Phytochemical investigation of the peels and pulp of *Citrus limetta* and *Citrus sinensis*

R. ANGELIN VINOTHA

(13PBC001)

A Thesis submitted to Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043

In Partial Fulfilment of the Requirement for the Degree of Master of Science in Biochemistry

March 2015

Certificate

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Signature of the Head of the Department

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Signature of the Supervisor

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Introduction

1.0 INTRODUCTION

Phytochemicals are the substances found naturally in all fruits, vegetables and medicinal plants that ingested daily or rarely, may exhibit a potential for modulating the human metabolism in a way favourable for the preclusion of chronic and degenerative diseases. These days, many studies are conducted on thousands of phytochemicals that may have important physiological and biochemical effects. Among phytochemicals, several compounds, including flavonoids, polyphenols, stilbenes, carotenoids and anthocyanins, are known to be important for a number of health promoting effects (Agarwal *et al*., 2015).

Secondary metabolites are small biomolecules considered to be non-essential for the life of the producer organisms. They provide the producer organism with survival advantages in various ways, for instance by improving nutrient availability, by protecting against environmental stressors, by enhancing competitive interactions with other organisms, or by acting as a metabolic defence mechanisms. Many secondary metabolites have great importance for humans. They are widely used as active drug ingredients in medicine. Many antibiotics, antitumor agents and antivirals are derived from secondary metabolites, which includes antipyretics like aspirin, hallucinogenics like LSD, and cholesterol-lowering drugs like lovastatin, as herbicides or phytotoxins in agriculture, as food additives (colour, flavours and sweeteners), fragrances, and even as precursors for the synthesis of plastics (Blumenthal *et al*., 2013).

In recent years, there is an increase in the areas of research related to development of novel drugs for the developments prevention of disease. Flavonoids, phenols and alkaloids are the most important groups of secondary metabolites or bioactive compounds in plants and are the good sources of natural antioxidants in human diets. They are also a kind of natural product and antioxidant substance capable of scavenging free superoxide radicals, reducing the risk of cancer and protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA (Saxena *et al*., 2013).

Medicinal plants are being used for centuries as remedies for human diseases because they contain chemical components of therapeutic value. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Javed *et al*., 2013).

Natural products, especially plants have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient times and an impressive number of modern drugs have been developed from them (Kaur *et al*., 2014).

Medicinal herbs play an important role in primary health care system among rural population, since synthetic anti-cancer remedies are beyond the reach of common man because of the cost factor. The herbal medicines have a vital role in the prevention and treatment of cancer which execute their therapeutic effect by inhibiting cancer activating enzymes and hormones, stimulating DNA repair mechanisms, promoting production of protective enzymes inducing anti-oxidant action and enhancing immunity. Siddha system of medicine provides a good base for scientific exploration of potent anti-cancer herbs (Gladys *et al*., 2013).

In India, several plants are inferred for various studies for their medicinal value. Phytochemistry reveals various antioxidant and antimicrobial activities which gains clinical significance in the field of drug therapeutics. The important products of *Citrus* fruits are the essential oil, which is obtained from *Citrus* peels. These oils are considered to be one of the potential sources for the screening of antimicrobial, antioxidant, and free radicals scavenging agents (Shinde *et al.,* 2013).

Natural plants are formulated to generate the different kinds of effective drugs to enhance anticancer activities. The complex synergistic interactions of the various constituents of anticancer herbs, which would help in the design to attack the cancerous cells of the body (Narah and Chandha, 2012). *Annona species* contain acetogenins, which possess significant cytotoxic activity against leukemia and sarcoma. Acetogenins are found to be effective in nasopharyngeal

carcinoma treatments. *Arctium lappa* contains anticancer factors that prevent mutations in the oncogenes. It has been effectively used in the treatment of malignant melanoma, lymphoma and cancers of the pancreas, breast, ovary, oesophagus, bladder, bile duct and the bone (Manju *et al.,* 2013).

Medicinal plants are rich sources of antimicrobial agents. Plants are used for medicinal purpose in different countries and they are the sources of potential and powerful drugs. A wide range of medicinal parts are used to get different rasayanas, which possess different medicinal properties against different microbes. Although, hundreds of plants species have been tested for antimicrobial properties, the majority of these have not been adequately evaluated (Vashit and Jindal, 2012).

Medicinal plants have been used for centuries as remedies for human diseases since the phytochemicals are abundant in fruits, because they contain chemical components of therapeutic value. The genus *Citrus* (*Rutaceae*) comprises of trees, shrubs and herbs of various. They are the most widespread arboreal plants in the world and represent one of the most important crops. *Citrus* plant is native to tropical Asia but it is also found in all tropical and subtropical countries. It is easily available plant showing a wide range of uses in treatment of various diseases. The major active biological constituents in *Citrus* herbs are flavonoids, phenolics and alkaloids with beneficial medicinal effects on human health. It has been used for their essential oil in foods and perfumes (Javed *et al.,* 2014).

In general, flavonoids components play important roles in the control of different human diseases. Much of guava's therapeutic activity is attributed to these flavonoids. The flavonoids have demonstrated antibacterial activity. Quercetin is thought to contribute to the anti-diarrhoea effect of guava; it is able to relax intestinal smooth muscle and inhibit bowel contractions. In addition, other flavonoids and triterpenes in guava leaves showed antispasmodic activity (Joseph *et al.,* 2014).

Medicinal applications of alkaloid compounds for their hypoglycaemic activity, which is of little importance compared to their cytotoxic effects. They have been used to treat diabetes, high blood pressure and the drugs have even been used as disinfectants. Nevertheless, the alkaloids are so important for being cancer fighters (Moudi *et al.,* 2013).

The present study was aimed to screen for the Phytochemicals constituents of *Citrus limetta* and *Citrus sinensis.*

Review of Literature

2.0 REVIEW OF LITERATURE

2.1 Phytochemicals

"Phyto" is the greek word of the plant. There are many families of phytochemicals and they help the human body in a variety of ways. Phytochemicals may protect human from various diseases, phytochemicals are non-nutrient plant chemicals, that have protective or disease preventive properties. Phytochemicals are basically divided in two groups that are primary and secondary metabolites (Santhi *et al*., 2012).

Phytochemicals have been proposed to offer protection against a variety of chronic ailments including cardiovascular diseases, obesity, diabetes, and cancer. As for cancer protection. It has been estimated that diets rich in phytochemicals can reduce cancer. The old saying "Prevention is always better than cure" is particularly true in the case of cancer where a cure, if at all possible, is associated with high cytotoxic loads and invasive procedures, it has become apparent that strategies which limit DNA damage and increase the probability of DNA repair by inhibiting aberrant proliferation will decrease cancer incidence (Manju *et al.,* 2012).

The plants exhibit a wide range of biological and pharmacological activities such as anti-inflammatory, diuretic, laxative, antispasmodics, antihypertensive and antimicrobial activities. These functions are performed due to chemical constituents comprising sugars, lipids, proteins, vitamins, minerals and phytochemicals (Okwu and Nnamdi, 2012).

Plants have limitless ability to synthesize aromatic substances mainly secondary metabolites of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. The synthesized aromatic substances are used by plants as defensive molecules against predation by microorganisms, insects and herbivores. However, some of which may involve in plant odour (terpenoids), pigmentation (tannins and quinines) and flavour. These defensive molecules give plants their medicinal value is accepted by human being of their great importance in health care of individuals and communities (Bolatito and Coolborn., 2012).

Phytochemicals are divided into two main groups that are primary and secondary constituents according to their function in the plant body. Secondary constituents consist of terpenoids and phenolics compounds. These compounds are believed to be involved in vital biochemical functions and as essential enzyme systems (Hussain *et al*., 2013).

2.2 Secondary Metabolites

Primary metabolites comprises of common sugars, amino acids, proteins and chlorophyll, while secondary metabolites are flavonoids, phenols, alkaloids, tannins, terpinoids, saponins and so on (Santhi *et al*., 2012).

Secondary metabolites are small biomolecules, considered to be non-essential for the life of the producer organism. They provide the producer organism with survival advantages in various ways, for instance by improving nutrient availability (e.g., in the form of chelating agents such as siderophores), by protecting against environmental stressors (e.g., pigments and osmoprotectants), by enhancing competitive interactions with other organisms (e.g., antibiotics, but also various signalling molecules), or by acting as a metabolic defence mechanism (e.g., many plant flavonoid and alkaloid toxins).

Many secondary metabolites have great importance for humans. They are widely used as active drug ingredients in medicine e.g., many antibiotics, antitumor agents and antiviral are derived from secondary metabolites, these are antipyretics like aspirin, hallucinogenic like LSD, and cholesterol-lowering drugs like lovastatin, as herbicides or phytotoxins in agriculture, as food additives (colour, flavours and sweeteners), fragrances, and even as precursors for the synthesis of plastics.

The rapid development of genomics in the last few years has helped us reveal that many organisms encode the potential to produce many more secondary metabolites than was originally expected. Most of these new secondary metabolites are only predicted by bioinformatics analysis of putative secondary metabolite gene clusters in sequenced genomes, but are not produced naturally under laboratory conditions or are present at levels that are too low to be detected by standard methods. In some cases, the production of such cryptic or sleeping secondary metabolites has been successfully induced by genetic manipulations. The emerging methods of Synthetic Biology have recently resulted in renewed interest in the discovery of novel bioactive secondary metabolites from a wide variety of sources. Metabolomics is a key component of the Synthetic Biology approach to secondary metabolite biology. It aims at discovering and characterizing secondary metabolites in their metabolic context in natural or engineered bio-systems, by simultaneously measuring as many low molecular weight compounds as possible. Comprehensive and detailed overviews of metabolomics methods applied to plant studies, Synthetic Biology and pathogens have been presented recently (Breitling *et al*., 2013).

2.2.1 Alkaloids

The term alkaloid was first proposed by Meissner in 1819 to characterize these alkali – like compounds found in plants, but it was not precisely defined. With time, the definition has changed to a compound that has nitrogen atom in a cycle ring. Numerous biological amines and halogenated cyclic nitrogen containing substances are in the term alkaloid. Morphine was the first discovered

alkaloid extracted from a terrestrial plant in 1805 as reported by Kappelmayer and Hordenine was the first alkaloid isolated from marine algae in 1969. Today approximately 2000 alkaloids are known (Giiven and Cassidy, 2010).

Around 12000 alkaloids of various types have been known to occur in all land plants, including more than 150 families. In plants, alkaloids generally exist as salt of organic acids like acetic, oxalic, citric, malic, lactic, tartartic and other acids. In angiosperms, alkaloids are common in dicots than in monocots. Pepperdine alkaloids such as conine and N-methyl coninne are present in *Conium maculatum*. Cocaine in erythroxylum coca was the first local anaesthetic to be discovered. Alkaloids currently in clinical use include the analgesics morphine, codeine, anticancer agent, vinblastine, the gout suppressant colchicines, the muscle relaxant tubocurarine, the antiarrythemicajmalicine, the antibiotic sanguinarine and sedative scopolamine (Remawat, 2012).

