CHAPTER – I

PHYTOCHEMICALS

1.1 Introduction

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga et al., 2005). Phytochemicals defined in the strictest sense, as chemicals produced by plants. However, the term is generally used to describe chemicals from plants that may enhance health status of organisms, but are not essential nutrients (Srivastava et al., 2011). There is ample evidence to support the health benefits of the diet in the form of fruits, vegetable, legumes, whole grains and nuts (Mojab et al., 2003). Because plant based foods are complex mixtures of bioactive compounds, information on the potential health of individual phytochemical is linked to information on the health effects of foods that contain those phytochemicals (Manjula et al., 2009).

The most important of these bioactive constituents of plants are alkaloids, tannins, flavanoids and phenolic compounds (Hill, 1952). Many of the indigenous medicinal plants are used as spices and food plants. They are also sometimes added to food for medicinal purposes for pregnant and nursing mothers (Okwu, 1999 and 2001). More than 2000 phytochemicals have been identified from plants (Taiz and Zeiger, 2006). Over 100 plant species are consumed world wide as vegetables, but of these, only about 20 species are grown globally and account for most of the vegetables produced and consumed (Siemonsma and Kasem, 1996). The phytochemical constituents of the medicinal plants were recorded by a number of workers (Joshi, 2000, Syed and Usha, 2005 and Ramasubbu
and Chandra Prabha, 2009). In India thousands of plant especially the angiosperms that all being exploited by the natives in tribal in variety of ways. The most important utilization of these plants is their application in medicines (Camciuc et al., 1998 and Felter et al., 2007).

In general plants contain flavonoids that can either occur as a glycones or as O- or C-glycosides. Recently, flavonoids have attracted interest due to the discovery of their pharmacological activities (Gurib-Fakim, 2006). The other phytochemicals generally present in plants include detoxifying agents like indoles, isothiocyanates, non-starch polysaccharids (NSP) or dietary fiber like gums hemicelluloses, mucilage, pectin, tannins and also alkaloids like coffin and non protein amino acids. They also bind to toxins in the food and helps protect the colon mucus membrane from cancers. In addition, a dietary fiber binds to bile salts (produced from cholesterol) and decreases their reabsorption, thus lower serum LDL cholesterol levels (Liu et al., 2006).

*Thespesia populnea* and *Abelmoschus esculentus* were studied to draw connections between their chemical properties and their use as treatments for conjunctivitis by local people. It was hypothesized that these plants contain active compounds that reduce swelling, enhance antibacterial activity, or act like antihistamines, and that the plants might have similar chemical compositions as seen in respective specific drugs (Bevans et al., 2001).

Flavanoids of popular vegetable have been intensively studied, but little is known about the flavanoid content of underutilized vegetable species. Many of which are partially domesticated or wild (Ray – Yu Yang et al., 2008). Flavonoids are 15-carbon compounds
generally distributed throughout the plant kingdom. To better understand the associations of flavanoids intake and health outcomes, analysis of flavanoids in plant foods, an intense area of research, clinical and epidemiological studies are required (Chun et al., 2007 and Daucher et al., 2006). It has long been recognized that fruits and vegetables are essential to a healthy and well balanced diet required for healthy living. It has also been recognized that high consumption of some fruits and vegetables is beneficial to health in combating the onset of cancer, coronary disease, inflammation, arthritis, immune system decline, brain dysfunction and cataracts (Shui and Lai Pang., 2004).

*Abelmoschus esculentus* L. is widely consumed as a fresh vegetable in both temperate and tropical countries. Although the seed pods are most often used, the mature seed is known to have superior nutritional quality. Rubatzky and Yamaguchi (1997) reported that the seed is a rich source of protein and oil. *A. esculentus* is rich in oleic, linoleic and palmitic acids (Sengupta et al., 1974) and mucilage (Malviya, et al., 2011). The high percentage of linoleic acid (42%) of okra (*A. esculentus*) seed oil and its amino acid pattern of the protein renders it an adequate supplement to legume or cereal based diets (Jambhale and Nerkar, 1998). Ndangui et al. (2010) stated *A. esculentus* seeds could be considered as good sources of protein and minerals and high unsaponifiable content. Phytonutrients provide crucial link between health and nutrition. A well balanced food that is rich in phytonutrients such as fresh fruits, herbs and vegetables can help to minimize free radical and reactive oxygen species (ROS) mediated diseases (Alamgir and Uddin, 2010). For humans, several health beneficial properties of dietary flavanoids are recognized for their antioxidant and anti proliferative effects which may protect the body from various diseases, such as Cancers, cardiovascular disease and inflammatory. (Middleton et al., 2005 and NIjvldt et al., 2001, Wildman, 2001 and Winkel, 2002). Anti
diabetic (Saha et al., 2011), antioxidants and antihypoxic (Shui and Lai Pang., 2004) diuretic activity and dental disease (Ndouenkeu et al., 1996), the role as stimulative, improved vision nature and its preventive role in heart vascular diseases (Kimbonguila et al., 2010) of A. esculentus were well documented.

