ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *PSIDIUM GUAJAVA* (GUAVA) MEDICINAL PLANT LEAVES USED IN FOLK MEDICINE FOR TREATMENT OF WOUNDS AND BURNS IN HUFASH DISTRICT AL MAHWEET GOVERNORATE–YEMEN

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**ABSTRACT**

**Objective:** *Psidium guajava* (PG) belongs to the family Myrtaceae that is believed to have active components that help to treat conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other condition. The objective of current study was phytochemical screening of chemical constituents of *Psidium guajava* extract.

**Methods:** In this study methanolic and aqueous extracts of one plant namely *Psidium guajava*, were screened for the presence of phytochemical constituents and tested for their antimicrobial and antioxidant activity.

**Results:** TLC tests conducted revealed Rf values in the leaves for alkaloids, Flavonoids, Tannins, Phenols and Saponins (0.96-0.97-0.99-0.97-0.99) respectively. The antimicrobial activity extracts against four bacterial isolates *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* sp. and a single fungal isolate *Candida albicans* with concentrations (0.5 mg/ml, and 1.0 mg/ml) of the extract were added to the disc and respective solvent was used as negative control. The antioxidant activity of leaf was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH), the results showed are 88.4%, highest from standard, ascorbic acid 87.5%.

**Conclusion:** The qualitative phytochemical analysis revealed the results showed presence of alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols, and amino acid were present in the methanol extract, with absence of glycosides, and amino acids in the aqueous extracts in leaves plant.

**Keywords:** Antimicrobial, antioxidative, phytochemical, *Psidium guajava*.

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cellulose, mineral salts, and a number of other fixed substances. In a study that attempted to investigate antioxidant activity of Psidium guajava leaves extract by DPPH 2, 2-diphenyl-1-pircyldihydrazyl free radical scavenging method using ascorbic acid as standard, it was found that the extract of P. Guajava leaves extract was found to possess strong antioxidant activity, this activity of P. Guajava extract may be attributed to their free radical-scavenging ability. The extent of antioxidant activity of P. Guajava extract was found significant as compared to standard value for P. Guajava leaves extract was found to be 45.5 μg/ml. Thus P. Guajava leaves possess moderate antioxidant activity as compared as standard.

| Table 1: Rf values of TLC solvent system for different extracts of Psidium guajava. |
|-----------------|-----------------|------------------|-----------------|-----------------|
| Phytochemical   | Mobile phase    | Confirmatory test | Extract          | Rf Value        |
| Alkaloids       | Acetone:water:26%ammonia (90:7:3) | Dragendorff reagent | 1 ml HCL+9 ml water | 0.96 |
| Flavonoids      | Chloroform: ethyl acetate (6:4) | Aluminum chloride reagent | 70% ethanol | 0.97 |
| Tannins         | Chloroform: ethyl acetate (6:4) | 10% FeCl3 reagent | 25 ml water | 0.99 |
| Phenols         | Toluene: Acetone: formic acid (60:60:10) | 10% KOH reagent | Methanol | 0.97 |
| Saponins        | Ethyl acetate | Vanillin sulfuric acid reagent | Methanol | 0.99 |

Table 2: Yields of Psidium guajava leaves extracts from methanolic and aqueous extracts.

<table>
<thead>
<tr>
<th>M</th>
<th>Powder of plants</th>
<th>Amount of samples used (g)</th>
<th>Solvent</th>
<th>Volume of the solvent used (ml)</th>
<th>Extract yield/(g)’*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Psidium guajava</td>
<td>100</td>
<td>Pure Methanol</td>
<td>400</td>
<td>32.40±0.08</td>
</tr>
<tr>
<td>2</td>
<td>Psidium guajava</td>
<td>100</td>
<td>distilled Water</td>
<td>400</td>
<td>27.62±0.06</td>
</tr>
</tbody>
</table>

Table 3: Phytochemical composition of the methanolic and aqueous leaves extracts of Psidium guajava.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Chemical Compounds/ Solvents</th>
<th>Alkaloids</th>
<th>Terpenoids</th>
<th>Glycosides</th>
<th>Resins</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Phenols</th>
<th>Amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psidium guajava L.</td>
<td>Methanolic extract</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Aqueous extract</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4: Antimicrobial activity of standard antibiotics discs against tested bacterial and fungal.

