Bactericidal Activity of *Psidiumguajava* Leaves Against Some Pathogenic Microbes

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Abstract: This study was carried out to determine the antimicrobial activity of *Psidium guajava*(PG) leaves extracts against some pathogenic microbes. The extracts of P.G leaves was fractionated and purified by preparative TLC technique, then subjected toGas Chromatography-Mass Spectroscopy (GC-MS) to identify the biologically active compounds. Thereafter, the toxicity of the pure fractions was assessed in vivo using experimental rats. The P.G leaves were extracted with 80% ethanol, then the extracts were fractionated with n-hexane, chloroform, ethyl acetate, n-butanol and water. The antimicrobial activities of the extracts and the fractions of P.G leaves were done on 3 clinical pathogenic strains namely, *Staphylococcus* (*S*)*aureus*, E. coli and Candida (*C*)*albicans*. The disc-diffusion and MIC & MBC tests were employed to evaluate the inhibitory effects of the extracts and the fractions. The result of this experiment showed that the ethanolic extract of P.G leaves had a significant (p< 0.01) inhibitory effect on *S. aureus* however the other strains were resistant. The n-hexane fraction of P.G leaves showed a significant (p< 0.01) inhibitory effect on the two tested bacterial strains compared to other fractions. However, *C. albicans* resisted all the fractions. The zones of inhibition of the sensitive strains significantly (p< 0.05) increased when the concentrations of the ethanolic extracts and fractions were increased. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic extracts of PG leaves were nearly equal. The PTLC of n-hexane fraction of P.G leaves showed 4 bands of different colors. These 4 bands were tested for antimicrobial activity and only band 1 gave highest antibacterial activity. Band 1 was further analyzed with GC-MS into variable biological compounds (n-hexadecanoic acid; 9-Octadecenoic acid; Octadecenoic acid; 9, 12-Octadecenoic acid (Z,Z)-; Palmitoyl chloride; Hexadecanoic acid; Cyclotridecanone; Eicosanoic acid; Oleoyl chloride; N,N-bis(trifluoracetetyl)-N,N'-ethylene- bis(stearamide); Glycidol stearate; 1,2-Benzenedicarboxylic acid; 1-Cyclohexylidimethylsilyloxybutane; 14-Beta-H- Pregna; Stigmasta-3,5-dien-7-one). The fractions were found nontoxic to rats. In conclusion, P.G leaves contain nontoxic active antibacterial compounds. Thus P.G leaves can be used as crude or in purified forms to treat patients suffering from intestinal infection caused by *S.aureus* and *E. coli*.

Keywords: Antimicrobial activity; biologically active; phytochemical components,*Psidium guajava*.

1. Introduction

The *Psidium guajava* has a long history of folk medicinal uses in Egypt and worldwide as a cough sedative (Karawya, et al., 1999). The problem of microbial resistance is growing and the microbes evolved to overcome these medicines action, therefore the future to overcome this problem is ambiguous. Furthermore, bacteria have the genetic ability to transmit and acquire resistance to medicines(Nascimento, et al., 2000). Unfortunately, development of an effective antibacterial agent has been accompanied by the emergence of drug-resistant organisms. The microbes become genetically versatile and resistant to antibiotic and capable to transfer their resistant genes horizontally(Amin and Shailendra, 2006; Aibinu, et al., 2007).

Roy, et al., (2006) reported that the aqueous extract of *P.G* has no antibacterial activity against the growth of *E. coli* and *S. aureus*. Different degrees of inhibitory effects of P.G on *S. aureus* and *E. coli* has been reported (Neivton, et al., 2005; Goncalves, et al., 2008; Joseph, et al., 2010; Metwally, et al., 2010; Balakrishnan, et al., 2011; Sushmita, et al., 2012). Also, many reports showed that the antifungal activity of P.G leaves on *Candida albicans* (Samy and Ignacimuthu, 2000; Stepanovic, et al., 2003; Metwally, et al., 2010; Adonu Cyril, et al., 2013). On the other hand, the hot water extracts of P.G were reported to have the least inhibitory effects on fungi (Amit and Shweta, 2011). The extracts of P.G can be used as food preservative to improve the shelf life and safety of foods (Rattanachaikunssonop and Phumkhachorn, 2007; Nwanneka, 2013). Additionally, Gutierrez, et al., (2008) reported that the P.G contains a number of chemical constituents which possess antimicrobial activities.