Most alkaloids are derivd from amines produced by the decarboxylation of aminoacids such as histidine, lysine, ornithine, tryptophan and tyrosine. Benzylisoquinoline alkaloids are a large and diverse group of pharmaceutical alkaloids with 2500 defined structures. Recent studies have also suggested that these alkaloids may be useful as novel medicine (Moudi *et al*., 2013).

2.2.2 Flavonoids

Over 4000 flavonoids have been identified to date and are widely distributed in the leaves, seeds, bark and flowers of plants. In plants, these compounds provide protection against UV radiation, pathogens and herbivores. Anthyocyanin which is present as copigments in flowers helps to attract pollinating insects. Flavonoids are benzo gamma pyrone derivatives constituting of phenolics and pyrane rings.

Many beneficial health effects are attributed to flavonoids, mostly due to their antioxidant and chelating abilities. Numerous studies have been conducted to prove flavonoids efficacy as antimycotic, antibacterial, antiviral, anti-inflammatory, antioxidant, immune modulator, enzyme inhibitor, mutagenic and toxic agents (Matejic *et al.,* 2012).

Flavonoids reduce the risk of stroke and heart disease. Flavonoids protect against related disorders, relieve hay fever, asthma symptoms, reduce inflammation in joints and muscles, reduce various veins and battle infections. A considerable number of pharmaceutical preparations containing flavonoids as active substances are commercially available today. For example: *Ginkgo biloba*leaf extract was used in early stages of Alzimers's disease, Vascular dementia and memory impairment. Quercetin, the most common dietery flavonoid, is generally used as dietery supplement (Malsev and Kuntic, 2011).

2.2.3 Phenols

Free radicals produced in the body react with various biological molecules namely lipids, proteins, deoxyribonucleic acids resulting in the imbalance between oxidants and antioxidants. Even though our body is safeguarded by natural antioxidant defence, there is always a demand for antioxidants from natural sources.

Phenolic compounds from medicinal plants possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free radicals. They are well known as radical's scavengers, metal chelators, reducing agents, hydrogen donors and singlet oxygen quenchers (Narayanaswamy and Balakrishnan, 2013).

Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenyl propane derived compounds which are in highest oxidant state. Catechol and pyrogallol are hydroxylated phenols shown to be toxic to microorganisms. Phenolic compounds possessing a 3-carbon side chain at a lower level oxidation and containing no oxygen are classified as essential oils and cited as antimicrobial. Eugenol is considered bacteriostatic against both fungi and bacteria (Sher*,* 2009).

2.2.4 Terpenoids

Terpenoids constitute one of the largest families of natural products accounting for more than 40,000 individual compounds of both primary and secondary metabolites. All organisms naturally produce some terpenoids as part of secondary metabolism but many produce terpenoids via secondary metabolism. Isopentyl diphosphate and its isomer Di Methyl Allyl Di Phosphate (DMAPP) are the universal five carbon precursors of all terpenoids. Terpenoids have been shown to be available for pharmacological applications. For example: artemisin and taxol as malaria and cancer methods (Saxena *et al.,* 2013).

The fragrance mixtures including terpenes found that scent chemistry of the emitted fragrance played a role in beetles and wasps pollinating an orchid species, *Satyrinum microrrhynchum.* Susceptibillty to terpense has been tested by Morales *et al* in which extracts from *Artemisia copa*showed inhibitory avtivity against yeast (*Candida albicans).*They also showed that some plant extracts containing terpenes showed biotoxicity effect against brine shrimp (*Artemiasalina)* (Zwenger and Basu, 2011). Terpenoids beta amyrin was isolated from a chloroform extract of *Euphorbia hirta.* This plant is a good source of terpenoids, used to treat colic troubles, chronic bronchitis, inflammations, dysentery, cough, asthma, worms and vomiting pulmonary disorders (Sharma and Sharma*,* 2011).

They also have some allelopathic potential. In recent years there is an increasing interest in sesquiterpene lactones, mostly because of their cytotoxic and anticancer properties. Sesquiterpene lactones are large secondary metabolites that belong to the group of C15 terpenoids. Till now 90% of identified lactones were also isolated from the family Asteraceae. Lipophilic solvents or liquid carbon oxide can be used for the extraction of sesquiterpene lactones from plant material and the purification is performed with the help of chromatographic techniques (Matejic *et al.,* 2012).

2.2.5 Saponins

Saponins comprise a large family of structurally diverse compounds containing a steroidal or terpenoidaglycone linked to one or more oligosaccharide moieties. The aglycone or non saccharide portion of the saponin molecule is called the genin or sapogenin. Depending on the type of genin present, the saponins can be divided into 3 classes that are triterpene glycosides, steroid alkaloid glycosides. The classical definition of saponin is based on their surface activity. Many saponins have detergent properties, give stable foams in water and show haemolytic activity and have a bitter taste and toxic to fish (Sezgin and Artik, 2012).

Most saponins show haemolytic and display a range of various biological and pharmacological properties, such as molluscicidal, anti-inflammatory, antimicrobial and cytotoxic activities. Commercial products containing saponins are available and used in the pharmaceutical, cosmetic and food industries. For example triterpenes saponins from *Quillaja saponaria* have immune stimulatory properties and included as adjuvants in vaccine formulation. They are also used to control nematode and insect development (Sanjay *et al.,* 2013).

Many saponins are present in higher plants in the form of glycosides of complex salicyclic compounds and show characteristic foaming properties in aqueous solutions. Many plants contain little or no saponin; in others the triterpenoid saponins predominate. The triterpenes are subdivided into 20 groups, depending on their particular structure. The basic structure found in the largest variety of medicinal plants is the oleanane-type triterpene is a substance of the therapeutic interest found in the Chinese herbal plant *Panax japonicum (*Kareru *et al.,* 2013).

2.3 Medicinal Plants and Herbs

Plants have been used to treat or prevent illness since before recorded history. Rig veda, one of the oldest available literatures written around 2000 BC mention the use of cinnamon, ginger and sandalwood etc not only in religious ceremonies but also in medical preparations. Plants and plant based medications

are the basis of many of the modern pharmaceuticals we use today for various ailments. The discovery of medicinal plants has usually depended on the experience of the population based on long and dangerous self experiment.

Progress over the centuries towards a better understanding of plant derived medicines has depended on two factors that have gone hand in hand, one has been the development of increasingly strict criteria of proof that a medicine really does what it is claimed to do and other has been the identification by chemical analysis of the active compounds in plant (Chetri *et al*,. 2013).

Natural Products, especially plants, have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs have been developed from them. The first written records on the medicinal uses of plants appeared in about 2600 BC from the Sumerians and Akkaidians. According to World Health Organization, 80 % of the people living in rural areas depend on medicinal herbs as primary healthcare system.

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Long practiced outside of conventional medicine, herbalism is becoming more main stream as up-to-date analysis and research show their value in the treatment and prevention of disease. In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. A number of medicinal plants, traditionally used for over 1000 years named rasayanas are present in herbal (Khan and Chisti*,* 2000).

In Indian systems of medicine most practitioners formulate and dispense their own recipes. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Germany, France, Japan and China have considerably improved quality of the herbal

medicines used in the treatment of cancer. Some herbs protect the body from cancer by enhancing detoxification functions of the body. Some herbs reduce the toxic side effects of chemotherapy and radiotherapy. Scientists all over the world are concentrating on the herbal medicines to boost immune cells of the body against cancer. By understanding the complex synergistic interaction of various constituents of anticancer herbs, the herbal formulations can be designed to attack the cancerous cells without harming normal cells of the body (Patel *et al.,* 2012).

The use of plants for treating diseases is as old as the human species. Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known. All over the globe, especially in South American countries, the use of medicinal plants has significantly supported primary health care. From 250 to 500 thousand plant species are estimated to exist on the planet, and only between 1 and 10% are used as food by humans and other animals. Brazil has the world's highest biodiversity, accounting for over 20% of the total number of known species. This country presents the most diverse flora, with more than 55 thousand described species, which corresponds to 22% of the global total. Such biodiversity is followed by a wide acceptance of the medicinal plant use. Most of the Brazilian population (80%) consumes only 37% of the commercially available drugs and depend almost exclusively on medicines of natural origin. Thus, phytotherapics entered the market promising a shorter and cheaper production, since basic requirements to use medicinal plants do not involve strict quality control regarding safety and efficacy compared to the other types of drugs (Chavez and Chavez, 2000).

India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Medicinal components from plants play an important role in conventional as well as western medicine. Plant derived drugs have been a part of the evolution of human, healthcare for thousands of years. Plant based drugs

are commonly used in India and China. Today a substantial number of drugs are developed from plants which are active against a number of diseases. The majority of these involve the isolation of the active ingredient (chemical compound) found in a particular medicinal plant and its subsequent modification. In the developed countries 25 percent of the medical drugs are based on plants and their derivatives and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries (Alam *et al.,* 2012).

Many higher plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives, and pesticides. The search for new plant derived chemicals should thus be a priority in current and future efforts toward sustainable conservation and rational utilization of biodiversity . In the search for alternatives to production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites (Walsh and Edward, 2009).

2.5 *Citrus* Plants

Citrus plant is native to tropical Asia but it is also found in all tropical and subtropical countries. It is an easily available plant showing a wide range of uses in treatment of various diseases. The major active biological constituents in *Citrus* herbs are flavonoids, especially hesperidin, naringenin and alkaloids, mainly synephrine, with beneficial medical effects on human health. It has been used for their essential oil in foods and perfumes. *Citrus* plants also used in herbal medicine as a stimulant and appetite suppressant. It has also been used in traditional Chinese medicine to treat nausea, indigestion, and constipation, cancer, cardio-vascular effect, of as a sedative. However, what has made bitter orange well known and popularized is the claim that it replaces the banned ephedra stimulant, without the ephedra side effects. Because of this, *C. aurantium* is a popular weight loss ingredient used in a wide variety of diet pills and fat. Bitter orange has been substituted into "ephedra-free" herbal weight-loss

products by dietary supplement manufacturers. The National Center for Complementary and Alternative Medicine found that "there is currently little evidence that bitter orange is safer to use than ephedra". It is easily available and comparatively safe (Jyotsna *and Suryawanshi,* 2012).

Citrus is one of the most important commercial fruit crops grown in all continents of the world. *Citrus* family had rich source of phytochemicals such as flavanones, polyphenols, anthocyanins and hydroxycinnamic acids which are beneficial to most pathological conditions which includes, high cholesterol and anti-inflammation; complications related to diabetes and cancer prevention. Having rich source of secondary metabolites like natural flavonoids, polyphenols, steroids, saponinsetc, in *Citrus* sinensis here pinpointing to screening the phytochemicals from different solvents and investigating on in vitro antioxidant activities of orange peel extract. The peel of *Citrus* fruit having abundant source of flavanones and many polymethoxylated flavones, which are very rare in other plants.

