World Journal of Science and Research. 2(4): 01-06 (2017)

ISSN: 2455 2208

Available online at http://www.harmanpublications.com

World Journal of Science and Research

Research Article Biochemistry

A STUDY ON PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *Citrus limetta*

A. Aneesh, K. Dharani, S. Uma maheswari, R. Vivetha and S. Josephinol *

1 Department of Biochemistry, Holy Cross College, (Autonomous), Trichirappalli, Tamil Nadu, S. India
2 Department of Biochemistry, Holy Cross College, (Autonomous), Trichirappalli, Tamil Nadu, S. India,
*Corresponding author

ABSTRACT

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc. In the present study, we investigate phytochemicals in *Punica granatum* peel. The qualitative analysis of *Citrus limetta* peel contain rich source of phytochemicals. Fluorescence behavior also proved in this study. Presence of phytochemicals further confirmed in histochemicals studies and UV visible spectral studies. Antimicrobial activity of *Citrus limetta* peel extract proved against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Candida albicans*.

***Citation*:** Aneesh A, Dharani K, Uma maheswari S, Vivetha R and Josephinol S. (2017) A study on phytochemical screening and antimicrobial activity of *Citrus limetta*. *World Journal of Science and Research*. 2 (4): 01-06.

Article Info:

Received 08 December 2017; Accepted 28 December 2017
Online January 2018

Keywords:
Citrus limetta peel, Phytochemicals, Histochemicals, Fluorescence Antimicrobial activity

Corresponding author
S. Josephinol
Assistant Professor
Department of Biochemistry, Holy Cross College, (Autonomous), Trichirappalli, Tamil Nadu, India.

INTRODUCTION

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg,1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Gibson *et al*., 1998: Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999) and About 150 phytochemicals have been studied in detail.

In wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices (Mathai, 2000). Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans, legumes, and soy foods are common sources (Moorachian, 2000).

Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, bark, bark, fruits or seeds (Costa *et al*., 1999). Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions (King and Young, 1999). Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc.

MATERIALS AND METHODS

Plant material and preparation of extract

The *Citrus limetta* peel was collected from Thanjavur on December 2017. The collected plant parts were cut into small pieces and shade dried at room temperature and makes a fine powder using grinder mixture. Plant tissue homogenization in solvent has been widely used by researchers. Dried or wet, fresh plant parts are grinded in a blender to fine particles, put in a certain quantity of solvent and shaken vigorously for 5 - 10 min or left for 24 h after which the extract is filtered. The filtrate then may be dried under reduced pressure and redissolved in the solvent to determine the concentration. Some researchers however centrifuged the filtrate for clarification of the extract.

Preliminary phytochemicals screening

Chemical tests were carried out on the different extracts using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Qualitative Analysis of Vitamins

Analysis of vitamin A, C, D and E of plant powder followed by the method of Pearson (1976) and Patel (2005).

Qualitative Analysis of Inorganic Elements

Ash of drug material (500mg) was prepared and treated with HNO3 and HCl (3:1 v/v) for 1 hour. After the filtration, the filtrate was used to perform the tests (Khandelwal 2006).

Fluorescence Behavior

The fluorescence studies were carried out as per the method of Bhattacharya and Zaman (2009).

Histochemical tests

The powder of *Citrus limetta* peel were treated with specific chemicals and reagents. The treated plant powder further analysed in light microscope. The *Citrus limetta* peel powder treated with phloroglucinol and diluted HCl gave red colour indicates lignin, treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids.

UV-Visible analysis

The extracts were examined under visible UV-Visible spectrum. The sample is dissolved in same solvent. The extracts were scanned in the wavelength ranging from 330-920 nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 50 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded.

Determination of Antibacterial Activity

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka et al., 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of *Citrus limetta* peel revealed the presence of chemical constituents represent in Table 1.

Vitamins

Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The word organic means that the molecules of the substance contain the element carbon. The term also means that vitamins can be destroyed and become unable to perform their functions in our bodies. Too much heat, certain kinds of light and even oxygen can destroy some vitamins. The amounts of vitamins ingested from food are measured in micrograms or milligrams (Okwu, 2004). The vitamins of the *Citrus limetta* peel investigated and summarized in Table-2.

