Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2018; 10(6); 246-253

ISSN: 0975-4873

Research Article

Spectral Studies and Biochemical Evaluation of *Citrus limon* Pulp

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Available Online:25th June, 2018

ABSTRACT

Novel chemical compounds synthesized from the active constituents of plants are of potential use in medicine and other useful applications. *Citrus limon* fruits have long been valued as part of a nutritious and tasty diet. Flavonoids are the important constituents present in the *Citrus limon* fruits. Flavonoids have gained recent attention because of their broad biological and pharmacological activities. The objective of this study was to identify the phytochemicals and to analyse flavonoids in the *Citrus limon* pulp. The crude methanol and chloroform extracts of *Citrus limon* were subjected to preliminary screening. The Phytochemical screening of the *Citrus limon* pulp extracts revealed the presence of polyphenols, alkaloids, flavanoids, terpenoids, amino acids and proteins. The DPPH free radical scavenging activity of *Citrus limon* pulp tissue shows a significant antioxidant property. The HPLC studies indicate that the quercetin is the major flavonoid present in the *Citrus limon* pulp juice. FT-IR and UV Spectral studies of *Citrus limon* pulp show the presence of different flavanoids in the *Citrus limon* pulp.

Keywords: *Citrus limon*, Phytochemical, flavonoids, DPPH, Quercetin.

INTRODUCTION

For thousands of years mankind is using plant sources to alleviate or cure illness[1]. Novel chemical compounds synthesized from the active constituents of plants which are of potential use in medicine and other useful applications. Herbal remedies are popular remedies for diseases used by a vast majority of the world's population[2]. Herbal plants having many pharmacologically active compounds like flavonoids, alkaloids, tannin, steroids, glycosides, phenols, fixed oils, which are stored in their specific parts of leaves, bark, flowers, seed, fruits, root etc[3]. Plants have been valuable and indispensible sources of natural products for the human and they have a great potential for producing new drugs[4].

According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. Epidemiological studies suggest that high dietary intake of phytochemicals, in particular of polyphenols, is associated with a reduced risk of a multitude of chronic diseases[5].

Scientific classification

Kingdom – Plantae, Angiosperms.
Order – Sapindales
Family – Rutaceae
Genus – *Citrus*
Species – *C. limon*
Binomial name - *Citrus limon*

Lemon grows as small, thorny trees which reach a height of 10 to 20 feet and produce fruits, which are full of fragrance, flavor and juice[6]. It has a rough, robust and bright color from green to yellow skin with epicarp or flavedo, which covers the fruits and protects from damages. The glands contain the essential oils that give the fruit its typical citrus fragrance. It consists of a white, thick and spongy mesocarp or albedo which together with the epicarp forms the pericarp or peel of the fruit. The internal part constitutes the pulp which is divided into separate segments or juice sacs by a thick radical film or endocarp. This part is rich in soluble sugars, ascorbic acid, pectin, fibers, different organic acids and potassium salt that give the fruit its characteristic citrine flavor.

The leaves of the lemon tree are dark green in colour and they are arranged alternately on the stem. The lemon has a white, fragrant flower with five petals. Lemons are oval citrus fruits with smooth porous skin. Some fruits have a pointed tip on the bottom of the fruit while other lemons are rounded at the base.

Citrus trees are evergreen trees. The exact origin of citrus fruits is not clearly identified, although most researchers place its origin to be South East Asia. Later, the citrus fruits were transported to America by the Spaniards, specifically to Mexico, Florida, Brazil and California, where we currently find the largest orange orchards in the world. Throughout the world, the fruit can be used for culinary and non-culinary purposes. Primarily it is used for its juice though the pulp is used mainly in cooking and baking. The top producers of lemon include India, Mexico, Argentina, Brazil, Spain, Peoples Republic of China, United States, Turkey, Iran and Italy.

Flavonoids are polyphenolic compounds that are ubiquitous in nature. More than 4,000 flavonoids have been recognised, many of which occur in vegetables, fruits

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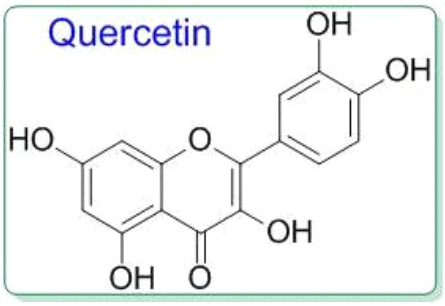
Figure 1: *Citrus limon* Fruit

Figure 2: *Citrus limon* Leaves



and beverages like tea, coffee and fruit drinks[7]. Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, antiinflammatory as well as antitumor activities. The best-described property of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species[8].

Plant flavonoids are a large group of very different compounds sharing the common feature of phenol moieties. They are, with a few notable exceptions, plant metabolites deriving from the shikimate pathway and the phenylpropanoid metabolism[9]. Flavonoids are aromatic secondary plant metabolites, which have been recognized as important due to their physiological[10] and pharmacological role and their health benefits[11].

