Chapter 1
Anti-Obesity Plants

Managing obesity without side effect is a real challenge. Medicinal plant preparations may enhance satiety, boost up metabolism, and also speed up weight loss. Herbs are most effective in the fight against obesity. They help in inhibition of lipase, down-regulation of adipogenesis, thermogenesis, metabolism of lipids and modulation of various signalling pathways leading to weight loss. Some anti-obesogenic herbs include *Acacia arabica*, *Aconitum heterophyllum*, *Aloe vera*, *Azadirachta indica*, *Betula utilis*, *Cinnamomum zeylanicum*, *Emblica officinalis*, *Moringa oleifera* Camellia sinensis, *Garcinia cambogia*, *Momordica charantia*, *Tinospora cordifolia*, *Curcuma longa*, *Cinnamomum zeylanicum*, *Trigonella foenum graecum*, *Zingiber officinals*, *Terminalia arjuna* and *Piper longum* Etc., (Chandrasekaran et al., 2012). Foods like cucumber, watermelon, oranges and grape fruit are extremely low calorie food, which keeps the stomach full for a longer time. Cucumber and water melon also belongs to cucurbitaceae family. So further study was performed with cucurbitaceae family with *Benincasa hispida* fruit.

*Benincasa hispida*

*Benincasa hispida cogn.* belongs to cucurbitaceae commonly known as wax gourd, ash gourd and winter melon. It is an important ingredient in kushmanda lehyam, in Ayurvedic medicine. It is used in the treatment of epilepsy and nervous disorders. *Benincasa hispida was* used for treatment of Schizophrenia and other psychological disorder (Sharma, 2005). *Momordica charantia* and *Lagenaria siceraria* fruits also belongs to cucurbitaceae family. These plants contains saponins and triterpenoids, which is reason for antihyperlipidemic and cardioprotective activity.

Crude drug which is obtained from many sources are subjected to pharmacognosy study to reveal the phyto-constituents. Pharmacognostical study is preliminary study for standardization of crude drugs. This study gives valuable information about crude drugs. The number of crude drugs from plant source has not been scientifically identified. Hence pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs.

Pharmacognosy is the branch of pharmacology. It deals with the study of medicines derived from natural sources. Natural sources are animals, microorganism
and plant species. Pharmacognosy is known as material medica. The word pharmacognosy is from Latin word. This word from ‘Pharmakon’, ‘a drug’, and gignosa. It means knowledge of science. The term "pharmacognosy" was first coined by the Austrian physician Schmidt in 1811 and 1815 by Crr. Anotheus Seydler in a work titled Analecta Pharmacognostica.

The growth of modern pharmacognosy was after the period 1934-1960 and branched into different disciplines like organic chemistry, biochemistry, biosynthesis, Pharmacology and technique of analytical chemistry including paper, thin layer, gas chromatography and spectrophotometry. Traditional Indian system of Ayurveda uses 85% of crude plant formulations for the treatment of various diseases. It has been currently derived from plant. These crude drugs are also used in cosmetic, textile and food industry.

Pharmacognosy includes identification of biological sources, geographical source, cultivation, collection and preparation, morphological character, microscopic character, identification of chemical constituent present in it, identification of its uses, adulterants and chemical tests.

Pharmacognostic study is as follows,

- Identification of biological sources
- Geographical source
- Cultivation collection and preparation
- Morphological character
- Microscopic characters
- Chemical constituents
- To identify the uses.

Phytochemicals are naturally occurring biologically active chemical compounds present in plants. It is abundant in fruit, vegetables, grains and other plant foods. More than 4000 compounds have been identified till date.

**Phytochemicals**

Phytochemicals is derived from the Greek word phyto which means plant. They are biologically active naturally occurring chemical compounds found in plants. This non-nutritive plant chemicals are either defensive or have disease protective properties against many diseases. Plants contain important phytochemicals like alkaloids, flavonoids, tannins, glycosides, phenolic compounds and saponins (Hussain et al.,...
Phytochemicals can be classified as carotenoids, phenolics, alkaloids, nitrogen containing compounds and organo sulfur compounds.

**Phenolics**

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups and are categorized as phenolic acids, flavonoids stilbenes, coumarins and tannins. There are three important groups of dietary phenolics which are flavonoids, phenolic acids, and polyphenols. A phenolic acid includes the widely distributed hydroxybenzoic and hydroxycinnamic acids. Phenolic polymers, commonly known as tannins, are compounds of high molecular weight that are divided into two classes hydrolyzable and condensed tannins.

Poly phenols are secondary metabolites mainly responsible for antioxidant property. Poly phenols contain two phenol subunits which are known as flavonoids and compound possessing three or more phenol subunits are called as tannins (hydrolysable and non-hydrolysable). Glycosides and esters are the functional derivatives of polyphenols that have one or more phenol groups in aromatic ring substituted with one hydroxyl group (*Harborne, 1989*).

Polyphenols have high free radical scavenging activity, which helps to reduce the side effects of diseases like neurodegenerative diseases, cardiovascular, cancer, liver cirrhosis and hepatitis (*Ames et al., 1993*). Phenols act as antioxidants (*Okuda and Ito, 2011*) based on hydrogen atom donating capacity.

**Carotenoids**

Carotenoids are organic pigments that are found in the chloroplasts of the plant. Carotenoids play an important role in photosynthesis and act as antioxidants. Carotenoids are tetra terpenoids (*Callow, 1936*). Carotenoids from the diet are stored in the fatty tissues of animals.

**Phenolic acids**

Phenolic acids are divided into two major groups, hydroxybenzoic acids and hydroxycinnamic acids. Hydroxy benzoic acid derivatives include p-hydroxy benzoic, protocatechuic, vanillic, syringic and gallic acids. They have complex structure like lignins and hydrozable tannins. Caffeic, ferulic acid, p-coumarics, procatechuic and vanillic acid are present in all type of plants. Curcumin is made up of two ferulic acids linked by methylene in a diketone structure and it is the major yellow pigment of
mustard. The simple phenolic acids are chlorogenic, coumaric, ferulic, gallic, gentisic and caffeic acids.