There is an enormous scope for tribal medicine based on plant products which are yet to be studied, analyzed and documented scientifically (Warrier et al., 1996 and Ramasubbu and Chandra Prabha, 2009). Plant and their parts and the pattern of administration vary from person to person. Hence, in the present study it was aimed to screen the phytoconstituents of A. esculentus.

1.2 MATERIALS AND METHODS

In the present study the plant A. esculentus (SPHB-9) was collected in early morning without much disturbance in and around Sivakasi agricultural field, Virudhunagar (District), TamilNadu, India using sterile polythene bag and knife and immediately transferred to the laboratory for further analysis. The plant material was shadow dried for one week, then it powdered with the help of mixer grinder and used for preparation of plant extraction.

Preparation of extracts

Ten gram of powdered plant material was taken in clean sterile Soxhlet apparatus and extraction was done with 100 ml of different solvents (low polar to high polar) like as hexane, butanol, ethanol, chloroform and water. After extraction the extracts were dried in room temperature until extract reach into solid form. From the solid extract suitable concentrations were made using Dimethyl sulfo-oxide (DMSO) for further analysis.
1.2.1 Preliminary phytochemical screening

Different organic solvent extracts of *A. esculentus* were used to screen the following phytochemicals like sterol, reducing sugar, sugar, alkaloids, phenolic compounds, flavanoids, tannins, saponins, aminoacids, glycosides and terpenoids. Phytochemical tests were carried out on the extract on the powdered specimens using standard procedures (Harborne, 1998). The various extract of *A.esculentus* was subjected to phytochemical tests to screen the following chemicals.

**Steroids**

The test extracts were treated with minimum quantity of chloroform, 3 to 4 drops of acetic anhydride and one drop of concentrated sulphuric acid. Purple color of the test content was changed into blue green. The result was qualitatively determined and recorded.

**Reducing sugar:**

The test extracts were treated with 2ml of Fehling’s reagent and 3ml of water. The test content was boiled and the development of red orange colour indicated the presence of reducing sugar.

**Sugar**

The test extracts were treated with minimum quantity of anthrone and a few drops of concentrated sulphuric acid and then heated. Change color from green to purple showed the presence of sugar.

**Alkaloids**

Aqueous layer was formed when the test extracts were treated with 2N hydrochloric acid. It was decanted and to which one or two drops of Mayer’s reagent was
added. The test content was changed into white turbidity or precipitate which indicated the presence of alkaloids.

**Phenolic compounds**

The test extract in alcohol was treated with a drop of neutral ferric chloride. Change of intense colour in the test content, shows positive result for phenolic compounds.

**Flavonoids**

A bit of magnesium and then one or two drops of concentrated hydrochloric acid were added. To the plant extract was heated and the development of red or orange color indicated the presence of flavonoids.

**Tannins**

The test extracts were treated with water and basic lead acetate. The presence of tannins in the test solution was noticed by the development of white ppt.

**Saponins**

The test extracts were treated with water shake well. The test solution was changed into foamyleather, if the test sample contains saponins.

**Amino acids**

After treating extract with ninhydrin in alcohol. The violet colour formed confirmed the presence of aminoacids.
Glycosides

Three mg of the extract was mixed with equal quantity of anthrone and treated with two drop of Conc. Sulphuric acid and heated gently on a water bath. Development of dark green colour indicated the presence of glycosides.

Terpenoids

Four mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid.

1.2.2 Thin Layer Chromatography (TLC)

TLC is a modified form of paper chromatography. Here, the adsorbent was spread over a supporting material (glass sheet) to form a thin layer of adsorbent rather than a paper. To achieve this, some material was added to bind the adsorbent particles together. Alternatively, the size of the particles used was so chosen that adhere to each other by physical forces. Once the sheet was coated and allowed to dry, the spotting of sample, developing in the solvent and detection of spots by spraying, were the same as in paper chromatography.