Flavonoids are potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity. On the other hand flavonoids such as luteolin and cathechins, are also the better antioxidants than the nutrients antioxidants such as vitamin C, vitamin E and β-carotene[8].

MATERIALS AND METHODS

Plant Source

The fruits of Citrus *limon* were collected from Kanchipuram district. The pulp of the fruits were separated and dried for 10-12 days in the shadow. Then the dried pulp was ground to powder and it was kept in an airtight container for further use.

Table 1: Qualitative analysis of the phytochemicals of methanol and chloroform extracts of C*itrus limon* pulp.

S.no	Phytochemicals	Methanol extract	Chloroform extract
1	Alkaloids	+	+
2	Flavonoids	+	_
3	Carbohydrates	+	+
4	Glycosides	_	_
5	Polyphenols	+	+
6	Tannins	+	_
7	Saponin	+	+
8	Terpenoids	_	+
9	Proteins	+	+
10	Aminoacids	+	+
11	Anthraquinone	_	_

Table 2: The levels of ascorbic acid and flavonoids in the C*itrus limon* pulp tissue.

S.no	Parameters	values
1	Ascorbic acid (mg/gm of tissue)	39.2
2	Flavonoids (µg/gm of tissue)	0.625

Preparation of Solvent Extracts

Methanol Extraction

5g of powdered pulp was used separately for the preparation of extract. Sample was packed between folds of filter paper and placed in Soxhelet apparatus, run between 60-80ºc using Methanol as solvent.

Chloroform Extraction

5g of powdered pulp was used separately for the preparation of extract. Sample was packed between folds of filter paper and placed in Soxhelet apparatus, run between 60-80ºc using Chloroform as solvent.

Qualitative analysis of phytochemicals

Qualitative analysis of phytochemicals were carried out on the methanol and chloroform extracts of the powdered pulp using standard procedures to identify the constituents as described by[12].

Quantitative analysis

The plant tissue was weighed, uniformly homogenized with 1.0ml of 0.5M phosphate buffer, pH 6.9 and the homogenate was used for the following Biochemical assays.

Estimation of Ascorbic acid (Vitamin C)

The level of ascorbic acid was estimated by the method of[13].

Estimation of flavonoids

The flavonoids level was estimated by the method as described in Harborne (1998).