**Stilbene**

Stilbene is diarylethene that is a hydrocarbon consisting of a Trans ethane double bond substituted with a phenyl group. The name stilbene is derived from the Greek word stilbos. Stilbene are present as stilbenoids in plants. Stilbene is used in the production of dyes and optical brighteners.

**Coumarins**

Coumarin is a fragrant organic compound in the benzopyrone chemical class. Coumarins are colourless substance used in certain perfumes and also used as fabric conditioner. Coumarins have bitter taste and can act as appetite suppressant.

**Tannins**

Tannins are a type of poly phenols. The multiple phenolic hydroxyl groups of tannins lead to the formation of complexes with proteins and lesser with metal ions, amino acids and polysaccharides. Tannins are divided into gallo tannin, ellagi tannins, complex tannins and condensed tannins. Tannins and flavonoids have been shown to have vital health protective benefits, which include lowering of blood lipids (Manach, et al., 2004). Tannin also has antioxidant or free radical scavenging activity and it plays an important role in promoting wound healing (Potterat, et al., 1997). Tannins, flavonoids and steroids show antibacterial activities (Lei et al., 2007; Prabavathy, 2013). Tannins exhibit significant inhibitory effect on pancreatic lipase activity and fat absorption from the intestine. Tannins also act as strong antibacterial, antiulcer, anti-inflammatory, anti-leishmanial, antimutagenic, enzyme regulating, blocking signal transduction pathways and apoptotic activities. Researches are carried out to exploit the benefit of tannin rich plants and agro industrial products in livestock feed. Some tannins are known to have strong anticarcinogenic and antioxidant activities.

**Flavonoids**

Flavonoids are widely distributed in plants and compared to other active plant compounds, they are less toxic. They act as biological response modifiers because of their inherent ability to modify the body’s reaction to allergens, viruses and carcinogens. Flavonoids like...
- **Flavones**
  Flavones are class of flavonoids. Flavones have backbone of 2-phenylchromen-4-one. Apigenin, chrysin and luteolin are flavones.

- **Flavonols**
  Flavonols are a type of flavonoids that have 3-hydroxyflavone as backbone. Flavonols are present in all types of fruits and vegetables. Quercetin, Kaempherol, myricetin, galangin and fisetin belongs to flavonols.

- **Flavanones**
  Flavanones are the type of flavonoid. They are glycosylated by a disaccharide at seventh position and give rise to flavanone glycosides. Flavanones are eriodictyol, Hesperidin and naringenin.

- **Flavanols**
  Flavanols contain catechin, epicatechin, epigallocatechin and gallate.

- **Isoflavonoids**
  Isoflavonoids are one of the categories of flavonoid phenolic compounds, which are biologically active. Iso flavonoids and their derivatives are phytoestrogen. Genistin, Daidzein, Glycitein and formononetein belongs to isoflavonoids.

- **Anthocyanidins**
  Anthocyanidins are common plant pigments. Cyanidin, pelargonidin and malvidin belongs to anthocyanidins.

  The flavonoids show anti-allergic, anti-inflammatory, antimicrobial (Rauha, et al., 2000; Cushnie and Lamb, 2005) and anticancer activity (Pouget et al., 2001). Flavonols and flavones are usually present in plants as glycosides. Flavonoids also have antioxidant property against reactive oxygen species (ROS).

- **Terpenoids**
  Terpenes originate from ‘turpentine’. Turpentine means resin of pine trees. It is otherwise known as terpenoids or isoprenoids. Natural products consist of 36,000 terpenes structure approximately. It plays a major role in plant processes such as growth, development, reproduction and defence mechanism. The classification of terpenoids is based on the number of isoprenoid units present in their structure. The largest classification is based on the compounds with two (monoterpenes), three (sesquiterpenes), four (diterpenes), five (sesterterpenes), six (triterpenes), and eight
(tetraterpenes) isoprenoid units. Terpenes are found to possess unique antioxidant activity when they interact with free radicals (Prakash et al., 2004; Prakash and Kumar, 2011; Nichenametla et al., 2006; Stahl, 2005).

**Alkaloids**

The alkaloids are alkaline in nature. About 21,000 alkaloids have been identified. Alkaloids contain basic nitrogen atoms and are produced by a large variety of organisms including bacteria, fungi, plants, and animals. Many alkaloids are toxic and often have a pharmacological effect, some alkaloids have a bitter taste Alkaloids have antimicrobial activity by inhibiting the DNA topoisomerase in the microorganisms (Suriyavathana and Praveena, 2013).

**Saponins**

Saponins are the class of secondary metabolites found in various plant species, and they are glycosides grouped by soap like foaming they produce when shaken in aqueous solutions and are composed of one or more hydrophilic glycoside moieties combined with lipohilic triterpenoid derivative (Hostettmann and Marston, 1995).

Saponins are the glycosides of 27 carbon atom steroids, or 30 carbon atom triterpenes in plants. They are found in various plant parts; leaves, stems, roots, bulbs, flowers and fruits. They are characterized by their bitter taste. Diet contains Saponins which have the property to decrease the plasma cholesterol level and also increase the bile acid production. Cardiac glycosides influence the sodium and potassium ion movement in cardiac membrane. It is also inhibits ATP-ase activity via regulation of sodium potassium pump. Saponins are believed to be useful in the human diet for controlling cholesterol and also strengthen the heart muscle causing the heart to pump more efficiently.

**Steroids**

Plant steroids are important cardiotonic and have insecticidal and anti-microbial properties. They are also used in herbal medicine and cosmetics. Several hundred of plant species is around the world. But less proportion of plants are investigated both phytochemically and pharmacologically. But still it is a big task for identification, characterization and isolation of bioactive compounds as plant contain thousands of plant constituents. It is essential to have efficient systems available for the rapid chemical and biological screening. For standardizing herbs and herbal formulation based on the chromatography techniques, chromatography techniques such as High
Performance Liquid Chromatography (HPLC), High-Performance Thin layer Chromatography (HPTLC), Thin layer chromatography (TLC) and Gas chromatography mass spectrum analysis are used for the identification of plant compounds.

