CHAPTER 3

PHARMACOGNOSTICAL AND PHYTOCHEMICAL SCREENING

3.1 Pharmacognostical investigation
3.2 Chemical group test
3.3 Thin Layer Chromatography screening

References
3.1 Pharmacognostical investigation

3.1.1 Introduction

The need of ensuring the quality of medicinal plant products by using current control techniques and applying appropriate standards has been emphasized in the resolution of World Health Assembly (Radha et al., 2008). Typical pharmacognostical studies are quite normal for quality control of herbal drugs (Panda, 2004). Pharmacognostical standardization of herbal drugs include macroscopic, microscopic, physio-chemical constants and fluorescence analysis of investigated parts, and to evolve standards for single drugs and compound preparations in order to validate genuineness of the crude drugs of plant, mineral and animal origin. The study includes to highlighting the macroscpical and microscopical characters as distinctive features for authentication and identification purposes (Ahmed & Dar, 2008; Shri et al., 2010). According to WHO (1998), before carrying out any test in medicinal plants, the first step towards establishing the identity and purity of the plants include the study of macroscopic and microscopic description of the medicinal plant (Balakrishna et al., 2010).

3.1.2 Methodology adopted for pharmacological investigation

3.1.2.1 Plant material

The plant, Alocasia decipiens Schott was identified in Central National Herbarium, Botanical Survey of India, Shibpur, Howrah-711103, West Bengal, India. The rhizome of the plant was selected for carrying out the experiment.

3.1.2.2 Macroscopical studies

The dried rhizome of Alocasia decipiens Schott were subjected to macroscopical studies which comprised of organoleptic characteristics of the plant part used viz., size, color, odour, taste and shape. The macroscopic character was useful in quick identification of plant material and also serves as an important standardization parameter.
3.1.2.3 Microscopical studies

Qualitative microscopic evaluation was carried out by taking transverse sections of rhizome of *Alocasia decipiens* Schott. Free hand sections of softened rhizome were carefully stained with safranin. The section (better one) was transferred to the slide and mounted on a glycerine and a cover slip was placed over it. The slide was then observed under microscope and photographs were taken.

3.1.2.4 Physicochemical parameters

Various physicochemical parameters such as total ash, water soluble ash, acid insoluble ash, water extractable matter, alcohol extractable matter, moisture content, were calculated.

**Determination of moisture content** (AOAC, 1990)

Moisture content was determined by drying 5g of sample at 105°C in a drying oven to a constant weight. The moisture content was calculated as percentage.


Total ash:- 5g of plant powder was ignited in an electric furnace at 600°C in silica crucible until the sample reaches a constant weight.

Water-soluble ash value:- Total ash obtained was heated up to 600°C with addition of 25ml of water for 10 mins. It was filtered in an ash less filter paper (Whatman No. 41) and the residue was ignited in the oven to get a constant weight.

Acid-insoluble ash value:- Total ash obtained was heated with addition of 25ml of dil.HCL for 10mins. It was filtered in an ash less filter paper (Whatman No. 41) and the residue was ignited in the oven to get a constant weight.

**Determination of solubility percentage** (Kokate, 1994)

**Alcohol**

5g of powdered material along with 100 ml of alcohol was shaken well occasionally for the first 6 hours and kept undisturbed for 18 hours. The liquified extract thus
obtained was concentrated and the percentage was calculated with the weight of the powder taken.

**Water**

The procedure adopted for the solubility percentage of the plant powder in alcohol is used with water instead of alcohol to get the water solubility percentage

### 3.1.2.5 Physical properties of the extract

The coarsely powdered shade dried rhizome of the plant was extracted sequentially with petroleum ether, bp 40-60 (abbreviated as PE), ethyl acetate (EA) and methanol (Me) in a Soxhlet apparatus. To clarify, the marc (i.e. plant material left after extraction with solvent and air dried) left after extraction with PE was extracted with EA. Finally, the marc left after extraction with EA was extracted with methanol. This led to the production of the three types of extracts namely petrol-soluble fraction (PE), ethyl acetate-soluble fraction (EA) and methanol-soluble fraction (Me). The physical properties like color, pH, density and specific gravity, of these three extracts were then evaluated.

