PHYTOCHEMICAL STUDY

Phytochemical characterization of plant material is important as it relates to the therapeutic actions. It is perhaps obvious that different species of plants would have different chemical constituents. However, these differences can extend to different varieties or even the same variety grown in different locations or harvested at a different time. Different parts of plant such as leaves, bark, seeds, roots, flowers and pods can also have different active constituents.
CHAPTER 5
PHYTOCHEMISTRY: ANALYTICAL STUDIES OF SOME INDIAN MEDICINAL PLANT

5.1 INTRODUCTION
Chemical diversity in natural product is an immensely rich source of new pharmaceuticals, cosmetics, agrochemicals and other economically important chemicals. Therapeutic potentials of herbal drug ranges from parts of plants, through simple extracts to isolated active constituents. Plants may be considered as biosynthetic laboratories in which various kinds of organic compounds are synthesized such as carbohydrates, proteins, lipids, flavonoids, glycosides, alkaloids, volatile oils, and tannins etc., which exert a physiological effect and are utilized as biologically active components by man since time immemorial. The medicinal value of any plant drug, however depends on the nature of chemical constituents present in it and is referred to as active principle. Phytochemical evaluation comprises of different chemical tests and chemical assay. The isolation, purification and identification of active constituents are chemical methods of evaluation. The phytochemical evaluation also covers phytochemical screening carried out in establishing chemical profile of crude drugs. The purity of crude drugs is ascertained by quantitative estimation of the active chemical constituents present in them. The method may be useful in determining single active constituents or the group of related constituents present in the same drug.

For our present study, we had taken Aegle marmelos (Corr.), Moringa oleifera (Lam.) stem bark and Melastoma malabathricum (Linn.), Paederia foetida (Linn.), leaves to extract the compounds and test the phytochemical constituents present in them.

5.2 Material and method for Aegle marmelos (Corr.)

5.2.1 Successive extractive value
Air dried stem bark powder of Aegle marmelos (Corr.) was taken in a soxhlet extractor and extracted successively with the following solvents (Petroleum ether, Ethyl acetate, Chloroform, Methanol and Water).

Each time before extracting with the next solvent, the powdered material was air dried first and then oven dried below 50°C. Each extract was concentrated by distilling off the solvent and then evaporated to dryness on the water bath. The percentage extracts of the drug with each solvent were calculated with the reference to the air dried drug (Trease and Evans, 1983; Kokate, 1994; Khandelwal, 2004).
5.2.2 Qualitative phytochemical analysis

All extracts were obtained from the successive extraction of *Aegle marmelos* (Corr.), *Moringa oleifera* (Lam.) stem bark and *Melastoma malabathricum* (Linn.), *Paederia foetida* (Linn.), leaves were subjected to various qualitative tests for the identification of phytoconstituents present in it (Trease and Evans, 1983; Kokate, 1994; Kokate et al., 2002; Khandelwal, 2004).

5.2.2.1 Test for Alkaloid

5.2.2.1.1 Dragendroff’s test: To 1 ml of extract, add 1 ml of Dragendorff’s reagent (potassium bismuth iodide solution), an orange-red precipitate indicates the presence of alkaloids.

5.2.2.1.2 Mayer’s test: To 1 ml of extract, add 1 ml of Mayer’s reagent (Potassium mercuric iodide solution), whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

5.2.2.1.3 Hager’s test: To 1 ml extract, add 3ml of Hager’s reagent (saturated aqueous solution of picric acid), a yellow coloured precipitate indicates the presence of alkaloids.

5.2.2.1.4 Wagner’s test: To the 1 ml of extract, add 2 ml of Wagner’s reagent (iodine in potassium iodide), the formation of reddish brown precipitate indicates the presence of alkaloids.

5.2.2.2 Test for Glycoside

5.2.2.2.1 Legal’s test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink, red to red color shows the presence of glycosides.

5.2.2.2.2 Baljet’s test: To 1ml of the test extract, add 1ml of sodium picrate solution and the formation of yellow to orange colour reveal the presence of glycosides.

5.2.2.2.3 Keller-Killiani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes and filtered. To the filtrate add 10ml of water and 0.5ml of strong solution of lead acetate and filter. Then the filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

5.2.2.2.4 Borntrager’s test: Add a few ml of dilute sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer is treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides.

5.2.2.3 Test for Saponin

Take small quantity of alcohol and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponin.
5.2.2.4 Test for Carbohydrate

5.2.2.4.1 Molisch’s test: To 2ml of the extract, add 1ml of α-napthol solution and add concentrated sulphuric acid through the side of the test tube. The formation of purple or reddish violet colour at the junction of the two liquids reveals the presence of carbohydrates.

5.2.2.4.2 Fehling’s test: To 1ml of the extract, add equal quantities of Fehling’s solution A and B. On heating formation of a brick red precipitate indicates the presence of sugars.

5.2.2.4.3 Benedict’s test: To 5ml of Benedict’s reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of a red precipitate shows the presence of sugars.

5.2.2.5 Test for Phenolic Compound and Tannin

Take the little quantity of test solution and mix with the basic lead acetate solution. Formation of white precipitate indicates the presence of tannins.

To 1ml of the extract, add the ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins.