Minerals

All human beings require a number of complex organic/inorganic compounds in diet to meet the need for their activities. The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water (Indrayan *et al*., 2005). Every constituent plays an important role and deficiency of any one constituent may lead to abnormal developments in the body. Plants are the rich source of all the elements essential for human

beings. The Minerals of the *Citrus limetta* peel investigated and summarized in Table-3.

Table.1: Phytochemical screening of *Citrus limetta* peel

S.No	Phytochemical analysis	Methanol extracts (100%)	Chloroform extracts (100%)	Ether extracts (100%)	Water extract (100%)
1	Tannin	+	-	+	++
2	Phlobatannins	-	-	-	-
3	Saponin	+	+	++	+
4	Flavonoids	-	+	-	+
5	Steroids	+	+	+	+
6	Terpenoids	++	+	+	++
7	Tri terpenoids	+	+	+	++
8	Alkaloids	+	+	-	+
9	Carbohydrate	-	+	-	+
10	Protein	+	+	+	+
11	Anthroquinone	-	+	-	+
12	Polyphenol	-	+	-	-
13	Glycoside	+	+	+	+

(+) Presence, (-) Absence, (++) Moderately present.

Table.2: Qualitative analysis of vitamins in *Citrus limetta* peel

S.No	Vitamins	Observation
1	Vitamins A	-
2	Vitamins C	+
3	Vitamins D	-
4	Vitamins E	+++

(+) Presence, (-) Absence, (+++) Highly moderately present

Table.3: Qualitative analysis of inorganic element in *Citrus limetta* peel

S.No	Inorganic Elements	Result
1	Calcium	+
2	Magnesium	+
3	Sodium	+
4	Potassium	+
5	Iron	-
6	Sulphate	+
7	Phosphate	+
8	Chloride	+
9	Nitrate	-

(+) Presence (-) Absence

Fluorescence analysis

The fluorescence analysis is a tool for the determination of constituents in the plant that gives a definite idea of the chemical nature. Similarly extracts were also subjected to UV chamber and fluorescence was observed and consistency was noted as an additional character for identification. Fluorescence analysis of the powdered drugs were performed and tabulated which helps to detect the adulteration, because phyto constituents exhibits characteristic fluorescence under ultraviolet light when they got mixed with the reagents. The fluorescence exhibited by the mixture was attributed to the chemical constituents present in the crude drug. Prior to the phyto chemical screening a rough estimation of phyto constituents was done by the behavior of powder drug with different chemical reagents which powdered drug showed different colours when it got mixed the particular reagents which reflects the presence phytochemicals in accordance with the colours obtained. Fluorescence behavior of *Citrus limetta* peel *p*owder was represented in table 4.

Histochemical Study

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues. Identification of localization of secondary metabolites in plant parts

which are using in the preparation of drug is an immense importance to prevent adulteration and also helpful in taxonomic hierarchy. Hence in the present study an attempt has been made to identification and localization of secondary metabolites of *Citrus limetta* peel in they were depicted below in table 5.

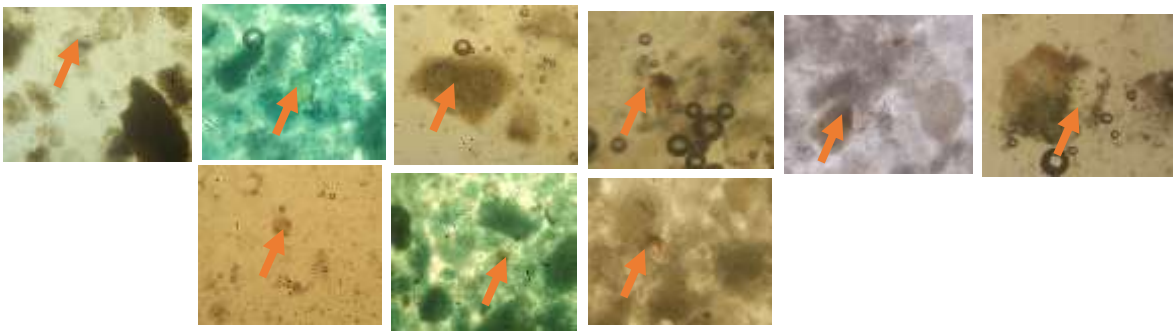
Table.4: Fluorescence behavior of *Citrus limetta* peel