Recently, there is an increasing interest in plants as a source of antimicrobial agents. Thus the objectives of this study are:-

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- investigate the antimicrobial activity of the ethanolic extracts and fractions of P.G against some pathogenic microbes.
- evaluate the safety of the most active compounds.
- isolate and characterize the biologically active ingredients.

II. Materials and Methods

2.1. Materials

2.1.1. Collection and Identification of Plant Materials

The Psidiumguajavad leaves were collect from domestic areas in Sudan. The leaves were collected in large quantity and washed with clear distilled water and dried in shade.

2.1.2. Extraction procedure

The dried clean leaves were taken and ground in a mortar with pestle under aseptic condition. Thereafter, 10 grams of the ground P.G leaves were suspended in 200ml of ethanol in Soxhlet apparatus at 78°C for 24hrs, the extract was evaporated aseptically (Nwinyi, et al., 2009; Haniyeh, et al., 2010). The yield percentage was calculated according to the following equation:

\[ \text{The yield} \% = \frac{\text{Extracted weight}}{\text{Sample weight}} \times 100 \]

Each 100 grams of ground P.G leaves gave an extract of about 28.3 grams.

2.1.3. Fractionation procedure

About 115 grams of the ethanolic extract of P.Gleaves were fractionated according to Sukhdev, et al., (2008). The yield of fractionation was calculated according to the previous equation. The weight of the fractions of n-hexane, chloroform, ethyl acetate, n-butanol and aqueous for the P.G leaves gave 4.574, 11.011, 4.338, 29.3 and 62.3 grams respectively.

2.1.4. Antimicrobial strains

The human clinical strains were obtained from the microbiology laboratory of King Khalid Hospital (K.K.H), Najran region, Saudi Arabia. These microorganisms include: Staphylococcus aureus, E. coli and Candida albicans. Furthermore, the Gram negative strain was further identified by microbact™ to ensure their purity (Mugg and Hill, 1981). The Gram positive and the fungal strains were further identified according to standard microbiological technique (James and Natalie, 2008; Barrow and Feltham, 1993).

2.2. Methods

2.2.1. Antimicrobial assay

The antimicrobial assay was carried out according to the standard methods of (Levan, et al., 1979; NCCLS, 2003; Zaidan, et al., 2005; Haniyeh, et al., 2010; Prasannabalaji, et al., 2012; Adonu, et al., 2013). Also, the MIC and MBC tests were determined by the macro broth dilution assay described elsewhere (Greenwood, 1989; NCCLS, 2008; Motamedi, et al., 2010). These experiments were repeated 4 times.

2.2.2. The phytochemical screening

The TLC plates were prepared according to Yrjönen, (2004). The PTLC was performed at the final step of the purification of the pure compound prior to the structure elucidation according to Kalpesh, et al., (2013). Also, the GC-MS technique was carried out as described elsewhere (John and Kumar, 2012; Srinivasan, et al., 2013; Poongodi, et al., 2015).

