7. PHYTOCHEMICAL ANALYSIS OF *URENA LOBATA*

7.1 Phytochemical screening and Metabolite analysis

7.1.1 Introduction

Plant possesses a wide variety of chemical compounds and is classified as primary metabolites and secondary metabolites. Primary metabolites are substances widely distributed in nature and most of them are occurring in one form or the other virtually in all organisms and perform the basic cell metabolism. Example of primary metabolites includes starch, cellulose, carbohydrates etc.

Secondary metabolism performs no apparent function in plants as primary metabolism but often have an ecological role. Examples of secondary metabolites includes alkaloids, steroids, terpenoids, flavonoids, quinoids, iridoids, coumarins, tannins, phenols and glycosides.

Secondary metabolites are synthesized in specialized cell type and distinct developmental stages making their extraction, purification difficult. As a result, secondary metabolites after isolation are subjected to structural elucidation and screened for activity and used commercially as biologically active compounds, known as phytopharmaceuticals.

Chemical evaluation comprises of different chemical tests and chemical assays, besides isolation, purification and identification of active constituents. The qualitative chemical tests are useful in detection of adulteration.
The systematic investigation of plant material for its phytochemical behaviour involves four different stages

1. The procurement of raw material and quality control.
2. Extraction, purification and characterization of the constituents of pharmaceutical interest and in process quality control.
3. Investigation of biosynthetic pathways of particular compounds.
4. Quantitative determination.

7.1.2 Materials and Methods

7.1.2.1 Chemicals used

Analytical grade chemicals were obtained from Loba, HI media, S.D. fine chemicals, E.Merck, Qualigens, Sigma chemicals and Fischer fine chemicals.

7.1.2.2 Preparation of Extract

Collection of Plant

The entire plant of *Urena lobata* was collected from Tambaram region, Chennai, Tamilnadu in the month of February, 2010 and authenticated by Prof. P. Jayaraman, Ph.D. Plant Anatomy Research Center (PARC), Chennai.

Procedure:

The fresh plant was shade dried and cleaned. Then it was homogenized.
**Preparation of various extracts**

**Procedure**

The entire plant was subjected to successive maceration with solvents of increasing polarity such as petroleum ether, chloroform, ethyl acetate and alcohol for 72 hours, 42 hours and 24 hours. They were then filtered, solvent distilled using rotary flash evaporator and dried under vacuum desiccator. The yield of various extracts are tabulated in Table 7.1. The hydo alcoholic extracts was prepared by macerating using 1:1 (alcohol : water) for 72 hours followed by 48 hours and 24 hours. The solvant was filtered, pooled and distilled under vacuum and reduced to 1/4th volume. Finally the traces of water were removed completely by evaporating over water bath and stored.

The extracts were designated as followed.

1. Pet.ether extract of *Urena lobata* (PEUL)
2. Chloroform extract of *Urena lobata* (CEUL)
3. Ethyl acetate extract of *Urena lobata* (EAUL)
4. Ethanolic extract of *Urena lobata* (ETUL)
5. Hydro alcoholic extract of *Urena lobata* (HAUL)
### Table 7.1 Percentage yield of various extracts

<table>
<thead>
<tr>
<th>S.No</th>
<th>Extracts</th>
<th>Percentage yield (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether</td>
<td>0.623</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform</td>
<td>0.787</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl acetate</td>
<td>0.632</td>
</tr>
<tr>
<td>4</td>
<td>Absolute alcoholic</td>
<td>2.936</td>
</tr>
<tr>
<td>5</td>
<td>Hydro alcoholic (1:1 water: alcohol)</td>
<td>1.923</td>
</tr>
</tbody>
</table>

#### 7.1.2.3 Chemical Analysis

The Chemical analysis of a herbal drug reflects its chemical nature and the quantity of the active principles that are responsible for its therapeutic activity.

#### 7.1.2.3.1 Qualitative Phytochemical Evaluation

Different chemical tests were performed in the pet ether, chloroform, ethyl acetate, ethanol and hydro alcoholic extracts asper Harborne, 1974.

