Analysis of Biochemical Parameters and Preliminary Phytochemical Screening of Two Medicinal Plants

By

Silpa, M

(Reg.No.15PBO007)

A Thesis Submitted To The

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore-641 043.

In Partial Fulfillment of the Requirements for the

Degree of Master of Science in Botany

April 2017

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A. Vijayalakshmi 8/4/17

Signature of the
Head of the Department

V. Mayakkari

Signature of the
Supervisor

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER I

INTRODUCTION

Medicinal plants have been extensively used for treatments of many diseases. Various parts of plants such as leaves, fruits, barks, roots and even the seeds are being used for preparation of medicine. Plants are one of the most important sources of natural medicine and number of modern drugs has been isolated from them. Over 80% of world population relies on the traditional form of medicine for their basic health care.

Use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceutical research. Epidemiological studies on medicinal plants support that the constituents such as phenols, flavonoids, alkaloids, tannins etc. are capable of exerting protective effect against oxidative stress. (Rajkumar *et al*., 2015).

Plants are being used as remedies for diseases from time immemorial. There is a tremendous increase in the consumption of herbs as an alternate source of medicine to maintain health and improve the quality of life. The chemical components in plants have diverse biological roles and are therefore of therapeutic value.

Phytochemicals, the compounds present in plants are valuable source of food and medicine. They are known to have various biological activities such as antimicrobial, antifungal, antioxidant activity, etc. The important bioactive components in plants are usually the secondary metabolites such as alkaloids, flavonoids, tannins and other phenolic compounds. One of the sources of pharmaceuticals for human ailments is plants, either as totally pure compounds or as synthetic analogs. Approximately 25% of drugs prescribed in the United States are plant derived natural products and 74% of the 119 most important drugs contain ingredients from plants used in traditional medicine. Hence, it can be stated that plants could be a source for the development of new molecules. Pharmaceutical research is now extensively focusing on natural compounds, for developing active molecules of plant origin.

Herbs are being used for the promotion of health, prevention and treatment of diseases in India from ancient times. Phytochemicals are products of plant metabolism, mainly used by the plants for their defense. Hence, attempts have been made to use them for therapeutic purposes. For example, saponins possess beneficial effects on the blood cholesterol, against cancer, immune system and also have antiviral property. Cardiac glycosides play a role in the treatment of falling heart disorders and are known to show beneficial effects on cardiac arrhythmias. Although phytochemicals are said to be useful to the human body, they may have some toxic effects as well, as seen in the case of alkaloids. Alkaloids are reported to have cytotoxic activity which may be used in treatment of cancer. Hence, it is desirable to know the phytochemical composition of the plant material before testing its efficacy for medicinal purpose (Vaishali *et al.,* 2013). The present work emphasizes on traditionally used clinically potential plants *Annona squamosa* (L.) and *Garcinia gummi-gutta* (L.) Roxb.

Annona squamosa L. belonging to the family Annonaceae, commonly known as custard apple is a native of West Indies. The cultivation is present throughout India, because of its edible nature. *Annona squamosa* is a tree with edible fruits that show medicinal value.

Annona, requires a tropical or subtropical climate with summer temperatures from 25 °C to 41 °C, and mean winter temperature above 15 °C. It is sensitive to cold and frost, being defoliated below 10 °C and killed by temperatures of a couple of degrees below freezing. It is only moderately drought tolerant, requiring at least 700 mm of annual rainfall and will not produce fruit well during droughts. It will grow from sea level to 2,000 meters and does well in hot dry climates, differing in its tolerance of lowland tropics from many of the other fruit bearers in the *Annona* family.

It is quite a prolific bearer and it produces fruit in as little as two to three years. A five-year-old tree can produce as many as 50 sugar apples. Poor fruit production has been reported in Florida, because there are few natural pollinators (honeybees have a difficult time penetrating the tightly closed female flowers); however, hand pollination with a natural fiber brush is effective in increasing

yield. Natural pollinators include beetles (Coleopteran) of the families Nitidulidae, Staphylinidae, Chrysomelidae, Curculionidae and Scarabeidae.

In traditional Indian, Thai and American medicine, the leaves are used in a decoction to treat dysentery and urinary tract infection. In traditional Indian medicine, they are also crushed and applied to wounds. In Mexico, the leaves are rubbed on floors and put in hens' nests to repel lice. Chemical constituent, the diterpenoid alkaloid Atisine is the most abundant alkaloid in the root. Other constituents of *Annona squamosa* include, the alkaloids oxophoebine, reticulate isocorydine, methylcorydaldine and the flavonoid quercetin-3-O-glucoside.

It is used as an antidiabetic, antioxidant, hepatoprotective, genetoxic, cytotoxic, antitumour and antilice agent. It is related to contain phytochemicals like alkaloids, carbohydrates, lipids, tannins and phenolics (Kaladhar *et al.,* 2014). The previous phytochemical investigations made on the plant have proved that they possess a wide variety of compounds like acetogenins which are responsible for anti-feedant, anti-malarial, cytotoxic and immunosuppressive activities. Diterpenes which was isolated from *Annona squamosa* possess anti-HIV principle and anti-platelet aggregation activity (Gajalakshmi *et al.,* 2011).

It is considered beneficial for cardiac disease, diabetes, hyperthyroidism and cancer. The root is considered as a drastic purgative. The crushed leaves are sniffed to overcome hysteria and fainting spells, they are also applied on ulcer and wounds. A leaf decoction was taken in the case of dysentery (Gajalakshmi *et al*., 2011). Leaves are used as poultice over boils and ulcers and also to kill lice. Bruised leaves with salt make a cataplasm to induce suppuration.

Garcinia gummi-gutta (L.) Roxb. is one of the most diverse species among Guttiferae family. It is an evergreen, small or medium-sized dioecious, understorey tree, 5 –20 m tall, about 70 cm dbh, with a rounded crown and horizontal or drooping branches. Female flowers occur singly or in clusters of upto 4, the stigmatic surface is normally enlarged, and there is no style. Pistillate flowers have rudimentary and nonfunctional staminodes. Neither male nor female flowers produce nectar. Fruit, a green, ovoid berry, 5 cm in diameter, yellow or red when ripe, with 6-8 grooves. Seed 6-8, smooth, large, about 5 cm long and 2 cm wide surrounded by a succulent aril.

Seed-grown plants start bearing fruit after 10-12 years, whereas, grafts bear fruit from the third year onwards and will attain the stage of full bearing at the age of 12-15 years. In India, flowering occurs in January-March and fruits mature in July. There are also reports of off-season bearers, bearing twice annually.The orange yellow mature fruits either drop from the tree or are harvested manually. Alkaloids, phenolic compounds, carbohydrates, steroids, proteins, terpenoids, tannins constitute the phytoconstituents of *Garcinia gummi- gutta*.

Pharmacological studies have revealed that *Garcinia gummi- gutta* possess anti-oxidant, anti-bacterial, larvicidal, antimalarial, anti-obesity, anti ulcer, anti-cancer and anti-cholinesterase activity. *Garcinia gummi-gutta* possess a great number of traditional uses. It is a wild subtropical and tropical plant.

The fruits of the plant are commercially important for its valuable chemical components. Mostly these species are found in forest. Most of plants are less cultivated in homes and extensively endemic to Western Ghats. *Garcinia gummi-gutta* is commonly known as kudampuli or malabar tamarind belonging to the family Clusiaceae. These families are a rich source of secondary metabolites.