Free Radical scavenging activity

The free radical scavenging activity of the *Citrus limon*

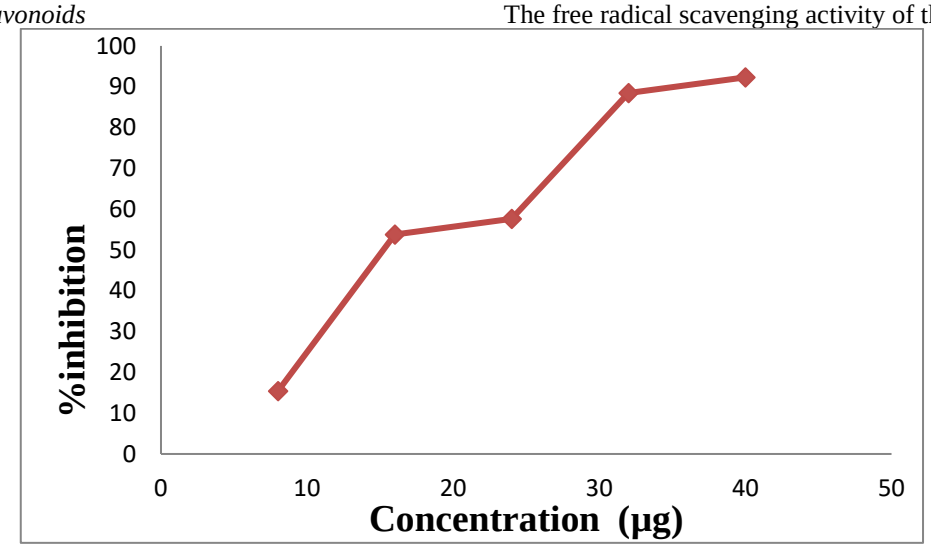


Figure 3: DPPH scavenging activity of C*itrus limon.*

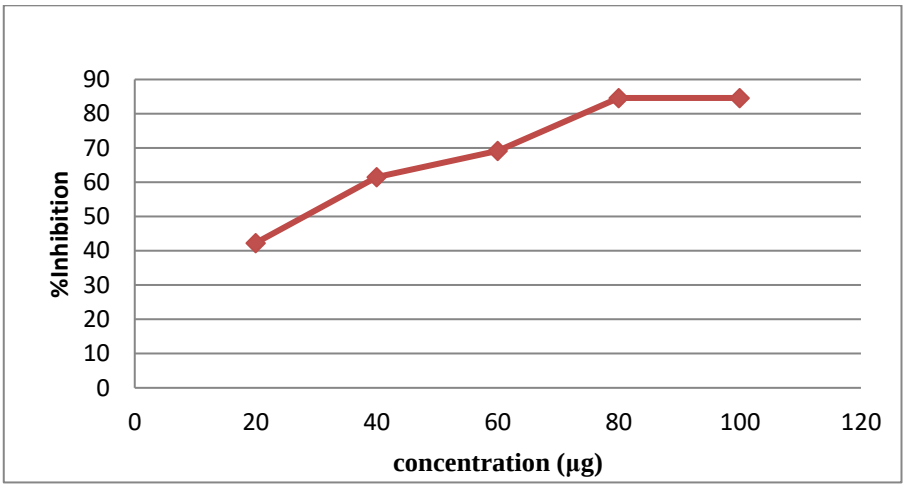


Figure 4: DPPH scavenging activity of ascorbic acid.

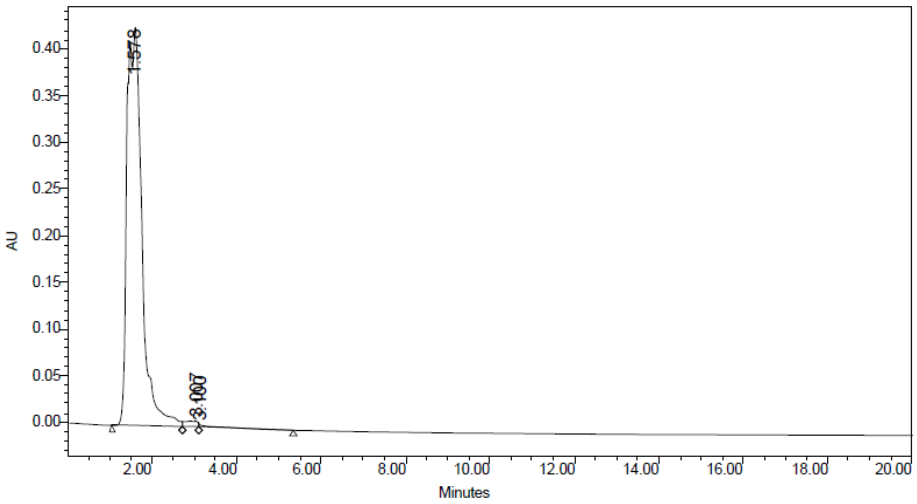


Figure 5: HPLC chromatogram of C*itrus limon* pulp juice at 280nm.

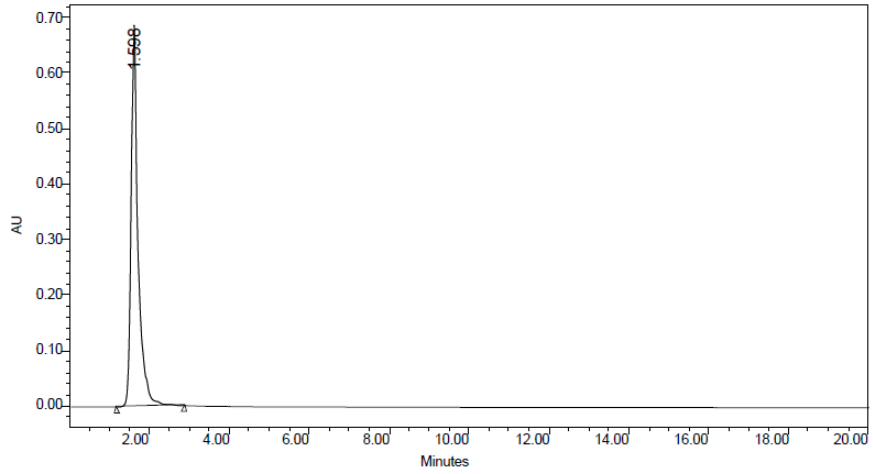


Figure 6: HPLC chromatogram of Quercetin at 280 nm.

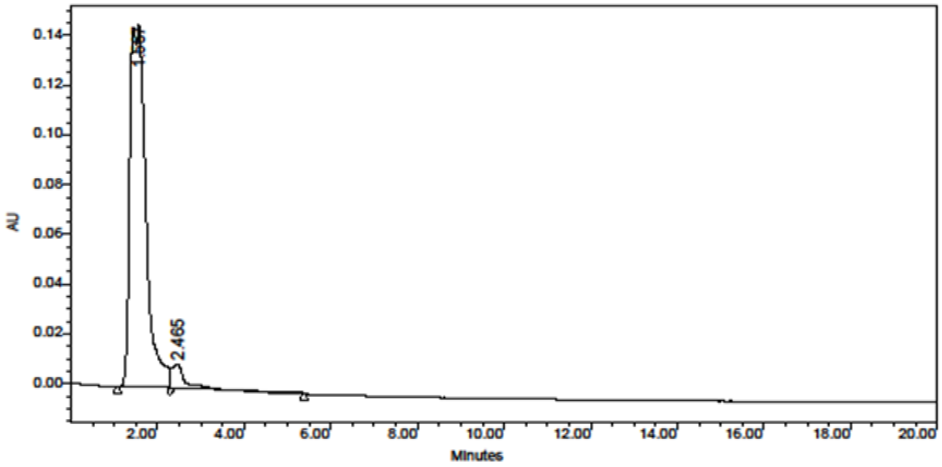


Figure 7: HPLC chromatogram of C*itrus limon* pulp juice at 325nm.