2.2.3. Toxicity of P.G Leaves in Rats

Thirty male and female rats were distributed and divided randomly into 3 groups each group containing 6 rats. Group 1 as control untreated, Group 2 and Group 3 received pure fraction of n-hexane of P.Gleaves at Dosing 5 and 2.5 ml/rat continued orally for 10 days respectively (Sunil, et al., 2001). Each Group of rats was then housed separately in a cage. Furthermore, the clinical signs were recorded. Blood samples were taken at day zero and after 10 days of the treatments. Thereafter, the rats were sacrificed. Also, the specimens of liver, kidney, spleen and heart were collected immediately after slaughtering and were fixed in formalin 70% for histopathology. Additionally, blood samples from rats were collected into clean dry bottles containing anti-coagulant heparin from the ocular vein. Also, the RBCs, Hb, MCV and MCHC were counted and calculated. However, the blood samples obtained from the ocular vein of rats were used to prepare sera for chemical methods such as enzymes AST, ALT and urea & serum metabolites of total bilirubin and albumin (Reitman and Frankel, 1957; Schmidt and Schmidt, 1963; Schalm, 1965).
2. 2. 4. Statistical Methods
Data were subjected to analysis of variance (ANOVA) followed by Fisher’s PLSD. Data are presented as means ± SE. Differences at P<0.05 (Snedecor and Cochram, 1989).

III. Results

3. 1. Effect of Ethanolic Extracts of P. G. Leaves on the Tested Clinical Strains
3. 1. 1. The Ethanolic Extract of P. G. Leaves

The ethanolic extract of P. G. leaves had a significant (p< 0.01) inhibitory effect on S. aureus only, while the remaining tested strains were resistant to the same extract. When the concentrations of ethanolic extract of P. G. leaves were increased, the zone of inhibition on the S. aureus significantly (p< 0.05) increased. The means of inhibitory zones of the different concentrations of ethanolic extracts of P. G. leaves on S. aureus was 8.75 ± 0.48, 10.00 ± 0.41, 10.50 ± 0.29 & 11.75 ± 0.75 for 300, 400, 500 and 600 µg/ml; respectively. Also, the inhibitory effect of P. G. leaf extracts on S. aureus was superior. Furthermore, the MIC and MBC of P. G. leaves extracts for S. aureus were equal (25 mg/ml).

3. 2. Effect of P. G. Leaves Fractions on Tested Clinical Strains
3. 2. 1. The n-hexane Fraction of P. G. Leaves

The n-hexane fraction of P. G. leaves had a significant (p< 0.01) inhibitory effect on all the tested bacterial strains, while the fungal (C. albicans) strain was resistant. When the concentration of n-hexane fraction was increased, the zone of inhibition on sensitive strains significantly (p< 0.01) increased. The means of inhibitory zones of the different concentrations of n-hexane fraction of P. G. leaves on S. aureus and E. coli were 13.00 ± 0.41, 14.00 ± 0.00, 14.00 ± 0.00 & 14.75 ± 0.25 and 12.75 ± 0.25, 13.50 ± 0.65, 14.75 ± 0.25 & 14.75 ± 0.25 for 300, 400, 500 and 600 µg/ml, respectively. Also, the inhibitory effect of n-hexane fraction was greater to that of other fractions (Fig. 1 a & b).

3. 2. 2. The Chloroform Fraction of P. G. Leaves

The chloroform fraction of P. G. leaves had a significant (p< 0.01) inhibitory effect on S. aureus only. However, this fraction had no effects on the other tested strains. When the concentrations of the same fraction were increased, the zone of inhibition on the S. aureus significantly (p< 0.01) increased. The means of inhibitory zones of the different concentrations of chloroform fraction of P. G. leaves on S. aureus was 8.50 ± 0.29, 8.50 ± 0.29, 9.00 ± 0.00, & 9.00 ± 0.00 for 300, 400, 500 and 600 µg/ml, respectively.