The least quantity of test extract is treated with potassium ferric cyanide and ammonia solution. A deep red color indicates the presence of tannins.

In the test extract, add strong potassium dichromate solution, a yellow color precipitate indicates the presence of tannins and phenolic.

5.2.2.6 Test for Flavonoids

The drug in alcoholic and aqueous solution with a few ml of ammonia is seen in U.V. and visible light; formation of fluorescence indicates the presence of flavonoids.

Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow color solution formed, disappears on addition of an acid indicates the presence of flavonoids.

5.2.2.7 Shinoda’s Test: The alcoholic extract when treated with magnesium foil and concentrated HCl gives intense cherry red colour which indicates the presence of flavonones or orange red colour which indicates the presence of flavonols.

The extract is treated with sodium hydroxide; formation of yellow colour indicates the presence of flavones. The extract is treated with concentrated H₂SO₄, formation of yellow or orange colour indicates the presence of flavones. The alcoholic and aqueous extract is treated with 10% sodium chloride; formation of yellow colour indicates the presence of coumarin.

5.2.2.8 Test for Steroid

5.2.2.8.1 Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride and 3ml of glacial acetic acid was added, warmed and cooled under
the tap and drops of concentrated sulphuric acid was added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

5.2.2.8.2 Salkowski test: Dissolve the extract in chloroform and add an equal volume of conc. H₂SO₄. Formation of bluish red to cherry color in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

5.2.2.9 Test for Protein and Amino Acid

5.2.2.9.1 Biuret test: Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO₄ solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet colour indicates the presence of proteins.

5.2.2.9.2 Xanthoprotein test: To 1ml of the extract, add 1ml of concentrated nitric acid. A white precipitate is formed, it is boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. The formation of orange color indicates the presence of aromatic amino acids.

5.2.2.9.3 Millon’s test: 1ml of test solution acidified with sulphuric acid is adding to Millon’s reagent and boils this solution. A yellow precipitate is formed indicates the presence of proteins.

5.2.2.10 Test for Triterpenoid

5.2.2.10.1 Noller’s test: Dissolve two or three granules of tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink colour indicates the presence of triterpenoids.

5.2.2.11 Test for fixed oil and fat

5.2.2.11.1 Spot test: Press a small quantity of extracts between the filter paper. Oil stains on the paper indicates the presence of fixed oils.

5.2.2.11.2 Saponification test: To 1ml of the extract, add few drops of 0.5 N; alcoholic potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

5.2.2.12 Test for Gum and Mucilage

Add about 10ml of aqueous extract slowly to 25ml of absolute alcohol with constant stirring. Filter the precipitate and dry in air. Examine the precipitate for its swelling properties and for the presence of carbohydrates.

5.2.2.13 Test for Lignin

With alcoholic solution of phloroglucinol and hydrochloric acid, the appearance of red colour shows the presence of lignins.
5.2.3 Isolation of stem bark of *Aegle marmelos* (Corr.)

### 5.2.3.1 Extraction of plant material

The shade dried stem bark of *Aegle marmelos* (Corr.) was powdered and 2 kg powder was extracted using methanol for 72 hours (Harborne, 1973; Lala, 1981).

### 5.2.3.2 Preparation of slurry

The concentrated methanolic extract of the *Aegle marmelos* (Corr.) was taken in the China dish and heated continuously on the water bath by gradually adding small portion of the methanol with continuous stirring, till desired consistency was obtained. A weighted quantity of silica gel (60-120 mesh, column chromatography) was then added slowly with continuous stirring with the help of the spatula until the whole methanolic solution of the plant extract adsorbed on silica gel particles. The methanolic extract adsorbed on silica gel dried in the air and pass through the sieve (No. 8) to get the uniform particles.

### 5.2.3.3 Packing of column

A column of 5.0 feet, height and 60 mm internal diameter was taken, cleaned properly and dried. The lower end of the column was plugged with the glass wool. The column was clamped and fitted in a vertical position on a stand. First of all half column filled with the petroleum ether (b.p. 40-60°C). Silica gel (for column, 60-120 mesh) was then poured in small portions and allowed to settle down and the dried plant extract slurry was loaded over the column and then eluted successively with different solvents, in increasing order of their polarity.

### 5.2.3.4 Extraction and isolation of compound

The shade dry stem bark of *Aegle marmelos* (Corr.) (2 kg) was extracted with methanol (5 L) at 45 °C for 72 h. After extraction total filtrate was concentrated to dryness in rotatory vacuum evaporator at 40°C to obtain a slurry (322 gm). The slurry was dissolved in small amount of methanol and was absorbed on silica gel (60-120 mesh). It is subjected to silica gel column using as a C₆H₁₄/CHCl₃/MeOH gradient system (1:0:0, 2:0:0, 4:0:0, 4:1:0, 1:1:0, 1:4:0, 1:6:0, 0:1:0, 0:48:0, 0:24:1, 0:48:2, 0:10:0, 0:10:1, 0:24:7, and 0:47:10; 3.0 L for each gradient system), yielding 22 fractions collected fraction spotted on pre coated silica gel TLC plate and the fraction having the same R_f value pooled together in 7 fractions. Fraction 2-4 (13.5 g) were combined, separated on a silica gel column (CHCl₃/MeOH, 3:1), and rechromatographed on a silica gel column (CHCl₃/MeOH, 6:1 to 3:1), yielding 7 subfractions. Compound I was separated first by a normal phase silica gel column (CHCl₃/MeOH, 1:2) and compound II was separated by a normal phase silica gel column (CHCl₃/MeOH 30:1).
5.2.3.5 **Characterization of isolated compound**

