Antibacterial Efficiency of Pomelo Peel Extract on Various Concentrations against Selected Microorganisms

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Abstract: This determined the phytochemical components and antibacterial efficiency of Citrus maxima (pomelo) peel extract on two concentrations, 75% and 95% against selected microorganisms. The phytochemical analysis revealed the presence of alkaloids, flavonoid, glycosides, saponins, sterols, tannins, and triterpines. Alkaloids were abundantly found while only traces of the other constituents were found. E. coli and P. aeruginosa both produce 10mm complete inhibition in 75% and 95% extract concentrations. On the other hand, S. aureus produces slight inhibitory activity with mean zone of inhibition of 10mm against the 75% extract concentration and again, S. aureus produced partial inhibitory activity with mean zone of inhibition of 10mm against 95% concentration. In comparison, the antibiotic Levofloxacin which served as appositive control for E. coli and P. aeruginosa, produced 17.92mm and 16.85mm complete inhibition for the 75% extract concentration. While on the 95% extract concentration, Levofloxacin produced 18.73mm and 18.70mm complete inhibition. For the positive control of S. aureus, a different antibiotic, Clindamycin, was utilized and it produced 16.30mm complete inhibition for S. aureus in the 75% concentration while 15.02mm complete inhibition for S. aureus was produced in the 95% extract concentration. These results showed that pomelo peel extract is effective in inhibiting the growth of bacteria and the difference in concentrations had a significant response for the S. aureus.

Keywords: phytochemical, antibacterial efficiency, Citrus maxima (pomelo) peel extract
Ever since the birth of mankind, there has been the relationship between life, disease and plants. *Citrus maxima* (pomelo), also known as Chinese grapefruit, belongs to the rue family (Rutaceae) and is the largest citrus fruit (Ding et al, 2013). It is native to Southeast Asia and the Indo-China regions (Cheong, 2013) and is one of the important horticultural crops growing extensively in tropical and subtropical southern regions of Asia (Lan-Phi and Vy, 2015). The pomelo tree grows 16-50 feet (5-15 meters) tall and it is a perennial shrub. Its peel may be greenish-yellow or pale-yellow while the pulp varies from greenish-yellow or pale-yellow to pink or red. The fruit’s taste varies from mildly sweet and bland or rather acid, and sometimes with a faint touch of bitterness (Cheong, 2013). Guo and Abeyesinghe as cited in Toh et al. (2013) state that the peel of the citrus fruit contains a higher amount of antioxidants as compared to its pulp, as the purpose of the peel is to protect the antioxidants in the fruit from oxidation. Therefore, it is recommended to consume the fruit together with its peel.

The pomelo fruit has been linked to several purposes. It is rich in vitamin C and has been used in indigenous medicine as a sedative, in nervous affections, convulsive cough, and in treatment of hemorrhagic disease and epilepsy (Vijaylakshmi and Radha, 2015). According to Arias and Ramon-Laca as cited in Caengprasath et al. (2012), the pulp of the pomelo was used as an appetizer, antitoxic, cardiac stimulant, and stomach tonic (Caengprasath et al, 2012). Furthermore, Mokbel and Suganuma as cited in Naradisorn and Ruenkum (2009) state that pomelo extracts have been revealed to have an antimicrobial activity against several bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Salmonella enteritidis* and *Escherichia coli*.

The human skin normally serves as a primary line of defense against infections caused by the aforementioned microorganisms. However, some bacteria are found in the normal flora which can be opportunistic once there’s breakage on the skin. Normal flora found in the skin includes *Staphylococcus aureus*, which is mostly localized in the nose and other orificial areas (Tognetti et al, 2012). *Pseudomonas aeruginosa* could infect people who have compromised immune systems and it could infect different areas of the body including the skin which can be life-threatening (Friedrich et al, 2015). *Escherichia coli* strains frequently are isolated from skin and soft tissue infections (Petkovsek, 2009). It is a major cause of diarrheal diseases, peritonitis, colitis, bacteremia, infant mortality, and urinary tract infections (Blount, 2015).

Commonly used as a sweet delicacy, pomelo is overlooked as a source of prevention in microorganism proliferation. A study by Sikander et al. (2013) showed that citrus fruits exhibited antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Further, the study in the King Mongkut’s University of Technology North Bangkok stated that the effect of crude extract from pomelo peel can be used to inhibit *Staphylococcus aureus* (Aichayawanich & Ngaouwthong, 2012).