3. 2. 3. The Ethyl acetate Fraction of P. G. Leaves

The ethyl acetate fraction of P. G. leaves had a significant (p< 0.01) inhibitory effect on all the tested bacterial strains, whereas the tested fungal (C. albicans) strain was resistant to this fraction. When the concentration of the same fraction was augmented, the zone of inhibition on the sensitive strains significantly (p< 0.01) increased. The means of inhibitory zones of the diverse concentrations of ethyl acetate fraction of P. G. leaves on S. aureus and E. coli were 11.50 ± 0.65, 12.25 ± 0.25, 12.75 ± 0.48, & 13.50 ± 0.50 and 9.25 ± 0.48, 9.25 ± 0.48, 10.50 ± 0.29 & 10.75 ± 0.48 for 300, 400, 500 & 600 µg/ml; respectively. The inhibitory effect of ethyl acetate fraction was greater than chloroform fraction (Fig. 1 a).

3. 2. 4. The n-butanol Fraction of P. G. Leaves

The fraction of n-butanol of P. G. leaves had a significant (p< 0.01) inhibitory effect on E. coli, whereas S. aureus, P. aeruginosa and C. albicans were resistant to the same fraction. When the concentrations of n-butanol fraction were increased, the zone inhibition on the E. coli strain significantly high (p< 0.05) increased. The means of inhibitory zones of the different concentrations of n-butanol fraction of P. G. leaves on E. coli was 9.75 ± 0.48, 10.75 ± 0.25, 11.00 ± 0.00 and 11.25 ± 0.25 for 300, 400, 500 and 600 µg/ml, respectively. Also, the inhibitory effect of n-butanol fraction was greater than ethyl acetate fraction (Fig. 1. b). Additionally, all the tested strains were resistant to water fraction of P. G (Fig. 1 (a, & b)).

3. 3. The MIC and MBC (mg/ml) of P. G. Leaves Fractions Against the Sensitive Clinical Strains

The MIC of n-hexane fraction of P.G leaves for S. aureus and E. coli were 50 and 100 mg/ml respectively, while the MBC of S. aureus was 100 mg/ml and E. coli (≥100 mg/ml). Furthermore, the MIC and MBC of chloroform fraction of P.G leaves for S. aureus were equal (25 mg/ml). The MIC of ethyl acetate fraction of P.G leaves for S. aureus was equal to the MBC (12.5). While, MIC of E. coli was (25 mg/ml) and MBC was 100 mg/ml (Table 1).
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Fig. (1). Inhibitory activity of P.G leaves fractions against the tested clinical strains.

a. S. aureus  

b. E. coli

Table (1). The mean of MIC and MBC of P.G leaves fractions (Sudan origin) against the tested clinical strains.

<table>
<thead>
<tr>
<th>P.G leaves fractions</th>
<th>n-hexane</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>n-butanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial spp</td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
</tr>
<tr>
<td>S. aureus</td>
<td>50.0 ± 0.0</td>
<td>100.0 ± 0.0</td>
<td>25.0 ± 0.0</td>
<td>25.0 ± 0.0</td>
<td>12.50 ± 0.0</td>
</tr>
<tr>
<td>E. coli</td>
<td>100.0 ± 0.0</td>
<td>&gt; 100.0 ± 0.0</td>
<td>-</td>
<td>-</td>
<td>25.0 ± 0.0</td>
</tr>
</tbody>
</table>

3. 4. The Preparative Thin Layer Chromatography (PTLC)

The PTLC of n-hexane fraction of P.Gleaves had several pure bands which were determined and examined for antimicrobial for the most active band. Furthermore, the retardation factor values of the PTLC of the n-hexane fraction of P.Gleaves were recorded in the bands (1, 2, 3, and 4) was 0.43, 0.77, 0.87, and 0.97 respectively and their colors were violet, green, red and blue respectively. Moreover, the most active pure band of the antimicrobial activity of the PTLC of P.Gleaves was band number 1 violet color.