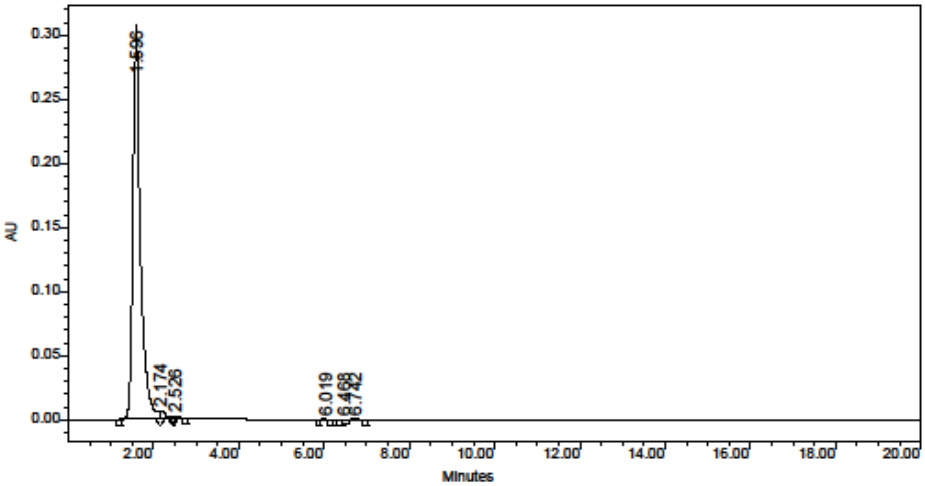


Figure 8: HPLC chromatogram of Quercetin at 325nm.

pulp was determined by DPPH method.

HPLC Analysis of Citrus limon

HPLC analysis was carried out by Kromasil 100 C18v reversed phase column (250 x 4.6mm) packed with 5µm diameter particles. The mobile phase was methanol:water:acetic acid (7:3:0.1 v/v/v). The mobile phase was filtered through a 0.45 µm membrane filter. Then deaerated ultrasonically prior to use. Flow rate and injection volume were 1 ml /min and 10 µl. The Chromatographic peaks of the analytes were confirmed by comparing their retention time and UV Spectra with those of the Quercetin. All chromatographic operations were carried out at ambient temperature.

FT- IR Analysis

The *Citrus limon* pulp was oven dried at 60ºC and ground into fine powder using a mortar and pestle. Two milligrams of the sample was mixed with 100mg KBr (FT-IR grade) and then compressed to prepare a salt disc (3mm diameter). The disc was immediately kept in the sample holder and FT-IR spectra were recorded in the absorption range between the wave number of 500 and 4000 cm^{-1}.

UV Spectroscopy analysis

The UV spectra of the *Citrus limon* pulp juice and standard quercetin were obtained by using UV spectrophotometer at the range of 200nm to 647nm.

RESULTS AND DISCUSSION

Table 1 shows the phytochemical compositions of the Methanol and chloroform extracts of *Citrus limon* pulp. The results illustrate the presence of various phytochemicals in *Citrus limon* pulp extracts. The Methanol and Chloroform extracts of *Citrus limon* pulp show the presence of alkaloids, flavanoids, proteins,

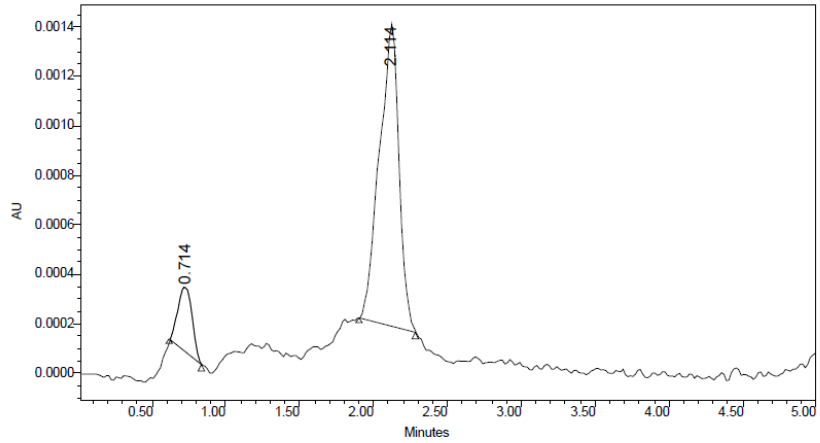


Figure 9: HPLC chromatogram of methanolic extract of *Citrus limon* at 280nm.