Thin layer plate preparation

The stationary phase was prepared as a slurry using silica with water or buffer at 1:2 and applied to a glass plate using a TLC applicator, (0.25 mm thickness for analytical separations and 2.5 mm thickness for preparative separations were prepared). Calcium sulphate, CaSO$_4$ $\frac{1}{2}$H$_2$O, (Gypsum) (10-15%) is incorporated to the adsorbent. After application of the adsorbent, the plates were air-dried for 10 - 15minutes and then oven-
dried for 10 -15 min at 100° C -110° C. This process was also known as activation of the adsorbent. The plates were used immediately or stored in desiccators.

**Sample application**

A line was drawn lightly with a pencil of about 1.5 - 2.0cm from the bottom. A scale was placed at the bottom and spotted at a distance of 1.5 cm (approx). The sample was spotted using capillary tubes.

**Plate development**

The chromatographic tank was filled with the developing solvent to a depth of 1.5 cm and equilibrated for about 60 min. Care was taken to place the plate so as the spot not touched the solvent directly. Due to capillary action the solvent ascendent and the separation of compounds was taken place. As the solvent front reaches about 1 - 2cm from the top of the plate, the plate was removed, solvent front was marked with a pencil immediately and allowed to air-dry by placing the plate upside down.

**Component detection**

For the detection of phytochemical component, the developed plates were exposed in iodine chamber. The numbers of spots were noted (Table 1.2.).

**Solvent system**

1. 96% Ethanol /Water (7:3)
2. Chloroform/Methanol/17% NH₄OH (2:2:1)
3. n-Butanol / Acetic acid /Water (8:2:2)
Spray Reagent

Spray the plate with 50% H₂SO₄, incubate for 20min (approx) in an oven. Violet spots are seen, or incubate the plate in desiccators containing a few crystals of iodine in a beaker. Aqueous slurry of gel was prepared by 30g of silica gel and 500mg of calcium sulphate in about 50 ml of water. This was coated uniformly over the glass plate. The coated plate was activated at 100° C for 1 hour in hot air oven and allowed to cool. Amino acid samples were spotted on the base of the plate. Then the plate was placed in the chamber, which is saturated with solvent, and the chromatogram was developed until the solvent front was close to the top of the plate. A line was drawn across the plate at this point and the chromatogram was removed and the plates were exposed in iodine chamber.

Calculation:

\[ R_f \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by the solvent}} \]

1.2.3 NMR Spectroscopy

The isolated compounds were identified using NMR spectroscopy. \(^1\)H NMR spectrum was recorded on a Bruker Advance 300 spectrum was operating at 300MHZ \(^1\)H.

Spectral data:

The Compound – 1 obtained by column chromatography method using Etanol and petroleum diethyl ether solvent. The active compound was separated in 60-70 fractions. Compound obtained from fraction 60:70 (Ethanol:Petroleum diethyl ether) was with brownish white.
RESULTS AND DISCUSSION

1.2.3 Phytochemicals

Hexane, butanol, ethanol, chloroform and aqueous extracts of the powdered *A. esculentus* were subjected to standard chemical test for the detection of different phytoconstituents. Results on the presence and absence of steroids, reducing sugar, sugars, alkaloids, phenolic compounds, flavonoids, tannins, saponin, aminoacids, glycosides and terpenoids were reported (Table 1.1). Among all the tested phytochemicals in test sample the composition arranged in the order of terpenoids, saponins, steroids, sugar, tannin, phenolic compound, aminoacids, alkaloids, flavanoids and glycosides. Similar phytoconstituents in the crude extracts were recorded in various plants by Edeoga *et al.*, 2005. The reducing sugar was non detectable in the fruit extracts of *A. esculentus*, of the present study. Similar report was recorded by Ganpati and Nivruti, 2010) in *Memecylon umbellantumburm*. Same kind of phytoconstituents were reported in *Lantana camara* due to various extracts (Barre *et al.*, 1997) aqueous extract of various plants (Edeoga *et al.*, 2005) and in the ethanol extract of *A.aspera* (Manjula *et al.*, 2009).
Table 1.1: Preliminary phytochemical investigation of various extracts of tender fruits of *A. esculentus*.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytoconstituents</th>
<th><em>A. esculentus</em> fruit extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
</tr>
<tr>
<td>1.</td>
<td>Sterols</td>
<td>++</td>
</tr>
<tr>
<td>2.</td>
<td>Reducing Sugar</td>
<td>_</td>
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<tr>
<td>3.</td>
<td>Sugar</td>
<td>_</td>
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<tr>
<td>4.</td>
<td>Alkaloids</td>
<td>_</td>
</tr>
<tr>
<td>5.</td>
<td>Phenol</td>
<td>++</td>
</tr>
<tr>
<td>6.</td>
<td>Flavanoids</td>
<td>_</td>
</tr>
<tr>
<td>7.</td>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>8.</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Amino acid</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Glycosides</td>
<td>_</td>
</tr>
<tr>
<td>11.</td>
<td>Terpenoids</td>
<td>+</td>
</tr>
</tbody>
</table>

**Note:** ++ indicate: Highly presence, + indicate: Presence, - indicate absence

Generally it was found that all the plants contain sterols and tannins. It should be noted that sterol compounds were of important and interest in pharmacy due to the relationship of such compounds with sex hormones (Okwu, 2001). In the present study also in all extracts of *A. esculentus* except butanol showed the presence of sterols.