These *Citrus* fruit having high natural antioxidant by helping prevent free radicals from damaging the DNA of cells and causing cancer. The chief flavonoids found in *Citrus* species are limonene, hesperidin, narirutin, naringin and eriocitrin etc. Recent studies explore that the beneficial activities of *Citrus* on dietary *Citrus* flavonoids reduce the risk of coronary heart diseases (Javed *et al.,* 2013).

The important aspects of these classe of compounds are due to their pharmacological activity as radical scavengers have been reported. Flavonoids of *Citrus* have been shown to be powerful antioxidant and free radical scavengers. There is lack of information regarding the inhibitory effects of orange peel extract on lipid oxidation. (Rekha *et al.,* 2013).

2.6 *Citrus limetta* and *Citrus sinensis*

The Sweet lime (*Citrus limetta* risso), commonly known as Mousambi Fruit in India, is a native plant of Asia, which is best cultivated in India, China, southern Japan, Vietnam, Malaysia, Indonesia and Thailand. The mousambi fruit is commonly eaten fresh or made as juice which is rich source of vitamin C and instant energy,

- ✓ *Citrus* juices are the most common among the fruit juices around the world and constitute a major portion of the food industry
- ✓ Sweet lime peel is source of flavonoids, pectin and essential oil
- ✓ The *Citrus* peel oils have a strong and desirable aroma with refreshing effect.

They have been used as flavoring in foods, beverages and pharmaceutical products. They also have been used as fragrance in perfumes, cosmetic and aromatherapy. Moreover, *Citrus* essential oils have been recognized as safe due to their wide spectrum of biological activities such as antimicrobial, antioxidant anti-inflammatory and anxiolytic (Mahendera and shah, 2014).

Citrus sinensis or sweet orange originated from south East Asia, but is consumed all over the world as an excellent source of vitamin C, a powerful natural antioxidant that builds the body immune system. Important phytochemicals like liminoids, synephrine, hesperidin flavonoid, polyphenols, pectin, and sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium are also present.

These biologically active compounds prevent arteriosclerosis, cancer, kidney stones, stomach ulcers and reduction in cholesterol level and high blood which promote human health.

The human diet contains important micronutrients namely vitamins C and E, carotenoids and flavonoids, essential for maintenance of human health. Multiple dietary sources of these compounds are present virtually in all plant material. The nutritional importance of foods is due to the presence of these functional food ingredients and antioxidant nutraceuticals or phytochemicals. Phytochemicals are present in edible fruits and vegetables and when eaten potentially modulate human metabolism in a favourable manner, thereby prevent chronic and degenerative diseases. Increase in fruits and vegetables consumption protect against degenerative pathologies such as cancer and atherosclerosis as epidemiological surveys had shown an inverse relationship between dietary flavonoids intake from *Citrus* and cardiovascular diseases (Etebu and Nwauzoma, 2012).

Thus it was imperative to compare the phytochemicals and their attribution towards therapeutic potential of the two selected *Citrus* species fruits. The present study focused on the isolation and identification of these phytochemicals in the methanolic extract peel and pulp of *Citrus limetta* and *Citrus limetta* fruits. The methods procedures adopted for the assays performed are discussed in the next chapter.

Materials and Methods

3.0 MATERIALS AND METHODS

World health organization has listed over many varieties of plant species used around the world for medicinal purposes. In India, many varieties of plant species belonging to more than 1000 genera are being used in indigenous system of medicine which symbolizes the rich tradition for herb and herbal remedies. From the ancient times, different cultures around the world have used herbs and plants as a remedy in different diseased conditions and for the maintenance of health. Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material. Scientific studies available on medicinal plants indicate that promising phytochemicals can be developed for many health problems (Trease and Evans, 2007). Thus present study also aimed to screen for the phytochemicals of *Citrus limetta* and *Citrus sinensis*.

3.1 Sample collection and preparation

3.2 Preliminary screening for phytochemicals

3.3 Spectral analysis

3.3.1 UV absorption spectral analysis

3.3.2 Thin Layer Chromatographic (TLC) analysis

3.3.3 HPTLC analysis

3.3.4 HPLC analysis

3.3.5 FT-IR analysis

3.1 Sample collection and preparation

Fresh, healthy and disease free *Citrus limetta* and *Citrus sinensis* were collected from fruit stall in Coimbatore, India. The fruit materials were identified and authenticated by Botanical survey of India, Coimbatore.

Firstly, *Citrus limetta* and *Citrus sinensis* have been washed with the running tap water and from the separated fruits; peels and pulp have been taken off.

And then, these peels were sliced into small pieces and pulp was taken separately. Then, peels and pulp were dried at 37℃ for 12hours in an incubator. These dried peels and pulp were made into power. Finally, the extract was obtained by dissolving methanol with powdered peels and pulp by allowing it to evaporation.

3.2 Preliminary Phytochemical screening

The methanolic extract of peel and pulp of *Citrus limetta* and *Citrus sinensis*, were screened for the presence of Phytochemicals according to the method of (Khandelwal, 2002).

(1) Detection of alkaloids

a) Mayer's test

A fraction of the extract was treated with Mayer's reagent (1.36g of mercuric chlorate and 5g of potassium iodide in 10ml of distilled water) and noted for a cream coloured precipitate.

b) Dragendroff's test

A fraction of extract was treated with Dragendroff's reagent and observed for the formation of reddish orange precipitate.

c) Wagner's test

A fraction of the extract was treated Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of distilled water) and observed for the formation of reddish brown precipitate.

2) Detection of flavonoids

a) Aqueous NaOH test

To a fraction of the extract 1N aqueous NaOH was added and observed for the formation of yellow- orange colour.

b) Concentrated H_2SO_4 test

To a small fraction of the extract, concentrated H_2SO_4 was added and observed for the formation of orange colour.

c) Schinido's test

To a small fraction of the extract, a piece of magnesium tuning was added, followed by Concentrated H_2SO_4 heated slightly and formation of dark pink colour was observed.

3) Detection of phenolics

a) Ferric chloride test

A fraction of the extract was treated with 5% $FeCl_3$ solution and observed for the formation of deep blue colour.

b) Lead acetate test

A fraction of the extract was treated with 10% lead acetate solution and observed for the formation of white precipitate.

4) Detection of saponins

a) Foam test

A fraction of the extract was vigorously shaken with water and observed for persistent foam.

b) Haemolytic test

A fraction of the extract was added with a drop of blood placed in a glass slide and observed for the haemolytic zone.

5) Test for steroids

a) Libermann- Buchard test

To a fraction of extract, 2ml of chloroform, followed by 10 drops of acetic anhydride and 2 drops of Concentrated H_2SO_4 were added. The appearance of rose red colour which quickly changes from blue to green indicated the presence of steroids.

6) Test for tannins

a) Braemer's test

To a fraction of extract, a few drops of 10% ferric chloride was added. A dark green blue or brown colour was observed, indicating the presence of tannins.

7) Test for terpenoids

a) Salkowski test

A fraction of extract was dissolved in chloroform and shaken well with an equal volume of Concentrated H_2SO_4. The appearance of red colour, in the chloroform layer and green fluorescence in the acid layer indicated the presence of steroid.

3.3 Spectral analysis

3.3.1 Ultraviolet Visible Spectrophotometer

An absorption spectral analysis was done by a survey scan of methanolic extract of peel and pulp of *Citrus limeitta* and *Citrus sinensis* in a nanospectrophotometer (Optizen). The instrument was set to scan mode and the absorption spectrum was obtained in the range of 200nm to 800nm.

3.3.2 Thin Layer Chromotography (TLC) analysis

The presence of alkaloids, phenols and flavonoids in the methanolic and Ethanolic extract of the peel and pulp of *Citrus limeitta* and *Citrus sinensis* were studied by TLC on silica gel G60 F_{254} plates. The solvent system used for the separation of alkaloids was Ethylacetate-Methanol-Water in the ratio of (10:1.35:1) whereas phenolics was Toluene-Chloroform-Acetone in the ratio of (5:8:10) and for flavonoids, Ethyl acetate-Butanol-Formic acid-Water in the ratio of (5:1:1).

The TLC plates were developed spraying Dragendroff's reagent for alkaloids, Folins reagent for phenolics, 1% ethanolic aluminium chloride for flavonoids. The R_f value was calculated as the ratio of the distance travelled by

the solute to the distance travelled by the solvent front. The TLC plates were derivatized using appropriate reagents. The image were captured in UV 366nm before and after derivatization.

3.3.3 HPTLC analysis

The Methanol and Ethanol extracts (10mg) of peel and pulp of *Citrus limetta* and *Citrus sinensis* were dissolved in the 1mg of Methanol and centrifuged at 3000rpm for 5minutes. Further assay was performed with the supernatant. The sample volume of 2µl was loaded using a Hamilton syringe in (CAMAG LINOMAT 5) instrument in the 10 x10 silica gel G60 F_{254} plate as 8mm band. The TLC plate loaded with sample and the reference sample were kept in a TLC twin trough developing chamber which was saturated with the respective mobile phase and developed up to 90mm.

3.3.4 HPLC analysis

The methanolic extract of *Citrus limetta* and *Citrus sinensis* peel and pulp were dissolved in an appropriate volume of HPLC grade methanol and 20µl of the sample was injected using Hamilton syringe into the reverse phase c18 column of the HPLC system (Sigma-Aldrich equipped with PDA detector). The sample analysis was performed at room temperature in the wavelength range of 210-440nm at1000psi and the mobile phase used was 100% HPLC grade methanol 60minutes at a flow rate of 1ml/minute.

3.3.5 FT-IR Spectral Analysis

IR was carried out in the methanolic extract of *Citrus limetta* and *Citrus sinensis* peel and pulp. When a methanolic extract of *Citrus limetta* and *Citrus sinensis* pulp was placed in the beam, it absorbs particular frequencies, so that their intensities are reduced in the inferogram and the Fourier Transformation is the infrared absorption of the sample.

Results & Discussion

4.0 RESULTS AND DISCUSSION

Phytochemicals are chemical compounds that occur naturally in plants and animals responsible for colour and organoleptic properties, such as the deep purple of blueberries and smell of garlic. The term is generally used to refer to those chemicals that may have biological significance but are not established as essential nutrients. Scientists estimate that there may be as many as 10,000 different phytochemicals having the potential to affect diseases such as cancer, stroke or metabolic syndrome. These Phytochemicals are abundant in fruits, vegetables and herbs (Ghasemzadeh, 2012).

Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, Phenolsic acids,lignins, stilbenes, tannins, flavonoids, Quinone's, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in their antioxidant activities. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities. The ingestion of natural antioxidants have been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing and in recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Bolatito and Coolborn, 2013).