**High-performance liquid chromatography-HPLC**

High-Performance Liquid Chromatography (HPLC) is a Quantitative method that is used mainly for the identification of volatile compounds. HPLC is an analytical technique that is used to separate the components in a mixture, to identify each component, and to quantify, each component. This technique is used for the detection of secondary metabolites in plants and also used for the detection of Vitamin D in serum and also used in pharmaceutical industries for production of bioactive compounds (Gerber et al., 2004). This method is rapid, reproducible, accurate and provides the optimum peak shape and theoretical plate number. According to the literature survey this method is used for diagnosis of serum, urine, fluids and tissue samples. Hormones like corticosterol is also identified using this method.

The aim of this chapter is to study the pharmacognostic features and carry out phytochemical analysis of *Benincasa hispida* fruit.

**Materials and Methods**

**Chemicals**

All the chemicals and solvents were purchased from Sigma – Aldrich, USA, Ltd.

**Collection of plant materials**

Fruit specimen of *Benincasa hispida* was collected from medicinal plant vendor. Healthy fruit was selected and selected fruit was authenticated. The *Benincasa hispida* fruit was authenticated by Dr. P. Jayaraman, Director of National Institute of Herbal science, Plant Anatomy Research Centre, Pharmacognosy Institute, Medicinal plant Research Unit, Chennai (Reg.No. PARC/2012/1225).

**Macroscopic and Microscopic studies of *Benincasa hispida* fruit extract**

The characteristics of the fruit such as colour, odour, taste, nature, texture were studied for morphological investigation.

**Collection of specimens**

The care was taken while selecting the healthy fruit. Rind and seeds were removed from the fruit and small piece were cut, from pulp and fixed in a FAA (mixture of Formalin-5ml and 70% Ethyl alcohol-90%). After 24 hours fixing, the specimens were dehydrated with the series of tertiary-Butyl alcohol as per the schedule given by
Sass, 1980. Infiltration of the specimens was carried out by the gradual addition of paraffin wax (melting point 50-60°C) until TBA solution attained super saturation. The specimen was cast into paraffin blocks.

**Sectioning**

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12µm. Dewaxing procedure was followed by customary procedure (Johansen, 1940). Then the section was stained with Toluidine blue (polychromatic stain) (O’Brien, 1964). The dye renders pink colour to the cellulose wall, blue to the lignified cells, dark green to suberin, violet to the mucilage and blue to the protein bodies.

**Photomicrographs**

Microscopic appearance of the specimen was done with micrographs. Photographs of the specimen were taken with Nikon lab photo 2 microscopic units. For normal observations, bright field was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnification is indicated by the scale bars in the figure. This procedure was done by standard methods (Esau, 1964).

**Preliminary phytochemical and pharmacognostic analysis**

**Physicochemical parameters**

The ash values, extractive values and loss on drying were performed according to the methods prescribed in Indian pharmacopeia (Indian Pharmacopoeia, 1996).

**Ash value**

Ash content is taken by incineration of the powder sample. Ash content usually represents the inorganic salts naturally occurring in the drug and adhering to it. Ash content varies based on the drug.

**Determination of Total ash**

Totally 2g of the powdered drug was weighed in silica crucible which was previously ignited and weighed. This powder was spread over the crucible. The crucible was incinerated at a temperature of 450°C until free from carbon. Then it was cooled and weighed. The procedure was repeated to get the constant weight.
Calculation

\[
\% \text{ of total ash} = \frac{\text{Weight of ash}}{\text{Weight of the sample}} \times 100
\]

Determination of acid insoluble ash:

The ash was boiled with 25 ml of concentrated hydrochloric acid for 5 minutes. The insoluble ash was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into pre-weighed silica crucible. The percentage of the acid insoluble ash was calculated.

Calculation

\[
\% \text{ of acid insoluble ash} = \frac{\text{Weight of acid insoluble residue}}{\text{Weight of the sample taken}} \times 100
\]

Extractive value

Alcohol soluble extractive value

5gm of air dried coarse powder of *Benincasa hispida* was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours. Shaking was done frequently at the intervals of 6 hours. It was filtered rapidly. Care was taken against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tare flat bottomed shallow dish dried at 105°C and weighed. The percentage of ethanol soluble extractive with reference to the air dried drug was calculated.

Calculation

\[
\% \text{ ethanol soluble extract} = \frac{\text{Weight of extract obtained}}{\text{Weight of the sample taken}} \times 5 \times 100
\]

Water soluble extractive value

5gm of fruit powder of *Benincasa hispida* was taken in closed flask, 95ml of water and 5 ml of chloroform was added. This was kept for 24 hours, shaking frequently for 6 hours and allowed to stand for 18 hours. It was filtered rapidly. Care was taken against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tare flat bottomed shallow dish dried at 105°C and weighed. The percentage of water soluble extractive with reference to the air dried drug was calculated.
Calculation

\[
\% \text{ Water soluble extract} = \frac{\text{Weight of extract obtained}}{\text{Weight of the sample taken}} \times 100
\]

Extraction

Preparation of extract

The *Benincasa hispida* fruit skin was peeled off and seeds were removed. The fruit pulp was taken, cut into pieces, dried under shade, segregated, pulverized by mechanical grinder and passed through a 40 mesh sieve. The powder was extracted with n-hexane, ethyl acetate, ethanol and water using soxhlet apparatus and concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. The extract was stored in a glass bottle in refrigerated condition throughout the period of experiment.

Preliminary phytochemical screening

Phytochemical analysis was done using powdered specimens with standard procedures to identify the constituents as described by Sofowara, 1993, Trease and Evans, 1989 and Harborne, 1973.

**Test for Carbohydrates**

To 2 ml of plant extracts, 1ml of Molisch’s reagent and few drops of concentrated sulphuric acid was added. Purple colour formation indicated the presence of carbohydrates.

**Test for Terpenoids**

To 0.5 ml of the plant extracts, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Red brown colour formation at the interface indicated the presence of terpenoids.

**Test for Triterpenoids**

To 1.5 ml of the plant extract, 1ml of Libermann - Buchard Reagent (acetic anhydride and concentrated sulphuric acid) was added. Blue or green colour formation indicated the presence of triterpenoids.