In view of previous findings regarding the high antimicrobial and anti-oxidant activities of a number of phytochemicals inherent in citrus fruits, the pomelo fruit can therefore be a potential replacement for synthetic preservatives as all citrus fruits have similar complex structures regardless of cultivars. Therefore, the pomelo holds potential for providing multiple benefits to consumers by way of its possible usage in the fields of medicine, therapeutics and food technology (Barrion et al, 2014). Due to the components found in the pomelo peel, the antibacterial properties may aid in eliminating the bacteria found in a person’s skin. This study was conceptualized to explore this potential and provide evidence of the fruit’s antibacterial efficiency.

**Objective**

This study determined the phytochemical components and antibacterial efficiency of *Citrus maxima* (pomelo) peel extract on two concentrations, 75% and 95%, against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

**Methods**

This study utilized an experimental research design. All the microorganisms were preserved and obtained from the Department of Science and Technology. The extract underwent Phytochemical
Analysis and Antimicrobial Activity Test using Disc Agar Diffusion method Kirby-Bauer Test on the three selected bacteria.

**Collection and Preparation of Pomelo Peels**

Pomelo fruit peels were collected and placed in a clean, air-tight container. The peels were thinly sliced for increased drying of moisture content. After slicing, the peels were placed in a container for weighing. Around 500 grams of pomelo peel were weighed. For drying, the peels were placed inside the Multi Commodity Heat Pump Dryer overnight. The peels were weighed again after an hour of drying to ensure the decrease in moisture content. The peels were placed in a blender and ground until they were in powdered form. They were then placed in a clean plastic container.

**Preparation of Pomelo Peel Extract**

One hundred grams of the ground plant material were weighed in an Erlenmeyer flask. Three hundred milliliters of 80% ethyl alcohol were then added to completely submerge the materials. The solution was stoppered and soaked for 24 hours. It was filtered through a Buchner funnel using gentle suction. The flask and the plant material with fresh portions of alcohol were rinsed. The washings with the first filtrate were combined. The plant residue was discarded.

The filtrate under vacuum was concentrated to a syrupy consistency or about 20 milliliters. The exact volume of the concentrated extract was measured. This is the strength of the extract expressed in grams of plant material per milliliter of the extract. The extract was stored in a tightly stoppered container in a cold environment (0-5 degrees Celsius). The extract was then considered ready for phytochemical and microbiological screening. The extract was weighed for quantitative determinations in biological tests.

For fresh plant material, 200g of the finely cut fresh material were used and soaked in 300 milliliters of 95% ethyl alcohol to completely submerge the material. Then the same procedure was followed.

**Phytochemical Analysis of the Pomelo Peel extract**

Following the extraction, the pomelo peels underwent phytochemical analysis to test for its chemical constituents. All the chemicals used for testing were purchased from the Department of Science and Technology and the process of analysis was based on the procedures of Standards of Testing Division where the test was conducted.

**Disc Agar Diffusion Method or Kirby–Bauer Test**

Kirby-Bauer test was performed under the standard conditions of the facility. The main purpose of this test was to calculate the inhibitory concentration for a given antibiotic by comparing the observed zone of inhibition’s size to known values.

**Findings and Discussion**

**Antimicrobial Activity Test Result**

Table 1 presents the antimicrobial activity test results of the pomelo (*Citrus maxima*) peel extract of 75% concentration against three microorganisms.

<table>
<thead>
<tr>
<th>Sample/Control</th>
<th><em>Escherichia coli</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
<th><em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Mean Zone of Inhibition Reactivity</td>
<td>Inhibitory Activity</td>
<td>Total Mean Zone of Inhibition Reactivity</td>
</tr>
<tr>
<td>Pomelo Peel Ethanolic</td>
<td>10</td>
<td>2</td>
<td>+++</td>
</tr>
</tbody>
</table>
The 75% ethanol extract showed that *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* are reactive organisms to the pomelo peel extract. The bacteria *Escherichia coli* produced complete inhibitory activity (++) with a total mean zone of inhibition of 10mm and had a mild reactivity rating (2) from the extract. This is in contrast with one study by Barrion et al (2014) where *E. coli* was observed to be resistant to all the sample extracts at all concentrations. The findings also show that *Pseudomonas aeruginosa* produced complete inhibitory activity (++) with a total mean zone of inhibition of 10mm and had mild reactivity (2) against the pomelo peel.