Flowers are either androecious or bisexual, thus it is an andromonoecious species. The fruit is a fleshy, globose, sub-globose to ovoid berry, green turning yellow, orangish or reddish when ripe, fluted with longitudinal grooves. The seeds are 6-8 numbers, smooth, pale brown, oval surrounded by a succulent reddish or whitish aril. *G. gummi-gutta* has been used in South India from time immemorial as a condiment for flavouring curries in place of tamarind or lime. *G. gummi-gutta* is mostly used in Kerala and Kanyakumari district of Tamil Nadu in cooking to add sour taste to fish curry.

It is found in semi-evergreen to evergreen forests. In India, it is commonly found in the evergreen and Shola forests of Western Ghats, Karnataka and Kerala. The tree is very much adapted to both hilltops and plain lands, but its performance is best in riverbanks and valleys. It also grows well in dry or occasionally water logged or flooded soils (Orwa *et al.,* 2009).

The present work on the two medicinal plants is mainly

i) To study the biochemical parameters such as chlorophyll, protein and carbohydrate
ii) Analyze the preliminary phytoconstituents present in the two medicinal plants.

CHAPTER II

REVIEW OF LITERATURE

Chitra *et al.* (2009) have studied the antibacterial and wound healing activity of the leaves of *Annona squamosa* Linn. The result revealed the presence of sterols, flavonoids, tannins in various extracts that was confirmed by preliminary phytochemical investigation, TLC and HPTLC methods.

Muley *et al*. (2009) have carried out studies on phytochemical constituents and pharmacological activities of *Calendula officinalis* Linn (Asteraceae). The extract of this plant as well as pure compounds isolated from it, have been demonstrated to possess multiple pharmacological activities such as anti-HIV, cytotoxic, anti-inflammatory, hepatoprotective, spasmolytic and spasmogenic, amongst others.

Sharma *et al.* (2009) has done pharmacognostical studies on the leaf of *Annona squamosa* Linn. Their studies indicate the use of *Annona* in the treatment of various diseases. The qualitative phytochemical fingerprint of the methanolic extract revealed the presence of alkaloids, terpenoids, phenolics, fats and waxes.

Maridass *et al.* (2010) have evaluated the phytochemical, pharmacognostical and antibacterial activity of *Garcinia gummi-gutta* leaves. Their study includes analysis of macroscopical characters, microchemical tests and behaviour of the powder on treatment with different chemical reagents, physical constant values, fluorescence analysis and preliminary phytochemical investigation.

Meena *et al.* (2010) have evaluated the physicochemical and preliminary phytochemical studies on the fruit of *Emblica officinalis* Gaertn. Amla is becoming increasingly well known for its unusually high levels of Vitamin C, which is resistant to storage and heat damage due to cooking. It is found natively in India. Indian gooseberry has been used as valuable ingredient for various medicines in India and abroad. The study revealed specific identities for the particular crude drug which will be useful in identification and control to adulterations of the raw drug.

Amador *et al.* (2010) have done phytochemical and pharmacological studies on *Mikania micrantha* H.B.K. (Asteraceae). Ethyl acetate extracts of this plant exhibited significant antibacterial and anti-inflammatory properties. Therefore, it could be used as a medicinal plant. Several species of this family contain polyacetylenic and thiophenic compounds that are used as taxonomic markers.

Devendran *et al*. (2011) have done qualitative phytochemical screening and GC-MS analysis in *Ocimum sanctum* L. leaves. The investigation was carried out to determine the qualitative analysis of phytoconstituents and the possible chemical components present in *Ocimum sanctum* L. leaves through GC-MS. GC-MS analysis of hydroalcoholic extract led to the identification of 10 compounds.

The study carried out by Usha *et al*. (2011) on preliminary phyto-chemical evaluation of the leaf extract of five *Cassia* species showed the presence of various phytoconstituents like alkaloids, tannins, saponins, anthraquinones, anthocyanosides, phenolic flavonoids, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides and phlobatannins.

Gajalakshmi *et al.* (2011) have studied the pharmacological activities of *Annona squamosa*. The plant also possess analgesic, anti-inflammatory, anti-microbial, cytotoxic, anti-oxidant, anti-lipidemic, anti-ulcer, molluscicidal properties, genotoxic effect, vasorelaxant, anti-tumour, hepatoprotective, larvicidal, insecticidal, anthelmintic activity, etc.

Hemshekhar *et al*. (2011) have done an overview on the phytochemical and therapeutic aspects of *Garcinia*. The result showed the presence of bioactive molecules like hydroxycitric acid (HCA), flavonoids, terpenes, polysaccharides, procyanidines and polyisoprenylated benzophen. The polyisoprenylated benzophenone and xanthone derivatives are known for their antioxidant, apoptotic, anti-cancer, anti-inflammatory, anti-bacterial, anti-viral, anti-fungal, antiulcer, anti-protozoal and HAT inhibiting properties.

Neha Pandey *et al.* (2011) have done phytochemical and pharmacological review on *Annona squamosa* Linn. The study showed that *Annona squamosa* Linn is a multipurpose tree with edible fruits and is one of the medicinal and industrial

products. *Annona squamosa* Linn is used as an antioxidant, antidiabetic, hepatoprotective, cytotoxic, genetoxic, antitumour activity and antilice agent.

Alagesaboopathi *et al.* (2011) have conducted phytochemical screening studies on the leaves and stem of *Andrographis neesiana* Wight - an endemic medicinal plant from India. It has been used in traditional medicine to treat antifungal and aphrodisiac. Phytochemical screening showed the presence of various components of therapeutic importance including tannins, saponins, phenolic compounds, glycosides, flavonoids, gums and mucilages, steroids and triterpenoids.

Mona Agrawal *et al.* (2012) have done phytochemical and HPTLC studies of various extracts of *Annona squamosa.* Their studies revealed the presence of alkaloids, flavonoids, carbohydrates, saponins, tannins and steroids. The TLC and HPTLC techniques were used for qualitative determination of possible number of components in the various extracts. Solvent systems for all the extracts were optimized in order to get maximum separation on plate.

The study carried out by Ashfaq *et al*. (2012) on the preliminary phytochemical screening of alcoholic and aqueous extracts of *Mentha longifolia* Linn. leaves showed many bioactive chemical constituents like alkaloids, flavonoids, cardiac glycosides, phenolics, saponins and terpenes, but, lack proteins and carbohydrate.

Wadood *et al.* (2013) have done phytochemical analysis of medicinal plants occurring in local area of Mardan. The result showed the presence of chlorophyll, proteins, sugar and amino acids and secondary metabolites such as alkaloids, terpenoids, phlobatannins, reducing sugar, flavonoids and alkaloids.

Suman Kumar *et al*. (2013) have done phytochemical screening of certain compounds using leaf extracts of *Holoptelea integrifolia* (Planch.) and *Celestrus emarginata* (Grah.) used by Gondu tribes at Adilabad district, Andhra Pradesh, India. The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, xantho proteins, glycosides, steroids, phenols, resins, carboxylic acid group in varying concentrations.

Sudipa *et al*. (2013) have carried out phytochemical analysis of methanolic extracts of leaves of some medicinal plants. The result showed the presence of secondary metabolites like starch, alkaloids, flavonoids, tannins, reducing sugars, amino acids and lignin.

Adachukwu *et al*. (2013) have done phytochemical analysis of paw-paw (*Carica papaya*) leaves. The qualitative phytochemical analysis showed the presence of alkaloid, flavonoid, saponin, tannin and glycosides.