**Test for Phenols**

To 1 ml of the plant extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of green colour indicated the presence of phenols.
Test for Tannins

To 1 ml of the plant extract, 2 ml of 5% ferric chloride was added. Formation of greenish black colour indicated the presence of tannins.

Test for Saponins

To 2 ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicated the presence of saponins.

Test for Flavonoids

5ml of dilute ammonia solution was added to a portion of aqueous filterate of plant extract followed by concentrated sulphuric acid. Appearance of yellow coloration indicated the presence of flavonoids.

Test for Alkaloids

To 2 ml of the plant extracts, 2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer’s reagent were added. Presence of green colour indicated the presence of alkaloids.

Test for Anthocyanins and Betacyanins

To 2 ml of the plant extracts, 1ml of 2N sodium hydroxide was added and heated for 5 minutes at 100°C. Formation of yellow colour indicated the presence of betacyanins.

Test for Quinones

To 1.0 ml of the plant extracts, 1 ml of concentrated sulphuric acid was added. Formation of red colour indicated the presence of quinones.

Test for Glycosides

To 2.0 ml of the plant extracts, 3 ml of chloroform and 10% ammonia solution was added. Pink colour formation indicated the presence of glycosides.

Test for Cardiac Glycosides

To 0.5 ml of the plant extracts, 2 ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was layered with 1ml of concentrated sulphuric acid. Brown ring formation at the interface indicated the presence of cardiac glycosides.
Test for Steroids and Phytosteroids

To 2.0 ml of the plant extract, 5 ml of chloroform was added and filtered; 2 ml of acetic anhydride was added to 2 ml of the filtrate with 2 ml of sulphuric acid. The colour changes from violet to blue or green and this indicates the presence of steroids.

Test for Phlobatannins

To 1.0 ml of the plant extract, few drops of 10% ammonia solution were added. Appearance of pink colour indicated the presence of phlobatannins.

Test for Anthraquinones

To 1.0 ml of the plant extract, few drops of 2% Hcl were added. Appearance of red colour precipitate indicates the presence of anthraquinones.

Test for Coumarins

To 1 ml of the plant extract, 1 ml of 10% sodium hydroxide was added. Formation of yellow colour indicated the presence of coumarins.

Test for Proteins and Aminoacids

To 2.0 ml of the plant extract, few drops of 0.2% Ninhydrin was added and heated. Purple colour formation indicated the presence of aminoacids and proteins.

Quantitative phytochemical analysis

Sample Preparation

100mg of ethanol, ethyl acetate and aqueous extract of Benincasa hispida was weighed, dissolved and made upto 100 ml with ethanol, ethyl acetate and water in a different standard flask (concentration: 1mg/ml). This was used for further study.

Estimation of Total Phenols

The total phenol content present in extracts of Benincasa hispida was determined by the method of Yu et al., 2002.

Reagents

- Standard gallic acid (1mg/ml)
- Folin ciocalteau reagent (1:2)
- 2% Sodium carbonate

Procedure

Extracts (100μL) was mixed thoroughly with 2 ml of 2% Na₂CO₃. After 2 minutes 100μl of Folin-Ciocalteau reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was
measured at 743 nm against the blank. Same procedure was followed for standard (gallic acid). The values were expressed in mg/g of sample.

**Estimation of Total Flavonoids**

The flavonoid content present in extracts of *Benincasa hispida* was determined by the method of **Chang et al., 2002**.

**Reagents**

- 2% aluminium chloride
- Ethanol
- Sodium acetate
- Standard Quercetin (1mg/ml)

**Procedure**

To 1 ml of extract, 3 ml of methanol, 0.2ml of 1 M potassium acetate, 0.2ml of 10% aluminium chloride and 5.6ml of distilled water was added and left at room temperature for 30 minutes. Absorbance of the mixture was read at 415 nm using UV spectrophotometer. Calibration curve was prepared using quercetin, as standard. The values are expressed as mg/g of sample.

**Estimation of Tannins**

The tannin present in extracts of *Benincasa hispida* was determined by the method of **Peri and Pompei, 1971**.

**Reagents**

- 35% Sodium carbonate
- Folin ciocalteau reagent(Diluted in the ratio of 1:2)
- Standard gallic acid (1mg/ml)

**Procedure**

1 ml of extract was taken. The volume was made upto 1 ml with distilled water and 1 ml of water serves as the blank. To this 0.5 ml of folin ciocalteau phenol reagent (1:2) followed by the 5 ml of 35% sodium carbonate was added and kept at room temperature for 5 minutes. Blue colour formed was read at 640nm. A standard graph (gallic acid) was plotted, from which the tannin content of the fractions was determined. The total tannin content was expressed in mg/g of extract.

**Estimation of Terpenoids**

The terpenoids present in extracts of *Benincasa hispida* was determined by the method of **Han Chien et al., 2009**.
Reagents

- Vannilin/glacial acetic acid (W/V)
- Perchloric acid
- Ursolic acid (1mg/ml)

Procedure

0.2 ml of extract was evaporated by placing the tubes in boiling water bath and to the residue, 0.3 ml of vannilin/glacial acetic acid (W/V), 1 ml of perchloric acid was added and incubated at 60°C for 45min. Tubes were cooled in ice and to the mixture 5ml of glacial acetic acid was added and the colour intensity was measured at 548nm using spectrophotometer. Vannilin/glacial acetic acid, perchloric acid and the solvent alone served as blank. Standard curve was drawn using ursolic acid and terpenoid content expressed as mg/g of extract.

Determination of Saponins

The saponin content present in extracts of *Benincasa hispida* was determined by the method of Han Chien et al., 2009.

Reagents

- 80% aqueous methanol
- Vanillin reagent (8%, w/v in 99.9% ethanol)
- Sulphuric acid (72% v/v)
- Saponin (1mg/ml)

Procedure

10mg of extract was dissolved in the 5 ml of 80% aqueous methanol and 50 µl of solution was taken in each test tubes. To this 0.25 ml of vanillin reagent (8% in 99.9% ethanol) was added. All the tubes were placed in ice-cold water bath and 2.5 of 72% sulphuric acid was added to the wall of the test tube. The content was mixed, kept for 3minutes and then tubes were kept in 60°C for 10 minutes. The colour developed was measured using spectrophotometer against reagent blank. Saponin was used as standard, and same procedure is followed for standard also.