The various chemical tests are given below

**Preliminary screening**

1. **Test for alkaloids**

   A. About 10 mg of the extract was mixed with 1gm of calcium hydroxide and 5 ml of water into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a waterbath. 20 ml of 90 % alcohol was added, mixed well and then refluxed for an hour on a water
bath. Then it was filtered and the alcohol was evaporated. To this, hydrochloric acid was added followed by 2 ml of the following reagents:

a. Mayer’s reagent – No cream coloured precipitate.
b. Dragendorff’s reagent – No reddish brown precipitate.
c. Hager’s reagent – No yellow precipitate
d. Wagner’s reagent – No reddish brown precipitate.

B. Murexide test for Purine Group

10 mg of extract was added with 1 ml of con. Hydrochloric acid and 3 – 4 drops of Conc. potassium chlorate. Evaporated and then treated with ammonia - No purple color.

2. Test for Carbohydrates

a) Molisch’s Test

The solution of the 10mg of extract was treated with alcoholic solution of α-naphthol in the presence of sulphuric acid. A purple colour ring was obtained which indicate the presence of carbohydrate.

b) Test for sugars

i) Fehling’s Test

The solution of the 10mg of extract after treatment with Fehling solution I and II by boiling reddish brown precipitate was produced indicating the presence of reducing sugars.

ii) Benedict’s Test

When boiling with Benedict’s solution the 10mg of extract showed reddish brown precipitate, indicates the presence of reducing sugars.
3. Test for Glycosides

a) Cyanogenetic glycosides

A small quantity of the 10mg of extract was placed in a stoppered flask with just sufficient water to cover it. A sodium picrate paper strip was inserted through the stopper, so that it was suspended in the flask and it was set aside for two hours in a warm place. No change in the colour of the sodium picrate paper was observed as black indicating the absence of cyanogenetic glycoside.

b) Test for Anthraquinone glycosides

i) Borntrager’s Test

The 10 mg extract was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly.

No pink color was observed in the ammoniacal layer showing the absence of Anthraquinone glycosides.

ii) Modified Borntrager’s Test

About 1g of the 10 mg extract was mixed with 10 ml of 5 % ferric chloride and 5 ml of solution of dilute hydrochloric acid. It was heated in a boiling water bath for 5 minutes and the solution was cooled and shaken gently with benzene for about one minute. The benzene layer was seperated and to it an equal volume of the dilute solution of ammonia was added.
No reddish brown layer acquiring bluish green color after standing was observed indicating the absence of deoxy sugars of cardiac glycosides.

4. Test for Phytosterols

The petroleum ether and alcoholic extracts were refluxed separately with solution of alcoholic potassium hydroxide till complete saponification takes place. The saponified mixture was diluted with distilled water and extracted with ether. The ether extract was evaporated and the residue (unsaponifiable matter) was subjected to the following tests.

a) Salkowski Test

To the residue few drops of concentrated sulphuric acid was added, shaken well and set aside. Chloroform was added and the chloroform layer turned red colour indicating the presence of sterols.

b) Liebermann-Burchard Test

The above residue was treated with concentrated sulphuric acid and acetic anhydride. A green color was developed, indicating the presence of sterols.

5. Test for Saponins

About 0.5 gm of 10 mg extract was boiled gently for 2 minutes with 20 ml of water and filtered while hot and allowed to cool. 5 ml of the filtrate was then diluted with water and shaken vigorously. Frothing was produced indicating the presence of saponins.
6. Test for Tannins

A small quantity of the 10 mg extract was extracted with water. To the aqueous extract few drops of ferric chloride solution was added. Colour was produced indicating the presence of tannins.

7. Test for Proteins and free amino acids:

a) Millon’s Test

10 mg extract was heated with Millon’s reagent. Change to red colour on heating indicates the presence of proteins.

b) Biuret Test

To the 10 mg extract one ml of dilute sodium hydroxide solution was added. Followed by this one drop of very dilute copper sulphate solution was added. Violet colour was obtained indicating the presence of proteins.

8. Test for Mucilage

Few ml of aqueous extract prepared from the powdered crude drug was treated with ruthenium red. Red color obtained indicated the presence of mucilage.

9. Test for Flavonoids

a) Shinoda Test

A little amount of the 10 mg extract was heated with alcohol and filtered. To the alcoholic solution a few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes. Red color indicated the presence of flavonoids.
10. Test for Terpenoids

10 mg extract was shaken with petroleum ether and filtered. The filtrate was evaporated and residue was dissolved in small amount of chloroform. To the chloroform solution tin and thionyl chloride was added. Pink color indicated the presence of terpenoids.