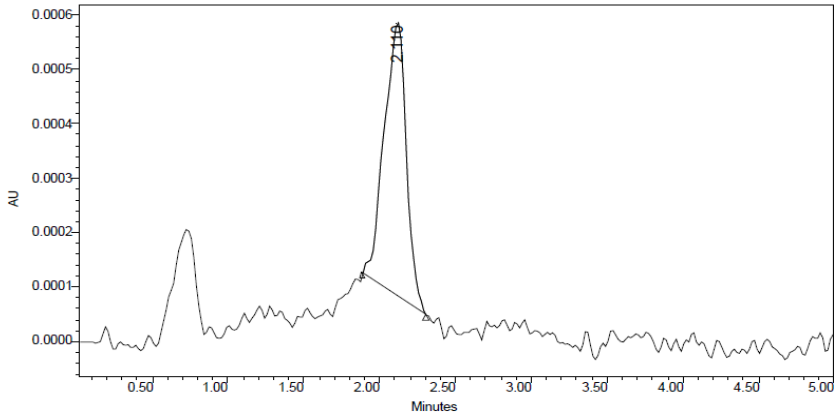


Figure 10: HPLC chromatogram of methanolic extract of *Citrus limon* at 325nm.

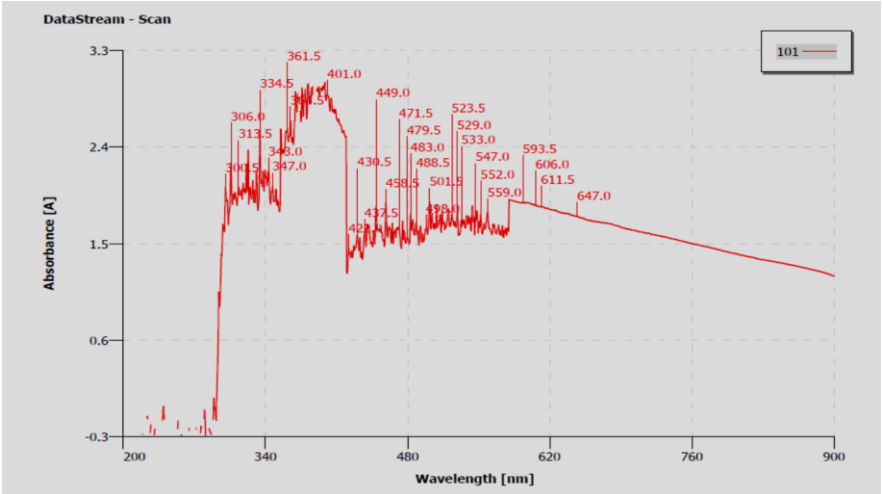


Figure 11: UV spectra of C*itrus limon* pulp juice.

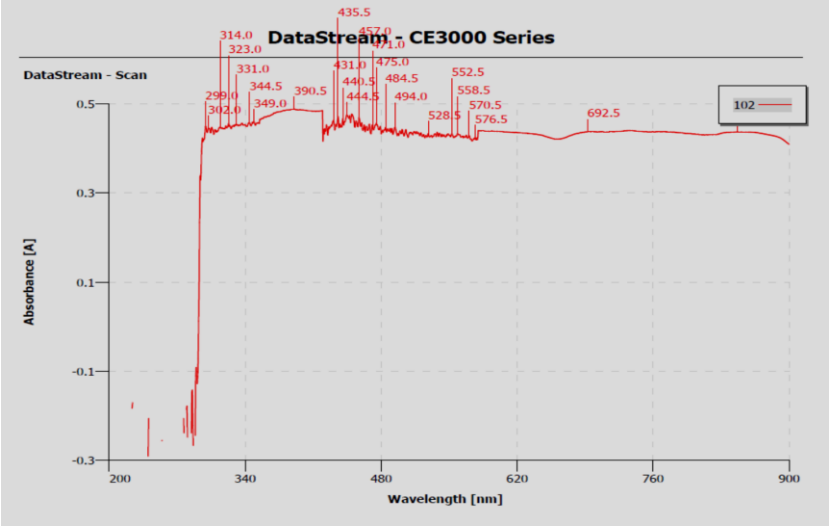


Figure 12: UV spectra of Quercetin.

