CHAPTER 5

EXPERIMENTAL RESULTS


## CHAPTER - 5: EXPERIMENTAL RESULTS

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<td>Analgesic Activity</td>
<td>153-161</td>
</tr>
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<td>5.16</td>
<td>Anti-inflammatory Activity</td>
<td>162-166</td>
</tr>
<tr>
<td>5.17</td>
<td>Anti-Cancer Activity</td>
<td>166-170</td>
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<td>171-179</td>
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CHAPTER -5: EXPERIMENTAL RESULTS

5.1 Extraction of extracts from different parts of *Citrus maxima*:

Extraction of *Citrus maxima* leaf, bark and fruit peel was carried out by using the soxhlet apparatus with different solvents (Acetone, Ethanol and Water) the percentage yield of each extract is given below in Table No.5.1.1, 5.1.2, 5.1.3.

**Table 5.1.1: Extractive Yield and Percentage Yield of *Citrus maxima* Leaf:**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Extracts</th>
<th>Yield in gm</th>
<th>Percentage Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acetone</td>
<td>38</td>
<td>3.8%</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>60</td>
<td>6%</td>
</tr>
<tr>
<td>3.</td>
<td>Water</td>
<td>72</td>
<td>7.2%</td>
</tr>
</tbody>
</table>

**Table 5.1.2: Extractive Yield and Percentage Yield of *Citrus maxima* Bark:**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Extracts</th>
<th>Yield in gm</th>
<th>Percentage Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acetone</td>
<td>32</td>
<td>3.2%</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>46</td>
<td>4.6%</td>
</tr>
<tr>
<td>3.</td>
<td>Water</td>
<td>54</td>
<td>5.4%</td>
</tr>
</tbody>
</table>

**Table 5.1.3: Extractive Yield and Percentage Yield of *Citrus maxima* Fruit Peel:**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Extracts</th>
<th>Yield in gm</th>
<th>Percentage Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acetone</td>
<td>42</td>
<td>2.8%</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>58</td>
<td>3.8%</td>
</tr>
<tr>
<td>3.</td>
<td>Water</td>
<td>65</td>
<td>4.3%</td>
</tr>
</tbody>
</table>
5.2 Preliminary Qualitative Phytochemical Studies:

It was observed that, CM-LF-ETH, CM-FP-ETH extracts contain more phytochemicals such as alkaloids, carbohydrates, flavonoids, phenols and tannins respectively. Whereas, rest of the extracts of the plant materials contain few of the phytochemicals such as CM-LF-ACET contains carbohydrates, phenols and tannins, CM-LF-WATE contains carbohydrates and glycosides, CM-BRK-ACET contains only phenols, CM-BRK-ETH contains alkaloids, phenols and tannins CM-BRK-WATE contains only carbohydrates, CM-FP-ACET contains carbohydrates and phenols and CM-FP-WATE extract contains carbohydrates and glycosides respectively. The results are summarized in Table nos. 5.2.1, 5.2.2, 5.2.3.

Table 5.2.1: Preliminary Phytochemical Screening of *Citrus maxima* Leaf Extracts

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Test</th>
<th>Acetone Extract</th>
<th>Ethanol Extract</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ii</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>iii</td>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>iv</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>v</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>vi</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>vii</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>viii</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

- Absent + Presence
Table 5.2.2: Preliminary Phytochemical Screening of *Citrus maxima* Bark Extracts

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Test</th>
<th>Acetone Extract</th>
<th>Ethanol Extract</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ii</td>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>iii</td>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>iv</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>v</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>vi</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>vii</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>viii</td>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

- Absent  + Presence

Table 5.2.3: Preliminary Phytochemical Screening of *Citrus maxima* Fruit Peel Extracts

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Test</th>
<th>Acetone Extract</th>
<th>Ethanol Extract</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ii</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>iii</td>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>iv</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>v</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>vi</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>vii</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>viii</td>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

- Absent  + Presence
5.3 Anti microbial studies reports:

The disc diffusion method was used to determine zones of inhibition of *Citrus maxima* extracts CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ETH, CM-FP-ACET and CM-FP- WATE. The CM-FP-ETH, CM-BRK-ETH, CA-BRK-ETH, CA-FP-ETH showed significant activity against *Staphylococcus aureus*, *Enterococcus faecalis* in gram positive and *Escherichia coli*, *Klebsiella pneumonia* in gram negative at the concentration of 75 µg. CM-LF-ACET, CM-LF- WATE, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ACET and CM-FP- WATE extracts showed less significant activity. The results are summarized in table no. 5.3.1.

In Anti fungal activity the CM-FP-ETH, CM-BRK-ETH, CA-BRK-ETH, CA-FP-ETH showed significant activity against *Candida albicans*, *Asperigillus fumigates*, *Dreschlera turcica* and *Fusarium verticillioides* at the concentration of 75 µg.

CM-LF-ACET, CM-LF- WATE, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ACET and CM-FP- WATE extracts showed less significant activity. These extracts were compared with Fluconazole which is used as a standard. The results are summarized in table no. 5.3.2.
Table 5.3.1: Anti bacterial Activity and Extracts of *Citrus maxima*:

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Enterococcus faecalis</em></th>
<th><em>Klebsiella pneumonia</em></th>
<th><em>Escherichia coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration in µg</td>
<td>75 50 25 10 5</td>
<td>75 50 25 10 5</td>
<td>75 50 25 10 5</td>
<td>75 50 25 10 5</td>
</tr>
<tr>
<td>CM-LF-ETH</td>
<td>19 16 14.5 12.5 10</td>
<td>18 15 12.5 10 9</td>
<td>20 17 14 12 10</td>
<td>21 18 16 13 11</td>
</tr>
<tr>
<td>CM-LF-ACET</td>
<td>13 10 - - -</td>
<td>14 9 - - -</td>
<td>12 9.5 - - -</td>
<td>14 11 10 9 -</td>
</tr>
<tr>
<td>CM-LF-WATE</td>
<td>17 14 12 11 9</td>
<td>16 13 11 9 -</td>
<td>15 12 10.5 - -</td>
<td>18 15 12 9.5 -</td>
</tr>
<tr>
<td>CM-BRK-ETH</td>
<td>18 14 12 9.5 9</td>
<td>17 15 12 9 -</td>
<td>14 10 - - -</td>
<td>16 13 12 - -</td>
</tr>
<tr>
<td>CM-BRK-ACET</td>
<td>14 11 10 - -</td>
<td>15 10 9 - -</td>
<td>13 11 9 - -</td>
<td>15 12 10 9 -</td>
</tr>
<tr>
<td>CM-BRK- WATE</td>
<td>16 14 12 10 9</td>
<td>17 14 10 9 -</td>
<td>14 12 10 9 -</td>
<td>17 14 12 10 -</td>
</tr>
<tr>
<td>CM-FP-ETH</td>
<td>18 16 15 13 9</td>
<td>18 16 13 10 9</td>
<td>19 14 10 9 9</td>
<td>19 15 13 11 9</td>
</tr>
<tr>
<td>CM-FP-ACET</td>
<td>14 11 9 - -</td>
<td>13 9 - - -</td>
<td>10 - - - -</td>
<td>14 13.5 10 9 -</td>
</tr>
<tr>
<td>CM-FP- WATE</td>
<td>19 15 14 10 9</td>
<td>17 16 13 10 9</td>
<td>15 12 10 - -</td>
<td>17 14.5 12.5 9 -</td>
</tr>
<tr>
<td>Standard (Ciprofloxacin)</td>
<td>22 20 18 17 14</td>
<td>21 20 18 16 15</td>
<td>22 19.5 17 15 13</td>
<td>23.5 19.5 17 16 14</td>
</tr>
</tbody>
</table>

*Data showing zone of inhibition in mm*
### Table 5.3.2: Anti Fungal Activity and Extracts of *Citrus maxima*:

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th><em>Candida albicans</em></th>
<th><em>Aperigillus fumigatus</em></th>
<th><em>Dreschlera turcica</em></th>
<th><em>Fusarium verticillioides</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration in µg</strong></td>
<td>75</td>
<td>50</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>CM-LF-ETH</td>
<td>17</td>
<td>14</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>CM-LF-ACET</td>
<td>14</td>
<td>11</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>CM-LF-WATE</td>
<td>14</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CM-BRK-ETH</td>
<td>15</td>
<td>13</td>
<td>11.5</td>
<td>9</td>
</tr>
<tr>
<td>CM-BRK-ACET</td>
<td>13</td>
<td>11</td>
<td>9.5</td>
<td>-</td>
</tr>
<tr>
<td>CM-BRK- WATE</td>
<td>14</td>
<td>12.5</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>CM-FP-ETH</td>
<td>18</td>
<td>16</td>
<td>14.5</td>
<td>11</td>
</tr>
<tr>
<td>CM-FP-ACET</td>
<td>12</td>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CM-FP- WATE</td>
<td>14</td>
<td>11</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td><strong>Standard (Flucanazole)</strong></td>
<td>20.5</td>
<td>17</td>
<td>15.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

*Data showing zone of inhibition in mm*
5.4 *In-vitro* Antioxidant Activity

Table 5.4.1: DPPH Radical Scavenging Activity of Different Extracts of *Citrus maxima*

It is observed that the CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET and CM-FP-WATE have been demonstrated dose dependent increase in the DPPH radical scavenging activity. Whereas, 250 µg Ascorbic acid (Std.) has 93.14% activity. However, 250 µg of CM-LF-ETH 90.14%, CM-BRK-WATE 91.42%, CM-FP-ETH 91.83% have shown scavenging activity. The results are summarized in table no. 5.4.1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Inhibition for Absorbance (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1.104 ± 0.008 (34.71%)</td>
</tr>
<tr>
<td>CM-LF-ETH</td>
<td>1.220 ± 0.005 (27.85%)</td>
</tr>
<tr>
<td>CM-LF-ACET</td>
<td>1.233 ± 0.008 (27.08%)</td>
</tr>
<tr>
<td>CM-LF-WATE</td>
<td>1.300 ± 0.005 (23.12%)</td>
</tr>
<tr>
<td>CM-BRK-ETH</td>
<td>1.328 ± 0.001 (21.46%)</td>
</tr>
<tr>
<td>CM-BRK-ACET</td>
<td>1.275 ± 0.002 (24.60%)</td>
</tr>
<tr>
<td>CM-BRK-WATE</td>
<td>1.255 ± 0.002 (25.78%)</td>
</tr>
<tr>
<td>CM-FP-ETH</td>
<td>1.243 ± 0.003 (26.49%)</td>
</tr>
<tr>
<td>CM-FP-ACET</td>
<td>1.273 ± 0.003 (24.71%)</td>
</tr>
<tr>
<td>CM-FP-WATE</td>
<td>1.183 ± 0.003 (30.04%)</td>
</tr>
</tbody>
</table>

Data represents the Mean ± SEM of three measurements. CM-*Citrus maxima*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
Table 5.4.2: Superoxide Anion Radical Scavenging Activity of Different Extracts of Citrus maxima

It is observed that CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET and CM-FP-WATE have demonstrated dose dependent increase in the superoxide anion scavenging activity. Whereas, 250µg Ascorbic acid (Std) has 83.62% activity. However, 250 µg CM-LF-ETH 76.66%, CM-BRK-ACET 72.87%, CM-FP-ETH 91.83% have shown superoxide anion radical scavenging activity. The results are summarized table no. 5.4.2.

Table 5.4.2: Superoxide Anion Radical Scavenging Activity of Different Extracts of Citrus maxima

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Inhibition for Concentration (Mean ± SEM)</th>
<th>50 µg/mL</th>
<th>100 µg/mL</th>
<th>150 µg/mL</th>
<th>200 µg/mL</th>
<th>250 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td></td>
<td>0.556 ± 0.003 (43.09%)</td>
<td>0.385 ± 0.002 (60.59%)</td>
<td>0.268 ± 0.004 (72.56%)</td>
<td>0.193 ± 0.003 (80.24%)</td>
<td>0.160 ± 0.002 (83.62%)</td>
</tr>
<tr>
<td>CM-LF-ETH</td>
<td></td>
<td>0.735 ± 0.002 (24.76%)</td>
<td>0.576 ± 0.003 (41.04%)</td>
<td>0.358 ± 0.004 (63.35%)</td>
<td>0.290 ± 0.005 (72.56%)</td>
<td>0.228 ± 0.001 (76.66%)</td>
</tr>
<tr>
<td>CM-LF-ACET</td>
<td></td>
<td>0.715 ± 0.002 (26.81%)</td>
<td>0.563 ± 0.003 (42.37%)</td>
<td>0.430 ± 0.005 (55.98%)</td>
<td>0.351 ± 0.002 (63.45%)</td>
<td>0.260 ± 0.002 (73.38%)</td>
</tr>
<tr>
<td>CM-LF-WATE</td>
<td></td>
<td>0.686 ± 0.003 (29.78%)</td>
<td>0.538 ± 0.004 (44.93%)</td>
<td>0.406 ± 0.004 (58.44%)</td>
<td>0.300 ± 0.005 (69.29%)</td>
<td>0.246 ± 0.003 (74.82%)</td>
</tr>
<tr>
<td>CM-BRK-ETH</td>
<td></td>
<td>0.810 ± 0.001 (17.09%)</td>
<td>0.710 ± 0.005 (27.32%)</td>
<td>0.570 ± 0.003 (41.65%)</td>
<td>0.440 ± 0.002 (54.96%)</td>
<td>0.370 ± 0.005 (62.12%)</td>
</tr>
<tr>
<td>CM-BRK-ACET</td>
<td></td>
<td>0.735 ± 0.002 (24.76%)</td>
<td>0.568 ± 0.004 (41.86%)</td>
<td>0.455 ± 0.005 (53.42%)</td>
<td>0.348 ± 0.006 (64.38%)</td>
<td>0.265 ± 0.002 (72.87%)</td>
</tr>
<tr>
<td>CM-BRK-WATE</td>
<td></td>
<td>0.761 ± 0.001 (22.10%)</td>
<td>0.636 ± 0.003 (34.90%)</td>
<td>0.486 ± 0.003 (50.25%)</td>
<td>0.378 ± 0.001 (61.31%)</td>
<td>0.285 ± 0.002 (70.82%)</td>
</tr>
<tr>
<td>CM-FP-ETH</td>
<td></td>
<td>0.673 ± 0.003 (31.11%)</td>
<td>0.545 ± 0.002 (44.21%)</td>
<td>0.368 ± 0.004 (62.33%)</td>
<td>0.263 ± 0.006 (73.08%)</td>
<td>0.180 ± 0.005 (81.57%)</td>
</tr>
<tr>
<td>CM-FP-ACET</td>
<td></td>
<td>0.735 ± 0.005 (8.01%)</td>
<td>0.575 ± 0.002 (28.03%)</td>
<td>0.398 ± 0.004 (50.18%)</td>
<td>0.270 ± 0.005 (66.20%)</td>
<td>0.198 ± 0.006 (75.21%)</td>
</tr>
<tr>
<td>CM-FP-WATE</td>
<td></td>
<td>0.713 ± 0.003 (10.76%)</td>
<td>0.635 ± 0.002 (20.52%)</td>
<td>0.476 ± 0.003 (40.42%)</td>
<td>0.348 ± 0.001 (66.20%)</td>
<td>0.276 ± 0.003 (75.21%)</td>
</tr>
</tbody>
</table>

Data represents the Mean ± SEM of three measurements. CM-Citrus maxima, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
Table 5.4.3: Hydroxyl Radical Scavenging Activity of Different Extracts of Citrus maxima

It was observed that CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET and CM-FP-WATE have demonstrated dose dependent increase in the hydroxyl radical scavenging activity. Whereas, 250 µg Sodium metabisulphite (Std.) has 80.20% scavenging activity. However HAEF at 250 µg CM-LF-ETH 74.43%, CM-BRK-WATE 72.68%, CM-FP-ACET 73.68% has shown of significant scavenging activity. The results are summarized table no. 5.4.3.