Essiett *et al*. (2013) have done comparative nutritional and phytochemical screening of the leaves and stems of *Acalypha fimbriata* Schum.Thonn. and *Euphorbia hirta* Linn. The phytochemical screening revealed the presence of tannins and flavonoids in both leaves and stems. Saponins were present in *Euphorbia hirta* leaves and stems.

Biba *et al.* (2013*)* have done phytochemical analysis is *Annona squamosa* seed extracts. The presence of appreciable to moderate amount of phytochemicals such as flavonoids, coumarins and alkaloids can be correlated with possible significant medicinal potential of the plant.

Karmakar *et al.* (2013) have analysed the antioxidant, anti-inflammatory, antimicrobial and cytotoxic properties of fungal endophytes from *Garcinia* species**.** The result indicated that some of these endophytes isolated from *Garcinia* plants are a potential source for bioactives and could be further exploited to foster the identity of the novel molecule.

Kavitha *et al.* (2013) have carried out studies on phytochemical screening and antioxidant activity of *Chromolaena odorata* and *Annona squamosa.* The result indicated the presence of active ingredients such as glycosides, steroids, saponins, phenols, flavonoids, terpenoids and tannins.

Kamaruz *et al*. (2013) have done pharmacognostical and phytochemical studies on the leaf and stem bark of *Annona reticulata* Linn. The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, haemorrhage, antibacterial infection, dysuria, fever and

ulcer. It is a highly apparent plant in Ayurvedic system of medicine for the treatment of various ailments.

Dipankar *et al.* (2013) have studied the toxicology, phytochemistry, bioactive compounds and pharmacology of *Parthenium hysterophorus.* The plant contains a large number of important bioactive compounds, mainly sesquiterpene lactones, flavonoid glycosides and pinenes. It has multiple pharmacologic properties such as, anticancer, anti-inflammatory, cardiotonic, antispasmodic, an emmenagogue, and used as an enema for worms.

Mamta *et al.* (2013) have done phytochemical studies in medicinal plants. The major classes of phytochemicals with disease-preventing functions are dietary fibre, antioxidants, anticancer, detoxifying agents, immunity-potentiating agents and neuropharmacological agents. Each class of these functional agents consists of a wide range of chemicals with differing potency. Some of these phytochemicals have more than one function.

Gowdhami *et al.* (2014) have done screening on phytochemicals and antibacterial activity of *Annona squamosa* extracts. The results of the phytochemical analysis indicated that the methanol and water extracts of seed and leaf had more positive results for alkaloids, oils, tannins, phenols and flavonoids.

Kaladhar *et al.* (2014) have carried out phytochemical analysis; antioxidant and antimicrobial activities from raw fruit peel crude extracts of *Annona squamosa* Linn. The phytochemical studies using methanol, ethanol, ethyl acetate and aqueous solution contain alkaloids, flavonoids, phenol and saponins.

The study carried out by Geetha *et al.* (2014) on quantitative analysis of primary and secondary metabolites of *Cymbopogan citratus* (DC). leaves from Kodaikanal hills, Tamil Nadu showed the presence of chlorophyll, carbohydrates, protein, lipids, phenol, tannin and flavonoids .

Santosh Kumar *et al*. (2014) have done pharmacognostic study and phytochemical screening on the leaves of *Adhatoda vasica*. The phytochemical screening showed the presence of tannins, alkaloids, saponins, steroids, flavonoids, glycosides and carbohydrates.

Cherish *et al.* (2014) have done whole plant screening for flavonoid and tannin contents in Castor plant (*Ricinus communis* L.) and also evaluated their biological activities. Flavonoids and tannins were found quantitatively in leaves, stems, seeds and roots, while capsules showed the presence of flavonoids alone.

Florence *et al.* (2014) have carried out screening of phytochemicals in certain flower extracts. The presence or absence of the phyto-constituents depends upon the solvent used and physiological property of the flowers.

Saidulu *et al. (*2014) have done preliminary phytochemical studies on *Withania somnifera*. Their study revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, xantho proteins, glycosides, steroids, phenols, resins, carboxylic acid groups in varying concentrations and the maximum solubility of all the phytochemicals was observed in methanol, water and chloroform extractions, but resins, coumarins were absent in the petroleum ether, acetone and also coumarins, carboxylic acid, quinines, xantho proteins were completely absent in the petroleum ether extracts.

Manjulika *et al*. (2014) have screened six medicinal plants used in traditional medicine for the presence of tannins, flavonoids, terpenoids, saponins, steroids, phlobatannins, carbohydrates, glycosides, coumarins, alkaloids, proteins, emodins, anthraquinones, anthocyanins and leucoanthocyanins using standard methods. It is evident from their study that *Swertia chirata* is of highest therapeutic efficacy possessing majority of phytochemical classes of compounds and *Phoenix dactylifera* is of lowest therapeutic potential due to the absence of majority of phytoconstituents.

Prabhakar *et al.* (2014) have done comparative phytochemical screening of six different plant species of Uttarakhand region. Plant extracts were prepared in ethanol, methanol and distilled water respectively and were tested for the availability of different bioactive components such as flavonoids, alkaloids, saponins, terpenoids, proteins, etc.

Jamuna *et al.* (2014) have carried out phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. The quantitative phytochemical analysis of this species

exhibited the presence of alkaloids, total phenolics, flavonoids, tannins, saponin, ascorbic acid, etc.

Kistamma *et al.* (2014) have carried out phytochemical screening in leaf extracts of *Cassia angustifolia* (vahl) grown in different soil treatments. The phytochemical screening has shown the presence of flavonoids, alkaloids, glycosides, phenols, quinones, steroids, saponins, tannins, resins, xantho proteins etc. in various concentrations.

Aziza Saif *et al.* (2015) have carried out work on total phenolics and antioxidant activity of crude extracts of *Annona squamosa* leaves traditionally used for the treatment of cancerous tumours. The result showed high amount of total phenolic content in crude extract of dry leaf powder.

Deva Krisna *et al.* (2015) have analysed total phenol and antioxidant activity of seed and peel of ripe and unripe fruit of Indonesian sugar apple (*Annona squamosa* L.) extracted with various solvents. Total phenol was determined by Folin-ciocalteau method. Total antioxidant was quantified by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method. There was a variation in total phenol content based on the level of ripening of the fruit and seeds had higher total phenol content than peels of the fruit.

Johnsy *et al.* (2015) have done preliminary phytochemical analysis of *Garcinia gummi-gutta* (L.) Robson grown in Western Ghats region of Kanyakumari District. The results suggested the presence or absence of phytoconstituents depending on the polarity of solvents used and the physiological property of the leaves and fruits.

Anu Saini (2015) has carried out studies in *Garcinia cambogia* (*Garcinia gummi-gutta*) – kodampuli (malabar tamarind). This study showed that *Garcinia cambogia* is an exotic fruit, which is popularly used in food industries, besides this is also very effective in the treatment of various diseases and different plant parts of *Garcinia cambogia* contain different health promoting compounds.

Rajkumar *et al.* (2015) have done phytochemical screening and antioxidant activity of leaves and stem bark extracts of *Garcinia imberti* - an endangered plant. Preliminary phytochemical screening of methanolic extracts of *Garcinia imberti* revealed the presence of various bioactive components like alkaloids, flavonoids, steroids, glycosides, phenols, saponins, terpenoids, resins, carbohydrates and tannins in both leaves and stem bark. Their study also suggests that the plant is a potential source of natural antioxidant that could have great importance as therapeutic agent in preventing or slowing the progress of oxidative stress related degenerative diseases.