<table>
<thead>
<tr>
<th>Antibiotic Organisms</th>
<th>AM (10ug)</th>
<th>CIP (25ug)</th>
<th>CF (30ug)</th>
<th>PZ (75ug)</th>
<th>PC (100ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>19</td>
<td>26</td>
<td>20</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>17</td>
<td>28</td>
<td>18</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>18</td>
<td>30</td>
<td>17</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>20</td>
<td>33</td>
<td>22</td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>21</td>
<td>31</td>
<td>20</td>
<td>19</td>
<td>22</td>
</tr>
</tbody>
</table>

AM=Amoxycillin, CIP=Ciprofloxacín, CF=cefazilín, PZ=Cefoperazone, PC=piperacilín

MATERIALS AND METHODS

Samples extraction: The Samples of 100g of the powdered powder were put in sterilized flasks together with 400 ml of pure methanol for methanolic extraction treatments, while for aqueous extraction treatments, samples of 100g of powdered powder were put in sterilized flasks with 400 ml of distilled water each. All flasks were covered with transparent nylon and tin and then all were put on a rotary shaker machine for 24 hours. The filtration process for each sample was carried out using filter paper to obtain a pure solution. The evaporation process for each methanol solution and distilled water was conducted separately in the evaporator methanol and distilled water solution. Then the obtained extracts were kept in dark conditions in the refrigerator at 4°C until used in the experiment.

Qualitative tests

Phytochemical screening of plant extracts

The methanolic and aqueous extracts subjected to phytochemical screening were alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols, and amino acids.

Alkaloids: Dragendorff’s test
In a test tube, 2-3 drops of Dragendorff’s reagent was added to 0.1 ml of the extract ,orange precipitate indicated the presence of alkaloids.

Terpenoids: Salkowski test
In a test tube 5ml of extract was mixed in 2ml of chloroform and then 3ml of concentrated sulfuric acid was added to form a layer. A reddish brown coloration forms at interface.

Glycosides: Keller-Killani test
Concentrated sulfuric acid in a test tube and extract sample were mixed with glacial acetic acid containing 1 drop of Ferric chloride (1:1:1 volume). A brown ring appears in the presence of glycosides.
Resins: Turbidity test
To 5ml extract 5ml distilled water was added, the occurrence of turbidity shows the presence of resins.

Saponins: Foam test
A 5ml extract was shaken with 2 ml of distilled water. If foams are produced and persists for ten minutes this indicates the presence of saponins.

Tannins: FeCl₃ test
A 4 ml extract was treated with 4 ml FeCl₃, the formation of green colour was taken as positive for tannin.

Flavonoids: Shinoda test
Extract was mixed with magnesium ribbon fragments, and concentrated hydrochloric acid was added drop wise. Orange, red, pink, or purple coloration indicates the presence of flavonoids.

Phenols: FeCl₃ test
Extract was mixed with 2 ml of 2% solution of FeCl₃. A blue-green or black coloration indicated the presence of phenols.

Amino acids: Biuret test
Extracts and 1 drop 2% Copper sulphate solution and 1 ml 95% ethanol excess of potassium hydroxide were mixed. Pink or yellow color in ethanol layer appears.

Thin Layer Chromatography.
One gram of *Psidium guajava*, powder was boiled with of with solvent system made from 15ml H₂SO₄ test for Alkaloids, 10ml 70% ethanol test for Flavonoids and Saponins, 25 ml water test for Tannins and phenols in rounded flasks. The TLC plate was prepared as such: (Layer: silica gel layers 0.25 mm thickness, 10 cm length and 5cm wide). The filtrate obtained was evaporated to dryness in a water bath at 37° C. The residue was dissolved by 0.2ml methanol. The solution was used for spotting the TLC by capillary tube by only one centered spot. The TLC plate was put inside a saturated tank, and development was waited. When the mobile phase reaches two thirds of plate’s length, the plate was lifted out from the tank and let to dry in air. The plate was examined by U.V. lamp at the wavelength 365nm. The colors of florescence appeared and recorded.

