CHAPTER-V QUALITATIVE PHYTOCHEMICAL EXAMINATION

5.1 INTRODUCTION

Now-a-days various types of extraction procedures are available for the purpose of research work. However effective extraction is done by proper selection of extraction process and proper solvent.

5.2 CONTINUOUS HOT PERCOLATION PROCESS OR SOXHLET EXTRACTION\(^1\)

Continuous hot percolation process is used for those drugs where the penetration of the menstruum into the cellular tissues is very slow and the solute is not readily soluble into the solvent and the quantity of menstrum is very less. In such cases Soxhlet extractor is used where small volume of hot menstrum is passed over the drug time and again to dissolve out the active constituents until the drug is exhausted. This process is known as Soxhlation.

The Soxhlet apparatus required for the continuous hot percolation is made from a very high grade of glass and consists of three parts.

(a) A flask in which the menstrum is boiled.

(b) An extraction chamber in which drug is filled is fitted with a side tube and a siphon.

(c) A condenser.
The drug to be extracted, in suitably comminuted form is usually packed in a ‘thimble’ made of filter paper which is then placed into the wider part of the extractor. ‘Thimble’ is used to prevent choking of the lower part of the extractor by drug particles. Menstrum is placed in the flask and boiled, the vapors are allowed to pass through the side tube to the condenser where they are condensed and fall on the packed drug, through which it percolates and extract out the active constituents. As the volume of menstrum in the extractor increases, the level of the liquid in the siphon also increases till it reaches the maximum point from where it is siphoned out into the flask. On further heating, the menstrum vaporizes while the dissolved active constituents remain behind in the flask. The alternate filling and emptying of the body of the extractor goes on continuously till the drug is exhausted. Thus the same quantity of menstrum is made to percolate repeatedly, about 14 to 15 times through the drug and the active constituents are collected in the flask.
5.3 QUALITATIVE PHYTOCHEMICAL SCREENING:

Obtained extracts were need to be check for the type of secondary metabolite presence like triterpenes, glycosides, alkaloids, flavonoids, essential oils. A series of qualitative tests were carried on the selected plant extracts, to detect various phyto constituents present in them.

5.3.1 Test for alkaloids:

About 50mg of solvent free extract was stirred with little quantity of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.

1. **Mayer’s test**: To a few ml of filtrate, two drops of Mayer’s reagent was added along the sides of the test tube. If the test is positive it gives white or creamy precipitate.

2. **Wagner’s Test**: To a few ml of the filtrate, few drops of Wagner’s reagent were added along with the sides of the test tube. Formation of reddish brown precipitate confirms test as positive.

3. **Hager’s Test**: To a few ml of filtrate 1 or 2 ml of Hager’s reagent was added. A prominent yellow precipitate indicates positive test.

4. **Dragendroff’s Test**: To a few ml of filtrate, 1or 2 ml of Dragendroff’s reagent was added. A prominent reddish brown precipitate indicates positive test.
5.3.2 Test for carbohydrates:

About 100mg of extract was dissolved in 5ml of distilled water and filtered. The filtrate was subjected to the following tests.

1. **Molisch’s test**: To 2ml of filtrate, two drops of alcoholic solution of α-naphthol was added. The mixture was shaken well and 1ml of concentrated sulphuric acid was added slowly along the sides of the test tube, it was cooled in ice water and allowed to stand. A violet ring at the junction of two liquids indicates the presence of carbohydrates.

2. **Fehling’s test**: 1ml of filtrate was boiled on water bath with 1ml each of Fehling’s solution A and B, formation of red precipitate indicates the presence of sugar.

3. **Barfoed’s test**: To 1ml of the filtrate, 1ml of Barfoed’s reagent was added and heated on a boiling water bath for 2 minutes. Red precipitate indicates the presence of sugar.

4. **Benedicts test**: To 0.5ml of filtrate 0.5ml Benedict’s reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic brick red precipitate indicates the presence of sugar.
5.3.3 Test for glycosides:

For detection of glycosides, about 50mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hrs on a water bath, filtered and the hydrolysate was subjected to the following tests.

1. **Brontrager’s Test:** To the 2ml filtrate 3ml of chloroform and shaken well, chloroform layer was separated and shaken with 10% ammonia. A rose pink to red colour formation in ammonical layer indicates the presence of Anthraquinone glycosides.

2. **Legal’s test:** About 50mg of the extract was dissolved in pyridine. Sodium nitro prusside solution was added and made alkaline using 10% sodium hydroxide solution. Presence of glycoside is indicated by a characteristic pink colour.

5.3.4 Test for Saponins:

1. **Foam or froth test:** A small quantity of extract was diluted with distilled water to 20ml. The suspension was shaken in a graduated cylinder for 15 minutes. A two centimeter layer of foam or froth which is stable for 10 minutes indicates the presence of saponins.
5.3.5 Test for phytosterols and triterpenoids:

1. **Libermann-buchard’s test:** The extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1ml of concentrated sulphuric acid was added along the side of the test tube. Red, pink or violet color at the junction of the liquids indicates the presence of steroids/triterpenoids and their glycosides.

2. **Salkowoski test:** Few drops of concentrated sulphuric acid was added to the chloroform extract shaken on standing, red colour in the lower layer indicates the presence of steroids and golden yellow color indicates the presence of triterpenoids.

