CHAPTER 4

RESULTS

4.1 Phytochemical Detection by (TLC) on Rhodomyrtus tomentosa extracts

Crude plant extracts are always composed of numerous compounds known as phytochemicals that are responsible for biosynthesis, metabolism and biological function in plant. In this study, analysis of phytochemical compounds in four different *R. tomentosa* extracts was carried out by means of thin layer chromatography (TLC). TLC is a useful method in fractionation, separation and detection of compounds. This method is used very widely for qualitative analysis of plant extracts. Special spraying reagents such as vanillin and dragendorff were used because of its ability to detect wide range of compounds. At the beginning of analysis, several mobile systems were tested to get the best separation for each extracts before the actual analysis can be done. A combination of different polarity solvents (toulene-ether, 70:30; toluene-ethylacetate, 60:40, chloroform-methanol, 50:50) were used as solvent system were found to be appropriate in separating these compounds from the extract. Alkaloids, phenols and terpenoids were indentified in all extracts solvent whereas chlorophylls were present only in methanol, chloroform and petroleum ether extracts.
Table 4.1.1: TLC analysis of crude water extract from the fruits of *Rhodomyrtus tomentosa*

<table>
<thead>
<tr>
<th>Labelled Compound</th>
<th>R_f in solvent</th>
<th>Visible light</th>
<th>Spraying reagents</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toulene-ether (70:30)</td>
<td></td>
<td>Dragendorffs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toluene-EtOAc (60:40)</td>
<td></td>
<td>10% Vanillin in H_2SO_4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chloroform-methanol (50:50)</td>
<td></td>
<td>Aniseldehydro in H_2SO_4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W11</td>
<td>-</td>
<td>-</td>
<td>Purple (+)</td>
<td>Terpenoid</td>
</tr>
<tr>
<td>W10</td>
<td>0.406</td>
<td>-</td>
<td>Purple (+++)</td>
<td></td>
</tr>
<tr>
<td>W9</td>
<td>0.382</td>
<td>Orange (+)</td>
<td>Blue (+++)</td>
<td>Phenol</td>
</tr>
<tr>
<td>W8</td>
<td>0.355</td>
<td>Yellowish green (+)</td>
<td>Blue (+)</td>
<td>Phenol</td>
</tr>
<tr>
<td>W7</td>
<td>-</td>
<td>Yellowish green (+)</td>
<td>Brown (+++)</td>
<td>Phenol</td>
</tr>
<tr>
<td>W6</td>
<td>0.197</td>
<td>-</td>
<td>Blue (+)</td>
<td>Phenol</td>
</tr>
<tr>
<td>W5</td>
<td>-</td>
<td>-</td>
<td>Purple (+++)</td>
<td>Terpenoid</td>
</tr>
<tr>
<td>W4</td>
<td>0.166</td>
<td>Orange (+)</td>
<td>Yellow (++)</td>
<td>Alkaloid</td>
</tr>
<tr>
<td>W3</td>
<td>-</td>
<td>-</td>
<td>Blue (++)</td>
<td>Phenol</td>
</tr>
<tr>
<td>W2</td>
<td>-</td>
<td>0.331</td>
<td>Brown (+)</td>
<td>Phenol</td>
</tr>
<tr>
<td>W1</td>
<td>0.100</td>
<td>0.125</td>
<td>Brown (++)</td>
<td>Phenol</td>
</tr>
</tbody>
</table>

Indication of colour intensity: Lightest (+) Light (++) Dark (+++) Darkest (++++)
Table 4.1.2: TLC analysis of crude methanol extract from the fruits of *Rhodomyrtus tomentosa*

<table>
<thead>
<tr>
<th>Labelled Compound</th>
<th>( R_f ) in solvent</th>
<th>Visible light</th>
<th>Spraying reagents</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toulene-ether (70:30)</td>
<td></td>
<td>Dragendorffs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toluene-EtoAc (60:40)</td>
<td></td>
<td>10% Vanillin in H(_2)SO(_4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chloroform-methanol (50:50)</td>
<td></td>
<td>Aniseldehyde in H(_2)SO(_4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M12</td>
<td>0.840</td>
<td>-</td>
<td>-</td>
<td>Bluish black (+++)</td>
</tr>
<tr>
<td></td>
<td>0.782</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>Orange (++++)</td>
<td>-</td>
</tr>
<tr>
<td>M11</td>
<td>0.756</td>
<td>-</td>
<td>-</td>
<td>Orange (++++)</td>
</tr>
<tr>
<td>M10</td>
<td>0.628</td>
<td>0.741</td>
<td>-</td>
<td>Yellow (++++)</td>
</tr>
<tr>
<td>M9</td>
<td>-</td>
<td>-</td>
<td>0.519</td>
<td>Orange (++++)</td>
</tr>
<tr>
<td>M8</td>
<td>0.589</td>
<td>0.594</td>
<td>Orange (++)</td>
<td>-</td>
</tr>
<tr>
<td>M7</td>
<td>-</td>
<td>0.473</td>
<td>0.499</td>
<td>Purple (++++)</td>
</tr>
<tr>
<td>M6</td>
<td>0.333</td>
<td>0.326</td>
<td>0.367</td>
<td>Purple (++)</td>
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<tr>
<td>M5</td>
<td>-</td>
<td>-</td>
<td>0.210</td>
<td>Green (++)</td>
</tr>
<tr>
<td>M4</td>
<td>0.200</td>
<td>0.205</td>
<td>-</td>
<td>Blue (++)</td>
</tr>
<tr>
<td>M3</td>
<td>-</td>
<td>-</td>
<td>0.178</td>
<td>Orange (++++)</td>
</tr>
<tr>
<td>M2</td>
<td>0.143</td>
<td>-</td>
<td>0.157</td>
<td>Green (++)</td>
</tr>
<tr>
<td>M1</td>
<td>0.125</td>
<td>0.199</td>
<td>0.136</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