The purified compounds were analyzed by GC-MS spectrometry in VG-Autospec spectrometer instrument at ionization energy of 70eV. The $^1$H and $^{13}$C NMR spectra of the isolated compounds were recorded on Bruker Avance II 400 NMR Spectrophotometer instrument using DMSO as solvent and TMS as an internal standard, respectively. All the $^{13}$C NMR spectra were recorded in the proton noise-decoupling mode. Functional groups of isolated compound were analyzed on Bio-Red FTIR Spectrophotometer using KBr pellets; $\nu_{\text{max}}$ values are given in cm$^{-1}$. UV $\lambda_{\text{max}}$ (DMSO) were recorded on Shimadzu UV-1700 and FT-IR (in 2.0 cm-1, flat, smooth, Abex) were taken on Perkin Elmer – Spectrum RX-I spectrophotometer.

5.2.4 **Result and discussion**

5.2.4.1 **Preparation of *Aegle marmelos* (Corr.) plant extract**

Air dried stem powder of the *Aegle marmelos* (Corr.), was taken in a soxhlet extractor and extracted successively with the following solvents like petroleum ether, ethyl acetate, chloroform, methanol and water. The result of the extract of *Aegle marmelos* (Corr.) and nature colour was presented in the table 5.2.1. The result showed the nature of the solvent and the extractive value of the solvent in the particular crude drug (Trease and Evans, 1983).

5.2.4.2 **Qualitative Phytochemical Tests**

The qualitative phytochemical test was performed for the identification of the chemical constituent present in the particular solvents. The extracts were subjected to qualitative phytochemical tests to find out the active constituents. The qualitative phytochemical test of the *Aegle marmelos* (Corr.) presented in the table 5.2.2. On the basis of the qualitative chemical test, it has been observed that chemically therapeutic compound like alkaloids, carbohydrates, glycosides, phenolic compound, tannin, saponin, sterol, gum and mucilage were present in the stem of the *Aegle marmelos* (Corr.) (Trease and Evans, 1983; Kokate, 1994; Kokate et al., 2002; Khandelwal, 2004).
### Table 5.2.1: Percentage extractive values along with colour of the extract and nature of the *Aegle marmelos* (Corr.) residue

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent</th>
<th>Colour of the Extract</th>
<th>Nature of Residue</th>
<th>Extractive Value (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether(60-80°C)</td>
<td>Light Yellow</td>
<td>Solid</td>
<td>0.14</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate</td>
<td>Pale Yellow</td>
<td>Sticky</td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>Slightly Orange</td>
<td>Sticky</td>
<td>0.43</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>Dark Brown</td>
<td>Thick resinous</td>
<td>6.15</td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>Green</td>
<td>Gummy</td>
<td>3.21</td>
</tr>
</tbody>
</table>

### Table 5.2.2: Qualitatively phytochemical test screening of *Aegle marmelos* (Corr.)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant constituents</th>
<th>Petroleum ether</th>
<th>Ethyl acetate</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Phenolic compounds and Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Sterols</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Gum and Mucilage</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
5.2.4.3 Characterization of Isolated compound I (BG I)