Estimation of Alkaloids

The alkaloid content present in ethanolic extract of *Benincasa hispida* was determined by the method of Harbone, 1973.

Reagents

- 20% Acetic acid in ethanol(v/v)
Ammonium hydroxide

Procedure

5g of sample was weighed into 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 hours. This was filtered and the extract was concentrated using a water bath to one quarter of original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration. The weight of the precipitate noted and expressed as mg/g of sample.

Nutritional Evaluation of Benincasa hispida.

Estimation of Total Protein

The protein content present in ethanolic fruit extract of Benincasa hispida was determined by the method Lowry et al., 1951.

Reagents

- Standard protein(BSA) solution(100µg/ml)
- Alkaline copper reagent
  - Reagent A: 2% sodium carbonate in 0.1% sodium hydroxide
  - Reagent B: 0.5% copper sulphate in 1% sodium potassium tartrate.

Before the experiment 50 ml of reagent A and 1ml of reagent B were mixed

- Folin–Ciocalteau reagent(1:2)

Procedure

0.2-1.0 ml of standard solution containing 20-100mg of protein was taken in different test tubes. Each test tube was made up to 1ml with water. 1ml of water alone served as blank. 5 ml of alkaline copper reagent was added to all the tubes, and allowed to stand for 10 minutes. 0.5 ml of folin ciocalteau reagent was added to all the tubes. Then tubes were incubated at room temperature for 30 minutes at room temperature. 0.1 ml of sample was treated similarly. The colour developed was measured at 680nm. The protein content was expressed as mg/g of extract.

Estimation of Total Sugars

The sugar content present in ethanolic fruit extract of Benincasa hispida was determined by the method Morales et al., 1973.

Reagents

- Standard glucose solution(100µg/ml)
0.2% Anthrone reagent

Procedure

0.1ml of sample was taken in test tube and the volume was made upto 1ml with distilled water. 1ml of distilled water alone serves as blank. 4ml of anthrone reagent was then added to all the tubes. The tubes were covered with marbles and heated in a boiling water bath for 10 minutes. The contents were cooled and the intensity of the colour was developed read at 620nm. Standard was also treated similarly. The amount of total sugar was expressed as mg/g.

HPLC analysis

HPLC analysis was carried out by the method of Hertog et al., (1992); Gennaro et al., (2002).

Instrument used

Company - Shimadzu
Detector - SPD -10AVP
S.No - C20 9944111453LP
Pump - LC -10 AT VP1
S.No - C20 974113558 N

Standards used

The following standards used for the phenol were Gallic acid, Coumaric acid, Ellagic acid, Ferulic acid, Mandelic acid and Vanillic acid. Standard used for the flavonoids are Rutin, Quercetin, Kaempferol andisorhamnetin,

Procedure

Column used for the HPLC analysis is C18 (reversed phase column Lichrospher 100 : RP18) length 4.6 mm x 25 cm, equipped with a pump (LC -10AT VP1), SIL-6A automatic injector furnished with a 50 Dl loop, detector (SPD -10AVP) set at 370 nm and C- R6A chromatography data station software. The extract sample was injected into the loop and the temperature was maintained at 40°C. The solvent system consists of 50 ml of methanol (A), 50 ml of phosphoric acid (B) and 1ml water (C) with a gradient system 50% of A in B. The solvents were used at a constant flow rate of 0.6ml /min. Sample peaks were quantified with the external standard method. The phenols and flavonoid were expressed as mg/ 100 g of fresh weight. Alcohol, water, and hydrochloric acid (50:20:8) mixture were used for the solvent extraction. Methanol, water, and phosphoric acid (100:100:1) mixture was used as mobile phase.
Chromatographic system
Detector : 270-nm
Column : 4.6-mm × 25-cm
Packing : L1
Flow rate : 1.5 ml per minute

Then 20μL of the Standard solutions and ethanolic fruit extract of *Benincasa hispida* were separately injected into the chromatograph, chromatograms were recorded, and the major peaks areas were measured. The percentage of phenols and flavonoids in the sample was calculated.

**Results and Discussion**

*Benincasa hispida* plant had various parts in which it contains beneficial compounds. Compounds present in fruit of *Benincasa hispida* was identified by various techniques. A whole plant of *Benincasa hispida* was traditionally used. Hence it could be used as a drug. The fruit of *Benincasa hispida* had anatomical components, pharmacological activity and the phytochemical aspects revealed the following results.

**Macroscopic analysis**

The macroscopic analysis of *Benincasa hispida* fruit was noted in **Table 1.1**. The colour of the fruit is yellowish white, taste was sweet and odour is very characteristic.

**Microscopic analysis**

Microscopic analysis of *Benincasa hispida* explained is shown in **Figure 1.1**.

**Anatomy of the fruit**

The fruit of *Benincasa hispida* was a large fleshy pepo. It consists of a thin skin of epidermis, fleshy and juicy mesocarp and swollen, thick placenta. The fruit was tricarpellary syncarpous and had peripheral placentation. The fruit consists of homogeneous parenchymatous tissue which was large, thin walled compact and the cells were variable in shape and size (**Figure 1.1: Plate 1.1**) Scattered in the ground tissue and vascular strand were a few xylem elements and fairly more number of phloem elements. The xylem and phloem were arranged in collateral position as shown in **Figure 1.1: Plate 1.2** respectively.

The xylem elements are thick walled and wide (**Figure 1.1: Plate 1.3**). These were so large, thick walled secretary cells, sparsely seen in the ground tissue (Figure
1.1:Plate 1.4). Calcium oxalate crystals were observed in the ground tissue. They are diffuse in distribution and it was 30μm in diameter (Figure 1.1: Plate 1. 5 and 1. 6).

**Physicochemical analysis of Benincasa hispida**

Plant materials are globally used as home remedies, folklore medicine and raw materials for pharmaceutical industries. Medicinal plant materials are authenticated according to macroscopic and microscopic characteristics. The identity and purity of the plant material should be confirmed.

