those bacteria. The present study presents promising compounds for treating mastitis caused by these bacteria; however, clinical trials showed be performed to estimate their therapeutic doses, safety, and effectiveness.

**Keywords:** Bacterial mastitis, guava extract, *Psidium guajava* L.

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Introduction

Mastitis is an infectious disorder of small-ruminant mammary glands that may inflict significant or total harm to udder, does not recover to normal activity, increases the body's weight and lowers the offspring's growth rate. Therefore, economical wastes occur via losing animals and treatment compounds. Clinical and sub-clinical mastitis refer to the irreversible obstruction of milk canals as a consequence of severe inflammatory transitory symptoms as a consequence of painful, toxic and bacteriological shifts in the mammalian gland. Factors such as maladministration, poor grooming and teat accidents are considered to be foreign enhancing the entry of pathogens. Pathogenic microorganisms can only be observed through lab experiments in mastitis. Subclinical illness of mastitis is one of the most severe and perceived to be a continuing source of infection for the entire flock and their ecosystem. Therefore, udder pathogens need not only to protect animals from, but also to protect the public consumers, to be avoided or identified in the early stages. The most important causative agents for mastitis in dairy animals are Staphylococcus aureus, Staphylococcus spp. and other bacterial infectious agents (1–11).

For the economic impacts of mastitis, in a period of one year between 2011 to 2012, US$ 19,132,35 had an economic effect. The economic effect was mainly affected by cattle slaughter (39.4%) and a decreased supply of milk due to subclinical and clinical mastitis (32.3% and 18.2% respectively). The two important elements were the decrease of milk production from mastitis (77.7%) and the dumping of milk (14.0%) utilizing actual mastitis indexes. The milk production decline was 27.2% above the reduction estimations. However, more deep estimations found that the financial influence was 91, 552, 69 US dollars. Most of the financial impacts come from using antibiotic treatments, disposal of milk, and animal deaths (12).

Using these antibiotics increases crises in different directions; one is via inserting antibacterial resistance into the system and developing side effects such as the reduction in milk production. Thus, finding alternative natural sources of antibacterial compounds may improve the overall welfare of the animal production and eventually the economics around the world. Here, guava (*Psidium guajava* L.) leaf extracts (GLE) of six solvents; n-hexane (HGLE), methanol (MGLE), ethanol (EGLE), ethyl acetate (EtGLE), and chloroform (CGLE) were used to evaluate their Mueller-Hinton-II based inhibition activities against *Bacillus cereus, Escherichia coli, Pseudomonas*
aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Salmonella spp. (isolated from milk of local cows in the current work).

Materials and methods

Preparation of Plant Extract:

Samples of the leaves have been collected on the guava trees which grow in Malaca, Malaysia. In plastic zip lock bags with the correct labeling, random leaf samples were gathered and placed in the ice-cooler up until transferred for to the laboratory.

Extraction methods:

The leafs were water-cleaned and dried and put in a powder make-blender. For the maceration extraction method, HGLE, MGLE, EGLE, EtGLE and CGLE were used. Twenty percent concentration of the leaf powder has been applied for each solvent and placed in Erlenmeyer containers coated in aluminum foil for 3 days at ~25°C. Then, the mixtures were centrifuged at 4,000 rpm for 10mins. Finally, the supernatants were collected and stored in a fridge until performing the experiments (13).

High performance liquid chromatography (HPLC):

HPLC via L-7455 (Hitachi) and C18 Column (Waters) wwere used at ml/min of a flow. The wavelegth used was 200-800nm depending on 100% ethyl acetate. Moreover, 80% METH/20%H₂O and 40% acetonitrile (CAN)/60% H₂O were used

Preparative purification HPLC was performed utilizing water 2695 HPLC, photodiode detector, and 5 C18 purse column (4.6x150mm). A volume of 100µL was used for each injection under 1.267 PSI, 1mL/min flow, 20-80% acetonitrile (mobile phase), and 25min period of time, figure 1.
Isolation of bacteria from cow milk:

Milk samples were collected from cows in a dairy farm in Al-Diwaniyah province, Iraq. Bacteria of *B. cereus*, *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. aureus*, *Salmonella* spp. were the targets of this study. Nutrient agar culture of each bacterium grown for 18 to 24hrs at 37°C was used to obtain colonies that were placed in sterile physiological
saline containing tubes and thoroughly vortex. Then, following 0.5 McFarland standard solution (containing about 1.5 × 10⁸ CFU/mL), the turbidity was estimated for each bacterium.