Indication of colour intensity: Lightest (+)  Light (++)  Dark (+++)  Darkest (++++)
Table 4.1.3: TLC analysis of crude chloroform extract from the fruits of *Rhodomyrtus tomentosa*

<table>
<thead>
<tr>
<th>Labelled Compound</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; in solvent</th>
<th>Visible light</th>
<th>Spraying reagents</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toluene-ether (70:30)</td>
<td></td>
<td>Dragendorffs</td>
<td>10% Vanillin in H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Toluene-EtoAc (60:40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chloroform-methanol (50:50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C13</td>
<td>-</td>
<td>0.81</td>
<td>-</td>
<td>Purplish blue (+)</td>
</tr>
<tr>
<td>C12</td>
<td>-</td>
<td>0.794</td>
<td>0.773</td>
<td>Purple (+)</td>
</tr>
<tr>
<td>C11</td>
<td>0.639</td>
<td>0.788</td>
<td>Yellow(++)</td>
<td>Yellow (+)</td>
</tr>
<tr>
<td>C10</td>
<td>-</td>
<td>0.755</td>
<td>-</td>
<td>Brown (+++)</td>
</tr>
<tr>
<td>C9</td>
<td>-</td>
<td>0.722</td>
<td>Yellow (+++)</td>
<td>Purple (++++)</td>
</tr>
<tr>
<td>C8</td>
<td>0.535</td>
<td>-</td>
<td>0.694</td>
<td>Orange (+++)</td>
</tr>
<tr>
<td>C7</td>
<td>0.511</td>
<td>0.672</td>
<td>0.686</td>
<td>Bluish black (+++)</td>
</tr>
<tr>
<td>C6</td>
<td>-</td>
<td>0.613</td>
<td>0.502</td>
<td>Green (++)</td>
</tr>
<tr>
<td>C5</td>
<td>-</td>
<td>-</td>
<td>0.446</td>
<td>Greenish blue</td>
</tr>
<tr>
<td>C4</td>
<td>0.487</td>
<td>0.522</td>
<td>-</td>
<td>Greenish blue</td>
</tr>
<tr>
<td>C3</td>
<td>0.395</td>
<td>0.266</td>
<td>0.350</td>
<td>Yellow (++)</td>
</tr>
<tr>
<td>C2</td>
<td>-</td>
<td>0.197</td>
<td>0.287</td>
<td>Orange (++)</td>
</tr>
<tr>
<td>C1</td>
<td>0.243</td>
<td>-</td>
<td>-</td>
<td>Purplish Orange (+)</td>
</tr>
</tbody>
</table>

Indication of colour intensity: Lightest (+) Light (++) Dark (+++) Darkest (++++)
Table 4.1.4: TLC analysis of crude petroleum ether extract from the fruits of *Rhodomyrtus tomentosa*

<table>
<thead>
<tr>
<th>Labelled Compound</th>
<th>R_f in solvent</th>
<th>Visible light</th>
<th>Spraying reagents</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toulene-ether (70:30)</td>
<td>Toluene-EtoAc (60:40)</td>
<td>Chloroform-methanol (50:50)</td>
<td>Dragendorffs</td>
</tr>
<tr>
<td>P9</td>
<td>0.912</td>
<td>0.900</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P8</td>
<td>-</td>
<td>0.855</td>
<td>Yellow</td>
<td>-</td>
</tr>
<tr>
<td>P7</td>
<td>0.741</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P6</td>
<td>-</td>
<td>0.617</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P5</td>
<td>0.570</td>
<td>0.544</td>
<td>0.447</td>
<td>-</td>
</tr>
<tr>
<td>P4</td>
<td>-</td>
<td>0.525</td>
<td>Brown</td>
<td>Orange (++++)</td>
</tr>
<tr>
<td>P3</td>
<td>0.340</td>
<td>-</td>
<td>-</td>
<td>Yellow (++)</td>
</tr>
<tr>
<td>P2</td>
<td>0.249</td>
<td>0.218</td>
<td>0.195</td>
<td>Green (++)</td>
</tr>
<tr>
<td>P1</td>
<td>0.188</td>
<td>0.153</td>
<td>0.160</td>
<td>Yellowish green (+)</td>
</tr>
</tbody>
</table>

Indication of colour intensity: Lightest (+) Light (++) Dark (+++) Darkest (++++)
4.11 Histology examination of Hematoxyline and Eosin (H and E)

4.11.1 Histology examination of aorta

Normal group

**Figure 4.11.1:** Cross section of aorta tissue specimen from normal group stained with H&E at LM X 10. TI = tunica intima TM = tunica media TA = tunica adventitia.