Table 5.4.3: Hydroxyl Radical Scavenging Activity of Different Extracts of Citrus maxima

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Inhibition for Concentration (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Sodium metabisulphite</td>
<td>0.205 ± 0.002</td>
</tr>
<tr>
<td>CM-LF-ETH</td>
<td>0.273 ± 0.001</td>
</tr>
<tr>
<td>CM-LF-ACET</td>
<td>0.291 ± 0.002</td>
</tr>
<tr>
<td>CM-LF-WATE</td>
<td>0.263 ± 0.004</td>
</tr>
<tr>
<td>CM-BRK-ETH</td>
<td>0.256 ± 0.004</td>
</tr>
<tr>
<td>CM-BRK-ACET</td>
<td>0.271 ± 0.001</td>
</tr>
<tr>
<td>CM-BRK-WATE</td>
<td>0.248 ± 0.002</td>
</tr>
<tr>
<td>CM-FP-ETH</td>
<td>0.276 ± 0.002</td>
</tr>
<tr>
<td>CM-FP-ACET</td>
<td>0.245 ± 0.002</td>
</tr>
<tr>
<td>CM-FP-WATE</td>
<td>0.268 ± 0.002</td>
</tr>
</tbody>
</table>

Data represents the Mean ± SEM of three measurements. CM-Citrus maxima, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
It is observed that the extracts CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET and CM-FP-WATE have demonstrated dose dependent increase in the nitric oxide anion scavenging property. Whereas, 250 µg Ascorbic acid (Std.) has 80.11% nitric oxide anion scavenging property. However HAEF at 250 µg CM-LF-ETH 73.39%, CM-BRK-ACET 72.80%, CM-FP-ETH 73.68% has shown of significant scavenging activity. The results are summarized table no. 5.4.4.

Table 5.4.4: Nitric Oxide Radical Scavenging Activity of Different Extracts of Citrus maxima

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Inhibition for Concentration (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.158 ± 0.004 (53.80%)</td>
</tr>
<tr>
<td>CM-LF-ETH</td>
<td>0.195 ± 0.002 (42.98%)</td>
</tr>
<tr>
<td>CM-LF-ACET</td>
<td>0.193 ± 0.001 (43.56%)</td>
</tr>
<tr>
<td>CM-LF-WATE</td>
<td>0.181 ± 0.001 (40.35%)</td>
</tr>
<tr>
<td>CM-BRK-ETH</td>
<td>0.196 ± 0.004 (42.69%)</td>
</tr>
<tr>
<td>CM-BRK-ACET</td>
<td>0.181 ± 0.001 (47.07%)</td>
</tr>
<tr>
<td>CM-BRK-WATE</td>
<td>0.186 ± 0.001 (45.61%)</td>
</tr>
<tr>
<td>CM-FP-ETH</td>
<td>0.176 ± 0.001 (48.53%)</td>
</tr>
<tr>
<td>CM-FP-ACET</td>
<td>0.191 ± 0.001 (44.15%)</td>
</tr>
<tr>
<td>CM-FP-WATE</td>
<td>0.201 ± 0.006 (41.22%)</td>
</tr>
</tbody>
</table>

Data represents the Mean ± SEM of three measurements. CM-Citrus maxima, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
5.5 Determination of Non enzymatic antioxidants

Table 5.5.1: Determination of Total Phenolic and Total Flavanoid Contents

*Citrus maxima* show that, most of the phenolics flavonoids are extracted with ethanol. The analysis of leaf, stem bark and fruit peel indicate that the phenolic and flavonoid content varies significantly among different plant parts. *Citrus maxima* the maximum phenolic content was found to be in ethanol extract of fruit peel. The phenolic content of ethanol extract of *Citrus maxima* is estimated as 178.8 mg/g, The flavonoid content of ethanol extract of *Citrus maxima* fruit peel is estimated as 31.2 mg/g. The values are tabulated in the Table 5.5.1.

Table 5.5.1: Determination of Total Phenolic and Total Flavanoid Contents

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of Phenolic Compound mg/g</th>
<th>Amount of Flavanoid Compound mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CM-LF-ETH</td>
<td>162</td>
<td>26.0</td>
</tr>
<tr>
<td>2. CM-LF-ACET</td>
<td>20.6</td>
<td>5.2</td>
</tr>
<tr>
<td>3. CM-BRK-ETH</td>
<td>124.6</td>
<td>23.0</td>
</tr>
<tr>
<td>4. CM-BRK-ACET</td>
<td>8.9</td>
<td>-</td>
</tr>
<tr>
<td>5. CM-FP-ETH</td>
<td>178.8</td>
<td>31.2</td>
</tr>
<tr>
<td>6. CM-FP.ACET</td>
<td>18.6</td>
<td>2.8</td>
</tr>
</tbody>
</table>

CM-*Citrus maxima*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone.

5.6 Analgesic Activity

5.6.1 Acetic acid Induced Writhing in Mice

Control and various treated groups were tested for analgesic activity against acetic acid induced writhing, which is nothing but the painful reaction. Thirty minutes after the treatment, each mouse was injected with 0.1 mL 0.7% v/v aqueous solution of acetic acid i.p. The
number of abdominal constrictions was cumulatively counted from 0 - 10 minutes. The % reduction of writhing in standard diclofenac sodium 10 mg/kg treated group was found to be 60.02% against control.

The mean response of control and standard was 41.50 ± 1.25 and 16.59 ± 0.92 respectively. The respective test compounds CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ETH, CM-FP-ACET and CM-FP- WATE in its 300 mg/kg dose, showed mean writhing responses as 23.00 ± 1.06, 26.33 ± 1.38, 26.17 ± 1.49, 23.83 ± 1.30, 28.33 ± 1.66, 25.59 ± 1.43, 22.67 ± 1.17, 27.83 ± 1.30 and 27.83 ± 1.30. In terms of percentage inhibition of writhing by diclofenac sodium was 60.02% while with the test compound it was CM-LF-ETH 44.57%, CM-BRK-ETH 42.57% and CM-FP-ETH 45.37% respectively. The values are tabulated in the Table 5.6.1 and shown in Figure 5.1.

**Table 5.6.1: Effect of *Citrus maxima* Plant Extracts on Acetic acid Induced Writhing in Mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Mean no of writhing ±SEM</th>
<th>% Inhibition of writhes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>41.50 ± 1.25</td>
<td>-</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diclofenac (10 mg/kg)</td>
<td>16.59 ± 0.92***</td>
<td>60.02%</td>
</tr>
<tr>
<td>Group-III</td>
<td>CM-LF-ETH (300 mg/kg)</td>
<td>23.00 ± 1.06***</td>
<td>44.57%</td>
</tr>
<tr>
<td>Group-IV</td>
<td>CM-LF-ACET (300 mg/kg)</td>
<td>26.33 ± 1.38***</td>
<td>36.55%</td>
</tr>
<tr>
<td>Group-V</td>
<td>CM-LF-WATE (300 mg/kg)</td>
<td>26.17 ± 1.49***</td>
<td>36.93%</td>
</tr>
<tr>
<td>Group-VI</td>
<td>CM-BRK-ETH (300 mg/kg)</td>
<td>23.83 ± 1.30***</td>
<td>42.57%</td>
</tr>
<tr>
<td>Group-VII</td>
<td>CM-BRK-ACET (300 mg/kg)</td>
<td>28.33 ± 1.66***</td>
<td>31.73%</td>
</tr>
<tr>
<td>Group-VIII</td>
<td>CM-BRK- WATE (300 mg/kg)</td>
<td>25.59 ± 1.43***</td>
<td>38.33%</td>
</tr>
<tr>
<td>Group-IX</td>
<td>CM-FP-ETH (300 mg/kg)</td>
<td>22.67 ± 1.17***</td>
<td>45.37%</td>
</tr>
<tr>
<td>Group-X</td>
<td>CM-FP-ACET (300 mg/kg)</td>
<td>27.83 ± 1.30***</td>
<td>32.93%</td>
</tr>
<tr>
<td>Group-XI</td>
<td>CM-FP- WATE (300 mg/kg)</td>
<td>27.33 ± 0.98***</td>
<td>34.86%</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. CM-*Citrus maxima*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-WATER.
5.6.2 Tail Flick Method in Rats

In the tail flick method, the increase in latency period at different time points significantly differed (P<0.001) compared to baseline values within the same drug treated groups. The CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET and CM-FP-WATE and diclofenac sodium caused significant increase (P<0.001) in the percentage reaction time whilst the control and dose of extracts (300 mg/kg). At all the specified time intervals, the percentage of tail flick elongation time differed significantly (P<0.001) between the extracts and diclofenac sodium at the doses of plant extracts, being greater for diclofenac sodium. At the peak of activity, CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH extracts showed (P<0.001) and significantly of tail
flick elongation time respectively, whilst diclofenac sodium gave (P<0.001) elongation of tail flicking time. The values are tabulated in the Table 5.6.2 and shown in Figure 5.2.
Table 5.6.2: Effect of *Citrus maxima* Plant Extracts on Tail Flick method in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Reaction Time (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>4.51 ± 0.29</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diclofenac (10 mg/kg)</td>
<td>4.71 ± 0.31</td>
</tr>
<tr>
<td>Group-III</td>
<td>CM-LF-ETH (300 mg/kg)</td>
<td>5.25 ± 0.48</td>
</tr>
<tr>
<td>Group-IV</td>
<td>CM-LF-ACET (300 mg/kg)</td>
<td>4.56 ± 0.48</td>
</tr>
<tr>
<td>Group-V</td>
<td>CM-WATE (300 mg/kg)</td>
<td>4.98 ± 0.38</td>
</tr>
<tr>
<td>Group-VI</td>
<td>CM-BRK-ETH (300 mg/kg)</td>
<td>4.21 ± 0.55</td>
</tr>
<tr>
<td>Group-VII</td>
<td>CM-BRK-ACET (300 mg/kg)</td>
<td>4.71 ± 0.48</td>
</tr>
<tr>
<td>Group-VIII</td>
<td>CM-BRK- WATE (300 mg/kg)</td>
<td>4.90 ± 0.69</td>
</tr>
<tr>
<td>Group-IX</td>
<td>CM-FP-ETH (300 mg/kg)</td>
<td>4.81 ± 0.59</td>
</tr>
<tr>
<td>Group-X</td>
<td>CM-FP-ACET (300 mg/kg)</td>
<td>4.86 ± 0.61</td>
</tr>
<tr>
<td>Group-XI</td>
<td>CM-FP- WATE (300 mg/kg)</td>
<td>4.51 ± 0.48</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. CM-*Citrus maxima*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
Fig. 5.2: Effect of *Citrus maxima* Plant Extracts on Tail Flick method in Rats
5.6.3 Hot plate Method in Mice

The standard pentazocine lactate (10 mg/kg) was given i.p., CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET and CM-FP-WATE extracts given orally, in a dose of 300 mg/kg, elicited a significant analgesic activity in the hot plate method as evidenced by increase in latency time in seconds as compared with vehicle control. The increase in latency time was dose dependant. Latency time was noted 30, 60, 90, 120 and 180 minutes after administration of vehicle, standard and plant extracts. The values are tabulated in the Table 5.6.3 and shown in Figure 5.3.
Table 5.6.3: Effect of *Citrus maxima* Plant Extracts on Hot Plate Method in Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Reaction time (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>2.66 ± 0.33</td>
</tr>
<tr>
<td>Group-II</td>
<td>Pentazocine (10 mg/kg)</td>
<td>2.50 ± 0.22</td>
</tr>
<tr>
<td>Group-III</td>
<td>CM-LF-ETH (300 mg/kg)</td>
<td>2.83 ± 0.40</td>
</tr>
<tr>
<td>Group-IV</td>
<td>CM-LF-ACET (300 mg/kg)</td>
<td>2.50 ± 0.34</td>
</tr>
<tr>
<td>Group-V</td>
<td>CM-LF-WATE (300 mg/kg)</td>
<td>3.33 ± 0.49</td>
</tr>
<tr>
<td>Group-VI</td>
<td>CM-BRK-ETH (300 mg/kg)</td>
<td>2.83 ± 0.30</td>
</tr>
<tr>
<td>Group-VII</td>
<td>CM-BRK-ACET (300 mg/kg)</td>
<td>3.83 ± 0.30</td>
</tr>
<tr>
<td>Group-VIII</td>
<td>CM-BRK-WATE (300 mg/kg)</td>
<td>2.83 ± 0.30</td>
</tr>
<tr>
<td>Group-IX</td>
<td>CM-FP-ETH (300 mg/kg)</td>
<td>3.33 ± 0.33</td>
</tr>
<tr>
<td>Group-X</td>
<td>CM-FP-ACET (300 mg/kg)</td>
<td>2.16 ± 0.16</td>
</tr>
<tr>
<td>Group-XI</td>
<td>CM-FP-WATE (300 mg/kg)</td>
<td>2.66 ± 0.33</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. CM-*Citrus maxima*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
Fig. 5.3: Effect of *Citrus maxima* Plant Extracts on Hot Plate Method in Mice
5.7 Anti inflammatory Activity

5.7.1 Acute Anti inflammatory Activity

5.7.1.1 Formalin-induced paw Oedema in Rats

All the test compounds were tested with the diclofenac sodium as a standard drug in the dose of 10 mg/kg for the anti-inflammatory activity.