Compound I first time isolated from the stem bark of *Aegle marmelos* (Corr.). The structure was elucidated by spectral methods that include UV, FTIR, FAB-MS, $^1$H NMR, $^{13}$C NMR, COSAY and NOSAY. Compound BG I obtained as yellowish colored semisolid compound (3 gm.). Compound exhibited UV absorption bands at 330 nm (log ε 3.1), indicating coumarin derivative. ESI-MS at m/z (rel. int.): 324 [M]$^+$ C$_{15}$H$_{16}$O$_{8}$ (1.8), $^1$H NMR (DMSO-d$_6$): 7.90 (1H, dd, J 9.6, 2.8 Hz, H – 6), 7.51 (1H, d, J=9.1 Hz, H - 4), 7.20 (1H, d, J=2.8 Hz, H - 8), 6.91 (1H, d, J=9.1 Hz, H - 3), 6.91 (1H, d, J=9.6 Hz, H - 5), 5.12 (1H, d, J=7.2 Hz, H - 1$^I$), 4.36 (1H, H - 5$^I$), 3.82 (1H, H - 2$^I$), 3.78 (1H, H - 3$^I$), 3.67 (1H, H - 4$^I$), 3.16 (2H, H - 6$^I$). $^{13}$C NMR (DMSO-d$_6$): 163.81 (C-2), 112.06 (C-3), 142.75 (C-4), 128.14 (C-5), 124.32 (C-6), 157.64 (C-7), 103.25 (C-8), 112.16 (C-9), 154.18 (C-10), 105.59 (C-1$^I$), 74.19 (C-2$^I$), 72.21 (C-3$^I$), 68.63 (C-4$^I$), 76.21 (C-5$^I$), 62.05 (C-6$^I$), IR $\gamma_{\text{max}}$ (KBr): 3435, 3390, 2936, 2851, 1702, 1607, 1515, 1468, 1425, 1337, 1278, 1224, 1115, 1071 cm$^{-1}$. IR absorption spectrum at 1702 cm$^{-1}$ and UV absorption spectrum at 330 NM for $\delta$-lactone ring suggested coumarins nature of the isolated compound. On the basis of $^{13}$C NMR and mass spectrum at m/z 324 consistent with the molecular formula of umbelliferon $\beta$-D-galactopyranoside C$_{15}$H$_{16}$O$_{8}$. The $^1$H NMR spectrum showed the presence of two AB-type double at $\delta$ 6.91 (J=9. 1 Hz) and 7.51 (J=9. 6 Hz) assigned to vinylic H-3 and H-4 protons, respectively. One-proton double douplet at $\delta$ 7.90 (J=7.2, 2.8 Hz) and two on –proton doublets at $\delta$ 7.20 (J=2.8 Hz) and 6.91 Hz (J=9.1) was ascribed to coumarin H-6, H-8 and H-5 protons, respectively. One-proton doublets at $\delta$ 5.12 (J=7.2 Hz) were accounted to $\alpha$-oriented anomeric H-1$^I$ protons, respectively. The other sugar protons resonated between $\delta$ 4.36 – 3.16. The $^{13}$C NMR spectrum displayed signals for nine coumarin carbons in the range of $\delta$ 163.81 – 103.25, anomeric carbon at $\delta$ 105.59 (C-1$^I$) and other sugar carbons between $\delta$ 74.19 – 62.05. The existence of an NMR H-2$I$ signal in the deshielded region at $\delta$ 3.82 and carbon C-2$I$ signal at $\delta$ 74.19 indicated (2$^I$→1$^{II}$) linkage of the sugar units. The HMBC spectrum of the coumarin showed interactions of H-6, H-8 and H-1$^I$ with C-7; H-3 and H-4 with C-2; and H-2$^I$. On the basis of spectral data analysis of this new compound has been elucidated as umbelliferone $\beta$-D-galactopyranoside.
Figure 5.2.1: Structure of as umbelliferone β-D-galactopyranoside
Figure 5.2.2: $^1$H NMR spectra of BG-I compound

Figure 5.2.3: $^1$H NMR spectra of BG-I compound
Figure 5.2.4: $^1$H NMR spectra of BG-I compound

Figure 5.2.5: $^1$H NMR spectra of BG-I compound
Figure 5.2.6: $^{13}$C NMR spectra of BG-I compound

Figure 5.2.7: $^{13}$C NMR spectra of BG-I compound
Figure 5.2.8: HMBC (Heteronuclear Multiple Bond Correlation) NMR spectra of BG-I compound

Figure 5.2.9: Correlation spectroscopy NMR spectra of BG-I compound
Chapter 5
Pharmacognostical and pharmacological evaluation of some medicinal plants