**Figure 4.11.2:** Cross section of aorta tissue specimen from normal group stained with H&E at LM X 20. TI = tunica intima IEL = internal elastic laminae.
Cholesterol group

Figure 4.11.3: Cross section of aorta tissue specimen from cholesterol group stained with H&E at LM X 4. TI = tunica intima TM = tunica media TA = tunica adventitia.

Figure 4.11.4: Cross section of aorta tissue specimen from cholesterol group stained with H&E at LM X 20. TI = tunica intima TM = tunica media.
**R. tomentosa group**

**Figure 4.11.5:** Cross section of aorta tissue specimen from *R. tomentosa* group stained with H&E at LM X 10. TI = tunica intima TM = tunica media TA = tunica adventitia.

**Figure 4.11.6:** Cross section of aorta tissue specimen from *R. tomentosa* group stained with H&E at LM X 20. TI = tunica intima TM = tunica media.
Simvastatin group

Figure 4.11.7: Cross section of aorta tissue specimen from simvastatin group stained with H&E at LM X 10. TI = tunica intima TM = tunica media TA = tunica adventitia.

Figure 4.11.8: Cross section of aorta tissue specimen from simvastatin group stained with H&E at LM X 20. TI = tunica intima TM = tunica media TA = tunica adventitia.
4.11.2 Histology examination of Kidney

Normal group

Figure 4.11.9: Cross section of kidney tissue specimen from normal group with H&E at LM X 10. B = Bowman’s spaces  R= Renal corpuscle

Figure 4.11.10: Cross section of kidney tissue specimen from normal group with H&E at LM X 20. M = mesangial cells
Cholesterol group.

**Figure 4.11.11**: Cross section of kidney tissue specimen from cholesterol group with H&E at LM X 10. R = renal corpuscle B = Bowman’s spaces I = Inflammatory cells

**Figure 4.11.12**: Cross section of kidney tissue specimen from cholesterol group with H&E at LM X 20. M = mesangial cells I = inflammatory cells
**R. tomentosa group**

**Figure 4.11.13:** Cross section of kidney tissue specimen from *R. tomentosa* group with H&E at LM X 10. R= renal corpuscle B= Bowman’s spaces I= Inflammatory cells BC = Blood capillary

**Figure 4.11.14:** Cross section of kidney tissue specimen from *R. tomentosa* group with H&E at LM X 20. M = mesangial cells  I = inflammatory cells
Simvastatin group

**Figure 4.11.15:** Cross section of kidney tissue specimen from simvastatin group with H&E at LM X 10. R = renal corpuscle B = Bowman’s spaces I = Inflammatory cells BV = Blood vessel

**Figure 4.11.16:** Cross section of kidney tissue specimen from simvastatin group with H&E at LM X 10. M = mesangial cells I = inflammatory cells
4.11.3 Histology examination of liver.

Normal group

Figure 4.11.17: Cross section of liver tissue specimen from normal group with H&E at LM X 10.

Figure 4.11.18: Cross section of liver tissue specimen from normal group with H&E at LM X 20.
Cholesterol group

Figure 4.11.19: Cross section of liver tissue specimen from cholesterol group with H&E at LM X 10. CV = central vein

Figure 4.11.20: Cross section of liver tissue specimen from cholesterol group with H&E at LM X 20.
R. tomentosa group

Figure 4.11.21: Cross section of liver tissue specimen from R. tomentosa group with H&E at LM X 10.

Figure 4.11.22: Cross section of liver tissue specimen from R. tomentosa group with H&E at LM X 10.
Simvastatin group

Figure 4.11.23: Cross section of liver tissue specimen from simvastatin group with H&E at LM X 10. CV = central vein

Figure 4.11.24: Cross section of liver tissue specimen from simvastatin group with H&E at LM X 20. CV = central vein
4.12 Evaluation of Atherosclerotic Area Macroscopically.

**Figure 4.12.1:** Histomorphoric analysis of intimal lesion area in different group
Figure 4.12.2: Aortas of Rabbit in Normal group which stained with Sudan IV without showing any atherosclerotic area
Figure 4.12.3: Aortas of Rabbit in Cholesterol group which stained with Sudan IV showing marked atherosclerotic area. Yellow arrows indicate the presence of atherotoma
Figure 4.12.4: Aortas of Rabbit in Tomentosa group which stained with Sudan IV showing atherosclerotic area. Yellow arrows indicate the presence of atherotoma.
Figure 4.12.5: Aortas of Rabbit in Simvastatin group which stained with Sudan IV showing atherosclerotic area. Yellow arrows indicate the presence of atherotoma.
Figure 4.2.13: ESI-MS at scan mode of water extract of *R. tomentosa* fruit TOF-MS 100-750
Figure 4.2.14: ESI-MS at scan mode of water extract of *R. tomentosa* fruit TOF-MS 100-165
Figure 4.2.15: ESI-MS at scan mode of water extract of *R. tomentosa* fruit TOF-MS 165-184
Figure 4.2.16: ESI-MS at scan mode of water extract of *R. tomentosa* fruit TOF-MS 184-215
Figure 4.2.17: ESI-MS at scan mode of water extract of *R. tomentosa* fruit TOF-MS 255-325
Figure 4.2.18: ESI-MS at scan mode of water extract of *R. tomentosa* fruit TOF-MS 334-365
Table 4.2.3: Identification of phytochemicals in water extracts from *R. tomentosa* fruit extract