A variety of techniques can be used to determine and estimate the presence of such phytoconstituents in medicinal plants. Analysis of small amount of chemicals has become effective, easier and in-expensive owing to the development of hyphenated techniques such as UV-Vis, FT-IR and GC-MS spectroscopic techniques, which can identify pure plant compounds. Chromatography and spectroscopic techniques are the most useful and popular tools used recently for this purpose (Gladys *et al*., 2013).

Citrus fruits are mainly used by juice processing industries while the peels are generally wasted in the industries. Since the juice yield of *Citrus* is less half of the fruit weight. From waste materials, there is always an increased attention in

bringing useful products and *Citrus* wastes are no exceptions. Suitable methods have to be adopted to utilize *Citrus* fruit peel and pulp for the conversion into value-added products here by; Environmental pollution can also be reduced. A very large amount of orange wastes are generated every year. The *Citrus* peels are rich in nutrients and contain many phytochemicals; they also can be efficiently used as drugs or as food supplements (Kaur, 2013).

4.1 Preliminary phytochemical analysis

The results of the preliminary phytochemical analysis of the methanol extract of the pulp and peel of *Citrus limetta* and *Citrus sinensi*s are tabulated in Table 1.

Table 1 Preliminary Phytochemical analysis of pulp and peel extract of *Citrus sinensis* and *Citrus limetta*

S.No.	COMPONENTS	RESULT			
		Citrus limetta		***Citrus sinensis***	
		Peel	**Pulp**	**Peel**	**Pulp**
1.	**ALKALOIDS**				
	Mayer's test	++	++	++	++
	Dragendroff's test	++	++	++	++
2.	**FLAVONOIDS**				
	Aqueous NaOH test	++	++	++	++
	Conc. Sulphuric acid test	++	++	++	++
	Schinado's test	++	++	++	++
3.	**STEROIDS**				
	Leibermann-Buchard test	+	+	+	+
	Salkowski test	+	+	+	+

S.No.	COMPONENTS	RESULT			
		Citrus limetta		*Citrus sinensis*	
		Peel	Pulp	Peel	Pulp
4.	**TERPENOIDS**				
	Buchard test	+	+	+	+
5.	**TANNINS**				
	Braemer's test	+	+	+	+
6.	**SAPONINS**				
	Froth test	-	-	-	-
7.	**PHENOLSS**				
	Ferrric Chloride test	++	++	++	++
	Lead acetate test	++	++	++	++

(+ +) - Present in high intensity (+) - Present in modern intensity (-) - Absence

The pulp and peel extracts were rich in phytochemical constituents as shown in Table 1. The preliminary analysis showed the presence of all the major phytochemicals such as alkaloids, flavonoids, tannins, phenols, steroids and terpenoids. The data showed that the concentration of alkaloids, flavonoids and phenols were a little higher than that of the other phyto-constituents and hence only these have constituents were further analysed in study. The spectral and chromatographic analyses were performed to substantiate the data obtained.

Ghonghade *(*2013) reported that both the aqueous and ethanolic extract of *Citrus karna*are a good source of phytochemicals.

Shoumya *et al*. (2011) compared the phytochemical constituets of 3 different *Citrus* fruits namely *Citrus sinensis* (orange), *Citrus maxima* (chakotara) and *Citrus reticulata* (kinnow). Their results showed that the orange extract was best among the three samples and confirmed the presence of alkaloids, tannins, steroids and flavonoids.

Our results are in tune with Mathew *et al*., (2012) who showed that the aqueous and ethanolic peel and pulp extracts of *Citrus limonum* are a rich source of phytochemical components. The phyto-constituents of the peel extracts were found to be more than that of the pulp fractions, but only the pulp fractions consisted of reducing sugars.

The phytochemical analysis performed on crude extracts obtained from the fruit of *Morindacitrifolia* in different solvents namely, ethanol and aqueous extract indicated the presence of a broad spectrum of secondary metabolites. Alkaloids, saponins and reducing sugar were predominantly found in all the three tested extracts followed by steroid, Phenols, tannin and terpenoids (Narayanaswamy and Balakrishnan, 2012).

Thus, the results of the preliminary phytochemical screening of the fruit extracts revealed that the peel extract of both the fruits (*Citrus limetta* and *Citrus sinensis)* were rich in phytochemical composition in comparison with the pulp extract. This difference may be because of the difference in the phytochemical composition in various part of the plant as reported by Mathew *et al. (*2012)

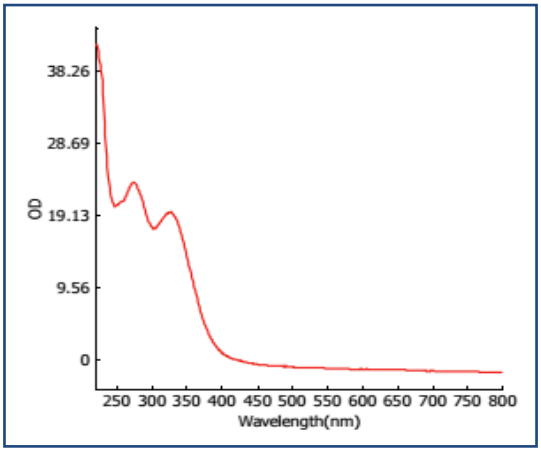
4.2 UV absorption spectral analysis

The presence of various phytochemicals present in the extract was identified using UV spectrophotometric analysis by co-elution with the standard.

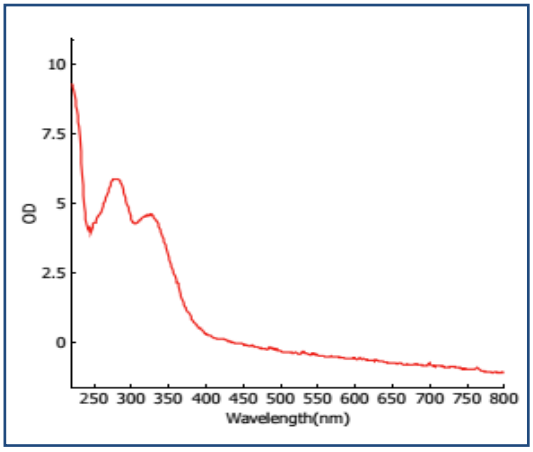
The methanol peel and pulp extract of the selected *Citrus* fruits were run in UV visble spectroscopy at the wavelength of 220 to 800nm. The results are given in Figure 1, and respectively.

Figure 1

UV absorption Spectra of Methanol Peel and Pulp extract of *Citrus limetta*



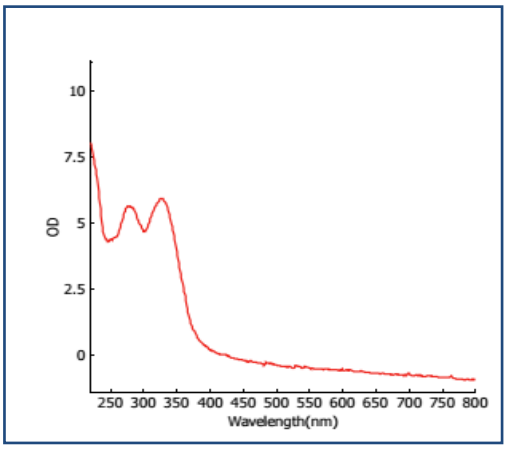
Peel of *Citrus limetta*



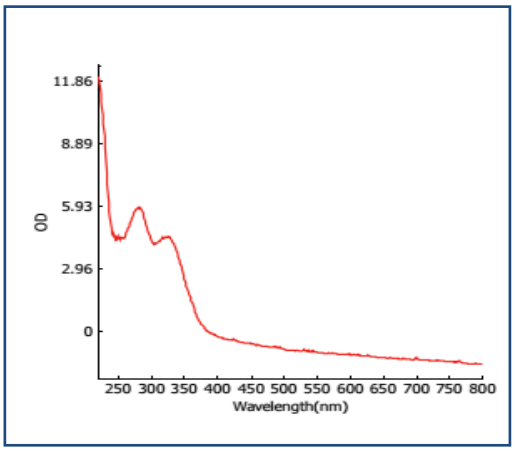
Pulp of *Citrus limetta*

Figure 2

UV absorption Spectra of Methanol Peel and of Pulp extract of *Citrus sinensis*



Peel of *Citrus sinensis*



Pulp of *Citrus sinensis*

Distinct peaks were observed in Figure 1 at 220nm, 276nm and 334nm with absorbance at 41.1, 23.45 and 19.43 in the pulp extract of *Citrus limetta.*

Peaks at 220nm, 282nm and 324nm with absorbance at 9.2, 5.8 and 4.7 respectively as seen in figure 1.The pulp extract of *Citrus limetta* showed a spectra in the peaks at 220nm.

The pulp extract of *Citrus sinensis* showed peaks at 220nm, 228nm and 284nm with absorbance of 12.2, 9.5 and 5.83 as observed in Figure 2.

Figure 2 Shows the UV- Vis spectral analysis of peel extract of *Citrus sinenesis* which showed peaks at 221nm, 277nm and 328nm with absorbance of 7.96, 5.65 and 5.9 respectively.

The UV- Vis spectral analysis of *Citrus reticulate* showed the peaks at 223.5nm, 258nm, 284nm, 303nm and 326.5nm with the absorbance of 5.086, 2.67, 3.489, 3.239 and 3.863 respectively (Showmya *et al.,* 2014).

The presence of distinct peaks in all spectrums confirms the presence of bio active compounds in the Methanolic extract of peel and pulp of *Citrus sinensis* and *Citrus limetta* fruits.

Janakiraman *et al.* (2011) studied the UV-VIS and FTIR spectroscopy of the different extracts of *Peristrophebicalyculata (Retz.) Nees.*

It proved to be a reliable and sensitive method for detection of bimolecular composition. The results of the study confirmed the presence of amides, ethers, alkanes, deuterated R-OH, organo - phosphoruscompounds, deuterated amines, aminoacids, aryl aldehydes, alkenes, ketones, sulfites, aliphatic esters, monosubstituted alkenes, sulfur, aldehydes, carboxylic acids, epoxides, alcohols, ketones, aminoacid hydrochlorides, halogen, nitroso compounds, benzene ring, silicon, boron, aliphatic nitro compounds, secondary alcohols and bromides in *P. bicalyculata*.

Though UV Spectrum confirms the presence of phytochemicals, further studies need to be carried out to confirm the value of the phytochemicals present.

4.3 TLC

The TLC chromatogram confirms the presence of various biomolecules in the peel and pulp extracts. It was found that alkaloids (Plate 1), flavonoids (Plate 2)and Phenolss (Plate 3) were present in both extracts.

The investigation of alkaloids showed two major peaks in the peel extracts corresponding to Rf value 0.36, 0.57, 0.71 respectively and the pulp extract showed faint bands with Rf value 0.42 and 0.54.

The TLC chromatogram of flavonoid showed 3 distinct bands in the chromatogram with Rf value 0.45, 0.56, 0.90 respectively in the peel extract. Prominent bands were not observed in the pulp extract.