Anti-bacterial activity of the guava extracts:

Using Mueller Hinton II plates and via the well-diffusion method, the test was performed following the National Committee for Clinical Laboratory Standards [CLSI]. Wells in the medium of 5mm diameter/each were made (3 plates for each solvent). Using a sterile cotton swab, streaking of the plate media was performed. Then, 50µL of each extract was inserted in each well. The parafilm-sealed plates were incubated for 24hrs at 37°C. After that, inhibition zones were measured (mm).

Results

The results showed that the CGLE was highly effective in decreasing the growth of the selected bacteria, followed by, EtGLE, MGLE, and EGLE when compared with the ciprofloxacin control. The findings revealed that HGLE did not show any activity in inhibiting those bacteria, table 1 and figure 2. Table 1: Antibacterial activity of the Psidium guajava leaf screened solvents extracts.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Ethyl Acetate</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Hexane</th>
<th>Ciprofloxacin</th>
<th>Chloroform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>8 ±0.1</td>
<td>9.5 ±0.2</td>
<td>6.12 ± 0.2</td>
<td>-</td>
<td>20.2 ± 0.1</td>
<td>10.5 ± 0.1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>6.5 ± 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.5 ± 0.12</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>12 ±0.2</td>
<td>7.2 ± 0.1</td>
<td>7.5 ±0.2</td>
<td>-</td>
<td>18.2 ± 0.1</td>
<td>10.5 ± 0.1</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>7.5 ± 2</td>
<td>8 ± 0.2</td>
<td>9.2 ± 0.1</td>
<td>-</td>
<td>14.5 ± 0.2</td>
<td>16 ± 0.2</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>9.5 ± 2</td>
<td>6.8 ± 2</td>
<td>6.3 ± 0.3</td>
<td>-</td>
<td>17.2 ± 0.1</td>
<td>13.5 ± 0.1</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>12 ±1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12 ±2</td>
<td>13 ± 1</td>
</tr>
</tbody>
</table>
Discussion

The dairy sector, which has an effect on milk yields and milk consistency, is impacted by mastitis. Many organisms correlated with mastitis cases have been identified, with *E. coli*, *Klebsiella* spp., *Streptococcus dysgalactiae* and *S. aureus* being the most specific identified bacteria linked to clinical mastitis. Identification of causative organisms allows for the effective diagnosis and proactive mastitis prevention using suitable antimicrobials utilized for mastitis and other bacterial illnesses impacting the dairy industry for prevention and control. There is also a general trend of reliance on antimicrobial agents in dairy farms. Resistance to antimicrobials exists in microorganisms that are in a role to counteract the impact of previously successful antimicrobial agents. This issue remains a major public health problem in Europe and is also one of the main environmental and food protection and growth challenges in the global economy (14–21). Finding alternative natural substances may help in improving the
resistance problem to the antibiotics and decreasing the spread of this health issue with successful treatment results using these alternatives.

The guava (*P. guajava*) contains some chemical constituents such as pentacyclic triterpenoid guajanoic acid, uvaol, beta-sitosterol, and ursolic acid, oleanolic acid (22). The GLE was found to be effective against the current study bacterial isolates which agrees with previous conducted work regarding this issue. It was detected that different types of GLE were successful in defeating the growth of resistant isolates of *S. aureus*, *P. aeruginosa*, *E. coli*, *Shigella* spp., and *Proteus* spp. Moreover, using various solvents such as methanol, acetone, and essential oils revealed great inhibitory effects against a wide range of Gram positive and negative bacterial microorganisms (23–25). The current study showed that methanol and other solvents but not hexane were effective in revealing antibacterial activity of the GLE against the employed bacteria. This completely agrees with Doltra *et al.*, (26) who found that methanol and ethanol extracts of guava leaves were the only ones in inhibiting the growth of *B. cereus* and *S. aureus*.

The present study presents promising compounds for treating mastitis caused by these bacteria; however, clinical trials showed be performed to estimate their therapeutic doses, safety, and effectiveness.

**References**