Presently diclofenac showed significant 87.14 % inhibition of inflammation at 5th hour (0.18 ± 0.01) when compared with control (1.40 ± 0.05) respectively.

The test compounds showed maximum percentage of inhibition of oedema at 5th hour significantly in respective dose level i.e., at 300 mg/kg the test compounds CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH showed 85.71%, 82.85% and 86.42%. The values are tabulated in the Table 5.7.1 and shown in Figure 5.4.
Table 5.7.1: Effect of *Citrus maxima* Plant Extracts on Formalin-induced paw Oedema (acute) in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>0.16 ± 0.01</td>
<td>0.78 ± 0.05</td>
<td>0.99 ± 0.05</td>
<td>1.20 ± 0.06</td>
<td>1.25 ± 0.07</td>
<td>1.40 ± 0.05</td>
<td>-</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diclofenac (10 mg/kg)</td>
<td>0.15 ± 0.01</td>
<td>0.37 ± 0.02***</td>
<td>0.55 ± 0.04***</td>
<td>0.36 ± 0.02***</td>
<td>0.30 ± 0.03***</td>
<td>0.18 ± 0.01***</td>
<td>87.14 %</td>
</tr>
<tr>
<td>Group-III</td>
<td>CM-LF-ETH (300 mg/kg)</td>
<td>0.17 ± 0.02</td>
<td>0.54 ± 0.02**</td>
<td>0.63 ± 0.03***</td>
<td>0.50 ± 0.02***</td>
<td>0.40 ± 0.01***</td>
<td>0.20 ± 0.01***</td>
<td>85.71%</td>
</tr>
<tr>
<td>Group-IV</td>
<td>CM-LF-ACET (300 mg/kg)</td>
<td>0.17 ± 0.02</td>
<td>0.63 ± 0.04*</td>
<td>0.76 ± 0.03**</td>
<td>0.65 ± 0.03***</td>
<td>0.49 ± 0.03***</td>
<td>0.42 ± 0.02 ***</td>
<td>70.00 %</td>
</tr>
<tr>
<td>Group-V</td>
<td>CM-LF-WATE (300 mg/kg)</td>
<td>0.17 ± 0.02</td>
<td>0.59 ± 0.03**</td>
<td>0.66 ± 0.01***</td>
<td>0.54 ± 0.01***</td>
<td>0.45 ± 0.01***</td>
<td>0.30 ± 0.01***</td>
<td>77.14%</td>
</tr>
<tr>
<td>Group-VI</td>
<td>CM-BRK-ETH (300 mg/kg)</td>
<td>0.18 ± 0.02</td>
<td>0.56 ± 0.04**</td>
<td>0.67 ± 0.02***</td>
<td>0.59 ± 0.03***</td>
<td>0.42 ± 0.02***</td>
<td>0.24 ± 0.03***</td>
<td>82.85%</td>
</tr>
<tr>
<td>Group-VII</td>
<td>CM-BRK-ACET (300 mg/kg)</td>
<td>0.13 ± 0.01</td>
<td>0.60 ± 0.04*</td>
<td>0.76 ± 0.05**</td>
<td>0.63 ± 0.03***</td>
<td>0.48 ± 0.02***</td>
<td>0.44 ± 0.05***</td>
<td>68.57%</td>
</tr>
<tr>
<td>Group-VIII</td>
<td>CM-BRK- WATE (300 mg/kg)</td>
<td>0.15 ± 0.01</td>
<td>0.63 ± 0.03*</td>
<td>0.72 ± 0.02***</td>
<td>0.59 ± 0.01***</td>
<td>0.50 ± 0.02***</td>
<td>0.39 ± 0.04 ***</td>
<td>72.14%</td>
</tr>
<tr>
<td>Group-IX</td>
<td>CM-FP-ETH (300 mg/kg)</td>
<td>0.15 ± 0.02</td>
<td>0.46 ± 0.023**</td>
<td>0.60 ± 0.02***</td>
<td>0.44 ± 0.02***</td>
<td>0.38 ± 0.02***</td>
<td>0.19 ±0.01***</td>
<td>86.42%</td>
</tr>
<tr>
<td>Group-X</td>
<td>CM-FP-ACET (300 mg/kg)</td>
<td>0.14 ± 0.02</td>
<td>0.67 ± 0.06ns</td>
<td>0.79 ± 0.03*</td>
<td>0.61 ± 0.02***</td>
<td>0.51 ± 0.02***</td>
<td>0.46 ± 0.01 ***</td>
<td>67.60 %</td>
</tr>
<tr>
<td>Group-XI</td>
<td>CM-FP- WATE (300 mg/kg)</td>
<td>0.13 ± 0.01</td>
<td>0.56 ± 0.03**</td>
<td>0.65 ± 0.03***</td>
<td>0.50 ± 0.02***</td>
<td>0.37 ± 0.02***</td>
<td>0.29 ± 0.03 ***</td>
<td>79.57%</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. CM-*Citrus maxima*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
Fig. 5.4: Effect of *Citrus maxima* Plant Extracts on Formalin-induced paw Oedema in Rats
5.7.2 Chronic Anti-inflammatory Activity

5.7.2.1 Formalin-induced paw Oedema in Rats

Formalin induced paw oedema is one of the most suitable test procedure to screen chronic anti-inflammatory agents. The results obtained as mean increase in paw volume (mL) and % inhibition are represented in Table 5.7.2.

The mean response of standard was 82.40% inhibition of increase in paw thickness after 6 days respectively. In this model at 300 mg/kg dose level of CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET and CM-FP-WATE extracts showed 65.66%, 20.60%, 54.07%, 59.22%, 28.75%, 20.60%, 60.94%, 27.03% and 39.48% inhibition of increase in paw thickness after 6 days. However, at CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH extracts showed 65.66%, 59.22% and 60.94% inhibition of increase in paw thickness after 6 days. All the results were compared with solvent control and diclofenac sodium reference drug control.
Table 5.7.2: Effect of *Citrus maxima* Plant Extracts on Formalin-induced Paw Oedema (chronic) in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Initial Paw Volume</th>
<th>Paw Volume After 6 Days</th>
<th>Increase in Paw Volume</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>1.28 ± 0.07</td>
<td>3.61 ± 0.12</td>
<td>2.33 ± 0.06</td>
<td>-</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diclofenac (100 mg/kg)</td>
<td>1.23 ± 0.04</td>
<td>1.65 ± 0.05</td>
<td>0.41 ± 0.07</td>
<td>82.40%</td>
</tr>
<tr>
<td>Group-III</td>
<td>CM-LF-ETH (300 mg/kg)</td>
<td>1.26 ± 0.03</td>
<td>2.00 ± 0.06</td>
<td>0.80 ± 0.12</td>
<td>65.66%</td>
</tr>
<tr>
<td>Group-IV</td>
<td>CM-LF-ACET (300 mg/kg)</td>
<td>1.21 ± 0.06</td>
<td>3.21 ± 0.24</td>
<td>1.85 ± 0.16</td>
<td>20.60%</td>
</tr>
<tr>
<td>Group-V</td>
<td>CM-LF-WATE (300 mg/kg)</td>
<td>1.25 ± 0.06</td>
<td>2.25 ± 0.16</td>
<td>1.07 ± 0.14</td>
<td>54.07%</td>
</tr>
<tr>
<td>Group-VI</td>
<td>CM-BRK-ETH (300 mg/kg)</td>
<td>1.31 ± 0.08</td>
<td>2.26 ± 0.10</td>
<td>0.95 ± 0.14</td>
<td>59.22%</td>
</tr>
<tr>
<td>Group-VII</td>
<td>CM-BRK-ACET (300 mg/kg)</td>
<td>1.23 ± 0.06</td>
<td>2.90 ± 0.14</td>
<td>1.66 ± 0.17</td>
<td>28.75%</td>
</tr>
<tr>
<td>Group-VIII</td>
<td>CM-BRK- WATE (300 mg/kg)</td>
<td>1.26 ± 0.06</td>
<td>3.11 ± 0.08</td>
<td>1.85 ± 0.11</td>
<td>20.60%</td>
</tr>
<tr>
<td>Group-IX</td>
<td>CM-FP-ETH (300 mg/kg)</td>
<td>1.28 ± 0.08</td>
<td>2.23 ± 0.17</td>
<td>0.91 ± 0.14</td>
<td>60.94%</td>
</tr>
<tr>
<td>Group-X</td>
<td>CM-FP-ACET (300 mg/kg)</td>
<td>1.28 ± 0.05</td>
<td>2.71 ± 0.23</td>
<td>1.70 ± 0.08</td>
<td>27.03%</td>
</tr>
<tr>
<td>Group-XI</td>
<td>CM-FP- WATE (300 mg/kg)</td>
<td>1.30 ± 0.07</td>
<td>2.86 ± 0.14</td>
<td>1.41 ± 0.19</td>
<td>39.48%</td>
</tr>
</tbody>
</table>

Results are expressed on mean + SEM (n=6) from four observations.

Paw Volume was measured after 6 days. CM-*Citrus maxima*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.

### 5.8 Anti-Cancer Activity

#### 5.8.1 Trypan Blue Dye Exclusion Method against by HeLa cell Line

**Table 5.8.1: Effect of *Citrus maxima* on HeLa cell Line against by Trypan Blue Dye Exclusion Method**

Table 5.8.1 depicts the effect of CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET and CM-FP-WATE on % of dead cells against HeLa cell line against by trypan blue dye exclusion method. The percentage
of dead cells was found to be 69.1%, 11.5%, 5.3%, 15.3%, 7.3%, 10.2%, 15.7%, 23.3% and 22.1% in HeLa cell line against CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET, CM-FP-WATE 300 mg/kg b.w p.o. respectively. CM-LF-ETH showed maximum % of dead cells 69.1% and CM-BRK-ETH and CM-FP-ACET showed 15.3% and 23.3% % of dead cells. The values are shown in Figure 5.5.

Table 5.8.1: Effect of *Citrus maxima* on HeLa cell Line against by Trypan Blue Dye Exclusion Method

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Number of Live cells</th>
<th>Number of Dead cells</th>
<th>Total Number of cells</th>
<th>% of Dead cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Control</td>
<td>230</td>
<td>11</td>
<td>241</td>
<td>4.6%</td>
</tr>
<tr>
<td>Group-II</td>
<td>CM-LF-ETH (300 mg/kg)</td>
<td>68</td>
<td>152</td>
<td>220</td>
<td>69.1%</td>
</tr>
<tr>
<td>Group-III</td>
<td>CM-LF-ACET (300 mg/kg)</td>
<td>246</td>
<td>32</td>
<td>278</td>
<td>11.5%</td>
</tr>
<tr>
<td>Group-IV</td>
<td>CM-LF-WATE (300 mg/kg)</td>
<td>232</td>
<td>19</td>
<td>251</td>
<td>5.3%</td>
</tr>
<tr>
<td>Group-V</td>
<td>CM-BRK-ETH (300 mg/kg)</td>
<td>209</td>
<td>38</td>
<td>247</td>
<td>15.3%</td>
</tr>
<tr>
<td>Group-VI</td>
<td>CM-BRK-ACET (300 mg/kg)</td>
<td>215</td>
<td>17</td>
<td>232</td>
<td>7.3%</td>
</tr>
<tr>
<td>Group-VII</td>
<td>CM-BRK-WATE (300 mg/kg)</td>
<td>228</td>
<td>26</td>
<td>254</td>
<td>10.2%</td>
</tr>
<tr>
<td>Group-VIII</td>
<td>CM-FP-ETH (300 mg/kg)</td>
<td>172</td>
<td>32</td>
<td>204</td>
<td>15.7%</td>
</tr>
<tr>
<td>Group-IX</td>
<td>CM-FP-ACET (300 mg/kg)</td>
<td>184</td>
<td>56</td>
<td>240</td>
<td>23.3%</td>
</tr>
<tr>
<td>Group-X</td>
<td>CM-FP-WATE (300 mg/kg)</td>
<td>53</td>
<td>186</td>
<td>239</td>
<td>22.1%</td>
</tr>
</tbody>
</table>

CM-*Citrus maxima*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
Figure No. 5.5: Effect of *Citrus maxima* on HeLa cell Line against by Trypan Blue Dye Exclusion Method

5.9 *In vivo* Anti Tumor Activity of *Citrus maxima* and Ascitic Tumor Model

**Table 5.9.1: Effect of *Citrus maxima* on Mean Survival Time in EAC Tumor Bearing Mice**

Table 5.9.1 depicts the effect of CYP, CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ETH, CM-FP-ACET and CM-FP-WATE on mean survival time and % ILS against EAC induced mice. In the EAC control group, the median survival time was 14 days and which increased significantly to 18 days (*p*<0.001) with CM-LF-ETH 300 mg/kg b.w p.o., to 22 days (*p*<0.001) with CM-FP-ETH 300 mg/kg b.w p.o., and (*p*<0.01) CM-LF-WTR, CM-BRK-ETH 300 mg/kg b.w p.o., which showed maximum increase in the life when compared with CM-LF-ACET, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ACET and CM-FP-WATE which was
found not significant when compared with EAC control group. CM-LF-ACET, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ACET and CM-FP-WATE 300 mg/kg b.w p.o., showed least effect in increasing the life span among the doses of test drugs on comparison. The median survival time of CYP treated was found to be 26 days \( (p < 0.001) \). The % ILS found to be 40.89%, 29.72%, 35.17% and 44.30%, in EAC induced animals with CM-LF-ETH, CM-LF-WATE, CM-BRK-ETH, CM-FP-ETH 300 mg/kg b.w p.o., and CYP 25 mg/kg i.p, respectively when compared with vehicle treated EAC animals. The values are shown in the Figure 5.6.