**Loss on drying**

Excess amount of water in the plant will enhance microbial growth and will lead to deterioration following hydrolysis. Therefore limits for water content should be accessed, especially for materials that readily absorb moisture or for those which have high rate of deterioration in the presence of moisture (WHO, 1998).

The test loss on drying determines both the water and the volatile matter that are present in the sample. It helps in detecting the net weight of a substance after drying at specific temperature for specified period of time. Loss on drying of powdered material of *Benincasa hispida* revealed the presence of 14.35 ± 0.9% of moisture (Table 1.2).

**Total ash**

The total ash was measured, the total amount of material remaining after ignition, and this includes physiological ash and non physiological ash, which is the residue of the extraneous matter (WHO, 1998). The total ash content of both the samples accessed falls within the limits set by *The Ayurvedic pharmacopeia of India, 2001*, which states that the total ash content of the materials should not be more than 12%. The *Benincasa hispida* has total ash value about 6.89 ± 0.18% (Table 1.2).

**Acid insoluble ash**

Acid insoluble ash was the residue that was obtained after boiling the total ash with diluted hydrochloric acid and igniting the remaining insoluble matter. Table 1.2 revealed that the negligible amount of acid insoluble siliceous was in *Benincasa hispida*. The acid insoluble ash content is 0.93 ± 0.08%. *The Ayurvedic pharmacopoeia of India, 2001*, which stated that acid insoluble content, should be not more than 1%.
Figure 1.1: Microscopic analysis of *Benincasa hispida* fruit

**Legends:**

Plate 1.1 and Plate 1.2 shows transverse section of *Benincasa hispida* fruit.

GP - Ground parenchyma, Ph - Phloem, X - Xylem

Plate 1.3 and Plate 1.4 Shows enlarged vascular strand

GT - Ground tissue, Ph - Phloem, VS - Vascular strand, X - Xylem

Plate 1.5 and Plate 1.6 Shows Distribution of calcium oxalate crystals.

Cr - Crystals, GP - Ground parenchyma
Alcohol soluble extractive value

The alcohol soluble extractive value of *Benincasa hispida* fruit was 12.58 ± 0.46%. The *Ayurvedic pharmacopoeia of India, (2001)* stated that the alcohol soluble extractive value of the materials tested should not be less than 10% (Table 1.2).

Water soluble extractive value

The water soluble extractive value indicates the presence of inorganic contents in the tested material. The water soluble extractive value of *Benincasa hispida* fruit was 28.49 ± 1.86%. The *ayurvedic Pharmacopoeia of India, (2001)* stated that the water soluble extractive value of the materials tested should be not less than 24%. The variation in the extractive values may be due to presence of specific compounds, solubility, soil condition and atmospheric condition and water content of the sample (Table 1.2).

Qualitative phytochemical analysis

Phytochemical screening of extracts like n-hexane, ethyl acetate, ethanol and aqueous were used for the analysis. Chemical reactions which can produce particular colour and based on the reaction, this colour formation are helpful in the identification of the phytochemicals present in the extract.

Phytochemicals present in *Benincasa hispida* fruit extracts like n-hexane, ethyl acetate, ethanol and aqueous extract was measured qualitatively and results was shown in Table 1.3. Ethanol, ethylacetate and aqueous extract of *Benincasa hispida* contains higher amount of phytochemicals like phenols, flavonoids, tannins, terpenoids, quinones, glycosides, carbohydrates, proteins, coumarins, alkaloids, saponins, amino acids and phytosterols. So quantitative analysis was done with these extracts.

Quantitative phytochemical analysis

Table 1.4 shows the quantitative analysis of total phenols, flavonoids, tannins, terpenoids and saponins of *Benincasa hispida* fruit extracts. The total phenols in ethyl acetate to aqueous extract ranges from 23.66 ± 1.77 to 80.71 ± 0.94mg/g of gallic acid equivalent. Ethanolic extract contain highest value of total phenols which is about 91.75 ± 0.92 mg of GAE/g followed by aqueous extract 80.71± 0.94 mg of GAE/g and Ethyl acetate was 23.66 ± 1.77 mg of GAE/g.
Table 1.1: Macroscopic characteristics of *Benincasa hispida* fruit

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Yellowish white</td>
</tr>
<tr>
<td>Odour</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Taste</td>
<td>Sweet</td>
</tr>
</tbody>
</table>

Table 1.2: Physicochemical parameters of *Benincasa hispida* fruit extract

<table>
<thead>
<tr>
<th>Physicochemical Parameters</th>
<th>Value in % (W/W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying</td>
<td>14.35 ± 0.9%</td>
</tr>
<tr>
<td>Total ash</td>
<td>6.89 ± 0.18%</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>0.93 ± 0.08%</td>
</tr>
<tr>
<td>Alcohol soluble extract value</td>
<td>12.58 ± 0.46%</td>
</tr>
<tr>
<td>Water soluble extract value</td>
<td>28.49 ± 1.86%</td>
</tr>
</tbody>
</table>

**Extractive value**
Table 1.3: Qualitative phytochemical analysis of *Benincasa hispida* fruit extract

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n-hexane</th>
<th>Ethyl acetate</th>
<th>Ethanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Anthocyanin &amp; Betacyanin</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins and amino acids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phytosteroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phalabatannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthroquinones</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Legends:** + denoted presence of metabolite, - denoted absence of metabolite.
Phenolic compounds have antioxidant property. Antioxidant activity was mainly due to the property to donate hydrogen atoms to free radicals. This played an important role in absorbing and neutralizing free radicals and quenching of singlet and triplet oxygen or decomposition of peroxides (Sulaiman et al., 2011; Kumar et al., 2012). Phenolic content reduce the risk of heart disease by slowing the progression of atherosclerosis. Phenolic compounds have antioxidant property towards LDL (Frankel et al., 1993; Kanner et al., 1994). Polyphenolic compounds in Clerodendron colebrookianum is used in prevention of hyperlipidemia (Devi et al., 2011).

Phytochemicals like saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti inflammatory effects (Liu et al., 2005; Manach et al., 1996) and Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activity (Bever, 1980).