7.1.2.3.2 Quantitative evaluation of primary metabolites

This involves the quantification of carbohydrate, proteins in ETU1 of *Urena lobata* using standard procedures.

Estimation of Carbohydrates (Morris *et al.*, 1948)

**Principle**

The carbohydrates present in the extract was evaluated by anthrone reagent and the formation of green color was estimated at 630 nm, which is the basis of determination of the total carbohydrate content of the plant extract.

**Requirements**

- Anthrone reagent-This was prepared by dissolving 0.2 gm of anthrone in 100 ml of sulphuric acid, made by adding 500 ml of concentrated acid to 200 ml of distilled water. It was allowed to stand for 30-40 minutes with occasional shaking until the solution was perfectly clear
- Stock glucose solution – 10mg of glucose was weighed accurately and dissolved in distilled water and the volume was made up to 10ml in standard flask (concentration 1 mg/ml).
Working standard solution – 1ml of stock solution was diluted to 10 ml with distilled water in a standard flask (concentration 100 µg/ml)

Preparation of sample- About 100 mg of the extract was hydrolyzed by boiling with 100 ml of 2.5 N HCl for 3 hours and then cooled to room temperature. This mixture was then neutralized using solid sodium carbonate until the effervescence ceased and its volume was made up to 100 ml. This was centrifuged at 3000 rpm for 15 minutes. The supernatant was decanted off and used for estimation.

Procedure

From the working standard solution 0.2, 0.4, 0.6, 0.8, 1 ml of solutions was pipetted out into series of the test tubes. 1 ml of supernatant of sample extract was pipetted out in triplicates. The volume in all test tubes were made up to 1 ml with distilled water. A test tube with 1 ml of water was used as the blank. 4 ml of anthrone reagent was added to each test tube including blank. It was heated for 8 minutes in a water bath and cooled. The absorbance of the developed green color was read at 630 nm. A standard graph of glucose was plotted from which the carbohydrate content of the extract was determined in terms of glucose equivalents.
Estimation of Total Protein (Lowry et al., 1953)

Principle

The aromatic amino acids present in the proteins react with Folin–cio calteau reagent, which contains phospho molybdic acid and tungstate to produce a blue colored complex which absorbs maximally at 620 nm, which is the basis of determination of the total protein content of plant extract.

Requirements

- Alkaline copper sulphate solution – 50 ml of 2 % w/v sodium carbonate in 0.1 N sodium hydroxide solution and 1 ml of copper sulphate in 1 % w/v potassium sodium tartarate were mixed prior to its use.
- Folin–cio calteau reagent – The reaction mixture was mixed with distilled water (1:2)
- Stock solution of Protein -50 mg of Bovine serum albumin was weighed accurately and dissolved in distilled water and made up to 50 ml in a standard flask.
- Working standard solution – 1 ml of the stock solution was diluted to 10 ml with distilled water in a standard flask (Concentration 100 µg/ml)
- Preparation of sample – About 100 mg of the extract was hydrolyzed by boiling with 100 ml of 2.5 N HCl for 3 hours and then cooled to room temperature. This mixture was then neutralized using solid sodium carbonate until the effervescence ceased and its volume was made up to 100 ml.
This was centrifuged at 3000 rpm for 15 minutes. The supernatant was decanted off and used for estimation.

**Procedure**

From the working standard solution 0.2, 0.4, 0.6, 0.8 and 1 ml of solution were pipetted into a series of test tubes. 1 ml of supernatant of sample extract was pipetted out in triplicates. The volume in all test tubes was made up to 1 ml with distilled water. A test tube with 1 ml of distilled water was used as the blank. 5 ml of the alkaline copper sulphate solution was added to each test tube including the blank. It was mixed well and allowed to stand for 10 minutes. To this 0.5 ml of prepared solution, Folin-cio calteau reagent was added and mixed well and incubated at room temperature in the dark for 30 minutes. Absorbance of developed blue color was read at 660 nm. A standard graph was plotted from which protein content of extract was determined in terms of bovine serum albumin equivalents.

**7.1.2.3.3 Secondary metabolites**

Secondary metabolites like phenols, tannins content of ETUL of the plant *Urena lobata* was quantified by the following methods.