In comparison, the antibiotic Levofloxacin (5 ug) which served as a positive control for *Escherichia coli* and *Pseudomonas aeruginosa* produced complete inhibitory activity (++) with a total mean zone of inhibition of 17.92 and 16.85 for for *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. Both resulted to a severe reactivity rating (4).

On the other hand, *Staphylococcus aureus* produced slight inhibitory activity (+) with a mean zone of inhibition of 10mm and had mild reactivity (2) against the extract concentration. For the positive control of the *Staphylococcus aureus*, a different antibiotic, Clindamycin (2 ug), was utilized. It produced complete inhibitory activity (++) with a total mean zone of inhibition of 16.30 and a severe reactivity rating (4) for *Staphylococcus aureus*.

Table 2
*Antimicrobial Activity Test: 95% Concentration of Ethanol Extract*

<table>
<thead>
<tr>
<th>Sample/Control</th>
<th><em>Escherichia coli</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
<th><em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Mean Zone of Inhibition</td>
<td>Reactivity</td>
<td>Inhibitory Activity</td>
</tr>
<tr>
<td>Pomelo Peel Ethanolic Extract 95% (10mm)</td>
<td>10</td>
<td>2</td>
<td>+++</td>
</tr>
<tr>
<td>Levofloxacin 5 ug (6-mm positive control)</td>
<td>18.73</td>
<td>4</td>
<td>+++</td>
</tr>
<tr>
<td>Clindamycin 2 ug (6-mm positive control)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Table 2 shows the antimicrobial activity test results of the pomelo (*Citrus maxima*) peel extract of 95% concentration against three microorganisms. The 95% ethanol extract showed that *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* are reactive organisms to the pomelo peel.

*Escherichia coli* produced complete inhibitory activity (+++) with a total mean zone of inhibition of 10mm and had a mild reactivity rating (2) from the extract. *Pseudomonas aeruginosa* produced complete inhibitory activity (+++) with a total mean zone of inhibition of 10mm and had mild reactivity (2) against the pomelo peel. On the other hand, *Staphylococcus aureus* produced partial inhibitory activity (+) with mean zone of inhibition of 10mm and had a mild reactivity (2) against the extract concentration.

In comparison, the antibiotic, Levofloxacin (5 ug), which served as a positive control for *Escherichia coli* and *Pseudomonas aeruginosa*, produced complete inhibitory activity (+++) with a total mean zone of inhibition of 18.73 and 18.70 for *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. Both resulted to a severe reactivity rating (4).

For the positive control of the *Staphylococcus aureus*, a different antibiotic, Clindamycin (2 ug) was utilized. It produced complete inhibitory activity (+++) with a total mean zone of inhibition of 15.02 and a moderate reactivity rating (3) for *Staphylococcus aureus*.

The results of the antimicrobial activity test mean that the 75% and 95% concentration of the pomelo peel extract have the capacity to completely inhibit the proliferation of the bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. This shows that the pomelo peel has positive antibacterial properties. These findings coincide with the study of Borah (2013) which determined that the plant extract of *Citrus maxima* showed significant antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. Both bacteria displayed mild reactivity to the extract.

It is to be noted, however, that the 75% concentration of the pomelo peel extract did not completely inhibit the proliferation of the *Staphylococcus aureus*. Hence, it may be concluded that the extract is not as effective when exposed to this microorganism which showed mild reactivity to the pomelo peel extract.

The antibiotics Levofloxacin and Clindamycin completely inhibited the growth of bacteria and all microorganisms tested either reacted moderately or severely to the aforementioned antibiotics. Comparing the extract with the positive control or antibiotics, it showed that the antibiotics were still more effective in killing and inhibiting the growth of bacteria. Upon seeing the results of the antimicrobial test, the higher concentration of the extract displayed a more effective inhibition of bacteria.

**Conclusion**

Based on the findings of the study, it was concluded that the pomelo peel extract showed positive results in inhibiting the growth of bacteria from the three selected microorganisms. The two different concentrations of pomelo peel extract has showcased its capability to completely inhibit the proliferation or spread of *Escherichia coli* and *Pseudomonas aeruginosa*. The extract exhibited a partial or slight inhibitory activity against *Staphylococcus aureus*. The higher concentration showed a better antibacterial effect on *S. aureus* which implies more components of the pomelo peel are essential in order to increase the effectiveness of the antibacterial properties from the pomelo peel extract.