<table>
<thead>
<tr>
<th>No</th>
<th>[M+H]$^+$ (m/z)</th>
<th>Experimental MS$^2$ (m/z)</th>
<th>Compound identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>133.0139</td>
<td>115, 89, 73, 71, 59</td>
<td>Malic acid</td>
</tr>
<tr>
<td>2</td>
<td>151.0658</td>
<td>136, 132, 119, 107, 101, 89, 71, 59</td>
<td>Unidentified</td>
</tr>
<tr>
<td>3</td>
<td>165.0402</td>
<td>147, 129, 75, 59</td>
<td>Unidentified</td>
</tr>
<tr>
<td>4</td>
<td>169.0142</td>
<td>151, 125, 108</td>
<td>Gallic acid</td>
</tr>
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<td>5</td>
<td>170.0173</td>
<td>126, 125, 108</td>
<td>Unidentified</td>
</tr>
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<td>6</td>
<td>171.1024</td>
<td>153,139, 127, 125, 111, 109, 108</td>
<td>Unidentified</td>
</tr>
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<td>7</td>
<td>179.0558</td>
<td>161, 135, 134, 99, 87, 75, 71, 59</td>
<td>Caffeic acid</td>
</tr>
<tr>
<td>8</td>
<td>181.0714</td>
<td>166, 163, 151, 137, 135,119, 109, 101</td>
<td>Dihydrocaffeic acid</td>
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<td>187.0975</td>
<td>169, 143, 125, 123, 97, 79, 57</td>
<td>Unidentified</td>
</tr>
<tr>
<td>10</td>
<td>191.0565</td>
<td>171, 137, 127, 109, 93, 87, 85, 81, 67, 59</td>
<td>Quinic acid</td>
</tr>
<tr>
<td>11</td>
<td>192.0597</td>
<td>Low intensity</td>
<td>Unidentified</td>
</tr>
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<td>12</td>
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</tr>
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<td>15</td>
<td>207.0510</td>
<td>147, 129, 109, 85, 72</td>
<td>Unidentified</td>
</tr>
<tr>
<td>16</td>
<td>208.0542</td>
<td>Low intensity</td>
<td>Unidentified</td>
</tr>
<tr>
<td>17</td>
<td>209.0300</td>
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<td>Unidentified</td>
</tr>
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<td>225.1128</td>
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<td>267.0716</td>
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<td>Unidentified</td>
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<td>21</td>
<td>291.0140</td>
<td>247, 219, 203, 191, 175, 171, 125, 80</td>
<td>Brevifolin carboxylic acid</td>
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<tr>
<td>22</td>
<td>301.0082</td>
<td>Low intensity</td>
<td>Unidentified</td>
</tr>
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<td>23</td>
<td>313.2376</td>
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<td>Unidentified</td>
</tr>
</tbody>
</table>
Table 4.2.3 Continued

<table>
<thead>
<tr>
<th>No</th>
<th>[M+H]$^+$ (m/z)</th>
<th>Experimental MS$^2$ (m/z)</th>
<th>Compound identified</th>
</tr>
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<tbody>
<tr>
<td>24</td>
<td>327.2169</td>
<td>Low intensity</td>
<td>Unidentified</td>
</tr>
<tr>
<td>25</td>
<td>329.2328</td>
<td>311, 293, 270, 229, 211, 201, 183, 171, 139, 127, 99</td>
<td>Octadecenoic acid</td>
</tr>
<tr>
<td>25</td>
<td>331.0663</td>
<td>313, 295, 271, 239, 211, 169, 168, 125, 124, 107, 89</td>
<td>Galloyl glucose</td>
</tr>
</tbody>
</table>
4.2 HPLC and GCMS analyses of *R. tomentosa* extract

Table 4.2.1: Retention time of standard for HPLC analysis.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Retention time at 280nm</th>
<th>Retention time at 360nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>4.938</td>
<td>4.941</td>
</tr>
<tr>
<td>Quercetine</td>
<td>31.942</td>
<td>32.506</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>4.981</td>
<td>4.968</td>
</tr>
</tbody>
</table>

Table 4.2.2: Retention time of *R. tomentosa* extract for HPLC analysis.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Retention time at 280nm</th>
<th>Retention time at 360nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>4.924</td>
<td>4.941</td>
</tr>
<tr>
<td>Quercetine</td>
<td>31.830</td>
<td>32.475</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>4.955</td>
<td>4.945</td>
</tr>
</tbody>
</table>
Figure 4.2.1: HPLC chromatogram of standard gallic acid at wavelength 280nm. Peak was marked with its retention time.

Figure 4.2.2: HPLC chromatogram of standard gallic acid at wavelength 360nm. Peak was marked with its retention time.
Figure 4.2.3: HPLC chromatogram of *R. tomentosa* at wavelength 280nm. Peak was marked with its retention time.

Figure 4.2.4: HPLC chromatogram of *R. tomentosa* wavelength 360nm. Peak was marked with its retention time.
Figure 4.2.5: HPLC chromatogram of standard quercetin at wavelength 280nm. Peak was marked with its retention time.