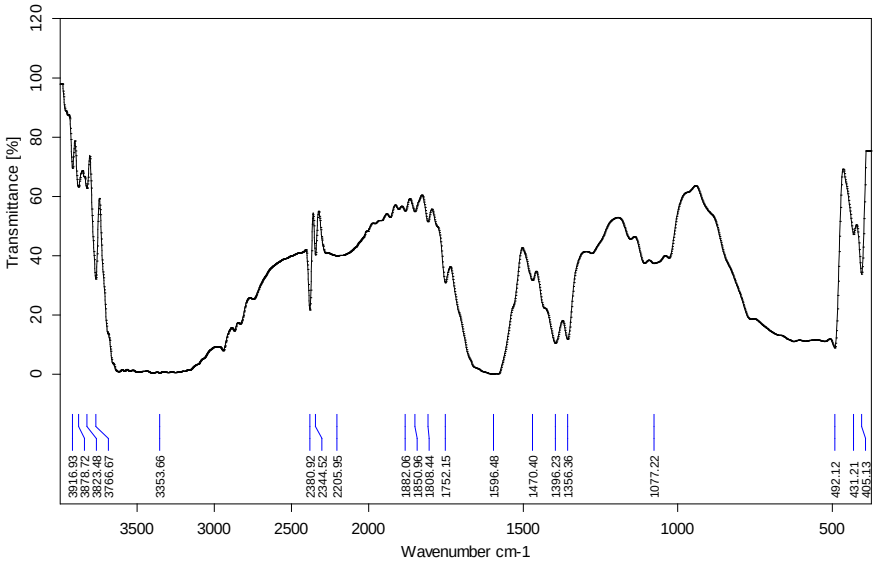


Figure 13: FT-IR spectrum of C*itrus limon* pulp.

carbohydrates, polyphenols, phytosterols, glycosides and terpenoids as phytochemical constituents.

Phytochemicals are non-nutritive plant chemicals possessing varying degrees of disease-preventive properties. Generally, phytochemicals are known to confer certain health benefits such as anti-inflammatory, antimicrobial, antihypertensive, and antidiabetic effects. The bioactive compounds are strongly associated with therapeutic properties including antiallergenic, antiatherogenic, anti-inflammatory, antimicrobial, anticarcinogenic, antithrombotic, cardioprotective, and vasodilatory effects[14]. Many of these pharmacological activities of citrus polyphenols are a consequence of their ability to scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS)[15].

The presence of phenol indicates that *citrus limon* could act as anti inflammatory, anti-clotting, antioxidant, immune enhancer and hormone modulators[16]. Phenols have been responsible in having the ability to block specific enzymes that causes inflammation. They modify the prostaglandin pathway and thereby protect platelets from clumping[17.]

Table 2 shows the levels of ascorbic acid and flavonoids in the *citrus limon* pulp tissue. Ascorbic acid is highly bioavailable and is consequently the most important water soluble antioxidant vitamin in cells, effectively scavenging reactive oxygen species (ROS). When relating the antioxidant activities of fruit juices to health and disease risk, it is important to consider the contribution of ascorbic acid in addition to that of phenolic compounds with antioxidant activity[18]. Natural ascorbic acid is crucial for the body performance. It aids in iron absorption from the intestine. It is required for the connective metabolism especially the scar tissue, bones and teeth. It is necessary as an anti stress and protect against cold.

Figures 3 & 4 show the DPPH scavenging activity of *Citrus limon* and standard ascorbic acid. DPPH is a stable free radical having maximum absorption at 517 nm that

accepts an electron or hydrogen atom to become a stable diamagnetic molecule. In the presence of a substance capable of donating a hydrogen atom, its free radical nature is lost and hence the reduction in DPPH radical was determined by the decrease in its absorbance at 517 nm[19].

Natural antioxidants that are present in plants are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Plants contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. Since oxidative stress is involved in various pathological conditions, the outstanding antioxidant role of natural polyphenols has received much attention from many researchers. In this regard, *Citrus* flavonoids have recently attracted considerable interest as potential therapeutic agents in numerous studies. Naringin, high levels of which occur in several varieties of citrus fruits and citrus byproducts, has demonstrated antiinflammatory, anticarcinogenic, lipid-lowering, and antioxidant activities[20]. Hesperidin, one of the main flavanone glycosides, which occurs in oranges, has been shown to exert a wide range of therapeutic effects such as antioxidant, antiinflammatory, and anticarcinogenic properties. Moreover, it has been found to significantly reduce ROS generation in cells and to restore mitochondrial enzyme activity[21].

The HPLC Chromatograms of the *Citrus limon* pulp juice and quercetin at 280nm are given in the figures 5&6. The results show that the retention times of the peaks in *Citrus limon* pulp juice and quercetin are 1.578min and 1.598min respectively. The HPLC Chromatogram of the *Citrus limon* pulp juice and quercetin at 325nm are given in the figures7 & 8. The results obtained show that the retention times of the peaks in *Citrus limon* pulp juice and quercetin are 1.567min and 1.596min respectively. It is evident from the results that the quercetin is the major flavanoid present in the *Citrus limon* pulp juice. R. S. Phani *et.al*., reported the presence of flavonoid in the *Citrus limon* by HPLC analysis.