**Estimation of Total Phenols** (Mc Donald *et al.*, 2001)

**Principle**

The phenolic hydroxyl groups present in the phenolic compound of the plant reacts with Folin’s Phenol reagent, to produce a blue color, which absorbs maximally at 765 nm, and form
the basis of determination of the total phenol content of plant extract.

**Requirements**

- **Folin Phenol reagent** - The reaction mixture was mixed with distilled water (1:2)
- **Sodium carbonate (7.5 % w/v)** – 7.5 gm of the accurately weighed sodium carbonate was dissolved in distilled water and volume was made up to 100 ml in a standard flask.
- **Stock solution of Gallic acid** – Accurately weighed 10 mg of Gallic acid was dissolved in methanol and made up to 10 ml in a standard flask (1 mg/ml).
- **Working standard** – 1 ml of the stock solution was diluted to 10 ml with methanol in a standard flask. (1 ml of this solution contains 100 µg of Gallic acid.)
- **Preparation of test extract** – 10 mg of extract was weighed and dissolved in 10 ml of methanol: water (70:30). From this 1 ml was used for estimation.

**Procedure**

From the working standard solution 0.2, 0.4, 0.6, 0.8 and 1 ml of solutions were pipetted out into a series of test tubes. 1 ml of sample extract with duplicates was pipetted out in two other test tubes. The volume in all the test tubes was made up to 1 ml with methanol. 1 ml of distilled water was used as a blank. To all the test tubes 5 ml of Folin’s phenol reagent (1:2) followed by 4 ml of 7.5 % sodium carbonate were added and kept at room temperature for
1.5 hour. with occasional shaking. A blue color was formed and its color intensity was read at 765 nm. A standard graph of gallic acid was plotted, from which the total phenol content of the extract was determined in terms of gallic acid equivalents.

**Estimation of Tannins** (Giner – Chavez, 1996)

**Principle**

The poly hydroxyl phenolic compounds of tannins present in plant extract reacts with Folin’s phenol reagent to produce a blue color, which absorbs maximally at 640 nm is the basis of determination of the total tannin content of plant extract.

**Requirements**

- **Folin’s Phenol reagent (1:2)** – The reagent was mixed with distilled water (1:2)
- **Sodium carbonate (35 % w/v)** – A supersaturated solution of sodium carbonate was prepared by dissolving accurately weighed 35 gm. of sodium carbonate in distilled water and the volume was made up to 100 ml with distilled water in a standard flask.
- **Stock tannic acid solution** – Accurately weighed 10 mg of tannic acid was dissolved in distilled water and made up to 10 ml in a standard flask (Concentration : 1mg/ml)
- **Working standard solution** 1 ml of the stock solution was diluted to 10 ml with distilled water in a standard flask. (1 ml of this solution contains 100 µg of Tannic acid).
Preparation of Extract - 10 mg of extract was weighed and dissolved in 10 ml of methanol: water (70:30). From this 1 ml was used for estimation.

Procedure

From the working standard solution 0.2, 0.4, 0.6, 0.8 and 1 ml of solutions were pipetted out into a series of test tubes. 1 ml of sample extract with duplicates was pipetted out in two other test tubes. The volume in all the test tubes was made up to 1 ml with distilled water. 1 ml of distilled water was used as blank. To all the test tubes 0.5 ml of Folin’s phenol reagent (1:2) followed by 3 ml of 35% sodium carbonate solution was added and kept at room temperature for 5 minutes. A blue color was formed and its color intensity was read at 640 nm. A standard graph of tannic acid was plotted, from which the total tannin content of extract was determined in terms of tannic acid equivalents.

7.1.2.3.4 Elemental Analysis by Atomic Absorption Spectrophotometry

About 250 mg of the alcoholic extract of *Urena lobata* was weighed and 5-10 ml of concentrated sulphuric acid was added to it. The acid digestion was further initiated by heating up to 440°C using a Digeadahl apparatus. The samples were made free of organic matter and the resulting solution was made colorless by adding 5-10 ml of H₂O₂. The digested material was made up to 100 ml for
elemental analysis in the AAS, Perkin Elmer. Hg and Se were estimated using a hydride generator attached to the AAS. Working standard solutions were prepared from stock standard solutions. Calibration was performed using appropriate standard solutions. Results were arrived from standard linear calibrations.