**Figure 5.2.10:** Mass spectra of BG-I compound

**Figure 5.2.11:** FTIR spectra of BG-I compound
5.2.4.4 Characterization of Isolated compound II (BG II)

The methanolic extract of stem bark powder of *Aegle marmelos* (Corr.) was subjected to column chromatography. Different solvent systems were used for the isolation of bioactive compound and fraction were collected subjected to thin layer chromatography (TLC) to determine the homogeneity of various fractions. Chromatographically identical fractions (having same R$_f$ values) were mixed together and concentrated. Fractions 40-60 were further purified by silica gel recolumn chromatography and the chromatography purified of these fractions led to the isolation of compound ‘BG II’ (500 mg). ESI-MS at m/z (rel. int.): 486 [M]$^+$ $C_{21}H_{26}O_{13}$ (2.2), $^1$H NMR (DMSO-d$_6$): δ 7.55 (1H, dd, J= 9.8, 2.8 Hz, H – 6), 7.47 (1H, d, J=9.2 Hz, H - 4), 7.20 (1H, d, J=2.8 Hz, H - 8) 6.83 (1H, d, J=9.2 Hz, H - 3), 6.40 (1H, d, J=9.8 Hz, H - 5), 5.27 (1H$_1$, d$_1$, J=3.6 Hz, H - 1$^i$), 4.99 (1H, d, J=3.6 Hz, H - 1$^{ii}$), 4.81 (1H, H - 5$^i$), 4.48 (1H, H - 5$^{ii}$), 4.31 (1H, H - 2$^i$), 4.02 (1H, H - 2$^{ii}$), 3.80 (1H, H - 3$^i$), 3.73 (1H, H – 3$^{ii}$), 3.68 (1H, H – 4$^i$), 3.62 (1H, H – 4$^{ii}$), 3.18 (1H, brs, H 2$^i$– 6$^i$), 3.04 (2H, brs, H 2$^{ii}$– 6$^{ii}$). $^{13}$C NMR (DMSO-d$_6$): 162.24 ( C-2), 112.51 (C-3), 140.86 (C-4), 122.86 (C-5), 131.20 (C-6), 158.15 (C-7), 106.37 (C-8), 116.48 (C-9), 153.06 (C-10), 103.80 (C-1$^i$), 82.31 (C-2$^i$), 72.68 (C-3$^i$), 69.88 (C-4$^i$), 77.89 (C-5$^i$), 61.05 (C-6$^i$), 99.61 (C-1$^{ii}$), 72.80 (C-2$^{ii}$), 71.53 (C-3$^{ii}$), 68.91 (C-4$^{ii}$), 74.04 (C-5$^{ii}$), 60.72 (C-6$^{ii}$); IR $\gamma_{max}$ (KBr): 3452, 3401, 3325, 2929, 2848, 1702, 1629, 1515, 1457, 1384, 1270, 1118, 1051 cm$^{-1}$, UV $\lambda_{max}$ 332 nm (log ε 3.1). The UV absorption maxima at 332 nm and IR absorption band at 1731 cm$^{-1}$ for δ-lactone ring suggested coumarin nature of the molecule. On the basis of mass spectrum and $^{13}$C NMR spectra the molecular ion peak of the compound was determined at m/z 486 consistent with the molecular formula of a coumarin diglycoside $C_{21}H_{26}O_{13}$. The $^1$H NMR spectrum showed the presence of two AB-type double at δ 6.83 (J=9. 2 Hz) and 7.47 (J=9. 2 Hz) assigned to vinylic H-3 and H-4 protons, respectively. A one-proton double doublet at δ 7.55 (J=9. 8, 2.8 Hz) and two one-proton doublets at δ 7.20 (J=2. 8 Hz) and 6.40 Hz (J=9. 8 Hz) were ascribed to coumarin H-6, H-8 and H-5 protons, respectively. Two one-proton doublets at δ 5.27 (J=3.6 Hz) and 4.99 (J=3.6 Hz) were accounted to α-oriented anomeric H-1$^i$ and H-1$^{ii}$ protons, respectively. The other sugar protons resonated between δ 4.81 – 3.04. The $^{13}$C NMR spectrum displayed signals for nine coumarin carbons in the range of δ 162.24 – 106.36, anomeric carbon at δ 103.80 (C-1$^i$) and 99.61 (C-1$^{ii}$) and other sugar carbons between δ 82.31 – 60.72. The existence of NMR H-2$^i$ signal in the deshielded region at δ 4.31 and carbon C-2$^i$ signal at δ 82.31 indicated (2$^i$→1$^{ii}$) linkage of the sugar units. The HMBC spectrum of the coumarin showed interactions of H-6, H-8 and H-1$^i$ with C-7; H-3 and H-4 with C-2; and H-2$^i$, H-2$^{ii}$ and H-3$^{ii}$ with C-1$^{ii}$. On the basis of spectral data analysis of this new compound has been elucidated as umbelliferon-7-O-α-D-glucopyranosyl-(2$^i$→1$^{ii}$) -α-D-glucopyranoside.
Figure 5.2.12: Structure of as umbelliferon-7-O-α-D-glucopyranosyl-(2₁→1₁₁)-α-D-glucopyranoside.
Figure 5.2.13: $^1$H NMR spectra of BG-II compound

Figure 5.2.14: $^1$H NMR spectra of BG-II compound
Figure 5.2.15: $^1$H NMR spectra of BG-II compound

Figure 5.2.16: $^1$H NMR spectra of BG-II compound
Figure 5.2.17: $^{13}$C NMR spectra of BG-II compound

Figure 5.2.18: $^{13}$C NMR spectra of BG-II compound
Figure 5.2.19: HMBC (Heteronuclear Multiple Bond Correlation) NMR spectra of BG-II compound

Figure 5.2.20: Correlation spectroscopy NMR spectra of BG-II compound
Figure 5.2.21: Mass spectra of BG-II compound

Figure 5.2.22: FTIR (Fourier transform infrared spectroscopy) spectra of BG-II compound
5.3 Material and method for *Moringa oleifera* (Lam.)

5.3.1 Successive extractive value

Air dried stem bark powder of *Moringa oleifera* (Lam.) was taken in a soxhlet extractor and extracted successively with the following solvents (Petroleum ether, Ethyl acetate, Chloroform, Methanol and Water).

Each time before extracting with the next solvent, the powdered material was air dried first and then oven dried below 50°C. Each extract was concentrated by distilling off the solvent and then evaporated to dryness on the water bath. The percentage extracts of the drug with each solvent were calculated with the reference to the air dried drug.

5.3.2 Qualitative phytochemical analysis

All extracts were obtained from the successive extraction of *Moringa oleifera* (Lam.) was subjected to various qualitative tests for the identification of phytoconstituents present in it. The method of the qualitative phytochemical analysis was mentioned in the 5.2.2 section.

5.3.3 Preparation of plant extract of *Moringa oleifera* Lam

The collected stem bark *Moringa oleifera* (Lam.) (3 kg) were blended and extracted with methanol (8 L) for 96 h at 60°C. After extraction, the extract was filtered with Whatmann No 1 filter paper and centrifuge at 8000 rpm (15 min) to remove the debris fragment and particulate substance from the extract. After centrifuging the plant extract was concentrated at reduced pressure in a rotatory vacuum evaporator give 145 gm of extract. The concentrated extract was stored at -20°C until use.