Flavonoids have been accepted as very important phytochemical mainly for their antioxidant properties. Flavonoids are potent water soluble antioxidants and scavenge free radicals, which in turn prevent oxidative cell damage and act as strong anticancer principle (Havsteen, 2002; Marimuthu et al., 2012). Flavonoids also reduce the risk of heart disease. Due to the antioxidant property of flavonoids, it can act as anti-inflammatory agent, anticancer and cardio vascular disease. Rhizome of Maranta arundinacea was rich in phenol and flavonoids. These compounds were responsible for the antioxidant property against the free radicals (Ruba Angel and Mohan, 2013).

Total flavonoids are higher in ethanolic extract and was about 70.46 ± 0.80 mg of QE/g, aqueous extract contain 65.65 ± 0.94 mg of QE/g, followed by ethyl acetate 16.76 ± 1.46 mg of QE/g were observed.

Phyllanthus amarus leaf extract exhibits hypoglycemic and hypolipidemic acitivity due to the presence phytoconstituents like flavonoids, alkaloids, saponins and tannins (Umbare et al., 2009). Flavonoids have different biological functions like hypolipidemic and cardioprotective activity (Anila and Vijayalakshmi, 2002).

Terpenoids are organic chemicals that were derived from the isoprene units and they are present in wide ranges in many plants. Terpenoids are lipid soluble by nature. Terpenoids are present in more amount in ethanol, aqueous and ethyl acetate 7.16±0.14 mg of UA/g, 4.27 ± 0.29 mg of UA/g, and 5.38 ± 0.24 mg of UA/g.

Tannins possessed anti-inflammatory and were also helpful in controlling gastritis and oesophaitis. Tannins have antiviral activity (Lu et al., 2004). Tannins were rich in ethanolic extract and tannin was about 14.75 ± 1.26 mg of GAE/g, aqueous
(12.31 ± 0.15 mg of GAE/g) and Ethyl acetate 0.92 ± 0.05 mg of GAE/g as shown in Table 1.4.

Tannins inhibited adipocyte differentiation in 3T3-L1 cell (Liu et al., 2005). Ziziphus mauritiana bark had tannin as phytochemical, which was responsible for the anti-obesity activity (Deshpande et al., 2013). Tannins also have antioxidant (Hong et al., 2001; Du et al., 2007), antiallergic (Bhattacharyya et al., 2007; Akiyama et al., 2005), anticancer (Choi et al., 2005; Yuste et al., 1992) and antidiabetic properties. Tannins have stringent properties, accelerate the wound healing and inflamed mucous membranes.

Syzygium cumini seeds have anti-inflammatory, anti-diabetic and analgesic activities due to the presence of secondary metabolite like alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and terpenoids (Kumar et al., 2009).

Saponins possess carbohydrate moiety, which was attached to triterpenoid or steroidal aglycone. Saponin reduces the glucose and cholesterol uptake from the gut. Saponins present in aqueous extract of Benincasa hispida fruit has highest level which was 21.46 ± 1.92 mg of saponin/g, followed by ethanolic extract (15.8 ± 0.45 mg/g) and Ethyl acetate 3.77 ± 0.22 mg/g (Table 1.4).

Rupasinghe et al., 2003 stated that saponins possess hypocholesterolemic and antidiabetic properties. Sapindus emarginatus have a lytic action on erythrocyte membrane and haemolytic action, in the presence of saponins. (Jeyabalan and Palayan, 2009). Saponins rich in Achyranthes aspera decreased the excretion of bile salt, while they bind with saponin. So saponin decreases the body weight gain. Achyranthes aspera contains high amount of saponins, Achyranthes aspera inhibits the lipid digestion and increase the absorption of triglycerides and cholesterol. (Lathaa et al., 2011)

Alkaloids are the final product of nitrogen metabolism in plant. It played a vital role in plant growth. Alkaloids related substances were used as neurotransmitor. Alkaloids also have antimalarial activity. The alkaloids obtained from medicinal plants have biological activities such as antimicrobial, antihyperglycemic, anti-inflammatory and antispasmodic activity. Equisetum ravens had higher alkaloid content, and its derivatives were used for analgesic, antispasmodic and bactericidal activity (Hussain et al., 2013).
Table 1.5 denoted that alkaloids present in ethanolic extract was found to be 3.9 ± 0.25mg/g, nutritive value of Benincasa hispida fruit contain sugar which was 30.33 ± 2.50mg/g and protein was 4.11±0.18mg/g.

Table 1.6, 1.7 and Figure 1.2, 1.3 showed the HPLC analysis of Phenolic compounds of standard and extract. The chromatogram which is obtained from standard and plant extract was compared. Peak in plant sample with RT value of 30.5 and 33.18 was correlated with standard such as gallic acid and coumaric acid. The area of percentage for gallic acid and coumaric acid was 45.5 and 45.4% (Figure 1.3) It showed that Benincasa hispida fruit extract contain gallic acid and coumaric acid. This result revealed the presence of gallic acid and coumaric acid. From the peaks present in ethanolic extract of Benincasa hispida, it can be inferred that Benincasa hispida fruit extract contains high amounts of gallic acid and coumaric acid.

Gallic acid had anti-inflammatory, antimutagenic, anticancer and antioxidant activity. It had beneficial effect in pulmonary and nephritic haemarrhages. It was also used in the treatment of diabetes and to prevent albuminuria. Coumaric acid was a metabolite of ubiquitous plant. This possessed antioxidant property (Abdel-Wahab et al., 2003). It is also possess anti-inflammatory, anticancer activity (Yoon et al., 2013) and hepatoprotective effect. It also acts as potential immune suppressive agent for treating autoimmune disease like rheumatoid arthritis (Pragasam et al., 2013). It gives protection against adverse effects of mutagenesis and oxidative damage.