Quercetin is a flavonol, it is plant derived flavonoid used as a nutritional supplement found in fruits and vegetables. Flavonoids are almost universal pigments of plants[22]. They are important parts of Human diet and considered as active principles of many medicinal plants. Quercetin is thought to have potent antioxidant, Antidiabetic, anti tumour, antiviral, and anti inflammatory benefits[23]. Quercetin is mainly found in many often consumed foods include green apple, onion, green tea, lemon as well as many seeds, flowers, barks, and leaves.

Figures 9 & 10 show the HPLC Chromatogram of the methanolic extract of *Citrus limon* pulp at 280nm and 325nm. The results indicate that the retention time of the major peaks of the methanolic extract of *Citrus limon* pulp at 280nm and 325nm are 2.114min and 2.110min respectively These results show that the flavanoid quercetin is present in higher concentration in the *Citrus limon* pulp juice than the methanolic extract of *Citrus limon* pulp. The presence of flavonoids may the reason for their use in herbal medicine for the treatment of capillary and vascular weakness (edema, varicose veins, blood clotting ,dysfunction). *Citrus limon* flavonoids are good digestive tonic with the appetizer effects and aids in digestion[24].

The UV spectra of *Citrus limon* pulp juice and quercetin are given in figure 11 & 12. Strong absorption peaks are obtained between 300nm and 361nm, 422nm to 559nm for *Citrus limon* pulp juice. Strong absorption peaks are obtained between 299nm and 390nm, 431nm to 576nm for standard quercetin. The UV spectra of flavanoids present in the Citrus juices were reported by Giuseppe Gattuso etal. It has been reported that the UV spectra of flavones and related glycosides show two strong absorption peaks commonly referred to as band I (300-380 nm) and band II (240-280 nm). It has also been reported that flavanones generally show a strong absorption peak in the range 270-295 nm[25].

Flavonoids present in *citrus limon* can function as direct antioxidants and free radical scavengers and have the capacity to modulate enzymatic activities and inhibit cell proliferation. In plants, flavonoids appear to play a defensive role against invading pathogens, including bacteria, fungi and viruses[26]. Flavonoids are generally present in glycosylated forms in plants, and the sugar moiety is an important factor determining their bioavailability. Quercetin may help to prevent cancer, especially prostate cancer. Scambia[27] reported quercetin inhibited human breast cancer cells (MCF–7 and MDA–MB–231) significantly.

The FTIR spectrum of *Citrus limon* pulp is given in Figure 13. Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. The bands corresponding to the vibration frequencies between 1077cm^{-1} and 1752cm^{-1} may due to the carbonyl groups, aromatic rings, C-OH deformation and C-OH stretching vibrations of flavanoids present in the *Citrus limon* pulp.

Citrus flavonoids may also exert neuroprotective effects since they are involved in the modulation of neuronal activities and mental health including brain plasticity, behaviour, mood, depression, and cognition. It has been demonstrated that hesperidin can protect neurons against various types of neurodegenerative diseases[28]. Natural flavonoids would therefore seem to have important potential as medicaments in the field of mental health, although their use in clinical practice is still a long way off[29].

CONCLUSION

The spectral studies of *Citrus limon* pulp reveal the presence of important flavonoids in this citrus fruit. The beneficial effects of flavonoids on human health is universally accepted. Citrus fruits and juices remain one of the most readily-available dietary sources for their intake. The studies have shown an inverse association between risk and intake level of some particular flavonoids, but further clinical trials are needed to assess a more precise

correlation between the level of flavonoids consumption and human health benefits. The *in-vitro* free radical scavenging assay of the *Citrus limon* pulp strongly indicates it's potent antioxidant activity. Further the antioxidant property of *Citrus limon* pulp confirms the necessity of *Citrus limon* in the dietary supplement. An in-depth knowledge of the valuable constituents in *Citrus limon* and their action has still to be achieved.