Figure 4.2.6: HPLC chromatogram of standard quercetin at wavelength 360nm. Peak was marked with its retention time.
Figure 4.2.7: HPLC chromatogram of *R. tomentosa* at wavelength 280nm. Peak was marked with its retention time.

Figure 4.2.8: HPLC chromatogram of *R. tomentosa* at wavelength 360nm. Peak was marked with its retention time.
Figure 4.2.9: HPLC chromatogram of standard tannic acid at wavelength 280nm. Peak was marked with its retention time.

Figure 4.2.10: HPLC chromatogram of standard tannic acid at wavelength 360nm. Peak was marked with its retention time.
Figure 4.2.11: HPLC chromatogram of *R. tomentosa* at wavelength 280nm. Peak was marked with its retention time.

Figure 4.2.12: HPLC chromatogram of *R. tomentosa* at wavelength 360nm. Peak was marked with its retention time.
4.4 Determination of Total Phenolic Contents (TPC)

**Table 4.4.1**: Total phenolic contents of *Rhodomyrtus tomentosa* extracts. Data presented are in mean ± standard error (n = 3)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total Phenolic Content (mg/g dry mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>66.515 ± 0.009</td>
</tr>
<tr>
<td>Methanol</td>
<td>40.000 ± 0.003</td>
</tr>
<tr>
<td>Chloroform</td>
<td>13.985 ± 0.006</td>
</tr>
<tr>
<td>Petroleum Ether</td>
<td>12.984 ± 0.002</td>
</tr>
</tbody>
</table>

Total phenol content (TPC) in this current experiment was expressed as gallic acid equivalent. Gallic acid was used as standard and the standard graph had $y = 0.0066x$ and $R^2$ with 0.9973. Water extract of *R. tomentosa* (65.515 ± 0.009mg/g dry mass) was higher than that on methanol extract (40.000 ± 0.003mg/g drymass). Followed by chloroform extract (13.985 ± 0.006mg/g dry mass) and lastly petroleum ether (12.984 ± 0.002mg/g dry mass).
Figure 4.4.1: Graph of standard gallic acid

\[ y = 0.0066x \]

\[ R^2 = 0.9973 \]
4.3 Determination of Antioxidant Activity of *Rhodomyrtus tomentosa* in different Crude Extracts

Determinations of antioxidant activity were carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and metal chelating ability assay. Ascorbic acid, Butylated hydroxyl toluene (BHT) and Ethylenediaminetetraacetic acid (EDTA) were standards in these assays respectively. The antioxidant activities were different between different extracts. All three assays showed that methanol extract had the most antioxidant capability followed by water extract, chloroform extract and lastly petroleum ether extract in. DPPH assay demonstrated that methanol extract had lower IC$_{50}$ (107µg/ml) compared to IC$_{50}$ of water extract (154µg/ml). The FRAP assay revealed methanol extract as the most antioxidant capability with absorbance of 0.162nm at concentration of 500µg/ml. In metal chelating ability assay also showed methanol extract with the highest chelating capability with 36% at concentration of 100µg/ml.
Figure 4.3.1: Free-radical scavenging activity of crude extract from fruits of *R. tomentosa* measured by DPPH assay. Data are mean ± SD (n=3)
Figure 4.3.2: Ferric Reducing Antioxidant Power of crude extract from fruits of *R. tomentosa*. Data are mean ± SD (n=3)
Figure 4.3.3: Metal Chelating Ability of the crude extract from the fruit of *R. tomentosa*. Data are mean ± SD (n=3)
4.5 Determination of Total Flavonoid Contents (TFC) in *Rhodomytus tomentosa* crude extract

**Table 4.5.1**: Total Flavonoid Contents of different *Rhodomyrtus tomentosa* extracts. Data presented are in mean ± standard error (n = 3)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total Flavonoid Contents (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1.828 ± 0.018</td>
</tr>
<tr>
<td>Methanol</td>
<td>1.602 ± 0.003</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.499 ± 0.003</td>
</tr>
<tr>
<td>Petroleum Ether</td>
<td>0.395 ± 0.010</td>
</tr>
</tbody>
</table>

Total flavonoid content (TFC) of water extract (1.828 ± 0.018mg/ml) was higher than that on methanol extract (1.602 ± 0.003mg/ml). Followed by chloroform extract (1.499 ± 0.003mg/ml) and lastly petroleum ether (0.395 ± 0.010mg/ml). Quercetin was used as standard and the standard graph had y = 0.5405x and R² with 0.9952.
Figure 4.5.1: Graph of standard quercetin
4.6 Brine Shrimp lethality Assay

Brine Shrimp Lethality Assay (BSLA) is a general bioassay which can be used for detecting in wide range of biological activities, pharmacological activities and chemical toxicity. BSLA was used because this assay provides the preliminary assay to evaluate the toxicity of plants (Meyer et al., 1982). *Artemia salina* is a simple zoological organism which has sensitivity to toxic environment. In vivo, lethality of such toxic environment can be used as a convenient method to monitor and screening bioactive compounds. *Artemia salina* brine shrimp has the advantages of being simple, rapid yet inexpensive. Furthermore it does not require any blood, tissues or large animals to be sacrificed in order to obtain toxicity effect of particular compounds. LC$_{50}$ for crude water extract, crude methanol extract, chlorofoam extract and petroleum extract were 616.083µg/ml, 316.228µg/ml, 100µg/ml and 31.623µg/ml respectively