HPLC analysis of flavonoids is shown in Table 1.8, 1.9 and Figure (1.4 and 1.5). HPLC analysis of ethanolic extract of Benincasa hispida fruit chromatogram contains RT value of 2.910 and 8.750. These RT correlated with standard flavonoids. The observation from the graph of standard was compared with fruit extract of Benincasa hispida fruit extract and it was observed that the presence of quercetin with
Table 1.4: Quantitative Phytochemical analysis of *Benincasa hispida* fruit extracts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ethyl acetate</th>
<th>Ethanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols (mg/g)</td>
<td>23.66 ± 1.77</td>
<td>91.75 ± 0.92</td>
<td>80.71 ± 0.94</td>
</tr>
<tr>
<td>Flavonoids (mg/g)</td>
<td>16.76 ± 1.46</td>
<td>70.46 ± 0.80</td>
<td>65.65 ± 0.94</td>
</tr>
<tr>
<td>Terpenoids (mg/g)</td>
<td>5.38 ± 0.24</td>
<td>7.16 ± 0.14</td>
<td>4.27 ± 0.29</td>
</tr>
<tr>
<td>Tannins (mg/g)</td>
<td>0.92 ± 0.05</td>
<td>14.75 ± 1.26</td>
<td>12.31 ± 0.15</td>
</tr>
<tr>
<td>Saponins (mg/g)</td>
<td>3.77 ± 0.22</td>
<td>15.85 ± 0.45</td>
<td>21.46 ± 1.92</td>
</tr>
</tbody>
</table>

Table 1.5: Alkaloids and nutritive value of Ethanolic fruit extract of *Benincasa hispida*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids (mg/g)</td>
<td>3.9 ± 0.25</td>
</tr>
<tr>
<td>Total Sugars (mg/g)</td>
<td>30.33 ± 2.51</td>
</tr>
<tr>
<td>Total Protein (mg/g)</td>
<td>4.11 ± 0.18</td>
</tr>
</tbody>
</table>

Figure 1.2: HPLC analysis of standard phenols
Table 1.6: Details of Standard phenols

<table>
<thead>
<tr>
<th>COMPONENT NAME</th>
<th>RT</th>
<th>AREA</th>
<th>AREA%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>30.6</td>
<td>13.5</td>
<td>0.38</td>
</tr>
<tr>
<td>Coumaric acid</td>
<td>33.2</td>
<td>125.600</td>
<td>3.6</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>35.7</td>
<td>546.700</td>
<td>15.31</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>37.4</td>
<td>1215.600</td>
<td>34.03</td>
</tr>
<tr>
<td>Mandelic acid</td>
<td>40.4</td>
<td>1560.600</td>
<td>43.65</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>43.2</td>
<td>108.5</td>
<td>3.03</td>
</tr>
</tbody>
</table>

Figure 1.3: HPLC analysis of *Benincasa hispida* fruit extract
Table 1.7: Details of phenols of *Benincasa hispida* fruit extract

<table>
<thead>
<tr>
<th>COMPONENT NAME</th>
<th>RT</th>
<th>AREA</th>
<th>AREA%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>30.5</td>
<td>10.3</td>
<td>45.5</td>
</tr>
<tr>
<td>Coumaric acid</td>
<td>33.18</td>
<td>198.400</td>
<td>45.4</td>
</tr>
</tbody>
</table>

Figure 1.4: HPLC analysis of standard flavonoids

Table 1.8: Details of standard flavonoids

<table>
<thead>
<tr>
<th>COMPONENT NAME</th>
<th>RT</th>
<th>AREA</th>
<th>AREA%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin</td>
<td>3.080</td>
<td>465.820</td>
<td>2.3</td>
</tr>
<tr>
<td>Quercetin</td>
<td>8.927</td>
<td>18009.48</td>
<td>88.5</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>12.560</td>
<td>841.105</td>
<td>4.1</td>
</tr>
<tr>
<td>Isorhamnetin</td>
<td>23.857</td>
<td>1043.843</td>
<td>5.1</td>
</tr>
</tbody>
</table>
Figure 1.5: HPLC analysis of Ethanolic fruit extract of *Benincasa hispida*

![HPLC analysis graph](image)

Table 1.9: Details of flavonoids of Ethanolic fruit extract of *Benincasa hispida*

<table>
<thead>
<tr>
<th>COMPONENT NAME</th>
<th>RT</th>
<th>AREA</th>
<th>AREA %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin</td>
<td>2.910</td>
<td>23331.59</td>
<td>32.5</td>
</tr>
<tr>
<td>Quercetin</td>
<td>8.750</td>
<td>4849.044</td>
<td>67.5</td>
</tr>
</tbody>
</table>
percentage was 67.5% and rutin (32.5%). These results proved that *Benincasa hispida* fruit extract contain quercetin and rutin.

Quercetin is a flavonoid which was most predominantly found in the form of glycosides. Quercetin is used in improvement of cardiovascular risk, cancer and also protection against osteoporosis (*Hertog et al., 1993; Yang et al., 2006*). Quercetin also gives inhibited adipogenesis and induced apoptosis in mouse pre adipocytes (*Strobel et al., 2005; Fang et al., 2008*). It also has protective effects against obesity-related inflammation (*Al Fayez et al., 2006; Chuang et al., 2010*).

Quercetin prevents free radical induced tissue damage and directly scavenges free radicals. Quercetin significantly inhibited tumour necrosis factor-alpha production and gene expression in a dose dependent manner as reported by *Nair et al., 2006*. This indicates that quercetin could modulate the immune response and had anti-inflammatory activity.

Rutin increases the ascorbic acid level intracellularly and decrease capillary permeability, fragility and inhibited bones destruction, scavange free radicals and also lower the risk of heart disease (*Kreft, et al., 1999*). Rutin inhibited leukemia and also had anticancerous effect (*Lin et al., 2012; Luo et al., 2008*).

The present chapter revealed that the ethanolic extract of *Benincasa hispida* fruit was rich in secondary metabolites like saponins, steroids, flavonoids, alkaloids, tannins, glycosides, Phenols, terpenoids, alkaloids, coumarins and triterpenoids. Quantitative analysis of various phytochemicals also proved that ethanolic extract contain greater amount of secondary metabolites suggesting ethanolic fruit extract of *Benincasa hispida* is a potent drug. The microscopic appearance and physicochemical parameters would be helpful for identification and authentication of *Benincasa hispida* fruit. HPLC analysis of ethanolic fruit extract of *Benincasa hispida* proved that extracts contains phenols such as gallic acid and coumaric acid and flavonoids like quercetin and rutin are present. These compounds present in ethanolic fruit extract of *Benincasa hispida* may be effective in modulating various diseases such as obesity.