REFERENCES

1. Kamboj, V.P. Herbal medicine, Current science; 2000; 78: 35- 51.
2. Pal S., Shukla. Y. Herbal medicine: Current status and the future, Asian pacific journal of cancer prevention; 2003; 4: 281-288.
3. Sharma A., Shankar.C., Tyagi, L., Singh, M., Rao,C. Herbal Medicine for Market Potential in India: An Overview. Academic journal of plant sciences; 2008; 2, 26-36.
4. Nascimento GG, Locatelli J, Freitas PC and Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria; Braz J Microbiol 2000; 31: 247–256.
5. Lucia Castro-Vazquez, María Elena Alañón, Virginia Rodríguez-Robledo.Bioactive Flavonoids, Antioxidant Behaviour, and Cytoprotective Effects of Dried Grapefruit Peels (Citrus paradisi Macf.); Oxidative Medicine and Celluar Longevity; 2016:1-12.
6. Mohanapriya M, Dr. Lalitha Ramaswamy and Dr.R. Rajendran. Medicinal properties of lemon (*citrus limonum*); International Journal of Ayurvedic and Herbal Medicine; 2013: 3:1 1095-1100.
7. Pridham JB. In: Phenolics in Plants in Health and Disease; Pergamon Press, New York; 1960: 34-35.
8. Tapas AR, Sakarkar DM and Kakde RB. Flavonoids as Nutraceuticals: A Review – Tropical; Journal of Pharmaceutical Research; 2008; 7: 1089-1099.
9. Stafford H. A. In Flavonoid Metabolism; CRC: Boca Raton, F; 1990: 1–59.
10. Buslig B. S, Manthey J. A. Eds, Flavonoids in cell function; Kluwer Academic/Plenum Publishers. New York NY; 2002.
11. Valenzuela A, Sanhueza J, Nieto S. Natural antioxidants in functional foods: from food safety to health benefits; Grasas Aceites;2003: 54: 295–303.
12. Harborne J.B. Phytochemical methods: A guide to modern techniques of plant analysis; third edition; London: Chapman and Hall; 1998: 107-108.
13. Omayae ST, et al. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids; Methods Enzymo; 1979: 62: 3-11.
14. Jeon S.-M, S.-H. Bok, M.-K. Jang et al., Antioxidative activity of naringin and lovastatin in high cholesterol-fed rabbits; *Life Sciences;* 2001: 69(24): 2855–2866.
15. Kulkarni A.P, H. S. Mahal, S. Kapoor, and S. M. Aradhya. In vitro studies on the binding, antioxidant, and cytotoxic actions of punicalagin; *Journal of Agricultural and Food Chemistry;* 2007; 55 (4): 1491–1500.
16. Okwu. D.E and O.D. Omodamiro,2005. Effects of Hexane Extracts and phytochemical content of *xylopia* aethiopica and *Ocimum gratissium* on the uterus of Guinea pig; *Bio-Research.*
17. Duke, J.A. Antibacterial and phenolic acids from *S. mombi;* Plant Medical; 2002: 60: 3-9.
18. Gardner P T, White T A C, McPhail D B and Duthie G G. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices; *Food Chemistry*; 2000: 68**:** 471-474.
19. Kekuda P T R, Shobha K S and Onkarappa R, *J Pharm Res.* 2010; 3(1): 26-29.
20. Vauzour D, K. Vafeiadou, A. Rodriguez-Mateos, C. Rendeiro, and J. P. E. Spencer. The neuroprotective potential of flavonoids: a multiplicity of effects; *Genes and Nutrition*; 2008; 3: 115–126.
21. Gaur V, Aggarwal A, and Kumar A. Protective effect of naringin against ischemic reperfusion cerebral injury: possible neurobehavioral, biochemical and cellular alterations in rat brain; European Journal of Pharmacology; 2009:616: 147–154.
22. Galeotti, F., Barile, E., Curir, P., Dolci, M. and Lanzotti, V. Flavonoids from carnation (Dianthus caryophyllus) and their antifungal activity; Phytochemistry Letters; 2008:1(1):44-48.
23. Spencer et al. Flavonoids: Modulators of brain function; British Journal of Nutrition; 2008: 99: 60–77.
24. Oliver-Bever, B. Medicinal plants in Tropical West Africa; Cambridge University Press,Cambridge; 1986: 123-125.
25. Datla K. P, M. Christidou, W. W. Widmer, H. K. Rooprai, and D. T. Dexter. Tissue distribution and neuroprotective effects of citrus flavonoid tangeretin in a ratmodel of Parkinson's disease; NeuroReport:2001: 12(17): 3871–3875
26. Sohn H.Y, K.H. Son, Know C.S and S.S. Kang. Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: Morusvalba L., Morus mongolica Schneider, Broussnetiavpapyrifera (L.) Vent, Sophora flavescens Ait and Echinosophora koreensis". Nakai. Phytomedicine; 2004: 11: 666-672.
27. Scambia G, Ranelletti FO, Panici PB. Quercetin potentiates the effect of adriamycin in a multidrugresistant MCF-7 human breast cancer cell line: Pglycoprotein as a possible target. Cancer Chemother; Pharmacol; 1994: 34: 459-464.
28. Kunimasa K, M. Ikekita, M. Sato et al. Nobiletin, a citrus polymethoxyflavonoid, suppresses multiple angiogenesisrelated endothelial cell functions and angiogenesis in vivo; Cancer Science; 2010: 101(11): 2462–2469.
29. Jeong K. H, U. J. Jung, and S. R. Kim. Naringin attenuates autophagic stress and neuroinflammation in kainic acid-treated hippocampus in vivo; Evidence-Based Complementary and AlternativeMedicine; 2015: 9.