S.No	Tests	Visible Light	Short UV 245	Long UV366
1	Plant powder (pp)	Yellowish brown	White	Black
2	PP with water	Yellowish brown	Light green	Violet
3	PP with Hexane	Yellowish brown	White	Black
4	PP with Chloroform	Yellowish brown	White	Black
5	PP with Methanol	Yellowish brown	White	Black
6	PP with acetone	Yellowish brown	White	Black
7	PP with IN Sodium hydroxide in water	Creamy white	Light green	Blackish brown + Green
8	PP with IN Hydrochloric acid	Light green	Creamy white	Violet
9	PP with 1N sulphuric acid	Yellow	Light green	Black
10	PP with 1N Nitric acid diluted	Yellow	Light Creamy white	Violet

Table.5: Histochemical studies of plant powder *of Citrus limetta* peel

S.No	Characterization	Observation	Result
1	Starch	Blue	+
2	Tannin	Blue /Green	+++
3	Flavanoids	Yellow	++
4	Glycoside	Brown	++
5	Terpenoids	Orange	+
6	Steriods	Violet To Blue Or Green	++
7	Saponin	Yellow	+++
8	Polyphenol	Blue Green/Red	+++
9	Alkaloids	Reddish brown	+

(+) Presence, (-) Absence. (+++) Highly moderately present



(Starch, Tannins, Flavonoids, Glycoside, Terpenoids, Steroids, Saponnin, Polyphenol, Alkaloids)

Plate.1: Histochemicals Analysis of *Citrus limetta* peel

Ultraviolet/visible (UV/VIS) spectroscopy

The relative percentage of scatter or absorption from the measured extinction spectrum depends on the size, shape, and composition and aggregation state of your sample. Your sample may absorb light, scatter light, or both. As a general rule, smaller particles will have a higher percentage of their extinction due to absorption. UV/Visible spectrum graph plotted.

Antimicrobial activity

Toxicity studies on pathogen opens a door for applications in medicine. A traditional method and the use of plant extract have a new awareness for the control of disease, besides being safe and no phytotoxic effects. Using medicinal plants were found to be highly toxic against different pathogenic

bacteria and Fungi of selected species. The plant of *Citrus limetta* peel shows against *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*. The inhibitory activities in culture media of the plant extract reported in table 6 were comparable with Bacterial standard antimicrobiotic viz. chloramphenicol and Fungi standard antimicrobiotic viz. Fluconazole.

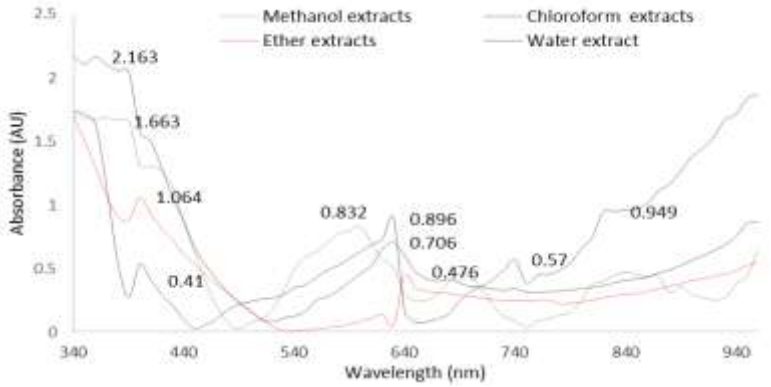
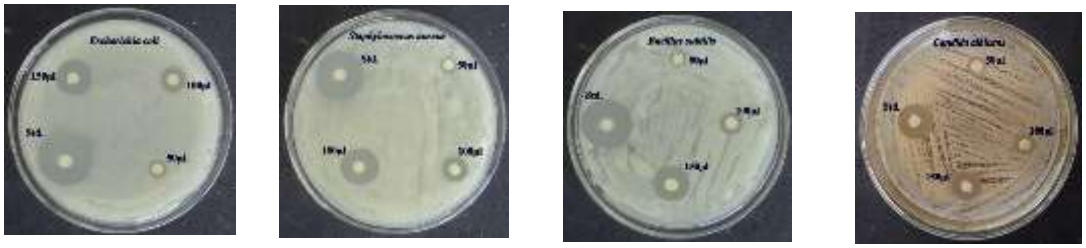