5.3.4 Extract standardization by HPTLC technique

5.3.4.1 Preparation of standard and sample solution

A stock solution of quercetin and ascorbic acid was prepared by dissolving 10 mg of standard quercetin in 10 ml of methanol (1000 μg/ml) and used as standard. The sample solution was prepared by extracting 2.0 g of dried, powdered crude drug with 50 ml of methanol. The methanolic extract was filtered through the Whatman filter paper and evaporated to dryness under reduced pressure. The residue obtained was re-dissolved in 1.0 ml of methanol and used for chromatography.

5.3.4.2 HPTLC instrumentation and procedure

The sample was carefully spotted in the pre-coated silica gel aluminum plate 60F-254 (10 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V (Switzerland) in the form of the bands of width 4.0 mm with a Camag microliter syringe with 150 nL/s constant application rate. A constant application rate 150 nL/s was employed. The slit dimension was kept at 4.0 mm × 0.45 mm and 20 mm/s scanning speed was employed. The mobile phase was composed of hexane: ethyl acetate: formic acid (5:4:1, v/v/v). The development of the HPTLC plate was carried out in ascending manner in
the twin trough glass chamber. The saturation time for the mobile phase 20 min in the saturation chamber and chromatogram was developed up to the length 80 mm of the HPTLC plate and dried in the room temperature. The scanning was done in the observance mode at 570 nm.

5.3.5 Result and discussion

5.3.5.1 Preparation of *Moringa oleifera* (Lam.) plant extract

Air dried stem powder of the *Moringa oleifera* (Lam.) was taken in a soxhlet extractor and extracted successively with the following solvents like petroleum ether, ethyl acetate, chloroform, methanol and water. The result of the extract of *Moringa oleifera* (Lam.) and nature, colour was presented in the table 5.3.1 (Trease and Evans, 1983; Kokate, 1994; Kokate et al., 2002; Khandelwal, 2004).

5.3.5.2 Qualitative Phytochemical Tests

The qualitative phytochemical test was performed for the identification of the chemical constituent present in the particular solvents. The extracts were subjected to qualitative phytochemical tests to find out the active constituents. The qualitative phytochemical test of the *Moringa oleifera* (Lam.) presented in the table 5.3.2. On the basis of the qualitative chemical test, it has been observed that a chemically therapeutic compound like alkaloids, glycosides, phenolic compound, tannin, saponin, sterol, gum and mucilage were present in the stem of the *Moringa oleifera* Lam (Trease and Evans, 1983; Kokate, 1994; Kokate et al., 2002; Khandelwal, 2004).

5.3.5.3 HPTLC analysis

The HPTLC analysis of *Moringa oleifera* (Lam.) extract showed the single compound with \( R_f \) 0.75 (ascorbic acid) (fig. 5.3.1). The presence of the ascorbic acid in a sample was confirmed by co-chromatography of standard ascorbic acid (98 %) procured from chromodex (fig. 5.3.2).
### Table 5.3.1: Percentage extractive values along with colour of the extract and nature of the *Moringa oleifera* (Lam.) residue

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent</th>
<th>Colour of the Extract</th>
<th>Nature of Residue</th>
<th>Extractive Value (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether(60–80° c)</td>
<td>Yellow</td>
<td>Solid</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate</td>
<td>Light Yellow</td>
<td>Sticky</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>Light Orange</td>
<td>Sticky</td>
<td>0.21</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>Light Brown</td>
<td>Semisolid</td>
<td>4.10</td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>Light Green</td>
<td>Gummy</td>
<td>1.12</td>
</tr>
</tbody>
</table>

### Table 5.3.2: Qualitatively phytochemical test screening of *Moringa oleifera* (Lam.).

| S. No. | Plant constituents          | Extracts | | | | |
|--------|----------------------------|----------|----------|----------|----------|----------|----------|
|        |                            | Petroleum ether | Ethyl acetate | Chloroform | Methanol | Water | |
| 1      | Alkaloids                   | -        | -        | +         | +        | -       | |
| 2      | Carbohydrates               | -        | -        | -         | -        | -       | |
| 3      | Glycosides                  | -        | -        | -         | +        | +       | |
| 4      | Phenolic compounds and      | -        | -        | -         | +        | +       | |
|        | Tannins                     |          |          |           |          |         | |
| 5      | Flavonoids                  | -        | -        | -         | +        | +       | |
| 6      | Saponin                     | -        | -        | +         | -        | +       | |
| 7      | Sterols                     | -        | -        | -         | -        | -       | |
| 8      | Gum and Mucilage            | -        | +        | -         | -        | -       | |
Figure 5.3.1: High performance thin layer liquid chromatography profiles of *Moringa oleifera* (Lam.).

Figure 5.3.2: High performance liquid thin layer chromatography profiles of standard Ascorbic acid and Quercetin.
5.4 Material and method for *Paederia foetida* (Linn.)

5.4.1 Successive extractive value

Air dried stem bark powder of *Paederia foetida* (Linn.) was taken in a soxhlet extractor and extracted successively with the following solvents (Petroleum ether, Ethyl acetate, Chloroform, Methanol and Water).

Each time before extracting with the next solvent, the powdered material was air dried first and then oven dried below 50°C. Each extract was concentrated by distilling off the solvent and then evaporated to dryness on the water bath. The percentage extracts of the drug with each solvent were calculated with the reference to the air dried drug (Trease and Evans, 1983; Kokate, 1994; Kokate et al., 2002; Khandelwal, 2004).