### 3.1.3 Results of the pharmacognostical investigation

**Macroscopical studies**

The macroscopic character was useful in quick identification of plant material and also serves as an important standardization parameter. The organoleptic evaluation of *Alocasia decipiens* Schott rhizome is shown in table 3.1

<table>
<thead>
<tr>
<th>Organoleptic characters</th>
<th>Observed characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Cylindrical</td>
</tr>
<tr>
<td>Color</td>
<td>Brownish</td>
</tr>
<tr>
<td>Odour</td>
<td>Unusual smell</td>
</tr>
<tr>
<td>Size</td>
<td>15-16 cm in length, 7-8 cm in thick</td>
</tr>
</tbody>
</table>
Microscopical studies

The T.S of rhizome shows single layered epidermis; cortex consist of ground tissue, which is a continuous mass of thin walled parenchymatous tissue and is provided with abundant intercellular spaces; vascular bundles are irregularly scattered in the ground tissue, these are conjoint, collateral and closed, each vascular bundle has a xylem towards the centre and phloem towards the periphery. The microphotographs of t.s. of rhizome are shown in fig 3.2
Fig 3.1 (A), (B) Powder microscopy of rhizome of *Alocasia decipiens* Schott

Fig 3.2 (A), (B) Microphotographs of T.S. of rhizome of *A.decipiens* Schott showing vascular bundle (1) scattered on ground tissue (2)
Physicochemical parameters

The results of the physicochemical parameters are given in the table 3.2

Table- 3.2 Physicochemical parameters of rhizome of *Alocasia decipiens* Schott

<table>
<thead>
<tr>
<th>Physicochemical parameters</th>
<th>Values (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>78.59±0.12</td>
</tr>
<tr>
<td>Total ash</td>
<td>3.80±0.20</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>1.76±0.15</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>0.82±0.29</td>
</tr>
<tr>
<td>Water soluble extractive</td>
<td>24±0.56</td>
</tr>
<tr>
<td>Alcohol soluble extractive</td>
<td>08.51±1.11</td>
</tr>
</tbody>
</table>

Physical properties of the extract

The physical properties of the extract are given in the table 3.3

Table- 3.3 Physical properties of the extract of rhizome of *Alocasia decipiens* Schott

<table>
<thead>
<tr>
<th>Extract</th>
<th>Color</th>
<th>pH</th>
<th>Density</th>
<th>Specific gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>Brownish</td>
<td>6.8</td>
<td>0.593</td>
<td>0.619</td>
</tr>
<tr>
<td>EA</td>
<td>Pale yellow</td>
<td>3</td>
<td>0.812</td>
<td>0.848</td>
</tr>
<tr>
<td>Me</td>
<td>Light orange</td>
<td>4</td>
<td>0.718</td>
<td>0.750</td>
</tr>
</tbody>
</table>

3.1.4 Conclusion

Pharmacognostical investigation is the initial step in any research of phytochemistry and drug discovery which includes study of macroscopic characters, microscopic characters, physicochemical parameters etc. of the plant part used. So, before stepping forward to carry other experiments, pharmacognostical investigation of rhizome of *Alocasia decipiens* Schott have been carried out.
3.2 Chemical group test

3.2.1 Introduction

Phytochemicals includes chemical compounds which are chemical present in plants and are biologically active. In plants, they act as a natural defense system and provide color, aroma and flavour. It has been estimated that till date more than 4000 phytochemicals have been discovered and it is expected that in future many more will be discovered (Tripathi et al., 2012). Phytochemicals are protective and disease-preventing particularly for some forms of cancer and heart diseases. The most important action of these chemicals in the body of human beings is that they function as antioxidants that react with the free radicals in our bodies. Free radicals can damage the cells of our bodies and therefore must be removed (Madhavan & Raphael, 2013).

3.2.2 Methodology adopted for chemical group test

3.2.2.1 Protocol for Qualitative chemical group test

To find out the presence of the active chemical constituents in the plant, the following preliminary tests have been done (Siddiqui & Ali, 1997).

**ALKALOID**

Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer’s reagent. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% HCl. After cooling, the mixture was filtered and treated with a few drops of Mayer’s reagent. Yellowish white precipitate confirms the presence of alkaloid.

**FLAVONOID**

4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated HCl was added. Red color was observed for flavonoids and orange for flavones.
GLYCOSIDES

To the solvent free extract, 2ml of distilled water and 2ml of 5% ferric chloride was added. The mixture was allowed to heat on water bath for 15mins., then cooled and lastly benzene was added, shaken. To the separated benzene layer, strong ammonia was added. Pink or red color was observed for the presence of glycosides.

SAPONIN

The solvent free extract was mixed with 20ml water, shaken. Formation of stable foam indicates the presence of saponin.

TANNIN

To 0.5ml extract solution, 1ml water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins.

ANTHAQUINONE

Solvent free extract was treated with 2ml water and few drops of dilute solution of sulphuric acid, heated on a water bath for 10 mins., to that 1ml dilute ammonia solution was added. Rose pink color was observed for the presence of anthraquinone.