5.3.6 Test for phenolic compounds and tannins

1. **Ferric chloride test:** About 50mg of extract was dissolved in distilled water and to this few drops of neutral 5% ferric chloride solution was added. Formulation of blue, green and violet color indicates the presence of phenolic compounds.

2. **Gelatin test:** To a little quantity of extract was dissolved in distilled water and 1% solution of gelatin containing 10% sodium chloride was added to it. Development of white precipitate indicates the presence of phenolic compounds.
3. **Lead acetate test**: A small quantity of extract was dissolved in distilled water and to this 3ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

5.3.6 **Test for flavonoids**:

1. **Alkaline reagents**: An aqueous solution of extract was treated with 10% ammonium hydroxide solution-yellow fluorescence indicates the presence of flavonoids.

2. **Shinoda test or magnesium-hydrochloric acid reduction**: A little quantity of extract was dissolved in alcohol and few fragments of magnesium turnings and conc. Hydrochloric acid (drop wise) were added. If any pink or crimson red color develops indicates the presence of flavonoids.
5.4 EXPERIMENTAL MATERIALS AND METHODS

5.4.1 Collection and authentication of plant materials

Based on exhaustive literature survey done on Vigna genus plants, 3 plants known as Vigna mung Linn, Vigna radiate Linn, Vigna ungingulata Linn were selected for the study.

The whole plant parts of Vigna mung Linn, Vigna radiate Linn, Vigna ungingulata Linn were collected from the nearby fields of Guntur district region, Andhra Pradesh and were authentified by Dr. D. Ramakanth raju, retire botanist, S.V. University, Tirupati and a voucher specimen for Vigna mung Linn (T.S.N-001, 13/12/2011), Vigna radiate Linn (T.S.N-002, 17/12/2011), Vigna ungingulata Linn (T.S.N-003, 9/12/2010), has been deposited in Pharmacognosy Department, Andhra university.

5.4.2 Extraction:

All the plant materials obtained were shade dried and made into coarse powder and passed through sieve #40, were successively extracted with petroleum ether, chloroform, ethanol by Soxhlet extraction method. (Fig: 5.01)
Selection of crude drug material and authentification

(V. Mung, V. Radiata, V. Ungiculata whole plant materials)

Shade drying of selected plants

Pulverized and passed through sieve 40#

Soxhlet extraction using pet ether solvent

Pet ether extract

Marc

Soxhlet extraction using chloroform solvent

Chloroform extract

Marc

Soxhlet extraction using ethanol solvent

Ethanol extract

Marc discarded
5.4.3 **Procedure:** The selected plants for the study were shade dried, pulverized to a coarse powder in a mechanical grinder, passed through 40# mesh sieve. 1kg of plant material was extracted in Soxhlet extractor consecutively using solvents of non polar to polar grade (Petroleum Ether, Chloroform, Ethanol) the crude extract were evaporated to dryness in a rotary evaporator.

The yield of different extracts for the selected plants is given below:

**Vigna mung Linn:**

- Petroleum ether extract: 10.5gms
- Chloroform extract: 17.9 gms
- Ethanol extract: 41 gms

**Vigna radiate Linn:**

- Petroleum ether extract: 11.3gms
- Chloroform extract: 20.9 gms
- Ethanol extract: 46gms

**Vigna unguiculata Linn:**

- Petroleum ether extract: 9.5gms
- Chloroform extract: 15.9gms
- Ethanol extract: 47gms
5.5 RESULTS AND DISCUSSION:

Table: 5.01 Results of Qualitative phytochemical tests of selected plant extracts

<table>
<thead>
<tr>
<th>S.no</th>
<th>Tests</th>
<th>V.mung</th>
<th>V.radiata</th>
<th>V.ungiculata</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Aminoacids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Mucilage</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Proteins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Starch</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Steroids &amp; triterpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Presence of metabolites, - = Absence of metabolites
Phytochemical Examination

Petroleum ether extract of *Vigna mung* Linn reveals the presence of alkaloids, aminoacids, carbohydrates, mucilage, proteins, starch, steroids and triterpenoids.

Chloroform extract of *Vigna mung* Linn reveals the presence of aminoacids, carbohydrates, mucilage, proteins, steroids and triterpenoids.

Ethanolic extract of *Vigna mung* Linn reveals the presence of alkaloids, aminoacids, carbohydrates, mucilage, proteins, starch, steroids and triterpenoids.

Petroleum ether extract of *Vigna radiate* Linn reveals the presence of alkaloids, aminoacids, proteins, steroids and triterpenoids.

Chloroform extract of *Vigna radiate* Linn reveals the presence of aminoacids, carbohydrates, mucilage, proteins, starch, steroids and triterpenoids.

Ethanolic extract of *Vigna radiate* Linn reveals the presence of alkaloids, aminoacids, carbohydrates, mucilage, proteins, starch, steroids and triterpenoids.

Petroleum ether extract of *Vigna ungiculata* Linn reveals the presence of carbohydrates, flavonoids, proteins, starch, steroids and triterpenoids.

Chloroform extract of *Vigna ungiculata* Linn reveals the presence of alkaloids, aminoacids, flavonoids, carbohydrates, proteins and starch.

Ethanolic extract of *Vigna ungiculata* Linn reveals the presence of alkaloids, aminoacids, flavonoids, carbohydrates, mucilage, starch and glycosides.
5.6 REFERENCES