**Table 5.9.1: Effect of *Citrus maxima* on Mean Survival Time in EAC Tumor Bearing Mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Mean survival time</th>
<th>ILS %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Mice</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>EAC + solvent 20 mL/kg</td>
<td>14.67±1.41</td>
<td>-</td>
</tr>
<tr>
<td>Group III</td>
<td>EAC + CYP 25 mg/kg</td>
<td>23.67±2.09</td>
<td>61.35%</td>
</tr>
<tr>
<td>Group IV</td>
<td>EAC + CM-LF-ETH (300 mg/kg)</td>
<td>20.67±1.382</td>
<td>40.89%</td>
</tr>
<tr>
<td>Group V</td>
<td>EAC + CM-LF-ACET (300 mg/kg)</td>
<td>17.17±1.621</td>
<td>17.04%</td>
</tr>
<tr>
<td>Group VI</td>
<td>EAC + CM-LF-WATE (300 mg/kg)</td>
<td>19.03 ±1.054( \times )</td>
<td>29.72%</td>
</tr>
<tr>
<td>Group VII</td>
<td>EAC + CM-BRK-ETH (300 mg/kg)</td>
<td>19.83±1.046( \times )</td>
<td>35.17%</td>
</tr>
<tr>
<td>Group VIII</td>
<td>EAC + CM-BRK-ACET (300 mg/kg)</td>
<td>15.33 ±1.202</td>
<td>4.50%</td>
</tr>
<tr>
<td>Group IX</td>
<td>EAC + CM-BRK-WATE (300 mg/kg)</td>
<td>18.33 ±2.06( \times )</td>
<td>24.94%</td>
</tr>
<tr>
<td>Group X</td>
<td>EAC + CM-FP-ETH (300 mg/kg)</td>
<td>21.17 ±1.75( \times )</td>
<td>44.30%</td>
</tr>
<tr>
<td>Group XI</td>
<td>EAC + CM-FP-ACET (300 mg/kg)</td>
<td>16.83 ±1.515</td>
<td>14.72%</td>
</tr>
<tr>
<td>Group XII</td>
<td>EAC + CM-FP-WATE (300 mg/kg)</td>
<td>17.45 ±0.73</td>
<td>18.95%</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. (n=6).

\( p \) values: \( p < 0.05, \ r < 0.001 \) as compared with EAC control + solvent. \( x < 0.05, \ y < 0.01, \ z < 0.001 \), as compared to CYP (by one way ANOVA followed by Dunnett’s multiple comparison test). 

*CM-Citrus maxima*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
Fig. 5.6: Effect of *Citrus maxima* on Mean Survival Time in EAC Tumor Bearing Mice

Table 5.9.2: Effect of *Citrus maxima* on body weight analysis and tumor growth response against EAC induced animals

Table 5.9.2 shows significant decrease \((p<0.001)\) in the body weight after treatment with CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ETH, CM-FP-ACET and CM-FP- WATE for 14 days. The percentage decrease was found to be 45.81%, 31.51%, 40.03%, 52.88%, 32.59%, 48.32%, 56.23%, 30.26%, 43.2% and 79.33% in EAC induced mice treated with CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ETH, CM-FP-ACET, CM-FP-WATE 300 mg/kg b.w p.o., and CYP 25 mg/kg b.w i.p respectively when compared with vehicle treated cancerous animals [Figure 5.7].

Treatment with CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ETH, CM-FP-ACET and CM-FP- WATE 300 mg/kg b.w p.o., and CYP 25 mg/kg b.w
i.p significantly ($p < 0.001$) decreased the tumor volume and packed cell volume when compared to that of EAC control group, these CM-LF-ETH, CM-BRK-ETH, CM-BRK-WTR, CM-FP-ETH, CM-FP-WTR at the dose of 300 mg/kg b.w p.o., showed the maximum decrease in tumor volume (Figure 5.8) and packed cell volume (Figure 5.9) when compared among test groups.

**Table 5.9.2: Effect of *Citrus maxima* on body weight analysis and tumor growth response against EAC induced animals**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Increase in body weight (g)</th>
<th>Tumor volume (mL)</th>
<th>Packed cell volume (mL)</th>
<th>% decrease in body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Mice</td>
<td>0.64 ± 0.26</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>EAC + solvent 20 mL/kg</td>
<td>8.95 ± 0.47 c</td>
<td>8.18 ± 0.26</td>
<td>4.8 ± 0.30</td>
<td>-</td>
</tr>
<tr>
<td>Group III</td>
<td>EAC + CYP 25 mg/kg</td>
<td>1.85 ± 0.56 r</td>
<td>1.20 ± 0.11 r</td>
<td>0.55 ± 0.20 r</td>
<td>79.33%</td>
</tr>
<tr>
<td>Group IV</td>
<td>EAC + CM-LF-ETH (300 mg/kg)</td>
<td>4.85 ± 0.34 c, r, z</td>
<td>4.95 ± 0.48 r, z</td>
<td>2.50 ± 0.19 r, z</td>
<td>45.81%</td>
</tr>
<tr>
<td>Group V</td>
<td>EAC + CM-LF-ACET (300 mg/kg)</td>
<td>6.13 ± 0.40 c, r, z</td>
<td>5.35 ± 0.47 r, z</td>
<td>2.95 ± 0.16 r, z</td>
<td>31.51%</td>
</tr>
<tr>
<td>Group VI</td>
<td>EAC + CM-LF-WATE (300 mg/kg)</td>
<td>5.37 ± 0.25 c, r, z</td>
<td>5.00 ± 0.38 r, z</td>
<td>2.58 ± 0.39 r, z</td>
<td>40.03%</td>
</tr>
<tr>
<td>Group VII</td>
<td>EAC + CM-BRK-ETH (300 mg/kg)</td>
<td>4.22 ± 0.36 c, r, y</td>
<td>4.70 ± 0.49 r, z</td>
<td>2.33 ± 0.21 r, z</td>
<td>52.88%</td>
</tr>
<tr>
<td>Group VIII</td>
<td>EAC + CM-BRK-ACET (300 mg/kg)</td>
<td>6.03 ± 0.65 c, r, z</td>
<td>6.03 ± 0.75 p, z</td>
<td>2.83 ± 0.45 r, z</td>
<td>32.59%</td>
</tr>
<tr>
<td>Group IX</td>
<td>EAC + CM-BRK-WATE (300 mg/kg)</td>
<td>4.63 ± 0.54 c, r, z</td>
<td>4.63 ± 0.56 r, z</td>
<td>2.15 ± 0.17 r, z</td>
<td>48.32%</td>
</tr>
<tr>
<td>Group X</td>
<td>EAC + CM-FP-ETH (300 mg/kg)</td>
<td>3.92 ± 0.65 c, r, x</td>
<td>3.93 ± 0.32 r, y</td>
<td>1.90 ± 0.07 r, y</td>
<td>56.23%</td>
</tr>
<tr>
<td>Group XI</td>
<td>EAC + CM-FP-ACET (300 mg/kg)</td>
<td>6.24 ± 0.28 c, r, z</td>
<td>6.23 ± 0.36 p, z</td>
<td>3.13 ± 0.31 r, z</td>
<td>30.26%</td>
</tr>
<tr>
<td>Group XII</td>
<td>EAC + CM-FP-WATE (300 mg/kg)</td>
<td>5.10 ± 0.28 c, r, z</td>
<td>4.75 ± 0.42 r, z</td>
<td>2.48 ± 0.15 r, z</td>
<td>43.2%</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. (n=6). *p* values: *p* < 0.05, *r* < 0.001 as compared with EAC control + solvent.

*x* < 0.05, *y* < 0.01, *z* < 0.001, as compared to CYP (by one way ANOVA followed by Dunnett’s multiple comparison test). CM-*Citrus maxima*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
Fig. 5.7: Effect of *Citrus maxima* on body weight response against EAC induced animals

Fig. 5.8: Effect of *Citrus maxima* on Tumor volume against EAC induced animals
Fig. 5.9: Effect of Citrus maxima on packed cell volume against EAC induced animals

Table 5.9.3: Effect of Citrus maxima on hematological parameters on 14th day in normal and EAC tumor bearing mice

Table 5.9.3 revealed the effect of CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET and CM-FP-WATE 300 mg/kg b.w p.o., and CYP 25 mg/kg b.w i.p on hematological parameters against EAC induced animals estimated on 14th day of treatment. CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH 300 mg/kg b.w p.o., showed better improvement in the hematological parameters than the rest of the doses among the compared groups. The total WBC count found increased in the EAC control group. All the test drugs when administered to the EAC bearing mice showed the significant ($p < 0.001$) decrease in the WBC count(Figure 5.10) when compared with the EAC control group,. RBC count (Figure 5.11) and Hb content (Figure 5.12) in the EAC groups were significantly ($p < 0.001$)
decreased as compared to the normal group. All the test drugs have showed the significant increase but CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH 300 mg/kg b.w p.o., showed the better activity compared to rest of the drugs and doses.

**Table 5.9.3: Effect of Citrus maxima on hematological parameters on 14th day in normal and EAC tumor bearing mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>WBC (x10^6/mL)</th>
<th>RBC (x10^9/mL)</th>
<th>Hb (g %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Mice</td>
<td>7.24 ± 0.23</td>
<td>5.67±0.24</td>
<td>12.9±0.44</td>
</tr>
<tr>
<td>Group II</td>
<td>EAC + solvent 20 mL/kg</td>
<td>21.78 ± 1.31</td>
<td>3.55±0.04</td>
<td>8.1±0.28</td>
</tr>
<tr>
<td>Group III</td>
<td>EAC + CYP 25 mg/kg</td>
<td>10.71 ± 0.27</td>
<td>5.26±0.09</td>
<td>12.08±0.40</td>
</tr>
<tr>
<td>Group IV</td>
<td>EAC + CM-LF-ETH (300 mg/kg)</td>
<td>14.76 ± 0.16</td>
<td>4.79 ± 0.11</td>
<td>9.25 ± 0.11</td>
</tr>
<tr>
<td>Group V</td>
<td>EAC + CM-LF-ACET (300 mg/kg)</td>
<td>16.82 ± 0.23</td>
<td>4.03±0.08</td>
<td>7.70±0.15</td>
</tr>
<tr>
<td>Group VI</td>
<td>EAC + CM-LF-WATE (300 mg/kg)</td>
<td>16.64 ± 0.29</td>
<td>4.32±0.05</td>
<td>8.97±0.14</td>
</tr>
<tr>
<td>Group VII</td>
<td>EAC + CM-BRK-ETH (300 mg/kg)</td>
<td>12.28 ± 0.28</td>
<td>4.91±0.02</td>
<td>11.29±0.09</td>
</tr>
<tr>
<td>Group VIII</td>
<td>EAC + CM-BRK-ACET (300 mg/kg)</td>
<td>15.59 ± 0.24</td>
<td>4.13±0.14</td>
<td>8.99±0.13</td>
</tr>
<tr>
<td>Group IX</td>
<td>EAC + CM-BRK-WATE (300 mg/kg)</td>
<td>14.13 ± 0.27</td>
<td>4.54±0.20</td>
<td>10.88±0.19</td>
</tr>
<tr>
<td>Group X</td>
<td>EAC + CM-FP-ETH (300 mg/kg)</td>
<td>11.93 ± 0.08</td>
<td>5.07±0.09</td>
<td>11.01±0.22</td>
</tr>
<tr>
<td>Group XI</td>
<td>EAC + CM-FP-ACET (300 mg/kg)</td>
<td>14.90 ± 0.20</td>
<td>4.23±0.05</td>
<td>9.34 ± 0.13</td>
</tr>
<tr>
<td>Group XII</td>
<td>EAC + CM-FP-WATE (300 mg/kg)</td>
<td>13.69 ± 0.31</td>
<td>4.50±0.07</td>
<td>10.19±0.29</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. (n=6).

*p* values:  
- p < 0.05, r < 0.001 as compared with EAC control + solvent.
- x < 0.05, y < 0.01, z < 0.001, as compared to CYP (by one way ANOVA followed by Dunnett’s multiple comparison test).

CM-*Citrus maxima*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
Fig. 5.10: Effect of *Citrus maxima* on WBC Count on 14\textsuperscript{th} day in normal and EAC tumor bearing mice

Fig. 5.11: Effect of *Citrus maxima* on RBC Count on 14\textsuperscript{th} day in normal and EAC tumor bearing mice
Fig. 5.12: Effect of *Citrus maxima* on Hemoglobin on 14th day in normal and EAC tumor bearing mice
5.10 Extraction of Extracts from different parts of *Citrus aurantium*:

Extraction of *Citrus aurantium* leaf, bark and fruit peel was carried out by using the soxhlet apparatus with different solvents (Acetone, Ethanol and Water) the percentage yield each extract is given below in Table No. 5.10.1, 5.10.2, 5.10.3.

**Table 5.10.1: Extractive Yield and Percentage Yield of *Citrus aurantium* Leaf:**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Extracts</th>
<th>Yield in gm</th>
<th>Percentage Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acetone</td>
<td>65</td>
<td>6.5%</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>78</td>
<td>7.8%</td>
</tr>
<tr>
<td>3.</td>
<td>Water</td>
<td>86</td>
<td>5.7%</td>
</tr>
</tbody>
</table>

**Table 5.10.2: Extractive Yield and Percentage Yield of *Citrus aurantium* Bark:**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Extracts</th>
<th>Yield in gm</th>
<th>Percentage Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acetone</td>
<td>48</td>
<td>4.8%</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>55</td>
<td>5.5%</td>
</tr>
<tr>
<td>3.</td>
<td>Water</td>
<td>50</td>
<td>5.0%</td>
</tr>
</tbody>
</table>

**Table 5.10.3: Extractive Yield and Percentage Yield of *Citrus aurantium* Fruit Peel:**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Extracts</th>
<th>Yield in gm</th>
<th>Percentage Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acetone</td>
<td>42</td>
<td>2.8%</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>64</td>
<td>4.2%</td>
</tr>
<tr>
<td>3.</td>
<td>Water</td>
<td>58</td>
<td>3.8%</td>
</tr>
</tbody>
</table>
5.11 Preliminary Qualitative Phytochemical Studies:

It was observed that, CA-LF-ETH and CA-FP-ETH extracts contain more phytochemicals such as alkaloids, carbohydrates, steroids, saponins, flavonoids, phenols and tannins respectively. Whereas, rest of the extracts of plant materials contain few phytochemicals, CA-LF-ACET extract contains flavonoids, phenols and tannins, CA-LF-WATE extract contains carbohydrates, steroids and glycosides, CA-BRK-ACET extracts only phenols, CA-BRK-ETH extract contains alkaloids steroids, phenols and tannins, CA-BRK-WATE extract contains only carbohydrates, CA-FP-ACET extract contains phenols and tannins and CA-FP-WATE extract contains only carbohydrates. The results are summarized in the Table nos. 5.11.1, 5.11.2, 5.11.3.