3.2.2.2 Quantitative estimation of the chemical constituents

ALKALOID

5g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume, Concentrated ammonium hydroxide was added drop wise to the extract until the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid. It was dried and weighed. The percentage of alkaloid was then calculated (Harborne, 1973).

FLAVONOID

10g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman
filter paper no.42. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight. The percentage of flavonoid was calculated (Bohm & Kocipai-Abyazan, 1994).

SAPONIN

The method used was that of Obadoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

TANNIN

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min. The tannin content was calculated as percentage (Van-Burden & Robinson, 1981).

GLYCOSIDES

The glycosides content of the extract was determined by dissolving 10.0g of the extract in 100ml of 50% H2SO4 in test tubes. The mixture was heated in boiling water for 15 mins. and 10ml of Fehling solution added and the mixture boiled. A red precipitate in extract tested indicated the presence of glycosides. The percentage glycoside was calculated (Malu et al., 2009).
3.2.3 Results of preliminary chemical group test

The results of the chemical group test of the plant are presented below in the table 3.4 & 3.5:

Table- 3.4 Qualitative analysis of phytochemical group present in *Alocasia decipiens* Schott

<table>
<thead>
<tr>
<th>Extract</th>
<th>Alkaloid</th>
<th>Flavonoid</th>
<th>Glycoside</th>
<th>Tannin</th>
<th>Saponin</th>
<th>Anthraquinone</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EA</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ME</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ indicates presence and - indicates absence

Table- 3.5 Quantitative estimation of phytochemical group present in *A.decipiens* Schott

<table>
<thead>
<tr>
<th>Alkaloid (%)</th>
<th>Flavonoid (%)</th>
<th>Saponin (%)</th>
<th>Glycoside (%)</th>
<th>Tannin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.60</td>
<td>7.7</td>
<td>1.40</td>
<td>1.01</td>
<td>1.9</td>
</tr>
</tbody>
</table>

3.2.4 Conclusion

The results of the chemical group test revealed the presence of alkaloid (0.60%), flavonoid (7.7%), glycosides (1.01%), tannin (1.9%), saponin (1.40%) in the rhizome of *Alocasia decipiens* Schott.
3.3 Thin Layer Chromatography Analysis of the extract

3.3.1 Introduction

Chromatography is the separation of two or more compounds or ions by the distribution between two phases, one which is moving (mobile phase) and the other which is stationary (stationary phase). These two phases can be solid-liquid, liquid-liquid or gas-liquid. Although there are many different variations of chromatography, the principles are essentially the same. Thin-layer chromatography or TLC, is a solid-liquid form of chromatography where the stationary phase is normally a polar absorbent and the mobile phase can be a single solvent or combination of solvents. In thin-layer chromatography, the stationary phase is a polar absorbent, usually finely ground alumina or silica particles. This absorbent is coated on a glass slide or plastic sheet creating a thin layer of the particular stationary phase. Almost all mixtures of solvents can be used as the mobile phase. By manipulating the mobile phase, organic compounds can be separated.

3.3.2 Methodology adopted for TLC screening

Each of the three extracts (PE, EA and Me) was checked by Thin Layer Chromatography (TLC) on analytical plates over silica gel (TLC- grade; Merck India). For each extract, four different solvent or solvent systems were used as developing systems. These were petroleum ether, hexane, PE-EA=9:1 and PE-EA=8:2.

3.3.3 Results of TLC analysis

The results of the TLC analysis are given in the table 3.6:
Table 3.6 Retention factor for each of the three extracts of rhizome of *Alocasia decipiens* Schott in different solvent system

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Developing solvent system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Petroleum ether</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>0.21</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>0.27</td>
</tr>
<tr>
<td>Methanol</td>
<td>Not visible</td>
</tr>
</tbody>
</table>

3.3.4 Relevance of TLC analysis

Thin Layer Chromatography analysis of the crude extract is important as it is a simple and quick procedure that is used to determine the number of components in a mixture, the identity of two substances, to monitor the progress of a reaction, to determine the effectiveness of purification, to determine the appropriate conditions for a column chromatographic separation, to monitor column chromatography, etc. Moreover, the behavior of an individual compound in TLC is characterized by a quantity known as Rf (retention factor) and is expressed as a decimal fraction. The Rf is calculated by dividing the distance the compound traveled from the original position by the distance the solvent traveled from the original position (the solvent front). The distance a compound travels indicates that compound's physical characteristics. The greater the similarity to the mobile phase, the further it will be pulled up through the stationary particles on the TLC plate. Therefore, the more a sample's components are like the eluting solvent the closer to a value of one the Rf will be for that component. Hence, known Rf values can be compared to those of unknown substances to aid in their identifications. Rf value is a constant for each component only under identical experimental condition.
References