Suneel *et al.* (2015) have determined phytochemical and phytotherapeutic properties of *Annona squamosa, Annona reticulata* and *Annona muricata*. The result showed that the plants have been used for centuries as traditional folk medicine for the treatment of various diseases and also used as an insecticide. The plants are considered to be a good source of vitamins, minerals, plant proteins, fibers, etc. as well as, the plant is supposed to have tremendous pharmacological importance.

Ranjani Sivapalan (2015) has carried out phytochemical studies on medicinal plant – *Sida cordifolia* Linn. Chemical tests performed using ethanol extract of leaves of *Sida cordifolia* showed the presence of alkaloids, flavonoids, lignin, glycosides, saponins, phytosterols and fixed oils.

Sharmila *et al.* (2015) have carried out phytochemical profile and *in vitro* antioxidant activity of *Garcinia gummi- gutta* (L.) peel extracts. The result indicated that methanolic peel extract possess more constituents compared to ethanolic and aqueous extract.

Sachet *et al. (*2015) have carried out pharmacognostic studies and preliminary phytochemical analysis of cold and hot extracts of leaf of *Tinospora malabarica* miers. The result showed the presence of carbohydrates, proteins, amino acids, glycosides, saponins, tannins and phenols in both the extracts and gums, protein containing sulfur and flavonoids were present only in cold extracts.

Varsha *et al.* (2015) have done phytochemical screening of *Cyanthellium cinereum* leaf extracts. Their study showed the presence of certain medically bioactive compounds, that possess medicinal property.

Prejeena *et al.* (2015) have determined qualitative phytochemical analysis of *Costus igneus* leaf extracts. Their study revealed the presence of compounds like alkaloids, steroids, terpenoids, glycosides, tannins, phenol, flavonoids, proteins and saponins.

Jayasudha *et al.* (2016) have analysed the phytochemical and *in vitro* antimicrobial evaluation of methanolic extracts of *Garcinia gummi-gutta*. The result indicated the presence of phenols, alkaloids, tannins, terpenoids, saponins, steroids, reducing sugars and phlobatannins in methanolic extract of leaf and fruit.

Ekta Singh *et al.* (2016) have carried out phytochemical screening of knol-khol (*Brassica caulorapa*) powder and juice. The study revealed the presence of many phytochemicals such as alkaloids, glycosides, flavonoids, saponin, tannin, steroids, terpenes and phytosterols.

Athiralakshmy *et al. (*2016) have done phytochemical screening in *Saraca asoca* and antimicrobial activity against bacterial species. Phytochemical analysis and antimicrobial activity of dried leaves and flowers of *Saraca asoca* were done with the samples extracted with acetone, diethyl ether, distilled water, methanol and petroleum ether. The extract showed the presence of alkaloids, flavonoids, tannins, saponins, glycosides and phenolic compounds.

Santhi *et al*. (2016) have carried out qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. Their study showed the presence of alkaloids, flavonoids, terpenoids, carbohydrates, protein and amino acids in the sample.

The study carried out by Victoriya *et al*. (2016) on the phytochemical analysis and antimicrobial activity of four different extracts from the leaves of *Murraya koenigii* showed the presence of tannins, saponins, flavonols, total phenol and aminoacids.

Sarini *et al*. (2016) have done phytochemical screening of the leaf and flower extracts of five *Ipomoea* species collected in and around Bangalore. The result showed the presence of various constituents like alkaloids, flavonoids, tannins,

saponins, lignin, terpenoids, glycosides, anthraquinones and phlobatannins. Alkaloids and flavonoids were invariably present in all the extracts.

Jayapriya *et al.* (2016) have done phytochemical screening on the leaf and flower extracts of *Thespesia populnea*. The leaf and flower extract showed good therapeutic efficacy, possessing majority of the phytoconstituents.

CHAPTER III

MATERIALS AND METHODS

The leaves of *Annona squamosa* L. and *Garcinia gummi- gutta* (L.) Roxb. were collected from the local area Calicut district in Kerala during the month of November . The taxonomic identification of plant material was done at Botanical Survey of India, Coimbatore. The fresh leaves were taken for chlorophyll, protein and carbohydrate studies.

Morphology of the plant

Annona squamosa L.

Systematic position

Kingdom	:	Plantae
Order	:	Magnoliales
Family	:	Annonaceae
Genus	:	*Annona*
Species	:	*A. squamosa*
Binomial Name	:	*Annona squamosa* L.

Description

Habit	:	Semi-deciduous tree, 3-7 m in height (Plate 1)
Trunk and Bark	:	Broad and light brown
Branches and branchlets	:	Open crown or irregularly spreading branches.
Leaves	:	6-17 x 3-6 cm, lanceolate or oblong lanceolate, Pale green on both surfaces and globate.
Flower	:	Greenish-yellow, fragrant, on slender hairy stalks, produced singly or in short lateral clusters about 2.5 cm long.

Fruit and Seed : Aggregate fruit formed from the numerous pistils of a flower. Each pistil forms a separate tubercle, mostly 1.3-1.9 cm long and 0.6-1.3 cm wide. Fruit is round, heart shaped ovate or conical, 5-10 cm.

Common names

English	:	Custard apple
Tamil	:	Sitaphalam
Malayalam	:	Seethapazham
Hindi	:	Sitaphal

Distribution

Annona squamosa is native to tropical America and West Indies, but the exact origin is unknown. It is now the most widely cultivated species of *Annona*, being grown for its fruit throughout the tropics and warmer subtropics, such as Indonesia, Thailand and Taiwan; it was introduced to Southern Asia before 1590.

Medicinal Uses

- Leaf, shoot, bark and root have been reported to have medicinal properties.
- The unripe fruit is astringent, and the root is a drastic purgative.
- The green fruit and seed have effective vermicide and insecticidal properties and are used as astringents in diarrhoea and dysentery.
- Crushed leaves are applied as an effective cure for ulcers and malignant sores.
- A poultice from fresh leaves is used for dyspepsia and when mixed with oil it is used for diseases of the scalp.

Plate 1

***Annona squamosa* L.**

HABIT

Garcinia gummi- gutta (L.) Roxb.

Systematic position

Kingdom	:	Plantae
Order	:	Malpighiales
Family	:	Clusiaceae
Genus	:	*Garcinia*
Species	:	*G. gummi-gutta*
Binomial name	:	*Garcinia gummi-gutta* (L.) Roxb.

Description

Habit	:	Tree up to 12 m tall (Plate 2).
Trunk and bark	:	Outer bark reddish brown, lenticellate; blaze reddish
Branches and branchlets	:	Drooping, glabraous.
Leaves	:	Simple, opposite decussate; petiole 5-16 cm long, lamina 5-13 or 2-6 cm, shape is variable from narrow elliptic, oblanceolate to obovate, apex usually acute.
Flowers	:	Polygamous, in axillary or terminal clusters; calyx cream, petals pink.
Fruit and seed	:	Berry, globose, 6-8 grooved, 5 cm in diameter; many seeded.

Common names

English	:	Malabar Tamarind
Tamil	:	Kottukkappuli
Malayalam	:	Kudampuli
Hindi	:	Kokam

Distribution

The tree is distributed in Western Ghats and Sri Lanka; in the Western Ghats throughout South and Central Sahyadris.

Medicinal uses

- *G. gummi-gutta* seeds, roots, leaves as well as their extracts possess medicinal value.
- They are useful in the treatment of various diseases and disorders.
- It contains various compounds that are beneficial for health. Such a composition makes it very different from other fruits.
- It is used to treat abdominal pain, infections and certain liver disorders.