5.4.2 Qualitative phytochemical analysis

All extracts were obtained from the successive extraction of *Paederia foetida* (Linn.) was subjected to various qualitative tests for the identification of phytoconstituents present in it. The method of the qualitative phytochemical analysis was mention in the 5.2.2 section (Trease and Evans, 1983; Kokate, 1994; Kokate et al., 2002; Khandelwal, 2004).

5.4.3 Preparation of plant extract of *Paederia foetida* Linn

The collected leaves of *Paederia foetida* (Linn.) were gently washed with tap water to remove the extraporeneous matter. After rinsing the leaves were dried in shade at room temperature and grounded 2 kg of powder was extracted with methanol in a Soxhlet apparatus for 5 days. The extract was filtered and the filtrate was concentrated under reduced pressure using a rotatory evaporator at 40°C until the extra solvent completely dried. The yield of methanolic extract was 30%. The extract was stored in the cooling condition in refrigerator at 4°C until further use. The *Paederia foetida* (Linn.) leaves extract was dissolved in 1% solution of carboxyl- methyl cellulose prepared in distilled water used for the animal studies.

5.4.4 Extract standardization by HPTLC technique

5.4.4.1 Preparation of standard and sample solution

A stock solution of quercetin and ascorbic acid was prepared by dissolving 10 mg of standard quercetin in 10 ml of methanol (1000 μg/ml) and used as standard. The sample solution was prepared by extracting 2.0 g of dried, powdered crude drug with 50 ml of methanol. The methanolic extract was filtered through the Whatman filter paper and evaporated to dryness under reduced pressure. The residue obtained was re-dissolved in 1.0 ml of methanol and used for chromatography.
5.4.4.2 HPTLC instrumentation and procedure
The sample was carefully spotted on the protected silica gel aluminum plate 60F-254 (10 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V (Switzerland) in the form of the bands of width 4.0 mm with a Camag microliter syringe with 150 nL/s constant application rate. A constant application rate 150 nL/s was employed. The slit dimension was kept at 4.0 mm × 0.45 mm and 20 mm/s scanning speed was employed. The mobile phase was composed of hexane: ethyl acetate: formic acid (5:4:1, v/v/v). The development of the HPTLC plate was carried out in ascending manner in the twin trough glass chamber. The saturation time for the mobile phase 20 min in the saturation chamber and chromatogram was developed up to the length 80 mm of the HPTLC plate and dried in the room temperature. The scanning was done in absorbance mode at 570 nm.

5.4.5 Result and discussion
5.4.5.1 Preparation of Paederia foetida (Linn.) plant extract
Air dried leaves powder of the Paederia foetida (Linn.) was taken in a soxhlet extractor and extracted successively with the following solvents like petroleum ether, ethyl acetate, chloroform, methanol and water. The result of the extract of Paederia foetida (Linn.) and nature, colour was presented in the table 5.4.1 (Trease and Evans, 1983; Kokate, 1994; Kokate et al., 2002; Khandelwal, 2004).

5.4.5.2 Qualitative Phytochemical Tests
The qualitative phytochemical test was performed for the identification of the chemical constituent present in the particular solvents. The extracts were subjected to qualitative phytochemical tests to find out the active constituents. The qualitative phytochemical test of the Paederia foetida (Linn.) presented in the table 5.4.2. On the basis of the qualitative chemical test, it has been observed that a chemically therapeutic compound like glycosides, phenolic compound, tannin, sterol, gum and mucilage were present in the leaves of the Paederia foetida (Linn.) (Trease and Evans, 1983; Kokate, 1994; Kokate et al., 2002; Khandelwal, 2004).

5.4.5.3 HPTLC analysis
The HPTLC analysis of Paederia foetida (Linn.) extract showed the single compound with Rf 0.77 (ascorbic acid) (fig. 5.4.1). The presence of the ascorbic acid in a sample was confirmed by co-chromatography of standard ascorbic acid (98 %) procured from chromodex (fig. 5.4.2).
Table 5.4.1: Percentage extractive values along with colour of the extract and nature of the *Paederia foetida* (Linn.) residue

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent</th>
<th>Colour of the Extract</th>
<th>Nature of Residue</th>
<th>Extractive Value (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether(60- 80° c)</td>
<td>Dark Yellow</td>
<td>Solid</td>
<td>0.372</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate</td>
<td>Greenish Yellow</td>
<td>Slightly sticky</td>
<td>0.210</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>Brown – Orange</td>
<td>Waxy</td>
<td>0.784</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>Brown</td>
<td>Thick resinous</td>
<td>11.203</td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>Green</td>
<td>Gummy</td>
<td>6.06</td>
</tr>
</tbody>
</table>

Table 5.4.2: Qualitatively phytochemical test screening of *Paederia foetida* (Linn.).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant constituents</th>
<th>Petroleum ether</th>
<th>Ethyl acetate</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Phenolic compounds and Tannins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>6</td>
<td>Saponins</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Sterols</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Gum and Mucilage</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Figure 5.4.1: High performance thin layer liquid chromatography profiles of *Paederia foetida* (Linn.) extract.

Figure 5.4.2: High performance thin layer liquid chromatography profiles of standard Ascorbic acid.
5.5 Material and method for *Melastoma malabathricum* (Linn.)