<table>
<thead>
<tr>
<th>Concentration Sample [µg/ml]</th>
<th>Total No of Shrimp</th>
<th>Number of Dead</th>
<th>Percentage Mortality (%)</th>
<th>LC$_{50}$ [µg/ml]</th>
<th>95 percent confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>10</td>
<td>6</td>
<td>60</td>
<td>616.083</td>
<td>141.674 – 1215910</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>316.228</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.6.1: LC$_{50}$ of crude water extract of *Rhodomyrtus tomentosa*
Table 4.6.2: LC$_{50}$ of crude methanol extract of *Rhodomyrtus tomentosa*

<table>
<thead>
<tr>
<th>Concentration Sample [µg/ml]</th>
<th>Total No of Shrimp</th>
<th>Number of Dead</th>
<th>Percentage Mortality (%)</th>
<th>LC$_{50}$ [µg/ml]</th>
<th>95 percent confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>10</td>
<td>7</td>
<td>70</td>
<td>316.228</td>
<td>75.036 – 14612.65</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.6.3: LC$_{50}$ of crude chlorofoam extract of *Rhodomyrtus tomentosa*

<table>
<thead>
<tr>
<th>Concentration Sample [µg/ml]</th>
<th>Total No of Shrimp</th>
<th>Number of Dead</th>
<th>Percentage Mortality (%)</th>
<th>LC$_{50}$ [µg/ml]</th>
<th>95 percent confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>10</td>
<td>8</td>
<td>80</td>
<td>100</td>
<td>11.413 – 876.213</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.6.4: LC$_{50}$ of crude petroleum ether extract of *Rhodomyrtus tomentosa*

<table>
<thead>
<tr>
<th>Concentration Sample [µg/ml]</th>
<th>Total No of Shrimp</th>
<th>Number of Dead</th>
<th>Percentage Mortality (%)</th>
<th>LC$_{50}$ [µg/ml]</th>
<th>95 percent confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>31.623</td>
<td>4.706 – 89.698</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>7</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.7 Maximum Tolerated Dose (MTD) of *Rhodomyrtus tomentosa* on rabbits

**Table 4.7.1:** Maximum tolerated dose of water extract of *Rhodomyrtus tomentosa* based on body weight effect of male rabbit.

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Concentration (mg/kg)</th>
<th>Duration of treatment (Week)</th>
<th>Weight Gain/Loss (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>2040 2080 2130 2230 2395</td>
<td>355 ± 63.522</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>2110 2255 2330 2375 2450</td>
<td>340 ± 57.909</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>2100 2180 2235 2300 2365</td>
<td>265 ± 46.027</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>2155 2180 2250 2315 2390</td>
<td>235 ± 43.261</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. No statistical difference between control and *R. tomentosa* treated group (p<0.05).

**Table 4.7.2:** Maximum tolerated dose of water extract of *Rhodomyrtus tomentosa* based on body weight effect of female rabbit

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Concentration (mg/kg)</th>
<th>Duration of treatment (Week)</th>
<th>Weight Gain/Loss (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>2115 2170 2245 2360 2435</td>
<td>320 ± 59.097</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>2205 2255 2330 2455 2500</td>
<td>295 ± 56.555</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>2140 2235 2380 2440 2505</td>
<td>365 ± 67.026</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>2105 2125 2205 2315 2476</td>
<td>371 ± 68.506</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. No statistical difference between control and *R. tomentosa* treated group (p<0.05).
Table 4.7.3: Maximum tolerated dose of methanol extract of *Rhodomyrtus tomentosa* based on body weight effect of male rabbit

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Concentration mg/kg</th>
<th>Duration of treatment (Week)</th>
<th>Weight Gain/Loss (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>2085 2120 2195 2235 2295</td>
<td>210 ± 37.98</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>2035 2090 2130 2165 -</td>
<td>130 ± 27.91</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>2010 2070 2140 2175 -</td>
<td>165 ± 36.76</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>2025 2060 2120 - -</td>
<td>95 ± 27.74</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. No statistical difference between control and *R. tomentosa* treated group (p<0.05).

Table 4.7.4: Maximum tolerated dose of methanol extract of *Rhodomyrtus tomentosa* based on body weight effect of female rabbit.

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Concentration mg/kg</th>
<th>Duration of treatment (Week)</th>
<th>Weight Gain/Loss (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>2010 2075 2115 2190 2235</td>
<td>225 ± 40.94</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>2015 2070 2100 2135 -</td>
<td>120 ± 25.41</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>2040 2095 2105 - -</td>
<td>65 ± 20.21</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>2055 2110 2145 - -</td>
<td>90 ± 26.19</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. No statistical difference between control and *R. tomentosa* treated group (p<0.05).