Plate 2

***Garcinia gummi-gutta* (L.) Roxb.**

HABIT

1. ESTIMATION OF CHLOROPHYLL CONTENT

Chlorophyll 'a', 'b' and total chlorophyll were analysed following the method of Arnon (1949).

Materials required

Analytical grade acetone was diluted to 80 % acetone.

Procedure

- One gram of freshly cut leaf sample was taken into a clean mortar.
- The leaf bits were ground into fine pulp with the addition of 20 ml of 80 % (W/V) acetone.
- The mixture thus obtained was centrifuged at 5000 rpm for 5 minutes.
- The supernatant was transferred to 100 ml volumetric flask .This procedure was repeated until the residue became colorless.
- Supernatant was collected and volume was made up to 100 ml in the flask with acetone.
- The absorbance of the solution was read in spectrophotometer at 645 and 663 nm against the solvent blank (80 % acetone).

Calculation

The amount of chlorophyll present in the extract was calculated (mg chlorophyll/gm tissue) using the formula,

1. mg chlorophyll 'a'/gm tissue $= 12.7A_{663} - 2.69\,A_{645} \times \frac{V}{1000 \times W}$

2. mg chlorophyll 'b'/gm tissue $= 22.9A_{645} - 4.68\,A_{663} \times \frac{V}{1000 \times W}$

3. mg total chlorophyll/gm tissue $= 20.2A_{645} + 8.02\,A_{663} \times \frac{V}{1000 \times W}$

where,

A = Absorbance at specific wave length

V = Final volume of chlorophyll extract in 80 % acetone.

W = Fresh weight of the tissue.

2. ESTIMATION OF PROTEIN

Protein was estimated following the method of Lowry *et al*., 1951.

Principle

The blue colour developed by the reduction of phosphomolybdic phosphotungstic components in the Folin ciocalteau reagent by the aminoacid tyrosine and tryptophan present in the reaction of the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured in the Lowry's method.

Materials required

- 2 % sodium carbonate in 0.1N sodium hydroxide (Reagent A)
- 0.5% copper sulphate ($CuSO_4.5H_2O$) in 1% potassium sodium tartarate (Reagent B).
- Alkaline copper solution:
  Mix 50 ml of reagent A and 1 ml of reagent B prior to use (Reagent C).
- Folin –ciocalteau reagent (Reagent D) :
  Reflux gently for 10 hours a mixture consisting of 100g sodium tungstate ($Na_2WoO_4.2H_2O$), 25g sodium molybdate ((Na_2MoO_4 $2H_2O$) in 700ml water, 50 ml of 85% phosphoric acid and 100 ml of concentrated hydrochloric acid in a 1.5L flask . Add 150 g lithium sulfate, 50 ml water and a few drops of bromine. Boil the mixture for 15 min without condenser to remove excess bromine. Cool, dilute to 1 L and filter. The reagent should have no greenish tint.
- Protein solution (stock standard):

Weigh accurately 50mg of bovine serum albumin (fraction v) and dissolve in distilled water and make up to 50ml in a standard flask.

- Working standard:

  Ten ml stock solution was diluted to 50 ml with distilled water in a standard flask. One ml of this solution contains 200 mg protein.

Procedure

Extraction is usually carried out with buffers used for the enzyme assay. Weigh 500 mg of the sample and grind well with a pestle and mortar in 5-10 ml of the buffer. Centrifuge and use the supernatant for protein estimation.

Estimation of protein

- Pipette out 0.2 ml, 0.4ml, 0.6ml, 0.8ml and 1ml of the working standard into a series of test tube.
- Simultaneously, different concentrations of the sample were pipetted out into other test tubes.
- Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of water serves as the blank.
- Add 5 ml of reagent C to each tube including the blank. Mix well and allow to stand for 10 min.
- Then add 0.5ml of reagent D, mix well and incubate at room temp in the dark for 30 min. Blue colour is developed.
- Take the readings at 660 nm.
- Draw a standard graph and calculate the amount of protein in the sample.

Calculation

The amount of protein present in the sample was expressed in mg/gm.

$$= \frac{\text{mg of protein}}{\text{Volume of test standard}} \times \text{Concentration of the standard}$$

3. ESTIMATION OF CARBOHYDRATE CONTENT

Carbohydrate was estimated using the method of Hedge and Hofreiter (1962).

Carbohydrate is an important component of storage and structural materials in the plants. They exist as free sugars and polysaccharides. The basic unit of carbohydrate is the monosaccharide which cannot be split by hydrolysis into more simple sugars. The carbohydrate content can be measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharide.

Anthrone method

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone, a green coloured product with an absorption maximum at 630 nm.

Materials required

- 2.5 N-HCl
- Anthrone reagent was prepared by dissolving 200mg anthrone in 100 ml of ice cold 95% H_2SO_4.
- Stock standard solution: 100 mg of glucose was dissolved in 100 ml of water.
- Working standard: 5 ml of stock standard solution was diluted to 100 ml using distilled water (50 mg⁄ml).

Procedure

- About 100mg of sample was taken in a boling tube.
- Hydrolysed by keeping it in boling water bath for 3 hours with 5 ml of 2.5 N HCl and cooled at room temperature.
- Then, it was neutralized with sodium carbonate until the effervescence ceases.
- Make up the volume to 100 ml and centrifuge.

- The supernatant was collected and 0.2, 0.4, 0.6 and 0.8 ml aliquots were taken for analysis.
- The standard was prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and a blank was maintained.
- The volume was made upto 1ml in all the tubes including the sample test tubes by adding distilled water.
- Then, 4 ml of anthrone reagent was added and heated for 8 min in a boling water bath.
- Then, it was cooled rapidly and blue green colour developed was read at 650 nm.
- A standard graph was drawn by plotting concentration of the standard on the X- axis versus absorbance on the Y-aixs.
- From the graph, the amount of carbohydrates present in the sample was calculated.

Calculation

Amount of carbohydrates present in 100 mg of the sample

$$= \frac{\text{mg of glucose}}{\text{Volume of the test sample}} \times 100$$

Preliminary phytochemical screening procedure

The preliminary phytochemical analysis of various primary and secondary plant metabolites were carried out using the method of Harborne (1984).

Preparation of plant extract

The leaves of both the medicinal plants taken for the present study were collected, cleaned and air dried under shade for almost three weeks. After drying, the leaves were then blended using a household electric blender. This fine powder (Plate 3 and 4) was analysed for phytochemical constituents present in it. The plant sample was soaked in water, ethanol and chloroform for overnight extraction and filtered through Whatmann No.1. filter paper. Qualitative tests were conducted on these extracts.

Plate 3

Leaf powder of *Annona squamosa* L.

Plate 4

Leaf powder of *Garcinia gummi-gutta* (L.) Roxb.

Test for Alkaloids

Mayer's test

To 1ml of extract, 2ml of Conc. HCl was added. Then, a few drops of Mayer's reagent was added. Green colour or white precipitate indicates the presence of alkaloids.

Test for Tannins

To 1ml of extract, 2ml of 0.1% Ferric chloride was added. Brownish green or blue black colouration indicates the presence of tannins.

Test for Flavonoids

To 1ml of extract, 1ml of neutral ferric chloride was added. The formation of brown colour confirmed the presence of flavonoids.

Test for Quinones

A small amount of the extract was treated with Conc. HCl and observed for the formation of yellow precipitate.