The results of TLC analysis of phenols showed 3 distinct bands at 0.38, 0.59 and 0.68 respectively. The bands of the pulp extract were not prominent.

The analyses of the plates showed that the concentration of the compounds separated in the TLC plate were more intense in the peel extracts than in the pulp. This showed that the peel extract was a potent source of phytochemicals than the pulp extract.

Plate 1

TLC profile of Alkaloid

Lane 1-*Citrus sinensis* peel methanol

Lane 2-*Citrus limetta* peel methanol

Lane 3-*Citrus sinensis* pulp methanol

Lane 4-*Citrus limetta*pulp methanol

Plate 2

TLC profile of Flavonoid

Lane 1-*Citrus sinensis* peel methanol

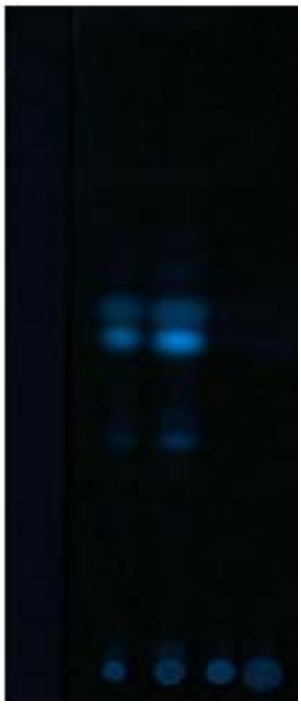
Lane 2-*Citrus limetta* peel methanol

Lane 3-*Citrus sinensis* pulp methanol

Lane 4-*Citrus limetta*pulp methanol

Plate 3

TLC profile of Phenols



Lane 1-*Citrus sinensis* peel methanol

Lane 2-*Citrus limetta* peel methanol

Lane 3-*Citrus sinensis* pulp methanol

Lane 4-*Citrus limetta*pulp methanol

The TLC profile of methanol extracts of three medicinal plants namely *Leucasaspera, Dilleniaindica and Enhydrafluctuans* in Chloroform: methanol, toluene: choloroform: acetone solvent systems confirmed the presence of biomolecules in the extract (Dutta*et al.,* 2013).

The TLC profiling of the stem, root and leaf extracts of *Centella asiatica* showed the presence of diverse groups of phytochemicals (Biradar and Rachetti, 2013).

The investigation reports of the fruit extracts of Physalis angulata reveal that the fruits possessed numerous phytochemicals that are indirectly or directly attribute biological activity of extracts. A TLC result indicates all the mobile phases are suitable to separate the compounds with good resolution which are present in those extracts (Parekh *et al.,* 2014).

TLC analysis of *Ficusracemosa* suggests the presence of different kinds of phytochemicals in leaf extract. TLC of plant extract in ethyl acetate and acetone reports three spots for various phytochemicals. The reported spots are separated with enough space and having various Rf values showing the presence of atleast three phytochemicals in ethyl acetate and acetone solvent extracts (Ganatra *et al*., 2012).

TLC allows a rapid analysis of unknown plant drug and allows an identification and assignation into a group on the basis of these constituents. TLC provide an idea about the polarity of various chemical compounds based on their Rf value in polar and non polar solvent systems and in the present study also, spots obtained for both the fruit extracts of the Rf values were comparable with standards were by confirms the presence of respective phytoconstituents.

4.4 HPTLC

Chromatograms of the standard Alkaloids, flavonoids and Phenols obtained at 366nm in the methanolic fruit extract are given in the Figure no 3, 4 and 5 respectively. The bands obtained in the peel and pulp extract was compared with the reference compound indicates the compounds identified from both the samples were similar to the reference standard and the Retention Factor of the samples are also comparable with that of the reference compound reiterates the similarity.

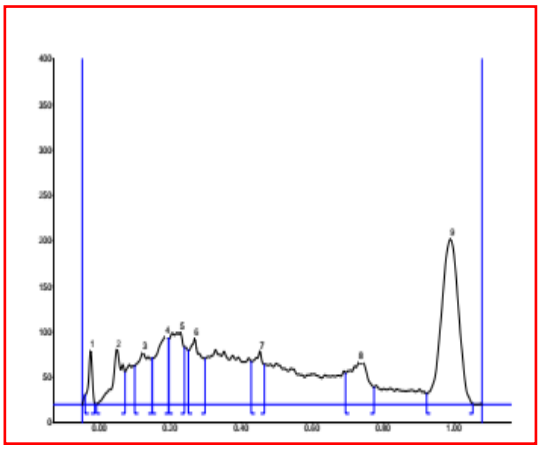
HPTLC profile shows the presence of alkaloids in both peel and pulp extracts exhibiting maximum number compared to pulp.

Peel extracts of both the fruits were found to be a rich source of flavonoids and the HPTLC profile of phenols confirmed the presence of phenols in both the peel and pulp extracts of *Citrus sinensis* and *Citrus limetta* which might be attributing for the therapeutic properties of fruits.