The results presented in the table 4.7.1, 4.7.2, 4.7.3 and 4.7.4 show maximum tolerated dose of water and methanol extracts of *R. tomentosa* on female and male rabbits. Toxicology was assessed on the mortality rate. Water extract of dosages of 50, 100, and 500mg/kg/day did not produce any mortality to the rabbits. Methanol extract at dosage of 50, 100 and 500mg/kg/day showed mortality at week 3 and 4. From this experiment it can be concluded that the rabbits did not have mortality even at maximum dosage of 500mg/kg/day. Therefore using multiple dose levels might not be necessary for further in vivo experiment.
4.8 Animal Organ Parameter

Heart

Figure 4.8.1: Dissected heart organs (A) = Normal group (B) = Cholesterol group (C) = Tomentosa group (D) = Simvastatin group
Figure 4.8.2: Dissected liver organs (A) = Normal group (B) = Cholesterol group (C) = Tomentosa group (D) = Simvastatin group
Figure 4.8.3: Dissected kidney organs (A) = Normal group (B) = Cholesterol group (C) = Tomentosa group (D) = Simvastatin group
Figure 4.8.4: Heart, Liver and Kidney weight of experimental rabbits. Different alphabets (a), (b), (c) and (d) represent significantly different between groups (p<0.05). Data are expressed as mean ± S.E (n=6).
In figure 4.8.4 showed that no significant difference of heart and kidney weight observed among normal, cholesterol, tomentosa and simvastatin group at the end of treatment. However, liver had a significant difference between groups (p<0.05). Cholesterol group had the highest liver weight while the normal group had the lowest liver weight. Supplementation of 1% cholesterol diet did give effect on the weight of the liver. The liver seen in the figure 4.8.2 had a whitish feature due to accumulation of cholesterol in the hepatocytes. Rabbits supplemented with R. tomentosa extract 50mg/kg/day and simvastatin 5mg/kg/day had a significantly lower liver weight (p<0.05) than the cholesterol group.
4.9 The Effect of *Rhodomyrtus tomentosa* aqueous extract on Body Weight

During the course of this experiment, normal group was given 100 g/day normal pellet. The cholesterol, simvastatin and *R. tomentosa* groups were given 100 g/day special feed pellet with 1% cholesterol. As seen in figure 4.9.1, the average readings of week 0 were taken as baseline body weight in this study. The range of body weight for all groups was between 2.0kg to 2.595kg with the average of 2.208 ± 0.036kg. As the experiment progressed to week 5, there was only a slight significant increase (p<0.05) effect on body weight in cholesterol group, *R. tomentosa* group and simvastatin group compared to normal group. However, towards the end of week 10, all groups had significant different of body weight (p<0.05). The cholesterol group was the heaviest group with mean average of 2.873 ± 0.061 kg.
Figure 4.9.1: The effect of *R. tomentosa* water extract on body weight at different week. Different alphabets (a), (b) and (c) represent significantly different between groups at week 5 (p<0.05). Different alphabets (d), (e), (f) and (g) represent significantly different between groups at week 10 (p<0.05). Data are expressed as mean ± S.E (n=6).
The Effect of *Rhodomyrtus tomentosa* aqueous extract on Serum Triacylglycerides (TG)

The estimation of TG level at week 0 shown in the figure 4.9.2 can be used as baseline value as they are no significant difference (p<0.05) in each group. The average level of TG at week 0 was 0.462 ± 0.028mmol/L with range of 0.400mmol/L to 0.533mmol/L. Towards week 5, cholesterol group showed significant difference (p<0.05) on the level of TG at 1.3 ± 0.150mmol/L compared with the normal group, *R. tomentosa* group and simvastatin group. Later in Week 10, all groups showed insignificant level of TG (p<0.05) with the highest level was in *R. tomentosa* group at 0.895 ± 0.223mmol/L. There was significant decrease (p<0.05) of triacylglycerides in cholesterol group when comparing between week 5 and week 10.
Figure 4.9.2: The effect of *R. tomentosa* water extract on Triacylglycerides (TG) at different week. Different alphabets (a) and (b) represent significantly different to its week 5 (p<0.05). Data are expressed as mean ± S.E (n=6).
The Effect of *Rhodomyrtus tomentosa* Aqueous Extract on Total Cholesterol (TC).

The average of serum total cholesterol level (TC) for all groups was taken at week 0 as baseline for estimation in this study. The average level at week 0 was found to be 1.204 ± 0.074 mmol/L with range between 0.950 mmol/L to 1.383 mmol/L. As shown in figure 4.9.3, the TC level at week 0 showed no significant different (p<0.05) in every control group (normal and cholesterol) and treatment group (*R. tomentosa* extract and simvastatin). At week 5, all groups except normal group exhibited significant high TC levels (p<0.05). Cholesterol group showed the highest level of TC among all groups at 24.833 ± 0.138 mmol/L. Followed by simvastatin group 15.967 ± 3.402 mmol/L and *R. tomentosa* group 12.633 ± 1.682 mmol/L. At week 10, *R. tomentosa* group (17.05 ± 0.419 mmol/L) and simvastatin group (16.067 ± 0.651 mmol/L) showed significant lower of TC level (p<0.05) compared to cholesterol group (25.983 ± 0.575 mmol/L).
Figure 4.9.3: The effect of *R. tomentosa* water extract on Total Cholesterol (TC) at different week. Different alphabets (a), (b) and (c) represent significantly different between groups at week 5 (p<0.05). Different alphabets (d), (e) and (f) represent significant different between groups at week 10 (p<0.05). Data are expressed as mean ± S.E (n=6).
The effect of *Rhodomyrtus tomentosa* Aqueous Extract on Serum Low Density Lipoprotein (LDL).