Test for Phlobatannins

To 1ml of extract, few drops of 1% aqueous hydrochloric acid was added. A red precipitate formed indicates the presence of phlobatannins.

Test for Phenol

To 1ml of extract 5ml of Folin ciocalteau reagent and 4ml of sodium carbonate was added. Appearance of blue colour showed the presence of phenol**.**

Test for Carbohydrates

a) To 1ml of extract, 5ml of Benedict's reagent was added and boiled for 5 minutes. Bluish green colour indicated the presence of carbohydrates.

b) Purple colour is seen with the addition of few drops of Molisch'reagent and Conc. H_2SO_4.

Test for Amino acids

To 1ml of filterate, few drops of 0.2% ninhydrin was added and heated for 5 minutes. Formation of blue colour indicated the presence of aminoacid.

Test for Steroids

To 1ml of the filterate, 10ml of Chloroform and 10ml of sulphuric acid was added slowly by the sides of the test tube. Upper layer turning red and the sulphuric acid layer turning yellow with green fluorescent indicates the presence of steroids.

Test for Terpenoids

To 1ml of filterate, 2ml of chloroform was added and few drops of concentrated sulphuric acid was added carefully. An interface with a reddish brown colouration is formed showing the presence of terpenoids.

Test for fats and oil

To 1 ml of extract, a few drops of Sudan III solution was added . A shining orange colour showed the presence of fixed oil and fat.

STATISTICAL ANALYSIS

The data obtained from various biochemical observations were subjected to statistical analysis as per the procedure of Panse and Sukhatme (1978).

CHAPTER IV

RESULTS AND DISCUSSION

The biochemical studies and preliminary phytochemical screening of the two medicinal plants *Annona squamosa* L. and *Garcinia gummi-gutta* (L.) Roxb. carried out showed the following results.

Biochemical analysis of the leaves of *Annona squamosa* L. and *Garcinia gummi-gutta* (L.) Roxb.

The biochemical parameters such as chlorophyll, protein and carbohydrate were analysed using fresh leaf samples.

Estimation of Chlorophyll 'a', Chlorophyll 'b' and Total chlorophyll content

Chlorophyll 'a', chlorophyll 'b' and total chlorophyll content was estimated for the two medicinal plants. The chlorophyll 'a' content of *Annona squamosa* L. and *Garcinia gummi-gutta* (L.) Roxb. was estimated to be 7.83 ± 0.71mg and 6.99 ± 0.75 mg respectively (Table-I and Figure-1). The chlorophyll 'b' content of *Annona squamosa* L. and *Garcinia gummi-gutta* (L.) Roxb. was found to be 13.29 ± 0.57mg and 12.84 ± 0.35 mg.

The total chlorophyll present in the medicinal plants was estimated to be 11.88 ± 0.50mg (*Annona squamosa)* and 11.47 ± 0.31mg (*Garcinia gummi-gutta).* Studies on the biochemical parameters of *Chromolaena odorata* (L.) R.M. King H. Rob. and *Adhatoda vasica* Nees have revealed a significantly higher Chlorophyll 'a', 'b' and total chlorophyll content in *Chromolaena odorata* (Gayathri and Bindhu, 2016).

Table I

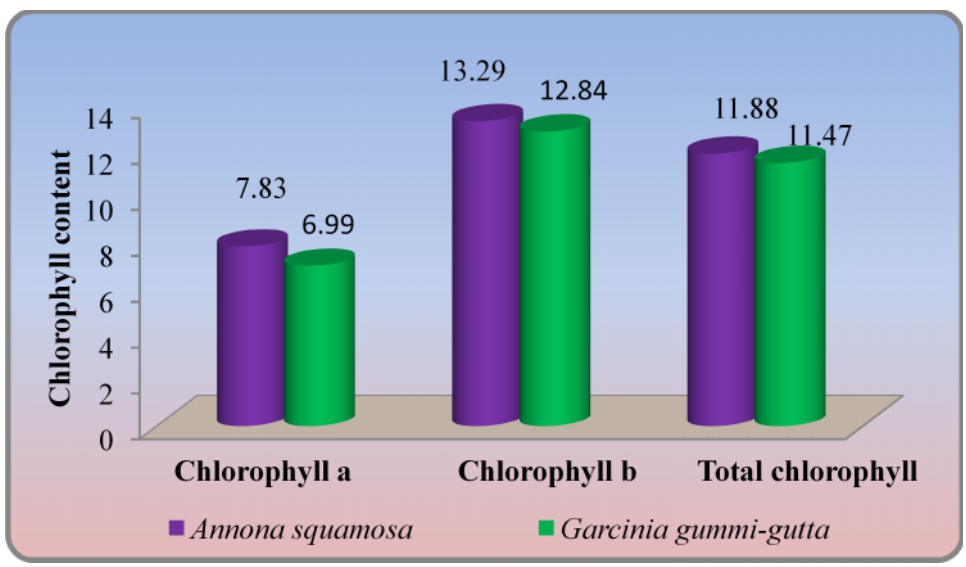
Chlorophyll 'a', chlorophyll 'b' and Total chlorophyll contents of the two medicinal plants

Name of the plant	Chlorophyll 'a' of leaf sample (mg/g)	Chlorophyll 'b' of leaf sample (mg/g)	Total chlorophyll of leaf sample (mg/g)
Annona squamosa L.	7.83 ± 0.71	13.29 ± 0.57	11.88 ± 0.50
Garcinia gummi-gutta (L.) Roxb.	6.99 ± 0.75	12.84 ± 0.35	11.47 ± 0.31

Values are mean ± SD of triplicates

Figure 1

Chlorophyll 'a', chlorophyll 'b' and Total chlorophyll contents of the two medicinal plants



Estimation of protein

Protein was estimated at different concentrations viz, 0.2, 0.4, 0.6 and 0.8 ml for both the medicinal plants *Annona squamosa* and *Garcinia gummi-gutta.* It was found to be significantly higher at 0.8 ml concentration in both *A. squamosa* and *G. gummi-gutta*. The values are presented in Table II, III and Figure 2, 3.

At 0.2 ml concentration, the protein content was 46.64±1.88 mg (*A. squamosa*) and 44.84±0.53 mg (*G. gummi-gutta*). It increased to 52.11±1.05 mg (*A. squamosa*) and 50.91±2.40 mg (*G. gummi-gutta*) at 0.8 ml concentration. The biochemical parameters studied in four medicinal plants namely *Rhodomyrtus tomentosa, Psidium guajava, Citrus aurantium* and *Citrus limonum* showed a significantly higher protein, carbohydrate and chlorophyll 'a' content in *C. aurantium*. The chlorophyll 'b' and total chlorophyll content was found to be significantly higher in *R. tomentosa* (Kiruba, 2014).

Estimation of carbohydrate

The carbohydrate content was estimated at different concentrations for both the medicinal plants *Annona squamosa* and *Garcinia gummi-gutta.* At 0.2 ml concentration, the carbohydrate was found to be 60.94±4.43 mg and 63.50±1.10 mg in *A. squamosa* and *G. gummi-gutta* respectively (Table IV, V and Figure 4, 5).

The carbohydrate level increased with increase in the concentration. Carbohydrate are the primary source of energy to our body and are often referred to as "fuel of life". The carbohydrate content was estimated to be higher in *Chromolaena odorata* at 0.1 ml concentration itself (Gayathri and Bindhu, 2016).

Watal *et al*. (2014) have evaluated the presence of carbohydrate, glycosides and coumarins in the plant parts that are known to exert a beneficial action on immune system by increasing the strength of the body and hence can be used as a valuable dietary supplement.