Table 5.11.1: Preliminary Phytochemical Screening of Citrus aurantium Leaf Extracts

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Test</th>
<th>Acetone Extract</th>
<th>Ethanol Extract</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ii</td>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>iii</td>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>iv</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>v</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>vi</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>vii</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>viii</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

- Absent  + Presence
Table 5.11.2: Preliminary Phytochemical Screening of *Citrus aurantium* Bark Extracts

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Test</th>
<th>Acetone Extract</th>
<th>Ethanol Extract</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ii</td>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>iii</td>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>iv</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>v</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>vi</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>vii</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>viii</td>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

- Absent    + Present

Table 5.11.3: Preliminary Phytochemical Screening of *Citrus aurantium* Fruit Peel Extracts

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Test</th>
<th>Acetone Extract</th>
<th>Ethanol Extract</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ii</td>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>iii</td>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>iv</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>v</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>vi</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>vii</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>viii</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

- Absent    + Present
5.12 Anti microbial studies reports:

The disc diffusion method was used to determine zones of inhibition of *Citrus aurantium* extracts CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ETH, CA-FP-ACET and CA-FP-WATE. The CA-FP-ETH, CA-BRK-ETH, CA-BRK-ETH, CA-FP-ETH showed significant activity against *Staphylococcus aureus*, *Enterococcus faecalis* in gram positive and *Escherichia coli*, *Klebsiella pneumonia* in gram negative at the concentration of 75 µg. CA-LF-ACET, CA-LF-WATE, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ACET and CA-FP-WATE extracts showed less significant activity. The results are summarized in table no. 5.12.1.

In Anti fungal activity the CA-FP-ETH, CA-BRK-ETH, CA-BRK-ETH, CA-FP-ETH showed significant activity against *Candida albicans*, *Asperigillus fumigates Dreschlera turcica* and *Fusarium verticillioides* at the concentration of 75 µg.

CA-LF-ACET, CA-LF- WATE, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ACET and CA-FP-WATE extracts showed less significant activity. These extracts were compared with Fluconazole which is used as a standard. The results are summarized in table no. 5.12.2.
Table 5.12.1: Anti bacterial Activity and Extracts of *Citrus aurantium*

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Enterococcus faecalis</em></th>
<th><em>Klebsiella pneumonia</em></th>
<th><em>Escherichia coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration in µgms</td>
<td>75 50 25 10 5</td>
<td>75 50 25 10 5</td>
<td>75 50 25 10 5</td>
<td>75 50 25 10 5</td>
</tr>
<tr>
<td>CM-LF-ETH</td>
<td>17 15.5 13.5 11 9.5</td>
<td>16 12.5 10 - -</td>
<td>16.5 12.5 11 9 9</td>
<td>19 17 15 13 10</td>
</tr>
<tr>
<td>CM-LF-ACET</td>
<td>12 10 9 - - -</td>
<td>13 11 9 - - -</td>
<td>11 - - - - -</td>
<td>12 9 - - - -</td>
</tr>
<tr>
<td>CM-LF-WATE</td>
<td>17 14 12 10 9</td>
<td>15 13 9 - - -</td>
<td>16 12.5 10.5 9.5 -</td>
<td>14 12 11 10 9</td>
</tr>
<tr>
<td>CM-BRK-ETH</td>
<td>18 16 15 12 10</td>
<td>15 12 11 10 - -</td>
<td>16 12 10.5 9 - -</td>
<td>15 12 10 9 - -</td>
</tr>
<tr>
<td>CM-BRK-ACET</td>
<td>11 9 - - - - -</td>
<td>14 12 10 9 - -</td>
<td>12 10 - - - - - -</td>
<td>11 9 - - - - -</td>
</tr>
<tr>
<td>CM-BRK-WATE</td>
<td>14 12.5 11 10 9</td>
<td>13 10 - - - -</td>
<td>12 10 - - - - - -</td>
<td>17 16 13 11 10</td>
</tr>
<tr>
<td>CM-FP-ETH</td>
<td>19 16 14 10 9</td>
<td>17 15.5 12 10 9.5</td>
<td>18 16 13 11 10</td>
<td>18 13 10 9 1</td>
</tr>
<tr>
<td>CM-FP-ACET</td>
<td>16 14 12 10 - - -</td>
<td>13 10 9 - - -</td>
<td>11 9 - - - - - -</td>
<td>16 12.5 10 - -</td>
</tr>
<tr>
<td>CM-FP-WATE</td>
<td>16 13 10 9 - - -</td>
<td>14 11 - - - -</td>
<td>13 10 - - - - - -</td>
<td>17 15 12 11 10</td>
</tr>
<tr>
<td>Standard (Ciprofloxacin)</td>
<td>22 20 18 17 14</td>
<td>21 20 18 16 15</td>
<td>22 19.5 17 15 13</td>
<td>23.5 19.5 17 16 14</td>
</tr>
</tbody>
</table>

*Data showing zone of inhibition in mm*
Table 5.12.2: Anti Fungal Activity and Extracts of *Citrus aurantium*

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th><em>Candida albicans</em></th>
<th><em>Aperigillus fumigatus</em></th>
<th><em>Dreschlera turcica</em></th>
<th><em>Fusarium verticillioides</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration in µgms</td>
<td>75</td>
<td>50</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>CM-LF-ETH</td>
<td>18</td>
<td>15.5</td>
<td>13.5</td>
<td>12</td>
</tr>
<tr>
<td>CM-LF-ACET</td>
<td>12</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CM-LF-WATE</td>
<td>15</td>
<td>12</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>CM-BRK-ETH</td>
<td>16</td>
<td>14</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>CM-BRK-ACET</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CM-BRK-WATE</td>
<td>13</td>
<td>12</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>CM-FP-ETH</td>
<td>19</td>
<td>17</td>
<td>14</td>
<td>12.5</td>
</tr>
<tr>
<td>CM-FP-ACET</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>CM-FP-WATE</td>
<td>15</td>
<td>12</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Standard (Flucanazole)</td>
<td>20.5</td>
<td>17</td>
<td>15.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

*Data showing zone of inhibition in mm*
5.13 In-Vitro Antioxidant Activity

Table 5.13.1: DPPH Radical Scavenging Activity of Different Extracts of Citrus aurantium

It is observed that the CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ETH, CA-FP-ACET and CA-FP-WATE have been demonstrated dose dependent increase in the DPPH radical scavenging activity. Whereas, 250 µg Ascorbic acid (Std) has 93.14% activity. However, 250 µg of CA-LF-ETH 86.22%, CA-BRK-ETH 80.72%, CA-FP-ETH 92.84% have shown scavenging activity. The results are summarized in table 5.13.1.
Table 5.13.1: DPPH Radical Scavenging Activity of Different Extracts of *Citrus aurantium*

| Sample            | % Inhibition for Absorbance (Mean ± SEM) | 50 µg/mL       | 100 µg/mL       | 150 µg/mL       | 200 µg/mL       | 250 µg/mL       |
|-------------------|------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                   |                                           |                |                |                |                |                |                |
| Ascorbic acid     | 1.104 ± 0.008 (34.71%)                   | 0.515 ± 0.002  | 0.195 ±0.002   | 0.137 ± 0.002  | 0.116 ± 0.001  | (93.14%)       |
| CA-LF-ETH         | 1.203 ± 0.003 (28.85%)                   | 0.833 ± 0.003  | 0.525 ± 0.002  | 0.366 ± 0.003  | 0.223 ± 0.006  | (86.22%)       |
| CA-LF-ACET        | 1.117 ± 0.008 (33.94%)                   | 0.873 ± 0.003  | 0.546 ± 0.003  | 0.366 ± 0.003  | 0.276 ± 0.003  | (83.67%)       |
| CA-LF-WATE        | 1.165 ± 0.002 (31.10%)                   | 0.821 ± 0.001  | 0.633 ± 0.003  | 0.408 ±0.001   | 0.248 ± 0.004  | (85.33%)       |
| CA-BRK-ETH        | 1.347 ± 0.003 (20.34%)                   | 1.017 ± 0.003  | 0.780 ± 0.005  | 0.533 ± 0.003  | 0.326 ± 0.003  | (80.72%)       |
| CA-BRK-ACET       | 1.280 ± 0.005 (24.30%)                   | 0.936 ± 0.003  | 0.738 ± 0.004  | 0.486 ± 0.003  | 0.340 ± 0.005  | (79.89%)       |
| CA-BRK-WATE       | 1.292 ± 0.001 (23.59%)                   | 0.955 ± 0.002  | 0.743 ± 0.003  | 0.615 ± 0.002  | 0.530 ± 0.005  | (68.65%)       |
| CA-FP-ETH         | 1.183 ±0.003 (30.04%)                    | 0.750 ± 0.005  | 0.455 ± 0.002  | 0.178 ± 0.004  | 0.121 ± 0.004  | (92.84%)       |
| CA-FP-ACET        | 1.215 ± 0.002 (28.14%)                   | 1.045 ± 0.002  | 0.745 ± 0.005  | 0.515 ± 0.002  | 0.328 ± 0.004  | (80.60%)       |
| CA-FP-WATE        | 1.168 ± 0.001 (30.92%)                   | 0.848 ± 0.004  | 0.666 ± 0.003  | 0.533 ± 0.001  | 0.413 ± 0.003  | (75.57%)       |

Data represents the Mean ± SEM of three measurements. CA-*Citrus aurantium*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water
Table 5.13.2: Superoxide Anion Radical Scavenging Activity of Different Extracts of *Citrus aurantium*

It is observed that CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ETH, CA-FP-ACET and CA-FP-WATE have demonstrated dose dependent increase in the superoxide anion scavenging activity. Whereas, 250 µg Ascorbic acid (Std.) has 83.62% activity. However, 250 µg CA-LF-ETH 77.48%, CA-BRK-ETH 70.31%, CA-FP-ETH 82.08% have shown superoxide anion radical scavenging activity. The results are summarized table 5.13.2.

Table 5.13.2: Superoxide Anion Radical Scavenging Activity of Different Extracts of *Citrus aurantium*

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Inhibition for Concentration (Mean ± SEM)</th>
<th>50 µg/mL</th>
<th>100 µg/mL</th>
<th>150 µg/mL</th>
<th>200 µg/mL</th>
<th>250 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td></td>
<td>0.556 ± 0.003 (43.09%)</td>
<td>0.385 ± 0.002 (60.59%)</td>
<td>0.268 ± 0.004 (72.56%)</td>
<td>0.193 ± 0.003 (80.24%)</td>
<td>0.160 ± 0.002 (83.62%)</td>
</tr>
<tr>
<td>CA-LF-ETH</td>
<td></td>
<td>0.671 ± 0.004 (31.32%)</td>
<td>0.541 ± 0.001 (44.62%)</td>
<td>0.408 ± 0.004 (58.23%)</td>
<td>0.278 ± 0.004 (71.54%)</td>
<td>0.220 ± 0.005 (77.48%)</td>
</tr>
<tr>
<td>CA-LF-ACET</td>
<td></td>
<td>0.731 ± 0.006 (25.17%)</td>
<td>0.613 ± 0.003 (37.25%)</td>
<td>0.485 ± 0.002 (50.35%)</td>
<td>0.421 ± 0.004 (56.90%)</td>
<td>0.341 ± 0.001 (65.09%)</td>
</tr>
<tr>
<td>CA-LF-WATE</td>
<td></td>
<td>0.688 ± 0.004 (29.58%)</td>
<td>0.520 ± 0.005 (46.77%)</td>
<td>0.416 ± 0.004 (57.42%)</td>
<td>0.321 ± 0.001 (67.14%)</td>
<td>0.238 ± 0.004 (75.63%)</td>
</tr>
<tr>
<td>CA-BRK-ETH</td>
<td></td>
<td>0.665 ± 0.002 (31.93%)</td>
<td>0.533 ± 0.003 (45.44%)</td>
<td>0.463 ± 0.003 (52.61%)</td>
<td>0.375 ± 0.002 (61.61%)</td>
<td>0.290 ± 0.005 (70.31%)</td>
</tr>
<tr>
<td>CA-BRK-ACET</td>
<td></td>
<td>0.726 ± 0.003 (25.68%)</td>
<td>0.645 ± 0.002 (33.98%)</td>
<td>0.516 ± 0.003 (47.18%)</td>
<td>0.435 ± 0.002 (55.47%)</td>
<td>0.318 ± 0.004 (67.45%)</td>
</tr>
<tr>
<td>CA-BRK-WATE</td>
<td></td>
<td>0.685 ± 0.003 (29.88%)</td>
<td>0.608 ± 0.004 (37.76%)</td>
<td>0.531 ± 0.006 (45.64%)</td>
<td>0.421 ± 0.004 (56.90%)</td>
<td>0.348 ± 0.004 (64.38%)</td>
</tr>
<tr>
<td>CA-FP-ETH</td>
<td></td>
<td>0.645 ± 0.002 (33.98%)</td>
<td>0.521 ± 0.001 (46.67%)</td>
<td>0.388 ± 0.004 (60.28%)</td>
<td>0.266 ± 0.004 (72.77%)</td>
<td>0.175 ± 0.002 (82.08%)</td>
</tr>
<tr>
<td>CA-FP-ACET</td>
<td></td>
<td>0.655 ± 0.002 (32.95%)</td>
<td>0.535 ± 0.002 (45.24%)</td>
<td>0.420 ± 0.005 (57.01%)</td>
<td>0.290 ± 0.005 (70.31%)</td>
<td>0.211 ± 0.001 (78.40%)</td>
</tr>
<tr>
<td>CA-FP-WATE</td>
<td></td>
<td>0.738 ± 0.004 (24.46%)</td>
<td>0.623 ± 0.001 (36.23%)</td>
<td>0.468 ± 0.004 (49.02%)</td>
<td>0.330 ± 0.005 (66.22%)</td>
<td>0.245 ± 0.002 (74.92%)</td>
</tr>
</tbody>
</table>

Data represents the Mean ± SEM of three measurements. CA-*Citrus aurantium*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
Table 5.13.3: Hydroxyl Radical Scavenging Activity of Different Extracts of *Citrus aurantium*

It was observed that CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ETH, CA-FP-ACET and CA-FP-WATE have demonstrated dose dependent increase in the hydroxyl radical scavenging activity. Whereas, 250 µg Sodium metabisulphite (Std) has 80.20% scavenging activity. However HAEF at 250 µg CA-LF-ETH 77.19%, CA-BRK-ETH 74.93%, CA-FP-ETH 74.43% has shown of significant scavenging activity. The results are summarized table 5.13.3.