3. 5. Phytocomponents Identified from the Active Pure Band 1 of n-hexane Fraction of P.Gleaves

As shown in Fig. (2 & 3) and Table (2) the GC-MS technique showed variable compounds from the pure band 1 of n-hexane fraction of P.Gleaves included the following: n-hexadecanoic acid C\textsubscript{16}H\textsubscript{32}O\textsubscript{2}; 9-Octadecenoic acid C\textsubscript{18}H\textsubscript{30}O\textsubscript{2}; C\textsubscript{37}H\textsubscript{70}O\textsubscript{2} Octadecenoic acid C\textsubscript{18}H\textsubscript{30}O\textsubscript{2}; C\textsubscript{37}H\textsubscript{70}O\textsubscript{2}; 9, 12-Octadecenoic acid (Z,Z)-C\textsubscript{18}H\textsubscript{30}O\textsubscript{2}; Palmitoyl chloride C\textsubscript{16}H\textsubscript{31}O\textsubscript{10}; Hexadecanoic acid C\textsubscript{16}H\textsubscript{32}O\textsubscript{2}; Palmitoyl chloride C\textsubscript{16}H\textsubscript{31}O\textsubscript{10}; Hexadecanoic acid C\textsubscript{16}H\textsubscript{32}O\textsubscript{2}; 9-Octadecenoic acid C\textsubscript{18}H\textsubscript{30}O\textsubscript{2}; C\textsubscript{37}H\textsubscript{70}O\textsubscript{2}; Cyclotridecanone C\textsubscript{13}H\textsubscript{24}O; Eicosanoic acid C\textsubscript{20}H\textsubscript{40}O\textsubscript{2}; Oleoyl chloride C\textsubscript{18}H\textsubscript{32}O\textsubscript{2}; Glycidol stearate C\textsubscript{21}H\textsubscript{40}O\textsubscript{3}; 1, 2-Benzenedicarboxylic acid C\textsubscript{12}H\textsubscript{26}O\textsubscript{4}; 1-Cyclohexyldimethylslyoxybutane C\textsubscript{29}H\textsubscript{46}O\textsubscript{4}; 14-Beta-H-Stigmasta-3, 5-dien-7-one C\textsubscript{29}H\textsubscript{46}O and one unknown compound. Also, the retention time, area percentage, molecular weight, compound nature and biological activity were shown in (Table 2).
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Fig. (2). Chromatogram of GC-MS analysis with pure n-hexane fraction of P.G Leaves.

Table (2). Activity of the phytocomponents identified from pure band 1 of n-hexane fraction of P.G Leaves.