Table II

Protein content of *Annona squamosa* L. at different concentrations

Concentration (in ml)	Protein content (mg/g f.wt.)
0.2ml	46.64±1.88
0.4ml	48.79±1.39
0.6ml	50.30±1.38
0.8ml	52.11±1.05
SEd CD ($p<0.05$)	1.8360 4.0909

Values are mean ± SD of triplicates

Figure 2

Protein content of *Annona squamosa* L. at different concentrations

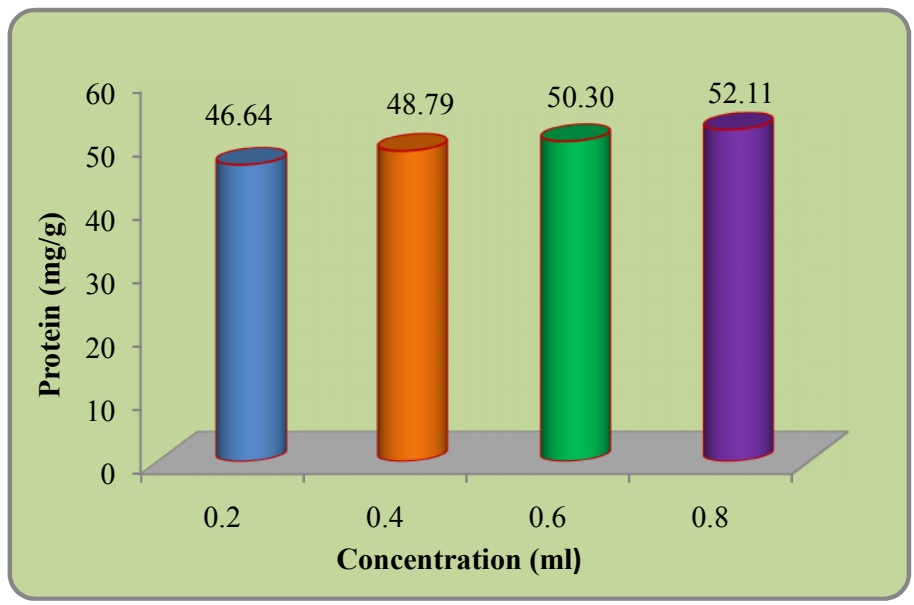


Table III

Protein content of *Garcinia gummi-gutta* (L.) Roxb. at different concentrations

Concentration (in ml)	Protein content (mg/g f.wt.)
0.2ml	44.84±0.53
0.4ml	47.57±1.39
0.6ml	48.79±1.39
0.8ml	50.91±2.40
SEd CD ($p<0.05$)	1.3606 3.0316

Values are mean ± SD of triplicates

Figure 3

Protein content of *Garcinia gummi-gutta* (L.) Roxb. at different concentrations

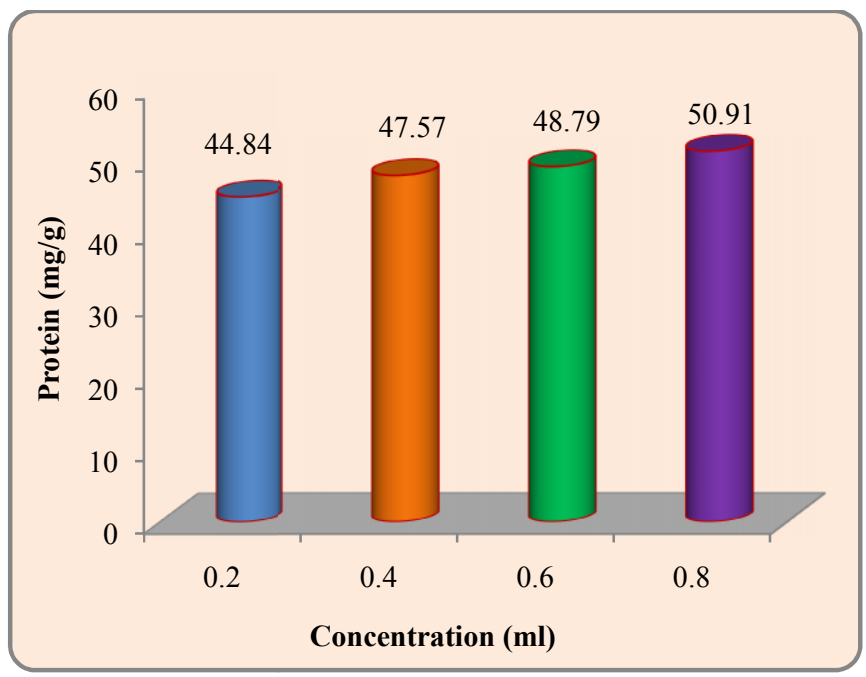


Table IV

Carbohydrate content of *Annona squamosa* L. at different concentrations

Concentration (in ml)	Carbohydrate content (mg/g f.wt.)
0.2ml	60.94±4.43
0.4ml	71.16±2.19
0.6ml	73.00±1.51
0.8ml	72.04±2.22
SEd CD ($p<0.05$)	2.3122 5.1518