**Table 5.13.3: Hydroxyl Radical Scavenging Activity of Different Extracts of *Citrus aurantium***

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Inhibition for Concentration (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>50 µg/mL</strong></td>
</tr>
<tr>
<td>Sodium metabisulphite</td>
<td>0.205 ± 0.002 (49.37%)</td>
</tr>
<tr>
<td>CA-LF-ETH</td>
<td>0.235 ± 0.002 (41.10%)</td>
</tr>
<tr>
<td>CA-LF-ACET</td>
<td>0.245 ± 0.002 (38.59%)</td>
</tr>
<tr>
<td>CA-LF-WATE</td>
<td>0.237 ± 0.002 (40.60%)</td>
</tr>
<tr>
<td>CA-BRK-ETH</td>
<td>0.256 ± 0.002 (36.59%)</td>
</tr>
<tr>
<td>CA-BRK-ACET</td>
<td>0.246 ± 0.003 (38.34%)</td>
</tr>
<tr>
<td>CA-BRK-WATE</td>
<td>0.266 ± 0.002 (33.33%)</td>
</tr>
<tr>
<td>CA-FP-ETH</td>
<td>0.247 ± 0.001 (38.09%)</td>
</tr>
<tr>
<td>CA-FP-ACET</td>
<td>0.271 ± 0.003 (32.08%)</td>
</tr>
<tr>
<td>CA-FP-WATE</td>
<td>0.255 ± 0.176 (36.09%)</td>
</tr>
</tbody>
</table>

Data represents the Mean ± SEM of three measurements. CA-*Citrus aurantium*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
Table 5.13.4: Nitric Oxide Radical Scavenging Activity of Different Extracts of *Citrus aurantium*

It is observed that the extracts CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ETH, CA-FP-ACET and CA-FP-WATE have demonstrated dose dependent increase in the nitric oxide anion scavenging property. Whereas, 250 µg Ascorbic acid (Std.) has 80.11% nitric oxide anion scavenging property. However HAEF at 250 µg CA-LF-ETH 69.88%, CA-BRK-ACET 69.00%, CA-FP-ETH 75.43% has shown of significant scavenging activity. The results are summarized table 5.13.4.

Table 5.13.4: Nitric Oxide Radical Scavenging Activity of Different Extracts of *Citrus aurantium*

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Inhibition for Concentration (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.158 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>(53.80%)</td>
</tr>
<tr>
<td>CA-LF-ETH</td>
<td>0.176 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>(48.53%)</td>
</tr>
<tr>
<td>CA-LF-ACET</td>
<td>0.178 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>(47.95%)</td>
</tr>
<tr>
<td>CA-LF-WATE</td>
<td>0.193 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>(43.56%)</td>
</tr>
<tr>
<td>CA-BRK-ETH</td>
<td>0.174 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>(49.12%)</td>
</tr>
<tr>
<td>CA-BRK-ACET</td>
<td>0.181 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>(47.07%)</td>
</tr>
<tr>
<td>CA-BRK-WATE</td>
<td>0.189 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>(45.32%)</td>
</tr>
<tr>
<td>CA-FP-ETH</td>
<td>0.170 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>(50.29%)</td>
</tr>
<tr>
<td>CA-FP-ACET</td>
<td>0.166 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>(52.63%)</td>
</tr>
<tr>
<td>CA-FP-WATE</td>
<td>0.187 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>(45.32%)</td>
</tr>
</tbody>
</table>

Data represents the Mean ± SEM of three measurements. CA-*Citrus aurantium*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water
5.14 Determination of Non enzymatic antioxidants

Table 5.14.1: Determination of Total Phenolic and Total Flavanoid Contents

_Citrus aurantium_ shows that, most of the phenolics flavonoids are extracted with ethanol. The analysis of leaf, stem bark and fruit peel indicate that the phenolic and flavonoid content varies significantly among different plant parts. _Citrus aurantium_ the maximum phenolic content was found to be in ethanol extract of fruit peel. The phenolic content of ethanol extract of _Citrus aurantium_ is estimated as 228.6 mg/g. The flavonoid content of ethanol extract of _Citrus aurantium_ fruit peel is estimated as 212 mg/g. The results are summarized in table 5.14.1.

Table 5.14.1: Determination of Total Phenolic and Total Flavanoid Contents

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Amount of Phenolic Compound mg/g</th>
<th>Amount of Flavanoid Compound mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>CA-LF-ETH</td>
<td>196</td>
<td>20.8</td>
</tr>
<tr>
<td>2.</td>
<td>CA-LF-ACET</td>
<td>18.2</td>
<td>4.1</td>
</tr>
<tr>
<td>3.</td>
<td>CA-BRK-ETH</td>
<td>90.4</td>
<td>90.4</td>
</tr>
<tr>
<td>4.</td>
<td>CA-BRK-ACET</td>
<td>8.6</td>
<td>8.6</td>
</tr>
<tr>
<td>5.</td>
<td>CA-FP-ETH</td>
<td>228.6</td>
<td>212</td>
</tr>
<tr>
<td>6.</td>
<td>CA-FP-ACET</td>
<td>12.2</td>
<td>2.7</td>
</tr>
</tbody>
</table>

_CA-Citrus aurantium_, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone.

5.15 Analgesic Activity

5.15.1 Acetic acid Induced Writhing in Mice

Control and various treated groups were tested for analgesic activity against acetic acid induced writhing, which is nothing but the painful reaction. Thirty minutes after the treatment, each mouse was injected with 0.1 mL 0.7% v/v aqueous solution of acetic acid i.p. The number of abdominal constrictions was cumulatively counted from 0-10 minutes. The % reduction of writhing in standard diclofenac
sodium 10 mg/kg treated group was found to be 60.02% against control.

The mean response of control and standard was 41.50 ± 1.25 and 16.59 ± 0.92 respectively. The respective test compounds CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK- WATE, CA-FP-ETH, CA-FP-ACET and CA-FP- WATE in its 300 mg/kg dose, showed mean writhing responses as 19.54 ± 1.31, 29.03 ± 1.42, 25.28 ± 1.79, 22.90 ± 1.50, 30.48 ± 1.78, 24.56 ± 1.63, 19.88 ± 1.56, 28.86 ± 1.40 and 26.30 ± 1.09. In terms of percentage inhibition of writhing by diclofenac sodium was 60.02% while with the test compound it was CA-LF-ETH 52.91%, CA-BRK-ETH 44.81% and CA-FP-ETH 52.09% respectively. The values are tabulated in the Table 5.15.1 and shown in Figure 5.13.

**Table 5.15.1: Effect of Citrus aurantium Plant Extracts on Acetic acid Induced Writhing in Mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Mean no of writhing ±SEM</th>
<th>% Inhibition of writhes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>41.50 ± 1.25</td>
<td>-</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diclofenac (10 mg/kg)</td>
<td>16.59 ± 0.92***</td>
<td>60.02%</td>
</tr>
<tr>
<td>Group-III</td>
<td>CA-LF-ETH (300 mg/kg)</td>
<td>19.54 ± 1.31***</td>
<td>52.91%</td>
</tr>
<tr>
<td>Group-IV</td>
<td>CA-LF-ACET (300 mg/kg)</td>
<td>29.03 ± 1.42***</td>
<td>30.04%</td>
</tr>
<tr>
<td>Group-V</td>
<td>CA-LF-WATE (300 mg/kg)</td>
<td>25.28 ± 1.79***</td>
<td>39.08%</td>
</tr>
<tr>
<td>Group-VI</td>
<td>CA-BRK-ETH (300 mg/kg)</td>
<td>22.90 ± 1.50***</td>
<td>44.81%</td>
</tr>
<tr>
<td>Group-VII</td>
<td>CA-BRK-ACET (300 mg/kg)</td>
<td>30.48 ± 1.78***</td>
<td>26.55%</td>
</tr>
<tr>
<td>Group-VIII</td>
<td>CA-BRK- WATE (300 mg/kg)</td>
<td>24.56 ± 1.63***</td>
<td>40.81%</td>
</tr>
<tr>
<td>Group-IX</td>
<td>CA-FP-ETH (300 mg/kg)</td>
<td>19.88 ± 1.56***</td>
<td>52.09%</td>
</tr>
<tr>
<td>Group-X</td>
<td>CA-FP-ACET (300 mg/kg)</td>
<td>28.86 ± 1.40***</td>
<td>30.45%</td>
</tr>
<tr>
<td>Group-XI</td>
<td>CA-FP- WATE (300 mg/kg)</td>
<td>26.30 ± 1.09***</td>
<td>36.62%</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. CA-Citrus aurantium, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone.
5.15.2 Tail Flick Method in Rats

In the tail flick method, the increase in latency period at different time points significantly differed (P<0.001) compared to baseline values within the same drug treated groups. The CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ETH, CA-FP-ACET and CA-FP-WATE and diclofenac sodium caused significant increase (P<0.001) in the percentage reaction time whilst the control and dose of extracts (300 mg/kg). At all the specified time intervals, the percentage of tail flick elongation time differed significantly (P<0.001) between the extracts and diclofenac sodium at the doses of plant extracts, being greater for diclofenac sodium. At the peak of activity, CA-LF-ETH, CA-BRK-ETH and CA-FP-ETH extracts showed (P<0.001) and significantly of tail.
flick elongation time respectively, whilst diclofenac sodium gave (P<0.001) elongation of tail flicking time. The values are tabulated in the Table 5.15.2 and shown in Figure 5.14.
Table 5.15.2: Effect of *Citrus aurantium* Plant Extracts on Tail Flick method in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Reaction Time (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>4.51 ± 0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group-II</td>
<td>Diclofenac (10 mg/kg)</td>
<td>4.71 ± 0.31</td>
</tr>
<tr>
<td>Group-III</td>
<td>CA-LF-ETH (300 mg/kg)</td>
<td>4.43 ± 0.36</td>
</tr>
<tr>
<td>Group-IV</td>
<td>CA-LF-ACET (300 mg/kg)</td>
<td>4.16 ± 0.56</td>
</tr>
<tr>
<td>Group-V</td>
<td>CA-LF-WATE (300 mg/kg)</td>
<td>4.50 ± 0.27</td>
</tr>
<tr>
<td>Group-VI</td>
<td>CA-BRK-ETH (300 mg/kg)</td>
<td>4.43 ± 0.44</td>
</tr>
<tr>
<td>Group-VII</td>
<td>CA-BRK-ACET (300 mg/kg)</td>
<td>4.82 ± 0.48</td>
</tr>
<tr>
<td>Group-VIII</td>
<td>CA-BRK- WATE (300 mg/kg)</td>
<td>4.70 ± 0.24</td>
</tr>
<tr>
<td>Group-IX</td>
<td>CA-FP-ETH (300 mg/kg)</td>
<td>4.35 ± 0.65</td>
</tr>
<tr>
<td>Group-X</td>
<td>CA-FP-ACET (300 mg/kg)</td>
<td>4.66 ± 0.55</td>
</tr>
<tr>
<td>Group-XI</td>
<td>CA-FP- WATE (300 mg/kg)</td>
<td>4.84 ± 0.38</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represent Not significant. CA-*Citrus aurantium*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone.
Fig. 5.14: Effect of *Citrus aurantium* Plant Extracts on Tail Flick method in Rats
5.15.3 Hot plate Method in Mice

The standard pentazocine lactate (10 mg/kg) was given i.p., CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK- WATE, CA-FP-ETH, CA-FP-ACET and CA-FP- WATE extracts given orally, in a dose of 300 mg/kg, elicited a significant analgesic activity in the hot plate method as evidenced by increase in latency time in seconds as compared with vehicle control. The increase in latency time was dose dependant. Latency time was noted 30, 60, 90, 120 and 180 minutes after administration of vehicle, standard and plant extracts. The values are tabulated in the Table 5.15.3 and shown in Figure 5.15.
Table 5.15.3: Effect of *Citrus aurantium* Plant Extracts on Hot Plate Method in Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Reaction time (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>2.66 ± 0.33</td>
</tr>
<tr>
<td>Group-II</td>
<td>Pentazocine (10 mg/kg)</td>
<td>2.50 ± 0.22</td>
</tr>
<tr>
<td>Group-III</td>
<td>CA-LF-ETH (300 mg/kg)</td>
<td>3.16 ± 0.47</td>
</tr>
<tr>
<td>Group-IV</td>
<td>CA-LF-ACET (300 mg/kg)</td>
<td>2.78 ± 0.44</td>
</tr>
<tr>
<td>Group-V</td>
<td>CA-LF-WATE (300 mg/kg)</td>
<td>3.15 ± 0.54</td>
</tr>
<tr>
<td>Group-VI</td>
<td>CA-BRK-ETH (300 mg/kg)</td>
<td>3.08 ± 0.36</td>
</tr>
<tr>
<td>Group-VII</td>
<td>CA-BRK-ACET (300 mg/kg)</td>
<td>3.20 ± 0.42</td>
</tr>
<tr>
<td>Group-VIII</td>
<td>CA-BRK- WATE (300 mg/kg)</td>
<td>2.98 ± 0.60</td>
</tr>
<tr>
<td>Group-IX</td>
<td>CA-FP-ETH (300 mg/kg)</td>
<td>3.00 ± 0.36</td>
</tr>
<tr>
<td>Group-X</td>
<td>CA-FP-ACET (300 mg/kg)</td>
<td>2.56 ± 0.26</td>
</tr>
<tr>
<td>Group-XI</td>
<td>CA-FP- WATE (300 mg/kg)</td>
<td>2.45 ± 0.38</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. *CA-Citrus aurantium*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone.
Fig. 5.15: Effect of *Citrus aurantium* Plant Extracts on Hot Plate Method in Mice
5.16 Anti-inflammatory Activity

5.16.1 Acute Anti-inflammatory Activity

5.16.1.1 Formalin-induced paw Oedema in Rat

All the test compounds were tested with the diclofenac sodium as a standard drug in the dose of 10 mg/kg for the anti-inflammatory activity.