The presence of terpenoids in various plants was reported by various researchers (Scortichini and Rossi, 1991; and Tsuchiya *et al.*, 1996) and that plants are widely used in herbal medicines (Hayashi *et al.*, 1993 and Edeoga *et al.*, 2005). Flavonoids have a number of nutritional functions and have been described as biological response modifiers’ most act as a antioxidant and some have a anti inflammatory properties (Tullanithi *et al*.2010)
From the present study it was concluded that the medicinal herb *A. esculentus* was found to rich source of phytochemical contents. This plant is also used to inhibit pathogens due to the presence of ample concentration of phytochemical, and that may be the reason responsible for its medicinal properties. *A. esculentus* belongs to the native of India, people use it for local pharmacopoeias and is also one of the economically important marketable vegetable. Scientific research proved the presence of rich phytochemical compounds in *A. esculentus* (Ndangai *et al.*, 2010). In the present study, the ethanol extract of *A. esculentus* found to have more amount of flavonoids.

1.3.2 TLC Profile of plant extracts:

TLC revealed the polarity of the chemical composition of the fruits of *A. esculentus* plants. The $R_f$ values show the complexity and diversity between the chemical profile of the fruit extracts of each solvents (Table 1.2). Each value corresponds to a chemical compounds spot seen on the TLC plate. Similar $R_f$ values seen in other part of the *A. esculentus* and *Thespesia populnea* indicating that several compounds have similar polarity and are found throughout a plant (Akhill and Rani, 1993 and Bevans *et al.*, 2001).

TLC profile of plant was studied using the different solvents namely hexane, butanol, ethanol, chloroform and water. The study revealed the polarity of the chemical composition of the tender fruits *A. esculentus*. The $R_f$ values of the present study showed the complexity and diversity between the chemical profile of the fruit extracts of each solvents and the each value corresponds to a chemical compound (Table 1.2).
Table 1.2: Separation of phytoconstituents in tender fruit of *A. esculentus* using various extract by TLC method.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Solvent system</th>
<th>No.of.Spots</th>
<th><em>A. esculentus</em> fruit extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hexane</td>
<td>Butanol</td>
<td>Ethanol</td>
</tr>
<tr>
<td>1.</td>
<td>Toluene – ethyl formate – formic acid (5:4:1)</td>
<td>R_f values</td>
<td>0.14</td>
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<td></td>
<td></td>
<td></td>
<td>0.17</td>
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<td>0.12</td>
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<td>0.15</td>
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</table>

In the present study, *A. esculentus* extracted with hexane, butanol, ethanol, chloroform and water showed different R_f values (Table 1.2). On comparing the R_f values of the various spots in different solvent system with standard R_f values, the various plant extracts may contain the phytoconstituents such as flavonoids and saponin. The result of the present study was similar to that of Tullanithi *et al.*, (2010). The various spots obtained with different solvent extracts like hexane, butanol, ethanol, chloroform and water were 3, 3, 4, 2 and 3 respectively. Similar results were reported by Mallikharjuna *et al.*, (2007) in the phytochemical studies on *Strychynos potatorum*.

Phytochemical analysis of the active extracts demonstrated the presence of common phytoconstituents like phenols, tannins, glycosides, flavonoids and alkaloids. The presence of these compounds was also detected by thin layer chromatography (TLC). TLC analysis also differentiated between monomeric and dimeric forms of flavonoids. Phytochemical analyses of the
The pytochemical present in the plant extract was confirmed as flavonoid group of compounds. The following structure was proposed for the compound isolated from *A. esculentus*. Similar group of flavonoids were reported in the leaves of *Urena lobata* belong to the same family (Adeloye et al., 2007).
Figure 1.1 Flavonoid compound obtained in the ethanol extract of *A. esculentus*.

3-hydroxy-2-(4-methoxycyclohexyl)-8-[[3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-2-pyranyl]oxy]-4H-4-chromenone