Figure 1: Antimicrobial activities (inhibition zones mm.) of standard antibiotics discs against tested bacterial and fungal.
Table 5: Antimicrobial activity of the methanolic extracts of leaves of (*Psidium guajava*) and standard antibiotics discs against tested bacterial and fungal.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Extract 0.5g/ml</th>
<th>Antibiotic AM (10ug)</th>
<th>CIP (25ug)</th>
<th>CF (30ug)</th>
<th>PZ (75ug)</th>
<th>PC (100ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>18</td>
<td>19</td>
<td>26</td>
<td>20</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>17</td>
<td>15</td>
<td>17</td>
<td>28</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>15</td>
<td>14</td>
<td>18</td>
<td>30</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td><em>Klebsiella sp.</em></td>
<td>14</td>
<td>15</td>
<td>20</td>
<td>33</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>15</td>
<td>17</td>
<td>21</td>
<td>31</td>
<td>20</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 6: Antimicrobial activity of the aqueous extract of leaves (*Psidium guajava*) and standard antibiotics discs against tested bacterial and fungal.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Extract 0.5g/ml</th>
<th>Antibiotic AM (10ug)</th>
<th>CIP (25ug)</th>
<th>CF (30ug)</th>
<th>PZ (75ug)</th>
<th>PC (100ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>13</td>
<td>15</td>
<td>19</td>
<td>26</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>14</td>
<td>16</td>
<td>17</td>
<td>28</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>11</td>
<td>15</td>
<td>18</td>
<td>30</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td><em>Klebsiella sp.</em></td>
<td>14</td>
<td>16</td>
<td>20</td>
<td>33</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>13</td>
<td>17</td>
<td>21</td>
<td>31</td>
<td>20</td>
<td>19</td>
</tr>
</tbody>
</table>

The plate was sprayed carefully reagent, and let to dry for 10 min. Then sprayed with solution. Then plate was examined under U.V. lamp at the wavelength 365nm. The iodine was used as the visualizing agent to detect the spot. A meter rule was used to measure the distance moved by the solvent and distance moved by spot, from which the retention factor (R_f) values of the various spots was calculated. TLC was performed for alkaloids, flavonoids, tannins and phenols solvent system and confirmatory tests are shown in Table 2. Calculation of RF of each spot was as follows:

\[ R_f = \frac{\text{Distance moved by solute from the origin}}{\text{Distance moved by solvent from the origin}} \]

![Figure 2](Image)

Figure 2: Antioxidant activities of the selected extracts and L-ascorbic acid using the (DPPH) free radical-scavenging assay.

Antimicrobial Activity of Plants extracts.

**Microbial Cultures:** Fresh plates of the four bacterial isolates *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella sp.* and a single fungal isolate *Candida albicans* were obtained from the National Center of Public Health Laboratories, Sana’a.

**Media Use:** The bacterial test were spread over the nutrient agar (56g/1000ML distilled water) was weight into separate flask and dispensed into distilled water make a total volume of 1 liter. Then the fungal test were spread over the sabouraud dextrose agar (65g/1000ML distilled Water) was weighted into separate flask and dispensed into distilled water to make a total volume of 1 liter. These powders were dissolved in distilled water and used for evaluation of their antibacterial and antifungal activities.

The mixture was heated in an electric water bath (GFC, 1083, Germany) until the Agar melted to form a homogenous solution. The prepared medium was separately transferred to Durum medium bottle and sterilized by autoclaving at 121°C for 30 minutes. The sterile medium was allowed to cool to about 45°C before being poured aseptically in an inoculation chamber (Ceslab England) in 15 ml portions, into sterile petri dishes to cool and gel into solids.

**Antimicrobial activity assay:** Two different concentrations (0.5 mg/ml, and 1.0 mg/ml) of the extract were added to the disc and respective solvent was used as negative control.