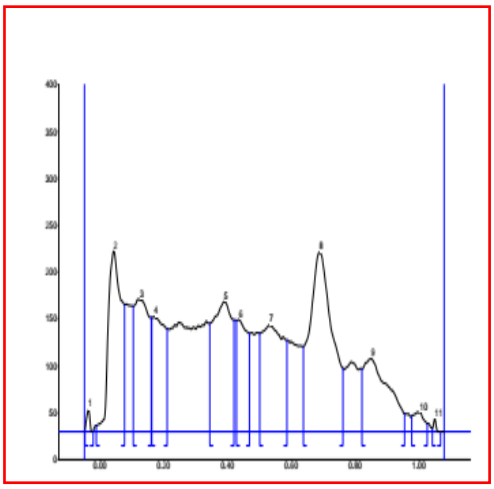
Figure 3

HPTLC profile of Alkaloids

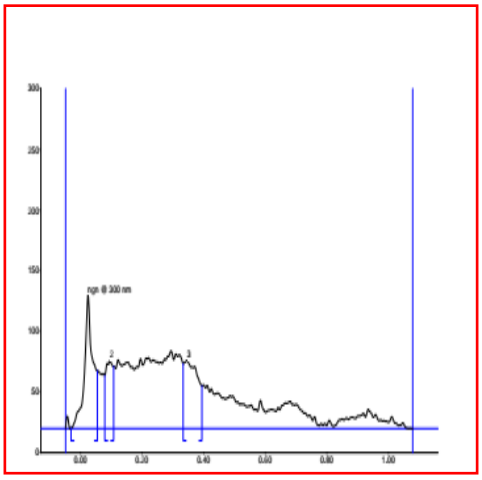
HPTLC of Alkaloids



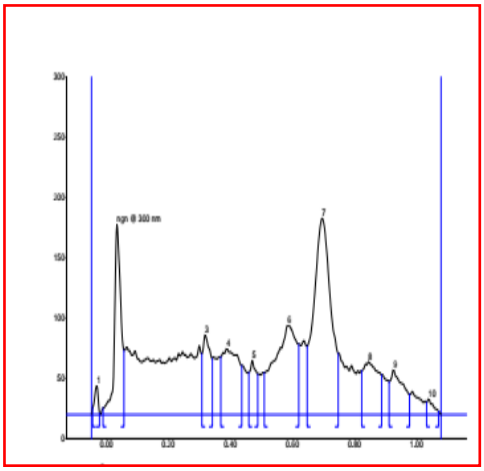
Colchicines



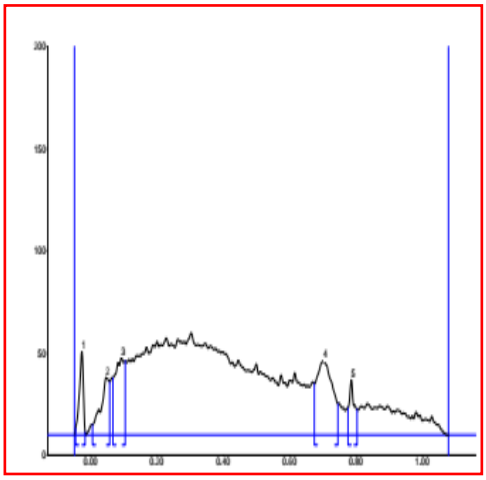
***Citrus limetta* Peel**



***Citrus limetta* Pulp**



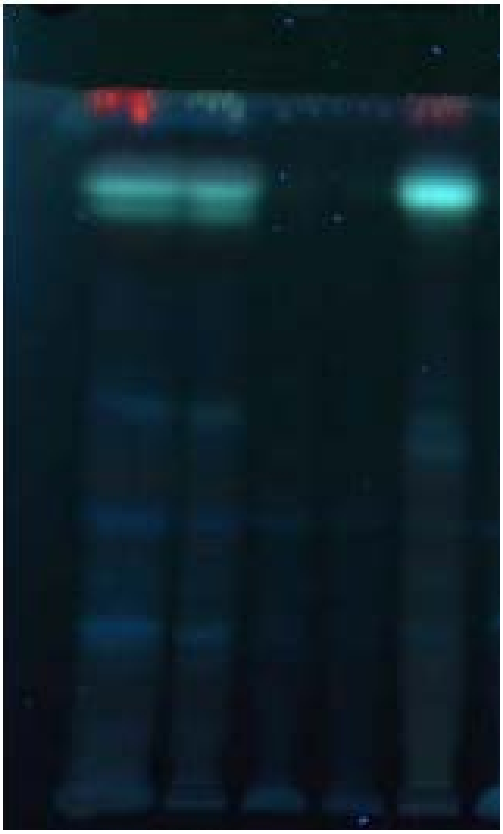
Citrus sinensis Peel



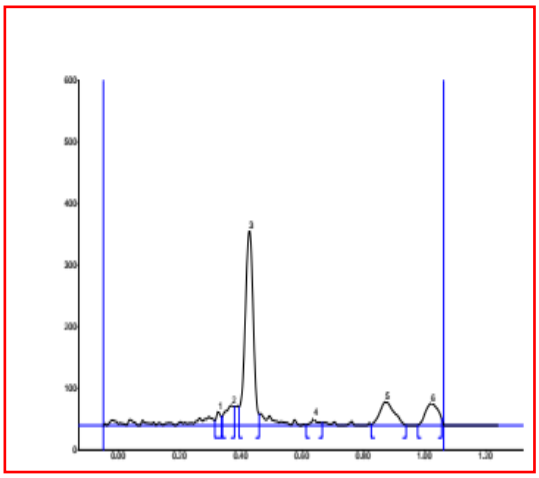
Citrus sinensis Pulp

Figure 4

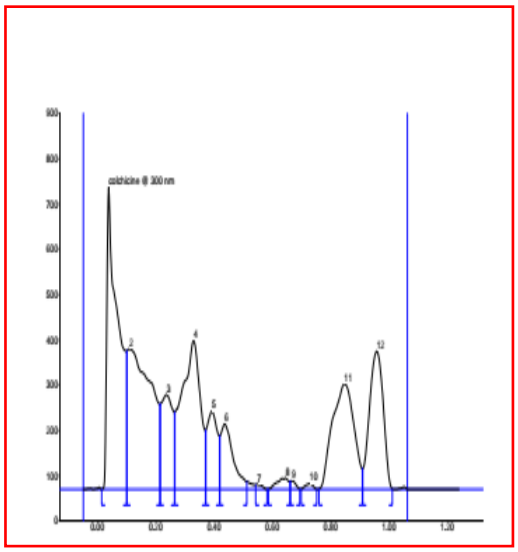
HPTLC profiles of Flavonoids



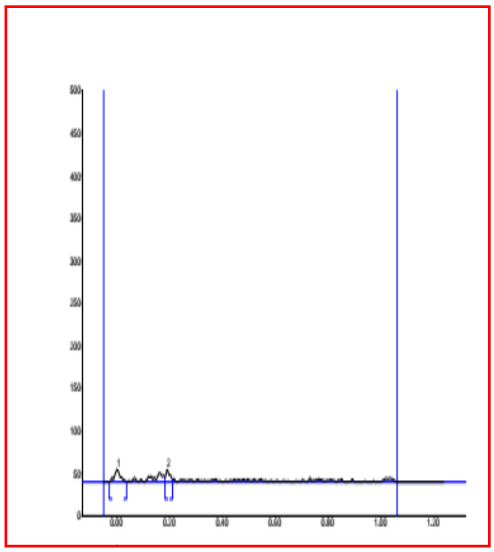
HPTLC of Flavonoids



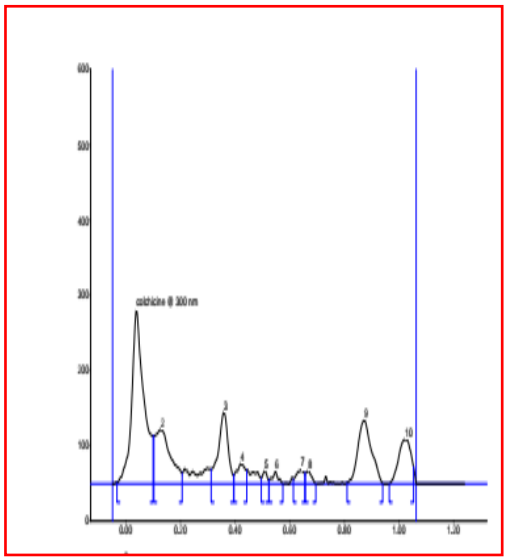
Naringenin



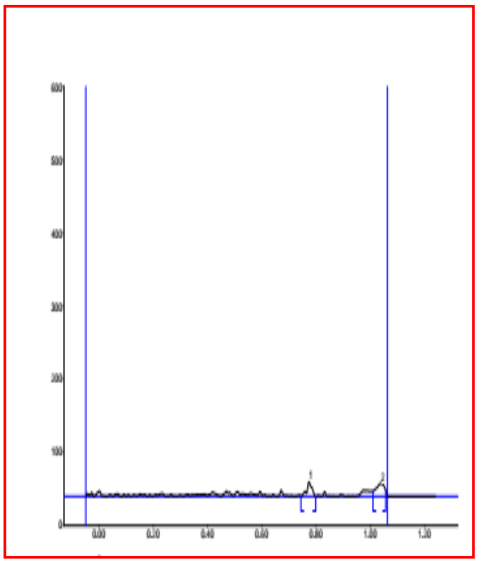
***Citrus limetta* Peel**



***Citrus limetta* Pulp**



***Citrus sinensis* Peel**

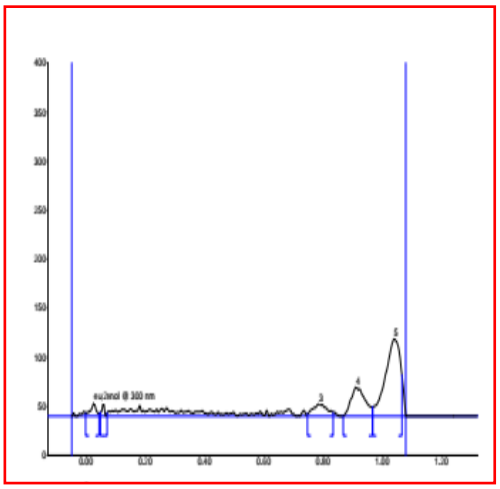


***Citrus sinensis* Pulp**

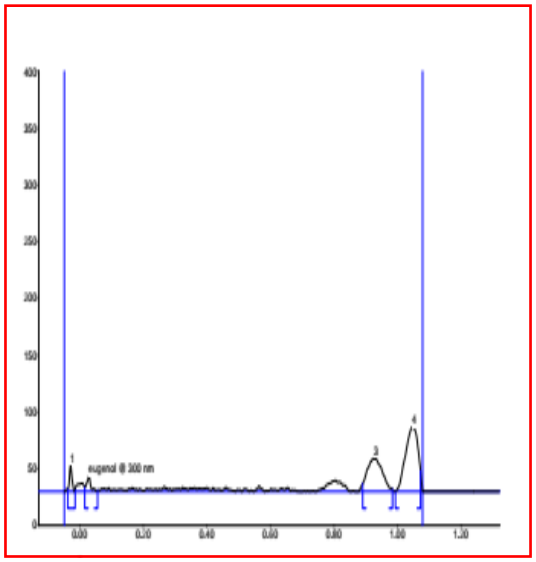
Figure 5

HPTLC profile of Phenols

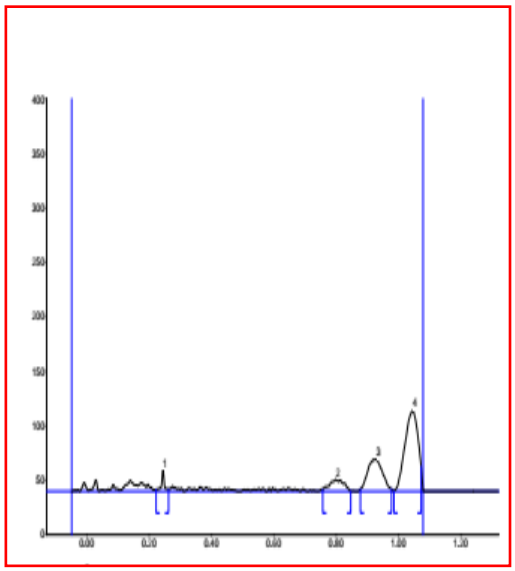
HPTLC of Phenols



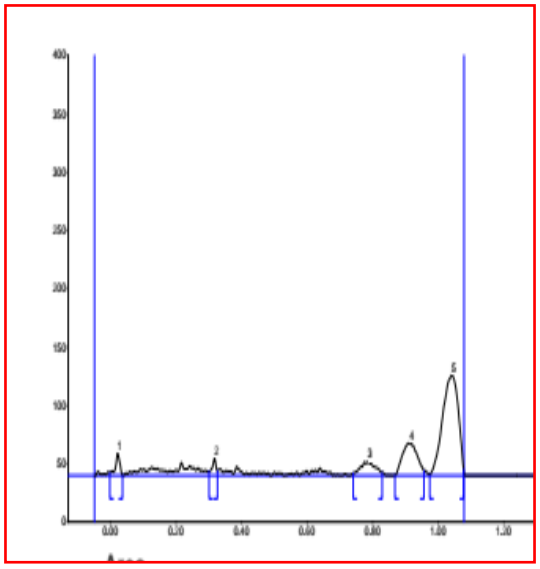
Eugenol



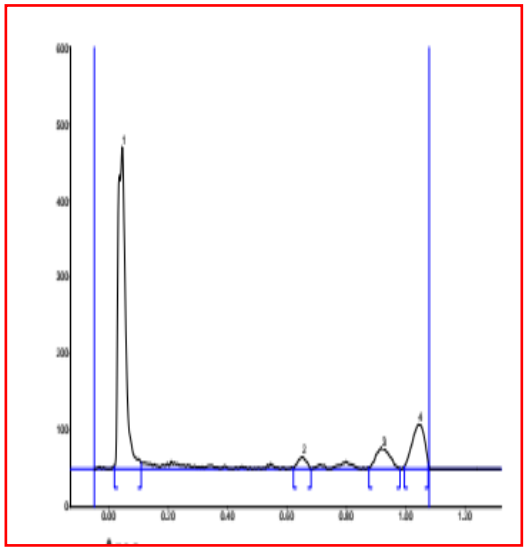
***Citrus limetta* Peel**



***Citrus limetta* Pulp**



***Citrus* sinensis Peel**



***Citrus* sinensis Pulp**

The presence of gallic acid in the methanolic extracts of leaf and flowers of *Saracaasoca*was confirmed with the help of HPTLC (Saha *et al.,* 2012).

HPTLC finger printing of acetone and methanol extract of greater cardamom fruit extracts separated 8 components and 11 components, respectively. It confirms the presence of protocatechuic acid in both extract. Here, acetone extract showed higher concentration of protocatechuic acid as compared to methanol extract (Shivanand and Mahalaxmi, 2010).

The results from HPTLC finger print for methanolic extract of *Wedelia chinensis* leaves showed the presence of alkaloid with Rf values (0.01 to 0.93). Rf value range of 0.97 confirmed the presence of flavonoids in the extracts. Phenols were confirmed with Rf value range 0.01 to 0.97. Tannin was confirmed in the extract by the Rf value range 0.01 to 0.94. These phytoconstituents in the methanolic extract had several visible colour spots on the TLC plate (Burda and Nagarajan, 2014).

4.4 HPLC

HPLC fingerprinting includes recording of the chromatograms, retention time of individual peaks and the absorption spectra (recorded with a photodiode array detector) with different mobile phases.