Presently diclofenac showed significant 87.14 % inhibition of inflammation at 5th hour (0.18 ± 0.01) when compared with control (1.40 ± 0.05) respectively.

The test compounds showed maximum percentage of inhibition of oedema at 5th hour significantly in respective dose level i.e., at 300 mg/kg the test compounds CA-LF-ETH, CA-BRK-ETH and CA-FP-ETH showed 82.14%, 79.28% and 84.28%. The values are tabulated in the Table 5.16.1 and shown in Figure 5.16.
Table 5.16.1: Effect of *Citrus aurantium* Plant Extracts on Formalin-induced paw Oedema in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>0 hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4 hr</th>
<th>5 hr</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>0.16 ± 0.01</td>
<td>0.78 ± 0.05</td>
<td>0.99 ± 0.05</td>
<td>1.20 ± 0.06</td>
<td>1.25 ± 0.07</td>
<td>1.40 ± 0.05</td>
<td>-</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diclofenac (10 mg/kg)</td>
<td>0.15 ± 0.01</td>
<td>0.37 ± 0.02***</td>
<td>0.55 ± 0.04***</td>
<td>0.36 ± 0.02***</td>
<td>0.30 ± 0.03***</td>
<td>0.18 ± 0.01***</td>
<td>87.14%</td>
</tr>
<tr>
<td>Group-III</td>
<td>CA-LF-ETH (300 mg/kg)</td>
<td>0.16 ± 0.02</td>
<td>0.48 ± 0.02**</td>
<td>0.61 ± 0.03***</td>
<td>0.49 ± 0.02***</td>
<td>0.38 ± 0.02***</td>
<td>0.25 ± 0.01***</td>
<td>82.14%</td>
</tr>
<tr>
<td>Group-IV</td>
<td>CA-LF-ACET (300 mg/kg)</td>
<td>0.17 ± 0.02</td>
<td>0.61 ± 0.03*</td>
<td>0.79 ± 0.03*</td>
<td>0.70 ± 0.03***</td>
<td>0.63 ± 0.03***</td>
<td>0.49 ± 0.02 ***</td>
<td>65.00%</td>
</tr>
<tr>
<td>Group-V</td>
<td>CA-LF-WATE (300 mg/kg)</td>
<td>0.16 ± 0.02</td>
<td>0.57 ± 0.03**</td>
<td>0.69 ± 0.03***</td>
<td>0.61 ± 0.03***</td>
<td>0.49 ± 0.01***</td>
<td>0.36 ± 0.01***</td>
<td>74.28%</td>
</tr>
<tr>
<td>Group-VI</td>
<td>CA-BRK-ETH (300 mg/kg)</td>
<td>0.17 ± 0.03</td>
<td>0.62 ± 0.03*</td>
<td>0.71 ± 0.02***</td>
<td>0.58 ± 0.02***</td>
<td>0.45 ± 0.01***</td>
<td>0.29 ± 0.02***</td>
<td>79.28%</td>
</tr>
<tr>
<td>Group-VII</td>
<td>CA-BRK-ACET (300 mg/kg)</td>
<td>0.18 ± 0.02</td>
<td>0.65 ± 0.04**</td>
<td>0.78 ± 0.04*</td>
<td>0.67 ± 0.03***</td>
<td>0.56 ± 0.02***</td>
<td>0.46 ± 0.04***</td>
<td>67.14%</td>
</tr>
<tr>
<td>Group-VIII</td>
<td>CA-BRK-WATE (300 mg/kg)</td>
<td>0.17 ± 0.01</td>
<td>0.61 ± 0.03*</td>
<td>0.73 ± 0.02***</td>
<td>0.61 ± 0.02***</td>
<td>0.52 ± 0.02***</td>
<td>0.41 ± 0.03 ***</td>
<td>70.71 %</td>
</tr>
<tr>
<td>Group-IX</td>
<td>CA-FP-ETH (300 mg/kg)</td>
<td>0.15 ± 0.01</td>
<td>0.52 ± 0.02**</td>
<td>0.63 ± 0.03***</td>
<td>0.42 ± 0.01***</td>
<td>0.33 ± 0.02***</td>
<td>0.22 ± 0.01***</td>
<td>84.28%</td>
</tr>
<tr>
<td>Group-X</td>
<td>CA-FP-ACET (300 mg/kg)</td>
<td>0.15 ± 0.02</td>
<td>0.68 ± 0.04**</td>
<td>0.80 ± 0.04*</td>
<td>0.66 ± 0.03***</td>
<td>0.57 ± 0.02***</td>
<td>0.47 ± 0.02***</td>
<td>66.42%</td>
</tr>
<tr>
<td>Group-XI</td>
<td>CA-FP-WATE (300 mg/kg)</td>
<td>0.16 ± 0.02</td>
<td>0.60 ± 0.02*</td>
<td>0.68 ± 0.03***</td>
<td>0.54 ± 0.02***</td>
<td>0.40 ± 0.03***</td>
<td>0.32 ± 0.02***</td>
<td>77.14%</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. *CA-Citrus aurantium*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone.
Fig. 5.16: Effect of *Citrus aurantium* Plant Extracts on Formalin-induced paw Oedema in Rats
**5.16.2 Chronic Anti inflammatory Activity**

**5.16.2.1 Formalin-induced paw Oedema in Rats**

Formalin induced paw oedema is one of the most suitable test procedure to screen chronic anti-inflammatory agents. The results obtained as mean increase in paw volume (mL) and % inhibition are represented in Table 5.16.2.

The mean response of standard was 82.40% inhibition of increase in paw thickness after 6 days respectively. In this model at 300 mg/kg dose level of CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK- WATE, CA-FP-ETH, CA-FP-ACET and CA-FP- WATE extracts showed 72.01%, 22.31%, 60.08%, 66.52%, 29.61%, 45.06%, 67.38%, 41.20% and 47.21% inhibition of increase in paw thickness after 6 days. However, at CA-LF-ETH, CA-BRK-ETH and CA-FP-ETH extracts showed 72.01%, 66.52% and 67.38% inhibition of increase in paw thickness after 6 days. All the results were compared with solvent control and diclofenac sodium reference drug control.
Table 5.16.2: Effect of *Citrus aurantium* Plant Extracts on Formalin-induced paw Oedema in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Initial Paw Volume</th>
<th>Paw volume after 6 days</th>
<th>Increase in Paw Volume</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Saline</td>
<td>1.28 ± 0.07</td>
<td>3.61 ± 0.12</td>
<td>2.33 ± 0.06</td>
<td>-</td>
</tr>
<tr>
<td>Group-II</td>
<td>Diclofenac (100 mg/kg)</td>
<td>1.23 ± 0.04</td>
<td>1.65 ± 0.05</td>
<td>0.41 ± 0.07</td>
<td>82.40%</td>
</tr>
<tr>
<td>Group-III</td>
<td>CA-LF-ETH (300 mg/kg)</td>
<td>1.27 ± 0.03</td>
<td>1.92 ± 0.05</td>
<td>0.65 ± 0.10</td>
<td>72.01%</td>
</tr>
<tr>
<td>Group-IV</td>
<td>CA-LF-ACET (300 mg/kg)</td>
<td>1.25 ± 0.08</td>
<td>3.06 ± 0.16</td>
<td>1.81 ± 0.13</td>
<td>22.31%</td>
</tr>
<tr>
<td>Group-V</td>
<td>CA-LF-WATE (300 mg/kg)</td>
<td>1.23 ± 0.05</td>
<td>2.16 ± 0.12</td>
<td>0.93 ± 0.10</td>
<td>60.08%</td>
</tr>
<tr>
<td>Group-VI</td>
<td>CA-BRK-ETH (300 mg/kg)</td>
<td>1.29 ± 0.06</td>
<td>2.07 ± 0.08</td>
<td>0.78 ± 0.08</td>
<td>66.52%</td>
</tr>
<tr>
<td>Group-VII</td>
<td>CA-BRK-ACET (300 mg/kg)</td>
<td>1.26 ± 0.06</td>
<td>2.90 ± 0.14</td>
<td>1.64 ± 0.15</td>
<td>29.61%</td>
</tr>
<tr>
<td>Group-VIII</td>
<td>CA-BRK-WATE (300 mg/kg)</td>
<td>1.24 ± 0.06</td>
<td>2.52 ± 0.07</td>
<td>1.28 ± 0.07</td>
<td>45.06%</td>
</tr>
<tr>
<td>Group-IX</td>
<td>CA-FP-ETH (300 mg/kg)</td>
<td>1.27 ± 0.07</td>
<td>2.03 ± 0.14</td>
<td>0.76 ± 0.11</td>
<td>67.38%</td>
</tr>
<tr>
<td>Group-X</td>
<td>CA-FP-ACET (300 mg/kg)</td>
<td>1.28 ± 0.04</td>
<td>2.65 ± 0.19</td>
<td>1.37 ± 0.06</td>
<td>41.20%</td>
</tr>
<tr>
<td>Group-XI</td>
<td>CA-FP-WATE (300 mg/kg)</td>
<td>1.31 ± 0.06</td>
<td>2.54 ± 0.10</td>
<td>1.23 ± 0.16</td>
<td>47.21%</td>
</tr>
</tbody>
</table>

Results are expressed on mean ± SEM from four observations. Paw Volume was measured after 6 days. CA-*Citrus aurantium*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.

5.17 Anti-Cancer Activity

5.17.1 Trypan Blue Dye Exclusion Method against by HeLa cell Line

Table 5.17.1: Effect of *Citrus aurantium* on HeLa cell Line against by Trypan Blue Dye Exclusion Method

Table 5.17.1 depicts the effect of CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ETH, CA-FP-ACET and CA-FP-WATE on % of dead cells against HeLa cell line against by trypan blue dye exclusion method. The percentage of dead cells was found to be 96.5%, 14.5%, 19.0%, 23.7%, 21.9%, 8.8%, 74.5%, 16.9% and 7.35% in HeLa cell line against with CA-LF-
ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ETH, CA-FP-ACET, CA-FP-WATE 300 mg/kg b.w p.o. respectively. CA-LF-ETH showed maximum % of dead cells 96.5% and CA-BRK-ETH and CA-FP-ACET showed 23.7% and 74.5% of dead cells. The values are in the shown in Figure 5.17.

**Table 5.17.1: Effect of *Citrus aurantium* on HeLa cell Line against by Trypan Blue Dye Exclusion Method**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Number of Live cells</th>
<th>Number of Dead cells</th>
<th>Total number of cells</th>
<th>% of Dead cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Control</td>
<td>230</td>
<td>11</td>
<td>241</td>
<td>4.6%</td>
</tr>
<tr>
<td>Group-II</td>
<td>CA-LF-ETH (300 mg/kg)</td>
<td>09</td>
<td>252</td>
<td>261</td>
<td>96.5%</td>
</tr>
<tr>
<td>Group-III</td>
<td>CA-LF-ACET (300 mg/kg)</td>
<td>211</td>
<td>36</td>
<td>247</td>
<td>14.5%</td>
</tr>
<tr>
<td>Group-IV</td>
<td>CA-LF-WATE (300 mg/kg)</td>
<td>238</td>
<td>56</td>
<td>294</td>
<td>19.0%</td>
</tr>
<tr>
<td>Group-V</td>
<td>CA-BRK-ETH (300 mg/kg)</td>
<td>196</td>
<td>61</td>
<td>257</td>
<td>23.7%</td>
</tr>
<tr>
<td>Group-VI</td>
<td>CA-BRK-ACET (300 mg/kg)</td>
<td>206</td>
<td>58</td>
<td>264</td>
<td>21.9%</td>
</tr>
<tr>
<td>Group-VII</td>
<td>CA-BRK-WATE (300 mg/kg)</td>
<td>217</td>
<td>21</td>
<td>238</td>
<td>8.8%</td>
</tr>
<tr>
<td>Group-VIII</td>
<td>CA-FP-ETH (300 mg/kg)</td>
<td>62</td>
<td>182</td>
<td>244</td>
<td>74.5%</td>
</tr>
<tr>
<td>Group-IX</td>
<td>CA-FP-ACET (300 mg/kg)</td>
<td>176</td>
<td>36</td>
<td>212</td>
<td>16.9%</td>
</tr>
<tr>
<td>Group-X</td>
<td>CA-FP-WATE (300 mg/kg)</td>
<td>228</td>
<td>18</td>
<td>246</td>
<td>7.35</td>
</tr>
</tbody>
</table>

*CA-Citrus aurantium*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water.
Fig. 5.17: Effect of *Citrus aurantium* on HeLa cell Line against by Trypan Blue Dye Exclusion Method
5.17.2 Microscopic study of HeLa cell line and Effect of *Citrus aurantium* extracts

Fig. 5.17.1 HeLa on 1\textsuperscript{st} day in Media control

Fig. 5.17.2 HeLa on 2\textsuperscript{nd} day Media control

Fig. 5.17.3 HeLa on 3\textsuperscript{rd} day in Media control

Fig. 5.17.4 HeLa in Media control- tryphan blue stain
Fig. 5.17.5 HeLa in vehicle control

Fig. 5.17.6 Tryphan blue stain
HeLa in IC_{50}-tryphan blue stain

Fig. 5.17.7 HeLa in IC_{100}-tryphan blue stain

Fig. 5.17.8 50% cell death
5.18 In vivo Anti Tumor Activity of Citrus aurantium and Ascitic Tumor Model

Table 5.18.1: Effect of Citrus aurantium on Mean Survival Time in EAC Tumor Bearing Mice