<table>
<thead>
<tr>
<th>P/</th>
<th>Compound Name</th>
<th>Mol Formula</th>
<th>R. time</th>
<th>Area%</th>
<th>Mol Weight</th>
<th>Compound nature</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-hexadecanoic acid</td>
<td>C_{16}H_{32}O_{2}</td>
<td>18.425</td>
<td>35.25</td>
<td>215</td>
<td>Fatty acid</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>2</td>
<td>9,10-decadienoic acid</td>
<td>C_{10}H_{14}O_{2}</td>
<td>19.554</td>
<td>29.48</td>
<td>212</td>
<td>Unsaturated F.A.</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>3</td>
<td>Octadecanoic acid</td>
<td>C_{18}H_{34}O_{2}</td>
<td>19.643</td>
<td>6.99</td>
<td>214</td>
<td>Fatty acid</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>4</td>
<td>9,12-Octadecadienoic acid (Z,Z)</td>
<td>C_{20}H_{36}O_{2}</td>
<td>19.748</td>
<td>1.81</td>
<td>280</td>
<td>Unsaturated F.A.</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>5</td>
<td>9,12-Octadecadienoic acid (Z,Z)</td>
<td>C_{20}H_{36}O_{2}</td>
<td>19.941</td>
<td>2.80</td>
<td>280</td>
<td>Unsaturated F.A.</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>6</td>
<td>Palmitoyl chloride</td>
<td>C_{16}H_{33}ClO_{2}</td>
<td>20.016</td>
<td>2.12</td>
<td>274</td>
<td>Fatty acid</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>7</td>
<td>Hexadecenoic acid</td>
<td>C_{16}H_{32}O_{2}</td>
<td>20.075</td>
<td>4.00</td>
<td>569</td>
<td>Un saturated F.A.</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>8</td>
<td>Cytochrome c</td>
<td>C_{63}H_{62}N_{4}O_{15}S_{6}</td>
<td>20.580</td>
<td>1.87</td>
<td>196</td>
<td>Fatty acid</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>9</td>
<td>Linolenic acid</td>
<td>C_{18}H_{30}O_{3}</td>
<td>20.809</td>
<td>0.24</td>
<td>312</td>
<td>Fatty acid</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>10</td>
<td>Oleic acid</td>
<td>C_{18}H_{36}O_{2}</td>
<td>20.930</td>
<td>3.21</td>
<td>300</td>
<td>Fatty acid</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>11</td>
<td>N,N-bis(2-methoxyethoxy)</td>
<td>C_{23}H_{45}N_{2}O_{4}</td>
<td>21.045</td>
<td>0.94</td>
<td>783</td>
<td>Fatty acid</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>12</td>
<td>n-Heptadecanoic acid</td>
<td>C_{17}H_{35}O_{2}</td>
<td>21.208</td>
<td>2.82</td>
<td>384</td>
<td>Fatty acid</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>13</td>
<td>Octadecanoic acid</td>
<td>C_{18}H_{34}O_{2}</td>
<td>21.315</td>
<td>0.90</td>
<td>240</td>
<td>Fatty acid</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>14</td>
<td>N-hexadecanoic acid</td>
<td>C_{16}H_{32}O_{2}</td>
<td>21.442</td>
<td>0.82</td>
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<td>Fatty acid</td>
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</tr>
<tr>
<td>15</td>
<td>1,2-Benzenedicarboxylic acid</td>
<td>C_{10}H_{8}O_{4}</td>
<td>21.654</td>
<td>2.10</td>
<td>273</td>
<td>Fatty acid</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>16</td>
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<td>C_{18}H_{34}O_{2}</td>
<td>22.174</td>
<td>0.45</td>
<td>312</td>
<td>Fatty acid</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>17</td>
<td>1-Cyclopentylcyclohexylpropanoic acid</td>
<td>C_{18}H_{32}O_{2}</td>
<td>22.261</td>
<td>0.60</td>
<td>214</td>
<td>Fatty acid</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>18</td>
<td>Oleic acid</td>
<td>C_{18}H_{36}O_{2}</td>
<td>22.437</td>
<td>1.90</td>
<td>300</td>
<td>Fatty acid</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>19</td>
<td>14-Diaza-2,2,6,6-tetramethylenepiperidine</td>
<td>C_{18}H_{34}N_{2}O_{2}</td>
<td>22.560</td>
<td>0.44</td>
<td>285</td>
<td>Fatty acid</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>20</td>
<td>9-Octadecenoic acid</td>
<td>C_{18}H_{34}O_{2}</td>
<td>22.656</td>
<td>0.80</td>
<td>356</td>
<td>Fatty acid</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>21</td>
<td>Stigmaster-3,5-dien-7-one</td>
<td>C_{20}H_{32}O_{2}</td>
<td>32.134</td>
<td>0.50</td>
<td>410</td>
<td>Steroids</td>
<td></td>
</tr>
</tbody>
</table>

*F.A. = Fatty acid, Mol = molecular, R = retention.
3.6. Acute Toxicity of Pure n-hexane Fractions of P.G leaves in Albino Rats

No toxic reactions or mortalities were recorded at any doses of pure fractions of n-hexane of P.Gleaves. All the rats were apparent alive healthy and active during the observation period. Also, there were no apparent signs of toxicity in all groups treated and no deaths. Also, there were no pathological changes in liver, kidney, heart and spleen in rats in all treatment groups. Moreover, the serum concentration of urea, total bilirubin, albumin and serum enzymes AST, ALT at day 11 were not significant (p > 0.05) in groups (Table 3).