**Table 7: Antioxidant activities of the selected extracts and L-ascorbic acid using the (DPPH) free radical-scavenging assay.**

<table>
<thead>
<tr>
<th>Particular</th>
<th>Antioxidant activity DPPH (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-ascorbic acid</td>
<td>87.5±0.05</td>
</tr>
<tr>
<td><em>Psidium guajava</em></td>
<td>88.4±0.20</td>
</tr>
</tbody>
</table>

Zone of inhibition: The bacteria plates were incubated at 37°C for 24hrs while the fungal plates were incubated at for 72 hours, and observed for the zone of inhibition of growth. The zones were measured with a transparent ruler and the result recorded.

**Determination of antioxidant activity**

The scavenging ability of the natural antioxidants of the leaves towards the stable free radical DPPH was measured by the specific method. The leaf extracts (20μl) were added to 0.5ml of methanolic solution of DPPH (0.3mM in methanol) and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 min. Methanol served as the blank and DPPH in methanol, without the leaf extracts, Served as the positive control. After 30 min of incubation, the discolouration of the purple colour was measured at 517 nm in a spectrophotometer. The radical scavenging activity was calculated as follows:
Radical Scavenging Activity

\[ \text{RSA \%} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100 \]

Statistical Analysis

Analysis of variance was made for all data using (SPSS) version (25) computer program.

RESULTS AND DISCUSSION

In this study methanolic and aqueous extracts of one plant namely Psidium guajava, were screened for the presence of phytochemical constituents and tested for their microbial and antioxidant activity.

Yield from different solvents

Yield of methanolic extract of Psidium guajava, extracted with 100% methanol produced 32.40(g). While yield of distilled water extract of Psidium guajava produced 27.62(g).

<table>
<thead>
<tr>
<th>Plant</th>
<th>Solvent</th>
<th>Yield (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psidium guajava</td>
<td>Methanol</td>
<td>32.40</td>
</tr>
<tr>
<td></td>
<td>Distilled Water</td>
<td>27.62</td>
</tr>
</tbody>
</table>

Phytochemical composition of the methanolic and aqueous leaves extracts.

The summarized phytochemical screening of chemical constituents of Psidium guajava extract is shown in Table 3. The results revealed the presence of active compounds in the two different extracts. As the table shows, the methanol and aqueous extracts indicate the presence alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols, and amino acid were present in the methanol extract, with absence of glycosides, and amino acids in the aqueous extracts in all three plants.

In a previous study, methanolic extracts of Psidium guajava revealed the presence of alkaloids, tannins, flavonoids and glucosides. Similarly other study has showed the presence of tannins, saponins, flavonoids, alkaloids and phenols as our study.

Thin Layer Chromatography (TLC)

Five secondary metabolites (alkaloids, flavonoids , tannins, phenols and saponins) were used for (TLC) thin layer chromatographic analysis. TLC tests conducted revealed Rf values in the leaves of Psidium guajava for alkaloids, Flavonoids, Tannins, Phenols and Saponins(0.96-0.97-0.99-0.97-0.99) respectively. In a study done through TLC profiling proved that different Rf values represent different chemical constituents present within methanol leaf extract of Psidium guajava. There were six visible spots. The Rf values (spot 1, RF=0.98), (spot 2, RF=0.78), (spot 3, RF=0.62), (spot 4, RF=0.54) (spot 5, RF=0.32) and

Plate 1: Inhibition zones observed with leaves methanolic extracts of Psidium guajava.

Mean values of the yield are presented as mean ± SEM. Values are statistically significant when p≤ 0.05. A similar investigation done in a study revealed that aqueous extracts (16.35%) of Psidium guajava gave high yields than of methanolic extracts (14.22%), which is contrary to our findings. Similarly, a previous study also reported a 16.35% yield in aqueous extracts from Psidium guajava. Yet the percentages of yields in both studies were less than of the present study.
Similar Rf values were in agreement with this investigation.