Table 5.18.1 depicts the effect of CYP, CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ETH, CA-FP-ACET and CA-FP-WATE on mean survival time and % ILS against EAC induced mice. In the EAC control group, the median survival time was 14 days and which increased significantly to 18 days \((p<0.001)\) with CA-LF-ETH 300 mg/kg b.w p.o., to 22 days \((p<0.001)\) with CA-BRK-ETH and CA-FP-ETH 300 mg/kg b.w p.o., and \((p<0.01)\) CM-FP-WTR 300 mg/kg b.w p.o., which showed maximum increase in the life when compared with CA-LF-ACET, CM-LF-WATE, CA-BRK-ACET and CA-FP-ACET which was found not significant when compared with EAC control group. CA-LF-ACET, CM-LF-WATE, CA-BRK-ACET and CA-FP-ACET 300 mg/kg b.w p.o., showed least effect in increasing the life span among the doses of test drugs on comparison. The median survival time of CYP treated was found to be 26 days \((p < 0.001)\). The % ILS found to be 49.28%, 40.01%, 24.33%, 46.21% and 30.19%, in EAC induced animals with CA-LF-ETH, CA-BRK-ETH, CM-BRK-WTR, CA-FP-ETH, CM-FP-WTR 300 mg/kg b.w p.o., and CYP 25 mg/kg i.p, respectively when compared with vehicle treated EAC animals. The values are in the shown in Figure 5.18.
Table 5.18.1: Effect of *Citrus aurantium* on Mean Survival Time in EAC Tumor Bearing Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Mean survival time</th>
<th>ILS %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Mice</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>EAC + solvent 20 mL/kg</td>
<td>14.67 ± 1.41</td>
<td>-</td>
</tr>
<tr>
<td>Group III</td>
<td>EAC + CYP 25 mg/kg</td>
<td>23.67 ± 2.09r</td>
<td>61.35%</td>
</tr>
<tr>
<td>Group IV</td>
<td>EAC + CM-LF-ETH (300 mg/kg)</td>
<td>21.90 ± 1.24r</td>
<td>49.28%</td>
</tr>
<tr>
<td>Group V</td>
<td>EAC + CM-LF-ACET (300 mg/kg)</td>
<td>17.50 ± 1.54</td>
<td>19.29%</td>
</tr>
<tr>
<td>Group VI</td>
<td>EAC + CM-LF-WATE (300 mg/kg)</td>
<td>17.97 ± 1.15</td>
<td>22.49%</td>
</tr>
<tr>
<td>Group VII</td>
<td>EAC + CM-BRK-ETH (300 mg/kg)</td>
<td>20.54 ± 1.13r</td>
<td>40.01%</td>
</tr>
<tr>
<td>Group VIII</td>
<td>EAC + CM-BRK-ACET (300 mg/kg)</td>
<td>16.03 ± 1.98</td>
<td>9.27%</td>
</tr>
<tr>
<td>Group IX</td>
<td>EAC + CM-BRK-WATE (300 mg/kg)</td>
<td>18.24 ± 1.35s</td>
<td>24.33%</td>
</tr>
<tr>
<td>Group X</td>
<td>EAC + CM-FP-ETH (300 mg/kg)</td>
<td>21.45 ± 1.10r</td>
<td>46.21%</td>
</tr>
<tr>
<td>Group XI</td>
<td>EAC + CM-FP-ACET (300 mg/kg)</td>
<td>15.77 ± 1.60</td>
<td>7.49%</td>
</tr>
<tr>
<td>Group XII</td>
<td>EAC + CM-FP-WATE (300 mg/kg)</td>
<td>19.10 ± 1.41y</td>
<td>30.19%</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. (n=6).

*p* values:  
- p < 0.05, r < 0.001 as compared with EAC control + solvent.  
- x < 0.05, y < 0.01, z < 0.001, as compared to CYP (by one way ANOVA followed by Dunnett's multiple comparison test).  
*CA-Citrus aurantium*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-water
Table 5.18.2: Effect of *Citrus aurantium* on body weight analysis and tumor growth response against EAC induced animals

Table 5.18.2 shows significant decrease \((p<0.001)\) in the body weight after treatment with CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK- WATE, CA-FP-ETH, CA-FP-ACET and CA-FP- WATE for 14 days. The percentage decrease was found to be 61.11%, 29.27%, 45.92%, 54.18%, 25.69%, 43.46%, 53.63%, 39.10%, 44.35% and 79.33% in EAC induced mice treated with CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK- WATE, CA-FP-ETH, CA-FP-ACET, CA-FP- WATE 300 mg/kg b.w p.o., and CYP 25 mg/kg b.w i.p respectively when compared with vehicle treated cancerous animals (Figure 5.19).

Treatment with CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK- WATE, CA-FP-ETH, CA-FP-ACET and CA-FP- WATE
300 mg/kg b.w p.o., and CYP 25 mg/kg b.w i.p significantly ($p < 0.001$) decreased the tumor volume and packed cell volume when compared to that of EAC control group, these CA-LF-ETH, CM-LF-WTR, CA-BRK-ETH, CM-BRK-WTR, CA-FP-ETH, CA-FP-WTR at the dose of 300 mg/kg b.w p.o., showed the maximum decrease in tumor volume (Figure 5.20) and packed cell volume (Figure 5.21) when compared among test groups.

### Table 5.18.2: Effect of *Citrus aurantium* on body weight analysis and tumor growth response against EAC induced animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Increase in body weight (g)</th>
<th>Tumor volume (mL)</th>
<th>Packed cell volume (mL)</th>
<th>% decrease in body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Mice</td>
<td>0.64 ± 0.26</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>EAC + solvent 20 mL/kg</td>
<td>8.95 ± 0.47 $^c$</td>
<td>8.18 ± 0.26</td>
<td>4.8 ± 0.30</td>
<td>-</td>
</tr>
<tr>
<td>Group III</td>
<td>EAC + CYP 25 mg/kg</td>
<td>1.85 ± 0.56 $^r$</td>
<td>1.2 ± 0.11</td>
<td>0.55 ± 0.20 $^r$</td>
<td>79.33%</td>
</tr>
<tr>
<td>Group IV</td>
<td>EAC + CM-LF-ETH (300 mg/kg)</td>
<td>3.48 ± 0.32 $^{c, r, z}$</td>
<td>3.74 ± 0.34 $^{r, z}$</td>
<td>1.85 ± 0.16 $^{r, z}$</td>
<td>61.11%</td>
</tr>
<tr>
<td>Group V</td>
<td>EAC + CM-LF-ACET (300 mg/kg)</td>
<td>6.33 ± 0.45 $^{c, r, z}$</td>
<td>5.86 ± 0.56 $^{r, z}$</td>
<td>3.08 ± 0.48 $^{r, y}$</td>
<td>29.27%</td>
</tr>
<tr>
<td>Group VI</td>
<td>EAC + CM-LF-WATE (300 mg/kg)</td>
<td>4.84 ± 0.65 $^{c, r, z}$</td>
<td>4.80 ± 0.38 $^{r, z}$</td>
<td>2.42 ± 0.25 $^{r, z}$</td>
<td>45.92%</td>
</tr>
<tr>
<td>Group VII</td>
<td>EAC + CM-BRK-ETH (300 mg/kg)</td>
<td>4.10 ± 0.23 $^{c, r, z}$</td>
<td>4.06 ± 0.29 $^{r, z}$</td>
<td>2.04 ± 0.31 $^{r, z}$</td>
<td>54.18%</td>
</tr>
<tr>
<td>Group VIII</td>
<td>EAC + CM-BRK-ACET (300 mg/kg)</td>
<td>6.65 ± 0.58 $^{c,r,x}$</td>
<td>5.96 ± 0.64 $^{r,x}$</td>
<td>2.88 ± 0.38 $^{r,x}$</td>
<td>25.69%</td>
</tr>
<tr>
<td>Group IX</td>
<td>EAC + CM-BRK-WATE (300 mg/kg)</td>
<td>5.06 ± 0.70 $^{c,r,x}$</td>
<td>5.25 ± 0.44 $^{p,z}$</td>
<td>2.23 ± 0.30 $^{r,z}$</td>
<td>43.46%</td>
</tr>
<tr>
<td>Group X</td>
<td>EAC + CM-FP-ETH (300 mg/kg)</td>
<td>4.15 ± 0.36 $^{c,r,z}$</td>
<td>3.83 ± 0.73 $^{r,z}$</td>
<td>1.93 ± 0.12 $^{r,z}$</td>
<td>53.63%</td>
</tr>
<tr>
<td>Group XI</td>
<td>EAC + CM-FP-ACET (300 mg/kg)</td>
<td>5.45 ± 0.33 $^{c,r,y}$</td>
<td>6.14 ± 0.48 $^{r,y}$</td>
<td>3.16 ± 0.40 $^{r,y}$</td>
<td>39.10%</td>
</tr>
<tr>
<td>Group XII</td>
<td>EAC + CM-FP-WATE (300 mg/kg)</td>
<td>4.98 ± 0.76 $^{c,r,z}$</td>
<td>5.34 ± 0.28 $^{p,r,z}$</td>
<td>2.56 ± 0.21 $^{r,z}$</td>
<td>44.35%</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. (n=6).

$p$ values: $p < 0.05$, $r < 0.001$ as compared with EAC control + solvent.

$x < 0.05$, $y < 0.01$, $z < 0.001$, as compared to CYP (by one way ANOVA followed by Dunnett’s multiple comparison test). CA-*Citrus aurantium*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-WATER.
Fig. 5.19: Effect of *Citrus aurantium* on body weight response against EAC induced animals

Fig. 5.20: Effect of *Citrus aurantium* on Tumor volume against EAC induced animals
**Fig. 5.21:** Effect of *Citrus aurantium* on packed cell volume against EAC induced animals

**Table 5.18.3: Effect of *Citrus aurantium* on hematological parameters on 14th day in normal and EAC tumor bearing mice**

Table 5.18.3 revealed the effect of CA-LF-ETH, CA-LF-ACET, CA-LF-WATE, CA-BRK-ETH, CA-BRK-ACET, CA-BRK-WATE, CA-FP-ETH, CA-FP-ACET and CA-FP-WATE 300 mg/kg b.w p.o., and CYP 25 mg/kg b.w i.p on hematological parameters against EAC induced animals estimated on 14th day of treatment. CA-LF-ETH, CA-BRK-ETH and CA-FP-ETH 300 mg/kg b.w p.o., showed better improvement in the hematological parameters than the rest of the doses among the compared groups. The total WBC count found increased in the EAC control group. All the test drugs when administered to the EAC bearing mice showed the significant \((p < 0.001)\) decrease in the WBC count (Figure 5.22) when compared with the EAC control group, RBC count (Figure 5.23) and Hb content (Figure 5.24) in the EAC groups were significantly \((p < 0.001)\) decreased as compared to the normal group. All the test drugs have showed the significant increase but CA-
LF-ETH, CA-BRK-ETH and CA-FP-ETH 300 mg/kg b.w p.o., showed the better activity compared to rest of the drugs and doses.

Table 5.18.3: Effect of *Citrus aurantium* on hematological parameters on 14th day in normal and EAC tumor bearing mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>WBC (x10⁶/mL)</th>
<th>RBC (x10⁹/mL)</th>
<th>Hb (g%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Mice</td>
<td>7.24±0.23</td>
<td>5.67±0.24</td>
<td>12.9±0.44</td>
</tr>
<tr>
<td>Group II</td>
<td>EAC + solvent 20 mL/kg</td>
<td>21.78±1.31²</td>
<td>3.55±0.04²</td>
<td>8.1±0.28²</td>
</tr>
<tr>
<td>Group III</td>
<td>EAC + CYP 25 mg/kg</td>
<td>10.71±0.27³,r</td>
<td>5.26±0.09³,r</td>
<td>12.08±0.40³,r</td>
</tr>
<tr>
<td>Group IV</td>
<td>EAC + CM-LF-ETH (300 mg/kg)</td>
<td>13.28±0.32³,r,z</td>
<td>5.03 ±0.15³,r,z</td>
<td>11.65±0.09³,r,z</td>
</tr>
<tr>
<td>Group V</td>
<td>EAC + CM-LF-ACET (300 mg/kg)</td>
<td>17.32±0.25³,r,x</td>
<td>3.96 ±0.09³,r,x</td>
<td>6.95±0.17³,q</td>
</tr>
<tr>
<td>Group VI</td>
<td>EAC + CM-LF-WATE (300 mg/kg)</td>
<td>16.70±0.31³,r,y</td>
<td>4.50±0.13³,r,z</td>
<td>9.70±0.20³,p,z</td>
</tr>
<tr>
<td>Group VII</td>
<td>EAC + CM-BRK-ETH (300 mg/kg)</td>
<td>15.04±0.23³,r,z</td>
<td>4.88±0.23³,r,z</td>
<td>10.87±0.16³,p,z</td>
</tr>
<tr>
<td>Group VIII</td>
<td>EAC + CM-BRK-ACET (300 mg/kg)</td>
<td>18.16±0.38³,r,x</td>
<td>4.03±0.07³,r</td>
<td>7.69±0.17³,r,y</td>
</tr>
<tr>
<td>Group IX</td>
<td>EAC + CM-BRK-WATE (300 mg/kg)</td>
<td>16.40±0.20³,r,y</td>
<td>4.34±0.20³,r,z</td>
<td>10.66±0.21³,r,y</td>
</tr>
<tr>
<td>Group X</td>
<td>EAC + CM-FP-ETH (300 mg/kg)</td>
<td>14.02±0.26³,r,z</td>
<td>5.02 ±0.05³,r,x</td>
<td>11.33±0.18³,q,z</td>
</tr>
<tr>
<td>Group XI</td>
<td>EAC + CM-FP-ACET (300 mg/kg)</td>
<td>16.82±0.14³,r,y</td>
<td>4.17±0.11³,r</td>
<td>7.88±0.20³,r,y</td>
</tr>
<tr>
<td>Group XII</td>
<td>EAC + CM-FP-WATE (300 mg/kg)</td>
<td>15.85± 0.18³,r,z</td>
<td>4.28 ±0.09³,r,z</td>
<td>10.23±0.24³,r,z</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. (n=6).

*p* values:  
- *p* < 0.05, *r* < 0.001 as compared with EAC control + solvent.
- *x* < 0.05, *y* < 0.01, *z* < 0.001, as compared to CYP (by one way ANOVA followed by Dunnett’s multiple comparison test).

*CA-Citrus aurantium*, LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone, WATE-WATER
Fig. 5.22: Effect of *Citrus aurantium* on WBC Count on 14th day in normal and EAC tumor bearing mice

Fig. 5.23: Effect of *Citrus aurantium* on RBC Count on 14th day in normal and EAC tumor bearing mice
Fig. 5.24: Effect of Citrus aurantium on Haemoglobin on 14th day in normal and EAC tumor bearing mice