After 10 days, no significant (p > 0.05) differences in RBCs count, WBCs, HGB, HCT, MCV, MCH and MCHC were detected in all groups and the control (Table 4). Additionally, histological sections of liver, kidney, heart and spleen of rats treated with pure fractions of n-hexane of P.Gleaves for 10 successive days showed no histological changes.

![Diagram](image-url)
IV. Discussion

4.1. Effect of Ethanolic Extracts of P.G Leaves on the Tested Clinical Strains

The present study showed that the ethanolic extract of P.G leaves has an inhibitory effect on the clinical strains of *S. aureus*. This result is in agreement with result of Egharevba, et al., (2010) who reported that the leaf extract of P.G has a broad spectrum antibacterial activity against *S. aureus*. Moreover, lower MIC and MBC were documented for ethanolic extract of P.G leaves on the clinical strains of *S. aureus*. This finding indicated that the ethanolic extract of P.G leaves has a good antibacterial activity. The same extract has no inhibitory effect on the clinical strains of *E. coli*. This finding is in disagreement with result of Balakrishnan, et al., (2011) who reported that the ethanolic extract of P.G leaves has higher activity on *E. coli* strains. This difference could be due to the environment where the P.G leaves had been grown (amplitude irrigation of P.G in Sudan may lead to dilution of the active ingredient) and/or may be due to the lower concentrations (µg/ml) used in this study and/or due to the genetic differences between the different strains.

The same extract has no inhibitory effect on the pathogenic clinical strain of *Candida albicans*. Opposing this finding Adonu Cyril, et al., (2013) and Metwally, et al., (2010) reported an inhibitory effect of ethanolic extract of P.G on *Candida albicans*. The difference may be due to the low concentrations (µg/ml) of P.G ethanolic extract used in this study. This finding is in agreement with result of Nwanneka, et al., (2013) who reported that the presence of saponins, tannins, glycosides and steroids in leaves of P.G are more bactericidal than fungicidal. When the concentrations of ethanolic extract of P.G leaves were increased, the zones of inhibition on the sensitive clinical strains of bacteria increased. This finding is in agreement with the findings of Mansour and Soudabe, (2012) who reported that higher concentrations induce greater inhibition.

4.2. Effect of P.G Leaves Fraction on the Tested Clinical Strains

This study indicated that the most active fractions are n-hexane & ethyl acetate and they have prominent inhibitory effect on all the tested clinical strains of bacteria. These findings are in disagreement with the findings of Gnan and Demello, (1999); Geidam, et al., (2007); Baby and Mini, (2010) who reported that n-hexane and ethyl acetate fractions of P.G have limit an inhibitory effect on the *S. aureus*. The ethyl acetate fraction has superior antibacterial activity against all tested bacteria. This is probably due to the properties of ethyl acetate fraction which contains flavonoids and tannins. These fractions are known to possess appreciable antibacterial activities (Narayana, et al., 2001). Furthermore, the n-hexane and ethyl acetate fractions induced higher MIC and MBC on the clinical strains of *E. coli*. This finding agrees with the results of Hassain, (2002) and Ogbonnia, et al., (2008) who reported that the quantity of active ingredients of plant origin that is required to cause inhibition of bacterial growth is not a problem, since medicinal plants have been reported to have little or no side effects.