Values are mean ± SD of triplicates

Figure 4

Carbohydrate content of *Annona squamosa* L. at different concentrations

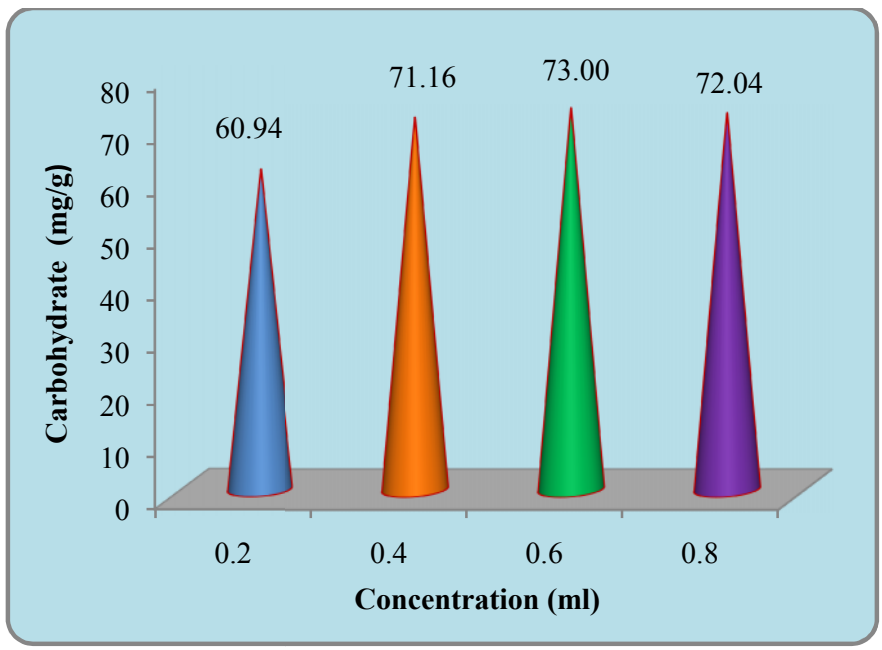


Table V

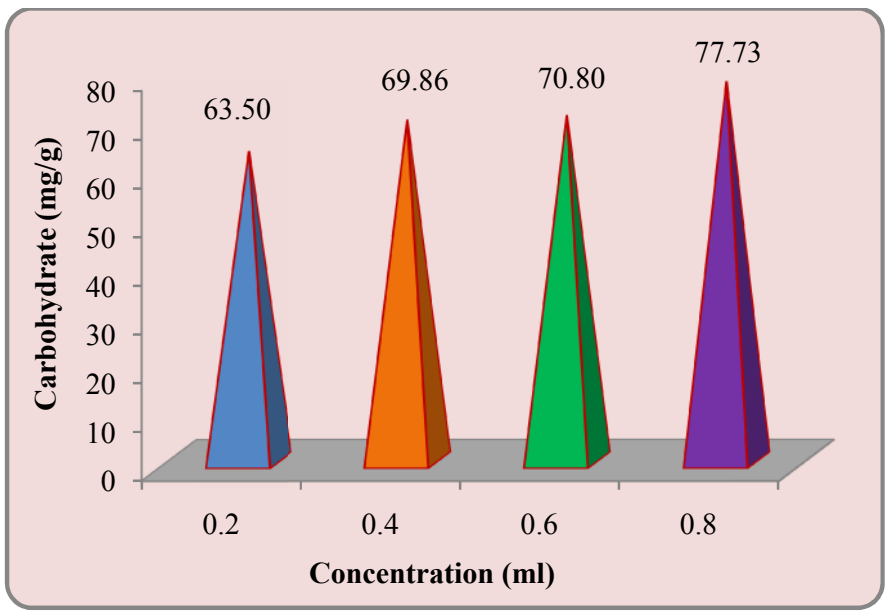
Carbohydrate content of *Garcinia gummi- gutta* (L.) Roxb. at different concentrations

Concentration (in ml)	Carbohydrate content (mg/g f.wt.)
0.2ml	63.50±1.10
0.4ml	69.86±1.72
0.6ml	70.80±4.14
0.8ml	77.73±3.29
SEd CD ($p<0.05$)	2.3921 5.3299

Values are mean ± SD of triplicates

Figure 5

Carbohydrate content of *Garcinia gummi- gutta* (L.) Roxb at different concentrations



Preliminary phytochemical analysis of leaves of *Annona squamosa* L. and *Garcinia gummi-gutta* (L.) Roxb.

In the present study, a preliminary phytochemical screening was done to analyze the presence of various secondary metabolites such as alkaloids, flavonoids, terpenoids, quinones, oil and fats, sterols, carbohydrates, aminoacids, tannins, phenols and phlobatannins in both the medicinal plants (Table VI and VII).

The dried and powdered leaves of *A. squamosa* and *G. gummi-gutta* were extracted using water, chloroform and ethanol and the extracts thus obtained were analyzed for the presence of various phytoconstituents. Carbohydrate and phenol was present in all the three extracts of *A. squamosa*. Alkaloid was present in water and chloroform extracts. Flavonoids, terpenoids, aminoacids and phlobatannin were present only in water extract.

Kaladhar *et al*. (2014) have carried out phytochemical analysis of raw fruit peel crude extracts of *Annona squamosa* L. The result of phytochemical studies showed the presence of alkaloids, flavonoids, phenols and saponins. Different species of *Annona* have been used for centuries as traditional folk medicine for the treatment of various diseases and also used as insecticides. The plants are considered to be a good source of vitamins, minerals, plant proteins, fibers, etc. as well as, the plant is supposed to have tremendous pharmacological importance (Suneel *et al.,* 2015).

In the case of *G. gummi-gutta,* only phenol was present in all the three extracts. Flavonoids, sterols, tannins and phlobatannin were completely absent in the three extracts of *G. gummi-gutta.* Terpenoids and quinones were present in water extract. Oil and fat was present in ethanol extract of *G. gummi-gutta.* Aja *et al.* (2010) have shown that the phytochemical analysis is very useful in the evaluation of some active biological compound from medicinal plants.

A study carried out by Sharma (2012) on preliminary phytochemical screening of leaf extracts of *Aegle marmelos, Annona squamosa, Ficus racemosa, Hibiscus rosa sinensis* and *Psidium guajava* revealed the presence of different type of compounds like alkaloids, coumarins, flavonoids and steroids that could be responsible for antidiabetic activities.

Table VI

Preliminary phytochemical analysis of *Annona squamosa* L.

Phytoconstituents	Water	Chloroform	Ethanol
Alkaloids	+	+	-
Flavonoids	+	-	-
Terpenoids	+	-	-
Quinones	-	-	-
Oil and Fats	+	-	+
Sterols	-	-	-
Carbohydrate	+	+	+
Aminoacid	+	-	-
Tannins	-	+	-
Phenols	+	+	+
Phlobatannin	+	-	-

Table VII

Preliminary phytochemical analysis of *Garcinia gummi-gutta* (L.) Roxb.

Phytoconstituents	Water	Chloroform	Ethanol
Alkaloids	+	+	-
Flavonoids	-	-	-
Terpenoids	+	-	-
Quinones	+	-	-
Oil and Fats	-	-	+
Sterols	-	-	-
Carbohydrate	+	-	+
Aminoacid	+	-	-
Tannins	-	-	-
Phenols	+	+	+
Phlobatannin	-	-	-

Earlier work by Gayathri and Kiruba (2014) have shown the presence of terpenoids, oil and fats, starch, carbohydrate and cellulose in four different solvent extracts of *Citrus limonum.*

G.gummi-gutta shows the presence of appreciable to moderate amounts of phytochemicals such as flavonoids, coumarins, alkaloid and terpenoids which can be correlated with the possible medicinal potential of the plant (Vijayalakshmi *et al*, 2014).

Chemical tests performed using ethanol extract of leaves of *Sida cordifolia* L. showed the presence of alkaloids, flavonoids, lignin, glycosides, saponins, phytosterols and fixed oils (Ranjani, 2015).

The phytochemical study of *S. nigrum* leaves showed the presence of almost all the secondary metabolites screened using five different solvents (Gayathri and Karthika, 2016).

Leaf, stem and flower extracts of *Sphenoclea zeylanica* has been analysed for the presence of secondary metabolites by Gowri *et al*. (2016). In their study, they have used four different solvents for extraction and the presence of flavonoids, alkaloids, steroids, etc. have been identified.

CHAPTER V

SUMMARY AND CONCLUSION

The present investigation on the two medicinal plants *Annona squamosa* L. and *Garcinia gummi-gutta* (L.) Roxb. has revealed the presence of various biochemical compounds such as chlorophyll, protein and carbohydrate.

Among the two medicinal plants taken for study, the chlorophyll 'a', chlorophyll 'b', total chlorophyll and protein content was found to be significantly higher in *Annona squamosa* L. The carbohydrate content was found to be higher in *Garcinia gummi-gutta* (L.) Roxb.

The preliminary phytochemical analysis on the two medicinal plants showed the presence of carbohydrate and phenol in all the extracts of *A. squamosa.* Alkaloids, flavonoids, terpenoids, aminoacids and phlobatannin were present in water extract of *A. squamosa.* In the case of *G. gummi-gutta,* terpenoids and quinones were present in water extract and oil and fat was present in ethanol extract. Plants are the valuable medicinal sources that are used in treatment of several diseases.

Phytochemicals act in numerous ways to assist the human body in combating disease and health problems. The preliminary phytochemical screening tests may be useful in the detection of bioactive principles and subsequently, may lead to drug discovery and development.

The presence of various phytoconstituents in the powdered leaves of the two medicinal plants indicate their potential use in drug synthesis or production. The present study is only a qualitative analysis of the medicinal plants. Further studies are required to analyze the various bioactive compounds present in the two medicinal plants that could contribute significantly in drug development and benefit human life.

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