**Antibacterial and antifungal activity of plants extracts**

Antimicrobial activity of standard antibiotics discs against tested bacterial and Fungal are displayed in Table 4 and Figure 1. The results of the study indicated that control Antibiotics against bacteria and Fungi showed different inhibitory zones. Antibiotics activity of AM (10ug), CIP(25ug) ,CF(30ug) , PZ (75ug) and PC (100ug) against *Staphylococcus aureus* were 19, 26, 20, 21, 20mm ; *E. coli* 17, 28, 18, 20, 19nm; *Pseudomonas aeruginosa* 18, 30, 17, 21, 18 mm; *Klebsiella sp* 20, 33, 22, 23, 17 mm, and *Candida albicans* 21, 31, 20, 19, 22 mm respectively.

![Image](https://via.placeholder.com/150)

**Table 4 and Figure 1**

The antimicrobial activity of the methanolic extracts of *Psidium guajava* compared to the selected antibiotics against selected microorganism Table 5 and Plate 2 showed that all antibiotics gave higher inhibition zones than the two extract concentrations. Yet, the activity of the two concentrations was closest to Amoxycillin activity, but much lower than the resistant *Staphylococcus aureus* and *Escherichia coli*. The antimicrobial activity of the aqueous extracts of *Psidium guajava* against selected microorganisms was less in activity compared to all the selected antibiotics Table 6 and Plate 2. This study showed that Ciprofloxacin (30µg) gave the highest inhibition zone among all antibiotics with the selected organisms 26, 28, 30 mm against *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* respectively. In other study Ciprofloxacin (25µg) gave high diameter of inhibition zone which reached up 19, 23, 23mm against *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* respectively. The majority of the antibacterial activity in this study was found in the methanolic rather than the aqueous extracts, and the highest activity was found in the methanolic extracts from *Psidium guajava*. Similar results were achieved in a previous study. Results from this study provide evidence for the medicinal values of the tested plants. It was showed that the antimicrobial activity of the methanol and aqueous extracts of *Psidium guajava* leaves achieved different diameters of the bacterial growth inhibition zone against *Klebsiella sp* and *E. coli*, while a study has mentioned that, the extract of *Psidium guajava* leaves had no any activity against *Klebsiella sp* and *E. coli*. 17 explained that the methanol extract of *Psidium guajava* leaves had an antibacterial activity with mean zones of inhibition of 12.3 mm, against *S. aureus*, while, in this study, high diameter of 17mm was achieved from methanol extract of *Psidium guajava* leaves Table 5 and Plate 1. In this
study, the results from water extract of *Psidium guajava* leaves against *E. coli*. Showed that the diameter of inhibition zone reached up 14mm Table 6 and Plate 2, these are similar result achieved by 18, who mentioned that, the antibacterial effects of water extracts from *P. guajava* (guava) leaves demonstrated mean exhibited zones of inhibition of 13.7mm on *E. coli*.

**Antioxidant activity**

Results showed are 88.4%, highest from standard, ascorbic acid 87.5% (Table 11 and Figure 2). These results revealed that the value of the *Psidium guajava* leaves extract was superior to the control (88.4%). Another study carried out by a previous study19. Results showed that the value of antioxidant activity in the guava extract was 94.4% at a concentration of 100 µg/ml, and the guava dried fruit extracts exhibited weaker antioxidant effects than did the leaf extracts. A study estimate the antioxidants in *Psidium guajava* leaves extract, showed a significant role of plant leaves as an antioxidant20. Similar results obtained another study6 where the antioxidants reached 82% in the full concentration of the leaves extract.

**CONCLUSION**

The present study showed that *Psidium guajava* are rich sources of useful secondary metabolites, it is strongly recommended of using them for general medicinal purpose and specially for treat wounds and burns diseases. It is strongly recommended of using them for production of effective pharmaceutical compounds and can be used as natural products of antimicrobial to treat wounds and burns diseases instead of chemical drugs. It is noticeable that the leaves of *Psidium guajava* are very rich in antioxidant content and therefore are good sources and safe and economical.

**CONFLICT OF INTEREST**

No conflict of interest associated with this work.

**AUTHOR’S CONTRIBUTION**

The manuscript was carried out, written, and approved in collaboration with all authors.

**REFERENCES**