This study also stated that the chloroform fraction has inhibitory effect on the clinical strains of *S. aureus*. This finding is in agreement with finding of Jaiarj, et al., (1999) who reported that the chloroform...
fractions of P.G has antibacterial activity on *S. aureus* isolated from clinical patients. The n-butanol fraction has a visible inhibitory effect on the *E. coli* clinical strain of bacteria. The effects obtained by these fractions are similar to those obtained by the ethanolic extract of P.G leaves but these fractions have more obvious inhibitory effects. The remaining Gram negative (*E. coli*) bacteria are not susceptible to chloroform fraction. This finding confirmed that the active ingredient that can affect Gram negative bacteria are not found in fraction of chloroform. The water fraction has no inhibitory effect on all the tested clinical strains of bacteria. This finding differs with the result of El-Mahmood, (2009) who reported that the aqueous extracts of P.G was more potent in inhibiting the growth of pathogenic *E. coli* and *S. aureus*. This difference may be due to low concentrations used in this study (µg/ml) and/or due to methods of fractionation used in this study; which known to be accurate than maceration method. When the concentration of all fractions was increased, the zone of inhibition on sensitive clinical strains increased. This result is in agreement with finding of Robbers, et al., (1997) who reported that higher concentrations of active chemical compounds in essential oils of P.G leaves explain their stronger inhibitory action.

4.3. The Preparative Thin Layer Chromatography (PTLC)

The present study showed that the analysis of PTLC plate of P.G leaves hexane fraction has four pure bands with difference *Rf* values and colors. The most active pure band was isolated for the antimicrobial activity of the preparative thin layer chromatography of P.G leaves is the band number 1 violet color with *Rf* 0.43. These finding is in disagreement with result of Hamid and Ali (2004) who reported that only two bands were isolated by preparative thin layer chromatography from *Peganum harmala* extract and the effective one with *Rf* of 0.33. This difference may be due to the variation in type of plant and/or solvents system used.

4.4. Phytocomponents Identified from the Active Pure Band 1 of n-hexane Fraction of P.G Leaves

New seven chemical compounds were discovered by analysis of pure n-hexane fraction of P.G leaves by GC–MS technique. These compounds are Palmitoyl chloride, Cyclotridecanone, N, N-bis(trifluoroacetyl)-N,N’-ethylene-bis(stearamide), Glycidol stearate, 1-Cyclohexylidimethylsilyloxybutane, 14-Beta-H- Pregna and Stigmasta-3,5-dien-7-one. All these new compounds were proofed to have antimicrobial activity. Also, the isolated chemical compounds of P.G leaves which have antimicrobial activity are 9-Octadecenoic acid (most abundant compound of P.G leaves), N-hexadecanoic acid, Eicosanoic acid, Oleoyl chloride, Hexadecanoic acid, Octadecanoic acid, 9, 12-Octadecanoic acid(Z,Z)- and 1, 2-Benzenedicarboxylic acid. This finding is superior to result of (Thenmozhi and Rajan, 2015) who reported antimicrobial activity on the 1, 2-Benzene dicarboxylic acid only.

4.5. Acute Toxicity of Pure Fraction of n-hexane of P.G Leaves in Albino Rats

The current study showed that there are no deaths and no signs of abnormal behavior observed in the rats treated with pure fractions of n-hexane of P.G leaves in the acute toxicity assay. Also, there is no appearance of pathological changes in the tested organs of rats in different groups compared to control. There is no significant change in serum constituents and hematological values in all different doses.

4.6. Conclusions

In conclusion, the present study showed that the extract of P.G leaves has an antibacterial activity on *S. aureus* and *E.coli*. Furthermore, increasing the extract concentrations increases its bactericidal effect. Additionally, fractions of P.G leaves produces more purifies chemical compounds that induces better inhibitory effect. These fractions contains new chemical compounds as antibacterial agents discovered (Palmitoyl chloride, Cyclotridecanone, N,N-bis(trifluoroacetyl)-N,N’-ethylene-bis(stearamide), Glycidol stearate, 1-Cyclohexylidimethylsilyloxybutane, 14-Beta-H- Pregna, Stearic acid chloride, Stigmasta-3,5-dien-7-one.

4.7. Recommendations

1. The new chemicals compounds of the fractionated from P.G leaves can be manufactured as new medicines.
2. Further investigation on the effect of P.G on *C. albicans* strain must be conducted to deny or to confirm its activity on this fungus.

References

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