7.1.3 Results and discussion

7.1.3.1 Preliminary phytochemical screening

The preliminary phytochemical screening of alcoholic extracts of *Urena lobata* was done by using cold maceration technique. The qualitative organic analysis has revealed the presence of following constituents in the extract. The alcoholic extracts consist of carbohydrate, phytosterols, flavonoids, saponins, tannins, terpenoids and mucilage and the results are tabulated in the table 7.1.

7.1.3.2 Metabolite analysis

Primary metabolite and secondary metabolite values were tabulated. Tannins in vascular plants occur as two types, condensed or hydrolysable tannins. Tannins are powerful antioxidants often characterized by reducing power and free radical scavenging activities (Zang and Lin, 2008). The preliminary phytochemical studies had revealed the presence of carbohydrates, phytosterols, flavonoids, saponins, tannins, terpenoids and mucilage. The observation is tabulated and result is represented in table 7.2.
7.1.3.3 Elemental analysis

The extract of *Urena lobata* was analyzed for presence of heavy metals using AAS (Table 7.3). The heavy metals with their respective amounts are: Fe-0.365 ppm; Zn-1.68 ppm; Cu-0.008 ppm; Mn-0.058 ppm; As-not detected; Hg- not detected; Se-not detected; and Co- not detected. The values obtained are well within the acceptable limits suggesting that extract is nontoxic and suitable for prescription to humans.

Table: 7.2 Preliminary phyto chemical tests of the ethanolic extract *Urena lobata.*

<table>
<thead>
<tr>
<th>Test for</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ALKALOID</td>
<td></td>
</tr>
<tr>
<td>a) Mayer’s Reagent</td>
<td>-</td>
</tr>
<tr>
<td>b) Dragendorff’s Reagent</td>
<td>-</td>
</tr>
<tr>
<td>c) Hager’s Reagent</td>
<td>-</td>
</tr>
<tr>
<td>d) Wagner’s test</td>
<td>-</td>
</tr>
<tr>
<td>se) Murexide test</td>
<td>-</td>
</tr>
<tr>
<td>2 CARBOHYDRATES</td>
<td></td>
</tr>
<tr>
<td>a) Molisch’s Reagent</td>
<td>+</td>
</tr>
<tr>
<td>b) Fehling’s solution</td>
<td>+</td>
</tr>
<tr>
<td>c) Benedict’s Reagent</td>
<td>+</td>
</tr>
<tr>
<td>3 GLYCOSIDES</td>
<td></td>
</tr>
<tr>
<td>a) Anthraquinone</td>
<td>-</td>
</tr>
<tr>
<td>b) Cardiac</td>
<td>-</td>
</tr>
<tr>
<td>Type of metabolite</td>
<td>Metabolite</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Primary metabolites</td>
<td>Total carbohydrates</td>
</tr>
<tr>
<td></td>
<td>Total proteins</td>
</tr>
<tr>
<td>Secondary metabolites</td>
<td>Phenols</td>
</tr>
<tr>
<td></td>
<td>Tannins</td>
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Table 7.4 Elemental analysis of extract of *Urena lobata*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Elements</th>
<th>Quantity (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Potassium(K)</td>
<td>3.26</td>
</tr>
<tr>
<td>2</td>
<td>Sodium (Na)</td>
<td>0.658</td>
</tr>
<tr>
<td>3</td>
<td>Calcium (Ca)</td>
<td>1.96</td>
</tr>
<tr>
<td>4</td>
<td>Magnesium (Mg)</td>
<td>2.98</td>
</tr>
<tr>
<td>5</td>
<td>Iron (Fe)</td>
<td>0.365</td>
</tr>
<tr>
<td>6</td>
<td>Zinc (Zn)</td>
<td>1.68</td>
</tr>
<tr>
<td>7</td>
<td>Manganese (Mn)</td>
<td>0.058</td>
</tr>
<tr>
<td>8</td>
<td>Copper (Cu)</td>
<td>0.008</td>
</tr>
</tbody>
</table>
References


7.2 DETERMINATION OF KAEMPFEROL AND QUERCETIN CONTENT IN THE EXTRACT BY HPLC

7.2.1 Introduction

HPLC has been widely used method for the phytochemical evaluation of the crude drugs, because of its simplicity and minimum sample requirement. Hence HPLC method has been developed for the present study to confirm the presence of Kaempferol, Quercetin in *Urena lobata*. It is an invaluable quality assessment tool for the evaluation of botanicals thus serving as a reference standard for the quality control of the extracts. It allows the analysis of a large number of compounds both efficiently and cost effectively. To confirm quantity of the flavonoids as Quercetin and Kaempferol was to be 0.21 and 0.27mg/ g fraction repectively.