5.5.1 Successive extractive value

Air dried stem bark powder of *Melastoma malabathricum* (Linn.) was taken in a soxhlet extractor and extracted successively with the following solvents (Petroleum ether, Ethyl acetate, Chloroform, Methanol and Water).

Each time before extracting with the next solvent, the powdered material was air dried first and then oven dried below 50°C. Each extract was concentrated by distilling off the solvent and then evaporated to dryness on the water bath. The percentage extracts of the drug with each solvent were calculated with the reference to the air dried drug (Trease and Evans, 1983; Kokate, 1994; Kokate et al., 2002; Khandelwal, 2004).

5.5.2 Qualitative phytochemical analysis

All extracts were obtained from the successive extraction of *Melastoma malabathricum* (Linn.) was subjected to various qualitative tests for the identification of phytoconstituents present in it. The method of the qualitative phytochemical analysis was mention in the 5.2.2 section (Trease and Evans, 1983; Kokate, 1994; Kokate et al., 2002; Khandelwal, 2004).

5.5.4 Extract standardization by HPTLC technique

5.5.4.1 Preparation of standard and sample solution

A stock solution of quercetin and ascorbic acid was prepared by dissolving 10 mg of standard quercetin in 10 ml of methanol (1000 μg/ml) and used as standard. The sample solution was prepared by extracting 2.0 g of dried, powdered crude drug with 50 ml of methanol. The methanolic extract was filtered through the Whatman filter paper and evaporated to dryness under reduced pressure. The residue obtained was re-dissolved in 1.0 ml of methanol and used for chromatography.

5.5.4.2 HPTLC instrumentation and procedure

The sample was carefully spotted on the protected silica gel aluminum plate 60F-254 (10 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V (Switzerland) in the form of the bands of width 4.0 mm with a Camag microliter syringe with 150 nL/s constant application rate. A constant application rate 150 nL/s was employed. The slit dimension was kept at 4.0 mm × 0.45 mm and 20 mm/s scanning speed was employed. The mobile phase was composed of hexane: ethyl acetate: formic acid (5:4:1, v/v/v). The development of the HPTLC plate was carried out in ascending manner in the twin trough glass chamber. The saturation time for the mobile phase 20 min in the saturation chamber and chromatogram was developed up to the length 80 mm of the HPTLC plate and dried in the room temperature. The scanning was done in absorbance mode at 570 nm.
5.5.4 Result and discussion

5.5.4.1 Preparation of Melastoma malabathricum (Linn.) plant extract
Air dried leaves powder of the Melastoma malabathricum (Linn.) was taken in a soxhlet extractor and extracted successively with the following solvents like petroleum ether, ethyl acetate, chloroform, methanol and water. The result of the extract of Melastoma malabathricum (Linn.) and nature, colour was presented in the table 5.5.1 (Trease and Evans, 1983; Kokate, 1994; Kokate et al., 2002; Khandelwal, 2004).

5.5.4.2 Qualitative Phytochemical Tests
The qualitative phytochemical test was performed for the identification of the chemical constituent present in the particular solvents. The extracts were subjected to qualitative phytochemical tests to find out the active constituents. The qualitative phytochemical test of the Melastoma malabathricum (Linn.) presented in the table 5.5.2. On the basis of the qualitative chemical test, it has been observed that a chemically therapeutic compound like phenolic compound, tannin, saponin, sterol, gum and mucilage were present in the leaves of the Melastoma malabathricum Linn (Brain and Turner, 1975; Trease and Evans, 1983; Kokate, 1994; Kokate et al., 2002; Khandelwal, 2004).

5.4.5.3 HPTLC analysis
The HPTLC analysis of Melastoma malabathricum (Linn.) extract showed the compound with Rf 0.78 (ascorbic acid) and 0.63 (quercetin) (fig. 5.3.1). The presence of the ascorbic acid in a sample was confirmed by co-chromatography of standard ascorbic acid (98 %) procured from chromodex (fig. 5.3.2).
Table 5.5.1: Percentage extractive values along with colour of the extract and nature of the *Melastoma malabathricum* Linn residue

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent</th>
<th>Colour of the Extract</th>
<th>Nature of Residue</th>
<th>Extractive Value (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether(60- 80° c)</td>
<td>Yellow</td>
<td>Solid</td>
<td>0.18</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate</td>
<td>Light Yellow</td>
<td>Sticky</td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>Light Orange</td>
<td>Sticky</td>
<td>0.26</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>Light Brown</td>
<td>Semisolid</td>
<td>6.22</td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>Light Green</td>
<td>Gummy</td>
<td>3.33</td>
</tr>
</tbody>
</table>

Table 5.5.2: Qualitatively phytochemical test screening of *Melastoma malabathricum* Linn.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant constituents</th>
<th>Petroleum ether</th>
<th>Ethyl acetate</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>2</td>
<td>Carbohydrates</td>
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<tr>
<td>3</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Phenolic compounds and Tannins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>5</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>7</td>
<td>Sterols</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Gum and Mucilage</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 5.5.1: High performance liquid chromatography profiles of *Melastoma malabathricum* (Linn.) extract traced at 570 nm.

Figure 5.5.2: High performance liquid chromatography profiles of standard Ascorbic acid and Quercetin traced at 570 nm.