In figure 4.9.4, the estimation of LDL level at week 0 can be used as baseline value as all level in each group showing no significant different (p<0.05). The average level of LDL was 0.36 ± 0.049mmol/L with range of 0.232 mmol/L to 0.448 mmol/L. At week 5, all groups except normal group showed significant increase (p<0.05) level of LDL. Cholesterol group had the significantly highest level (p<0.05) of LDL 23.295 ± 0.136mmol/L, followed by *R. tomentosa* group 11.647 ± 1.732mmol/L and simvastatin group 15.305 ± 3.159mmol/L. The LDL level at week 10 showed significant lower (p<0.05) in *R. tomentosa* group and simvastatin group compared with cholesterol group but slightly higher when compared to week 5 for each groups. The LDL level for *R. tomentosa* and simvastatin at week 10 was at 17.572 ± 0.341mmol/L and 15.293 ± 0.675mmol/L.
**Figure 4.9.4:** The effect of *R. tomentosa* water extract on Low Density Lipoprotein (LDL) at different week. Different alphabets (a), (b), and (c) represent significantly different between groups at week 5 (p<0.05). Different alphabets (d), (e) and (f) represent significantly different between groups at week 10 (p<0.05). Data are expressed as mean ± S.E (n=6).
The effect of *Rhodomyrtus tomentosa* Aqueous Extract on Serum High Density Lipoprotein (HDL).

In figure 4.9.5, the estimation of HDL level at week 0 may be used as baseline as all level in each group showed no significant different (p<0.05). The average of HDL level was 0.591 ± 0.024mmol/L in range of 0.527mmol/L to 0.670mmol/L. At week 5, the data showed no significant difference (p<0.05) in HDL level in all groups even though the *R. tomentosa* group and simvastatin group had slightly increased HDL level with 0.628 ± 0.039mmol/L and 0.612 ± 0.062mmol/L respectively. After week 10, the level of HDL in cholesterol group was decrease significantly (p<0.05) with value of 0.423 ± 0.038mmol/L, meanwhile *R. tomentosa* group had HDL level of 0.520 ± 0.047mmol/L which is higher than the cholesterol group. The simvastatin group had HDL level of 0.4967 ± 0.053mmol/L which also significantly higher than cholesterol group. Overall the average of HDL level in all groups at week 10 was 0.493 ± 0.030mmol/L.
Figure 4.9.5: The effect of *R. tomentosa* water extract on High Density Lipoprotein (HDL) at different week. Different alphabets (a) and (b) represent significantly different to its week 10 (p<0.05) Data are expressed as mean ± S.E (n=6).
4.10 The Effect of *R. tomentosa* Aqueous Extract on Serum Lipid Peroxidation Index, TBARs-Malondialdehyde (MDA).

Studies have proven that lipid oxidation by products are MDA (Uchida, 2006) can bind and modify to various macromolecules such as proteins and lipoproteins (Yamada et al, 2001). Modifications of proteins or lipoproteins have many harmful effects in a number of diseases including atherosclerosis. This binding and modification of proteins and lipoproteins usually leads to onset of atherosclerosis (Fu *et al.*, 1998). In atherosclerotic lesions (Palinski *et al.*, 1989; Yla-Herttuala *et al.*, 1989) and circulations (Holvoet *et al.*, 1995; Palinski *et al.*, 1996) of blood, there have been detections of modified proteins and lipoproteins. The current experiment is important because MDA is one of the most important biomarker produced during lipid peroxidation. Lipid peroxidation occurs when cells are exposed to reactive oxygen species causing cell walls to rupture and membrane lipids to degrade to the end-product that is MDA.

As shown in figure 4.10.1, the levels of malondialdehyde (MDA) consist of different values among different groups. The level of MDA at week 0 cannot be considered as basal line of experiment because each group has different multiple variables. This condition occurs maybe due to any internal or external factor during assessing the MDA level in lab or maybe the subjects condition itself was not stable as the rabbits were quite young.

The average level of MDA at week 0 was 0.452 ± 0.001nmol/ml. In week 5, cholesterol group showed significant different (p<0.05) in MDA level compared to other groups. The level at week 5 was at 0.072 ± 0.003nmol/ml. Later in week 10, cholesterol group still showed significantly higher (p<0.05) in MDA level compared with other group. The level of MDA in *R. tomentosa* group and simvastatin group was at 0.043 ± 0.001nmol/ml and 0.046 ± 0.001nmol/ml. Generally, all groups showing different rate in MDA level with the exception of simvastatin group with
slightest increase. *R. tomentosa* group show the lowest level followed by simvastatin group, normal group (0.048 ± 0.003nmol/ml) and lastly cholesterol group (0.068 ± 0.001nmol/ml). The supplementation of 50mg/kg/day of *R. tomentosa* resulted in significantly lower (p<0.05) of MDA respectively compared to cholesterol group.
Figure 4.10.1: The effect of *R. tomentosa* water extract on lipid peroxidation index, indicated by malondialdehyde (MDA) at different time. Alphabet (a) and (b) represent significant difference at week 5 (p<0.05), (c) and (d) represent significant difference at week 10 (p<0.05). *#* significant different within group. Each point represents a mean ± S.E (n=6)