Fig.1: UV-Visible spectrum analysis of *Citrus limetta* peel

Table.6: Antimicrobial activity of *Citrus limetta* peel

Microorganism	50µl	100µl	150µl	Standard (30µl)
Escherichia coli (mm)	0.60±0.04	2.40±0.16	5.30±0.37	9.10±0.63
Staphylococcus aureus (mm)	0.30±0.02	2.10±0.14	4.90±0.34	8.70±0.60
Bacillus subtilis (mm)	0.20±0.01	1.80±0.12	4.50±0.31	8.80±0.61
Candida albicans (mm)	0.30±0.02	1.50±0.10	4.10±0.28	8.20±0.57

Values were expressed as Mean ± SD
Bacterial standard : Chloramphenicol
Fungi standard : Fluconazole



Escherichia coli ***Staphylococcus aureus*** ***Bacillus subtilis*** ***Candida albicans***

Plate. 2: Antimicrobial activity of *Citrus limetta* peel

CONCLUSION

The results of the present study concluded that *Citrus limetta* peel may be a good source of phytochemicals, vitamins, minerals, histochemical, fluorescence, UV-Visible and antimicrobial activity. Supplementation of this *Citrus limetta* peel may be useful for human health associated emerging diseases such as diabetes, hypertension and cancer.

REFERENCES

Awoyinka O A, Balogun I O and Ogunnow A A. (2007) Phtochemical screening and in vitro bioactivity of *Cnidoscolus aconitifolius* (Euphorbiaceae). *J. Med. Plant* Res, 1: 63-95

Bhattacharya and Zaman. (2009) Fluorescence microscopy of cereals in, new frontiers in food micostruture, Ed. By American Association of cereal chemists, Ottawa Canada. pp1 67-75.

Costa M A, Zia Z Q, Davin L B and Lewis N G. (1999) Chapter Four: Toward Engineering the Metabolic Pathways of Cancer-Preventing Lignans in Cereal Grains and Other Crops. In Recent Advances in Phytochemistry, vol. 33, Phytochemicals in Human Health Protection, Nutrition, and Plant Defense, ed. JT Romeo, New York; 67-87.

Gibson EL, Wardel J AND Watts CJ. (1998) Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children. Appetite; 31: 205-228.

Harborne J B. (1973) Phytochemical Methods; A guide to modern techniques of plant Analysis.2nd Edition, London New York. pp. 49-188.

Hasler C M and Blumberg J B. (1999) Symposium on Phytochemicals: Biochemistry and Physiology. *Journal of Nutrition*, 129: 756-757.

Indrayan A K, Sharma S, Durgapal D, Kumar N and Kumar M. (2005) Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. Current Sci, 89, 1252- 1255.

Khandelwal K R. (2006) Practical Pharmacognosy (16th ed.,) Nirali Prakashan, Pune. p 98-106.

King A and Young G. (1999) Characteristics and Occurrence of Phenolic Phytochemicals. Journal of the American Dietetic Association; 24: 213-218.

Mathai K. (2000) Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.

Meagher E and Thomson C. (1999) Vitamin and Mineral Therapy. In Medical Nutrition and Disease, 2 Nd ed.,G Morrison and L Hark, Malden, Massachusetts: Blackwell Science Inc,; 33.

Moorachian M E. (2000) Phytochemicals: Why and How? Tastings. 4-5.

NCCLS. (1993) National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility tests. PA: NCCLS Publications 25.

New Wall C A, Anderson L A and Phillipsan J D. (1996) Herbal medicines a guide for healthcare professionals. The Pharmaceutical Press, London.

Okwu D E. (2004) Phytochemical and vitamin content of indigenous spices of south eastern Nigeria. *J. Sustain Adric. Environ*. 6(1): 30-37.

Patel K K. (2005) Master dissertation. Shorea robustra for burn wound healing and antioxidant activity. Department of Pharmacology, KLESS College of Pharmacy, Karnataka, India, 33.

Pearson D. (1976) The Chemical Analysis of Food, 17th ed. Churchill Livingstone, London, pp 3-4.

Sofowara A. (1993) Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. p. 289.

Trease G E and Evans W C. (1989) Pharmacognsy. 11th edn. Brailliar Tiridel Can. Macmillian publishers.

Source of support: Nil;
Conflict of interest: None declared