Figure 6

HPLC of *Citrus limetta* peel

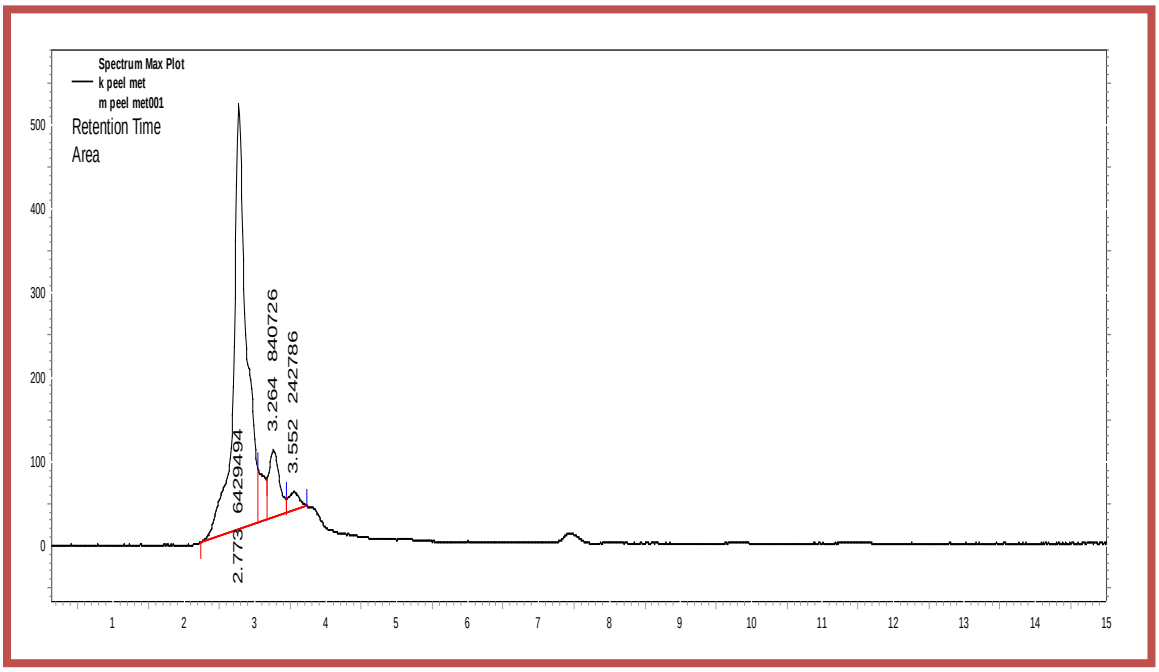


Figure 7

HPLC of *Citrus limetta* pulp

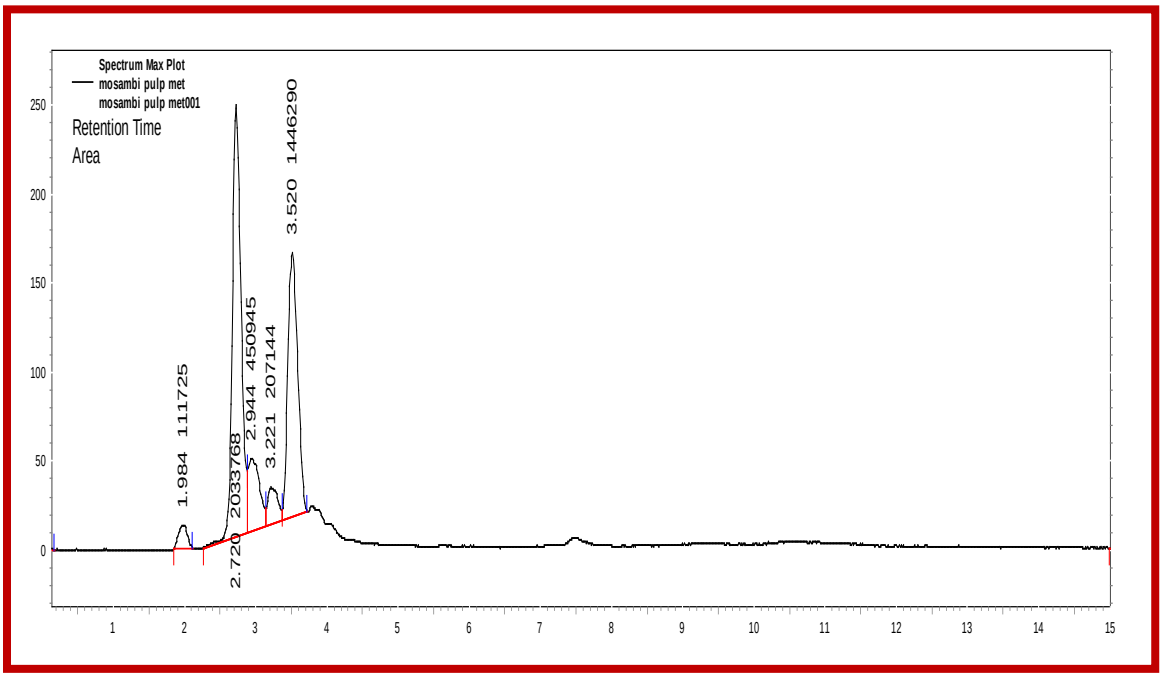


Figure 8

HPLC of *Citrus* sinensis peel

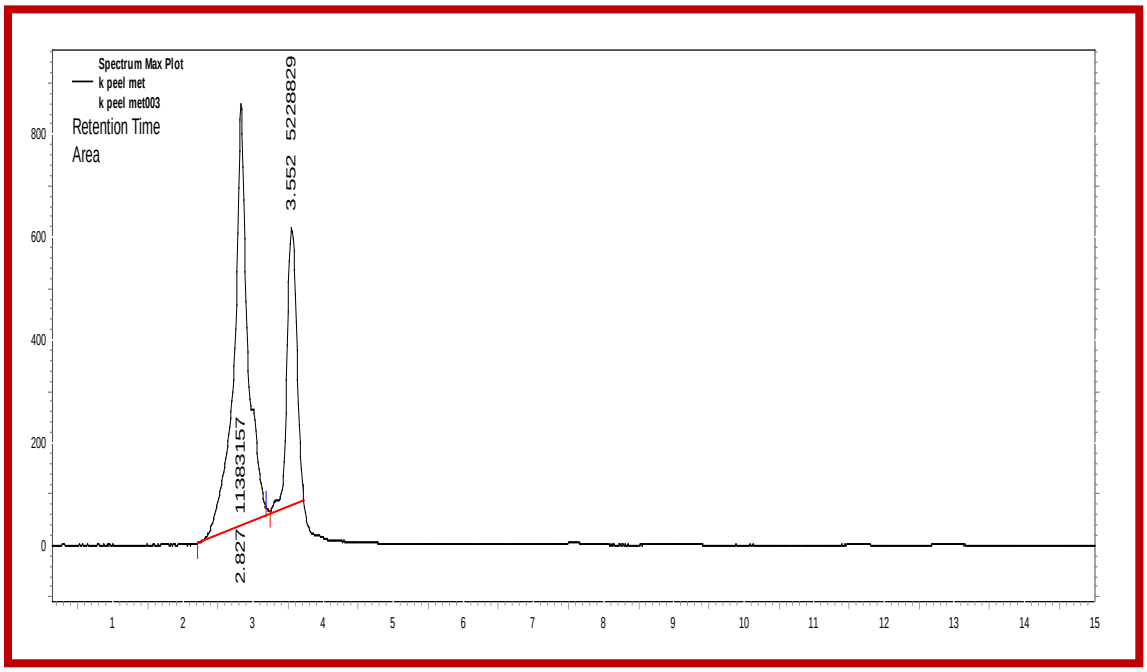
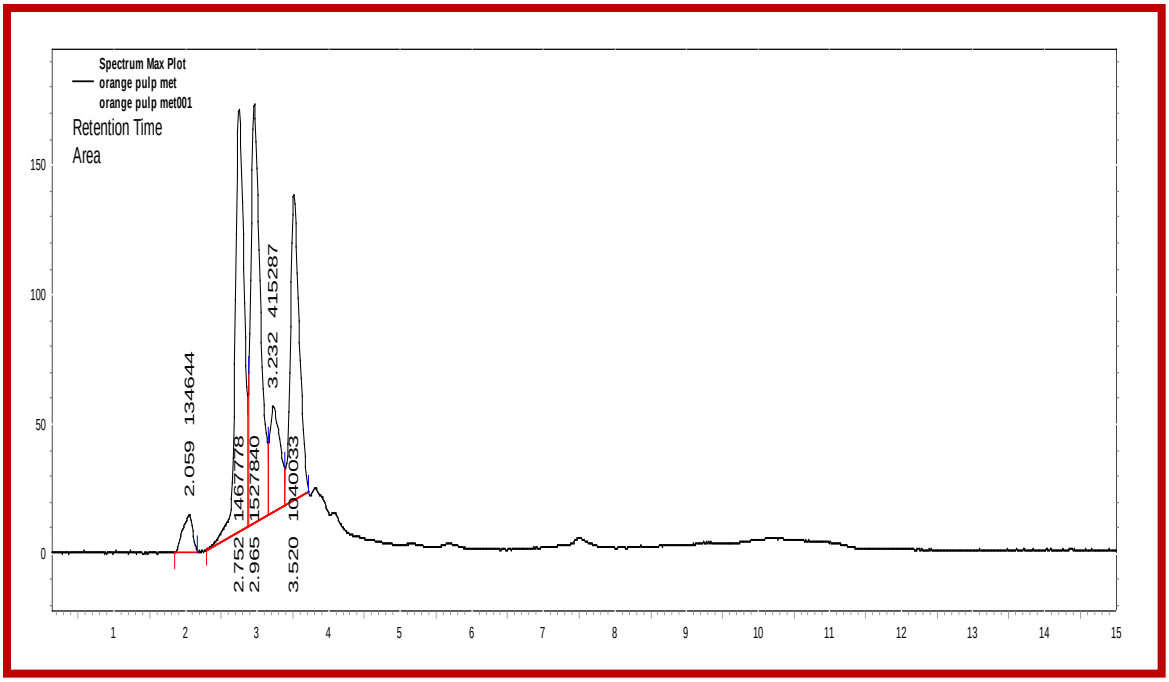


Figure 9

HPLC of *Citrus* sinensis pulp



The HPLC compositional fingerprint analysis of the methanol extract was performed and showed the presence of various phytochemical constituents of the *Citrus* fruits.

The *C. limetta* extract showed the presence the presence of one major peak and one minor peaks as observed in Figure 6. The results of the pulp extract of *C. limetta* showed 2 major and 3 minor peaks in Figure 7. Figure 8 showed the presence of two major peaks in *Citrus sinensis* peel extract, Figure 9 showed 5 peaks in the orange pulp extract.

This observation confirmed the presence of phyto components in the extract.

The HPLC and FTIR of aqueous extract *Terminalia chebula* confirmed the presence of phyto components in extracts and fabric respectively (Sathish et al., 2012).

HPLC analysis of the methanol extract of *Ixoracoccinea* showed several peaks. One among them is quercetin, a flavonoid which has various biological actions is also present (Showmya *et al.,* 2014)

The compositional fingerprint analysis of neotropical blueberries showed that the extracts contained flavonoids and cinnamic acid derivatives, some of which were identified and quantitated (Diplock *et al.,* 2011).

The HPLC analysis of *Ficuscarica* Linn,ethanolic extract was carried out which exhibited 7 prominent peaks as well as the presence of Quercetin was also confirmed (Dutta, 2013).

4.5 FTIR

FTIR is used foridentifying types of chemical bonds (functional groups). The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.