7.2.2 Materials and methods

7.2.2.1 Plant material and the extraction

*Urena lobata* aerial parts were collected and authenticated and were extracted with ethanol after processing as powder.

7.2.2.2 Instrumentation

The HPLC experiment was performed using a Waters Alliance system equipped with a vacuum degasser, quaternary detector. The UV spectra were collected across the range of 200–900 nm, extracting 360 nm for chouromatograms. Empower software was utilized for instrument control, data collection and data processing. The column was an ACE C18 (4.6×250mm, 5 μm). The mobile phase was a linear gradient with O-phosphoric acid 0.25%
(A)- acetonitrile (B) for 42 min starting with A:B (95:5) for 2 min, changing to A:B (90:10) for 5 min, A:B (85:15) for 3 min, A:B (80:20) for 13 min, A:B (70:30) for 5 min, A:B (50:50) for 4 min with equilibrating for 10 min. The flow rate was 1 ml/min. The injection volume for all samples and standard solutions was 10 μL.

7.2.2.4 Procedure

The ethanolic extract of *Urena lobata* was filtered, concentrated under reduced pressure.

- The concentrated extract (25 mg) was dissolved in water and then extracted with n-hexane followed by n-butanol successively.
- The concentrated n-butanol fraction (14 mg) was chromatographed on silica gel column and eluted with chloroform–methanol of increasing polarity to obtain A–1.
- This A–1 fraction was repeatedly passed through silica gel column with chloroform–methanol of increasing polarity to give kaempferol.
- The isolated kaempferol was subjected to HPLC analysis qualitatively and hence confirmed the presence of kaempferol in the plant material *Urena lobata*.

7.2.3 Results and Discussion

Reversed-phase HPLC has been used in a number of occasions for the analysis of flavonoids in plants, it was used to distinguish species based on the quantitative variation of flavonoids among them. It has been applied especially for the identification of
flavonoid derivatives. In the present investigation, flavonoids were quantified at 254nm using peak area by comparison to a calibration curve derived from the quercetin. Comparing the HPLC chouromatograms from the aerial parts of the *Urena lobata*, the main difference was in peak eluted at 2.133 min. External flavonoid aglycones were already analysed using HPLC method in various plant extracts. The peaks in this study has shown marked increase in peak area in case of *Urena lobata* and compared with standard Quercetin (Fig-7.1 and 7.2). From the calibration curve results, the amount of Quercetin, in the sample injected was calculated.

**Table 7.5.** Qualitative identification of the isolated compounds.

<table>
<thead>
<tr>
<th>Color of the compound</th>
<th>Color of TLC spots in presence of ammonia</th>
<th>Rf Values</th>
<th>λmax.(nm) (MeOH)</th>
<th>Melting Point</th>
<th>Name of the isolated compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td>Brown</td>
<td>0.75</td>
<td>252,267,306,370</td>
<td>315°C</td>
<td>Quercetin</td>
</tr>
<tr>
<td>Yellow</td>
<td>Brownish yellow</td>
<td>0.82</td>
<td>253,268,324,367</td>
<td>277°C</td>
<td>Kaempferol</td>
</tr>
</tbody>
</table>

**Table 7.6.** Quantitative Estimation of Total Flavonoids in isolated fractions of *Urena lobata*

<table>
<thead>
<tr>
<th>Fractions Isolated</th>
<th>Flavonoids (mg/g.fraction wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q</td>
</tr>
<tr>
<td>Fraction I absolute alcohol</td>
<td>0.21</td>
</tr>
</tbody>
</table>
Fig. 7.1 HPLC chromatogram of the standard Quercetin

Figure 7.2 HPLC chromatogram of the Quercetin in the plant *Urena lobata*
Fig: 7.3 HPLC chromatogram of the Kaempferol in the plant *Urena lobata*
References