The FTIR profile of the fruit extracts showed the presence of various secondary metabolites identified based on the fuctional groups. The sample showed (Figure 10, 11, 12 13) the presence of alkyl, carboxylic acid, alcohols, alkanyl groups in the extract of both *Citrus sinensis* and *Citrus limetta* on observed in figure 10, 11, 12 and 13

Figure 10

FTIR profile of Methanol peel extract of *Citrus limetta*

Figure 11

FTIR profile of Methanol pulp extract of *Citrus limetta*

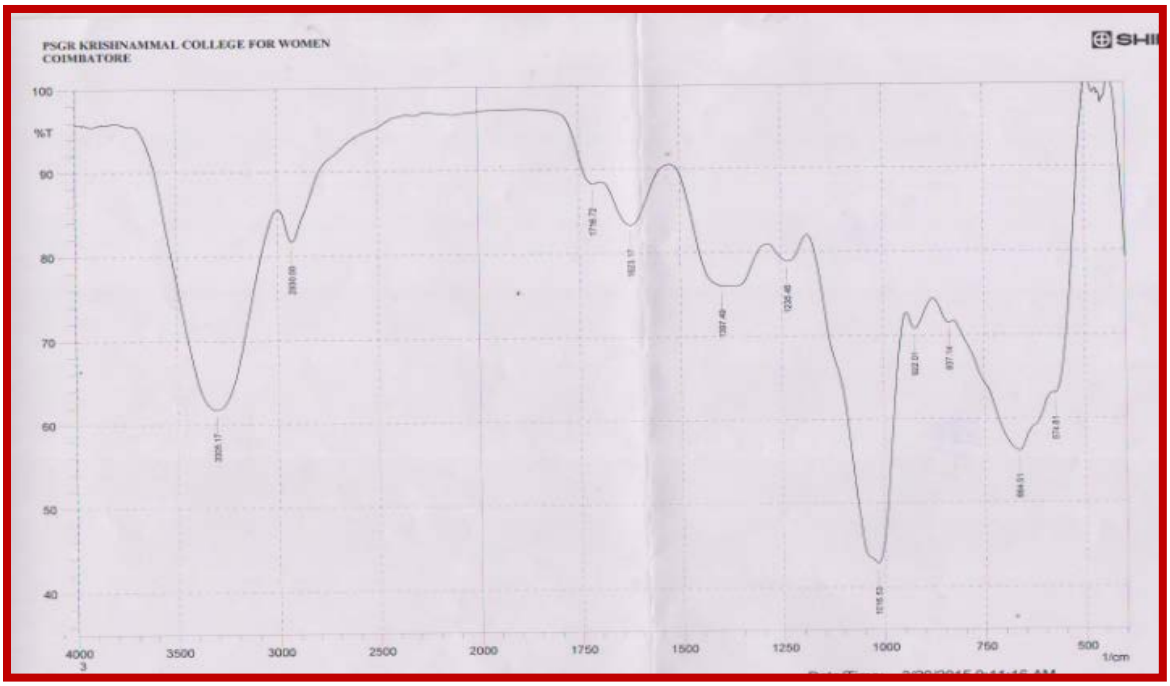
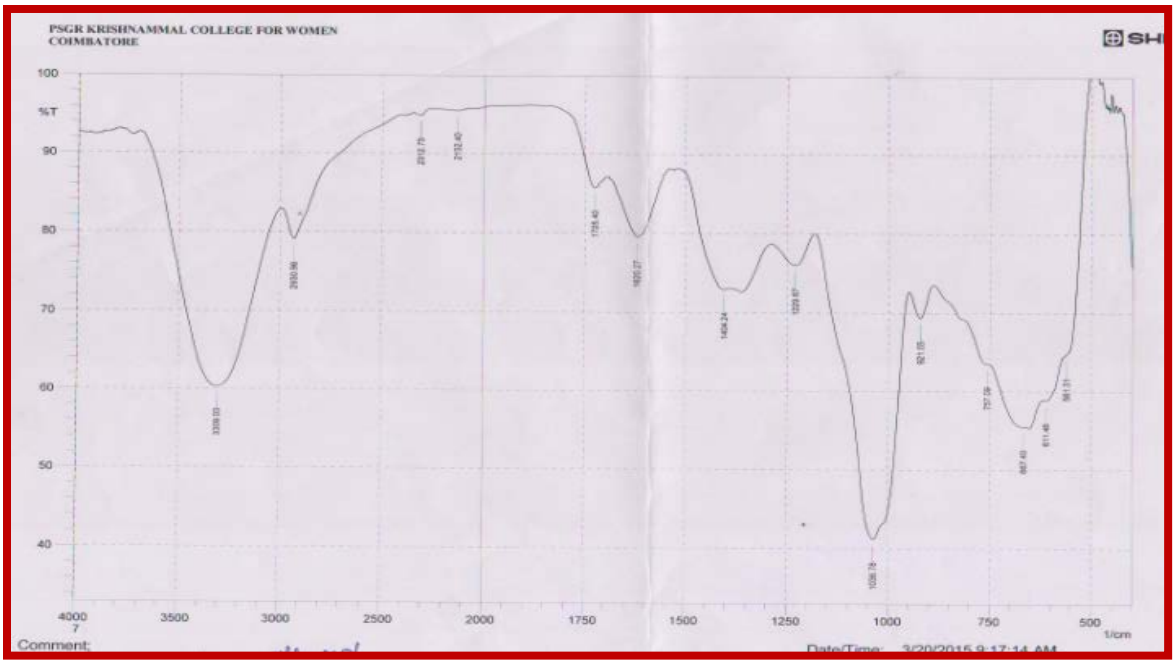


Figure 12

FTIR profile of Methanol peel extract of *Citrus sinensis*

Figure13

FTIR profile of Methanol pulp extract of *Citrus* sinensis



The result of the FTIR analysis of *Citrus karna shows* the presence of various secondary metabolites identified through the presence of various functional groups. The distribution of functional groups provides a basis for comparison of compositional differences between isolates and among samples (Ghonghade*,* 2013).

Nehadali *et* al., (2014) performed the phytochemical and FTIR analysis on different extracts of *Terminaliabellerica*. The FT-IR analysis has revealed the presence of Phenols, alcohol, amines and carboxylic acid as functional groups in *Terminaliabellerica.*

The FTIR spectrum of the methanol extract of *Citrus reticulate* confirmed the presence of alcohols, Phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines (Shoumya *et al*., 2014a).

The results confirm that the *Citrus* fruits are a rich source of phytochemicals. The study shed light to the fact that the peel extract are a much more potent source of phytochemicals than the pulp extracts.

The *Citrus* pulp has the medicinal value which lies in bioactive phytochemical that produce definite physiological action on the human body. The Alkaloid and glycoside components of the fruit can be responsible for the anticancer activity which can be further used as drug supplement (Mathur *et al.,* 2011). The most important flavonone in oranges is hesperidin which has been reported to lower high blood pressure as well as cholesterol in animal studies and have strong anti-inflammatory properties. The flavanones, flavones and flavonols are the three types of flavonoids that occur in *Citrus* peel and polymethoxylated flavones are the unique to *Citrus* family involving in anti-inflammatory, anticarcinogenic, antibacterial, antioxidant and antiatherogenic properties (Chanda *et al.,* 2006).

It is evident from the above citations, that there secondary metabolites play vital role in the treatment of various disease and disorders. The results of the present study suggests that though both peel and pulp extracts of both the *Citrus* fruit samples exhibited sufficient phytochemical constituents in their

methanolic extract. Peel extract constitutes more components compared to the pulp extract. Therefore, necessary steps can be taken to utilise the peel sample waste resources for medicinal preparations. This can even be used to develop formulation or drugs to combat a variety of disease.

Summary & Conclusion

5.0 SUMMARY AND CONCLUSION

The term phytonutrients refer to plant nutrients with particular biological activities in supporting human health. Phytonutrients are mainly natural bioactive compounds from plants with general benefits to human health. The secondary metabolites of plants provide humans with numerous biologically active products, which have been used extensively as food additives, flavours, colours, insecticides, drugs, fragrances and other chemical. These plant secondary metabolites include several classes such as, flavonoids, *alkaloids* and phenolics compounds having diverse chemical structures and biological activities and exist widely in *Citrus* fruits.

The term *Citrus* fruit includes different types of fruits and products. Although oranges are the major fruit in the *Citrus* fruits group, accounting for about 70% of *Citrus* output, the group also includes small *Citrus* fruits (such as Tangerines, Mandarins, Clementine and Satsuma), lemon and lime and grapefruits. The leading processed form in the group is orange juice (Armanbor *et al.,* 2000).

The study was conducted to assess the phytochemical profiles of methanol extract of peel and pulp of two varieties of *Citrus* fruits namely *Citrus sinensis* and *Citrus limetta.* The summary of the conclusions derived from study is given below

The first step in the study was to determine the presence and absence of the various phytochemicals in the sample. The study showed that the sample was a good source of phytochemicals. The study also pointed to the fact that alkaloids, flavonoids and phenols were predominantly present over the other phytocompounds. The preliminary screening also indicated that the peel extracts was better source than the pulp. With these results further spectral and chromatographic analysis were performed to study the alkaloids, flavonoid and phenol components only as these were adequately present.

The UV- vis spectral analysis was performed at the wavelength as 220 to 800nm, which showed the presence of the various peaks indicates the presence

of multiple compounds such as alkaloids, flavonoids and phenols in the extracts by co elution with the standards. This was observed as peaks in the graph.

The TLC helps in rapid analysis of the extract and gives an account of the various phytocompounds present in the extract. The TLC showed the presence of the various phytocompouds and it reiterated the result of preliminary analysis that peel extract was better than pulp based on the intensity of the bands in the chromatogram.

The HPTLC analysis of the plant extract was useful to justify the TLC results as the study showed the presence of alkaloids, flavonoids and phenols in the extract. This was confirmed by the comparison of the extracts with suitable standards namely naringenin, colchicine and eugenol. The results were confirmed based on the chromatogram and Rf value on comparison with the standard.

The HPLC analysis showed the presence of various alkaloids, flavonoids and phenols which were observed as peaks in the chromatogram.

The FTIR analysis was performed to identify the functional groups present in the extract and were useful in identify the nature of compounds present.

CONCLUSION

Spectroscopic technique has become a powerful and analytical tool for the qualitative and quantitative analysis of pharmaceutical and biological materials. *Citrus Sinensis and Citrus limetta* peel is rich in its bioactive compounds than the fruit. It has antioxidant, anti-cancer activity etc. About 34% of *Citrus* fruits are made into juices, therefore; large amounts of residues are formed resulting in an environmental pollution and loss of many valuable pharmaceutical components present in *Citrus* fruits peels. Waste is always not a waste, *Citrus* fruit peels is considered as waste so far, but the present finding reveals that fruit peel can be an alternative use in pharmaceuticals and food industries.

Further work is needed to refine the techniques and to quantitatively analyse the compounds and structurally investigate the different active constituents present in the peel and pulp of different *Citrus* fruits.

Wealth can be obtained from waste.

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