ANALYSIS OF BIOACTIVE COMPOUNDS IN *CITRUS* FRUIT PEELS

Amutha.R[1] ,Kavusik.T[1], & Sudha.A[2*]

1.Department of Biotechnology,
Periyar University PG Extension Center, Dharmapuri.
2. Department of Microbiology,
Vivekanandha College of Arts and Sciences for Women, Tiruchencode,

Abstract

In history for a long period plants are the sources of natural products for the health of human beings and they have a great potential for producing new drugs. In plant chemotherapy, the utilize of naturally occurring antimicrobial substances is gaining more significance and have higher important values . Citrus fruit is an important medicinal plant belongs to the family Rutaceae . Citrus fruits are highly nutritious, medicinal and are found to be commonly in cultivation throughout the tropics. Citrus fruits constitute only 0.9% of total daily calories and 1.7% of daily carbohydrate intake although the peel waste are highly perishable and seasonal which could be a wealth for the farmers if the processing industries and monitoring agencies are evolve new methodologies to use them and take attention in bringing useful products from citrus waste materials. This study was aimed to focus on waste minimization in fruit juice processing industry and also evaluate the biomolecules and antibacterial activity of various citrus fruit peel . C.sinensis showed the high content of protein(100mg,). C.sinensis showed the high content of free fatty acids(19.63). C.limon showed the high amount of sugar(28mg,). C.limetta have high content of cholesterol (117.5mg). Phytochemical analysis showed the presence of phenols, flavonoids, saponins etc. This study showed the importance of different citrus fruit peel extracts and their significant in antibacterial activity.

Key words: Antibacterial, Biomolecule, cholesterol flavonoids. Rutaceae,

Sudha.A
Department of Microbiology,
Vivekanandha College of Arts and Sciences for Women, Tiruchencode
E.mail: sudha29.a@gmail.com

1.Introduction

Citrus is a common term and genus (***Citrus***) of flowering plants in the rue family, Rutaceae. Citrus is believed to have originated in the part of Southeast Asia bordered by Northeastern India, Myanmar (Burma) and the Yunnan province of China[1]. Citrus fruit has been cultivated in an ever-widening area since ancient times; the best-known examples are the oranges, lemons, grapefruit, and limes Citrus is one of the most important commercial fruit crops grown in all continents of the world[2].Citrus importance is attributed to its diversified use and growing world demand with about 102.64 million tones total world production and probably stands first largest among the produced fruit[3].

Citrus fruits and juices are the important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids phenolic compounds and pectins that are important to human nutrition. The main flavonoids present in citrus species are Hesperidine ,Narirutin, Naringin and Eriocitrin. Citrus byproducts also represent rich source of naturally occurring flavanoids. The peel which represents almost one half of the fruit mass contains the highest concentration of flavanoids in the citrus fruits[4]. The peel of Citrus fruit is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants [5].

The antimicrobial abilities of essential oils, among which citrus oils are shown to be a particularly interesting field for applications within the food and cosmetic industries. Among the citrus fruits Lemon is an important medicinal plant of the family Rutaceae[6].

Orange (Citrus sinensis L.), a hesperidium belonging to the Rutaceae family, is the most widely grown and commercialized citrus specie. Orange is composed by an external layer (peel) formed by flavedo (epicarp or exocarp) and albedo (mesocarp), and an inner material called endocarp that contains vesicles with Juice, flavedo and albedo account for about 50, 10 and 25% (w/w) respectively of the whole fruit . Besides sugars, acids, and polysaccharides, oranges are an important source of phytochemicals such as phenolics, vitamin C and carotenoids. These compounds also known as nutraceuticals provides health benefits due to a risk reduction of chronic illness such as cancer and cardiovascular disease[7].

Citrus fruits are among the most accepted and well preferred fruits in the world not only due to their flavour, but also for its taste and benefits in general health. They have been valued for a very long time because of their nutrition and as part of a tasty diet. Citrus and its products are a very good source of vitamins, minerals and dietary fiber that are needed for growth, development and overall nutritional well-being [8].

This study was aimed to focus on waste minimization in fruit juice processing industry. The combined efforts of waste minimization during the production process and recovery of valuable product substantially reduce the amount of waste, as well as boost the environmental profile of fruit juice processing industry. This study investigates the antibacterial activity and anti oxidant analysis for the use of peels of citrus fruits by determining the chemical constituents as well as quantifying the yield percentage of crude phytochemicals.

2. Materials and Methods

2.1extraction of Various Citrus Peels

Extraction carried out by soxhlet method. 25 g of dried peel powder of *C.sinensis, C. limon, C.limetta* were added to 200 ml of ethanol solvent and allowed for extraction. After extraction, condensation of extraction through the rotator evaporation. The prepared extracts are used for further analysis [9].

2.2 Phytochemical Analysis

Phytochemical analysis of ethanol extract of various *citrus* peels (*C.sinensis,C.limon,C.limetta)* carried out by the standard method[10].

2.3 Estimation of Biomolecules

2.3.1 Estimation of protein:

Pipetted out 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard(BSA) into a series of test tubes. Pipetted out 0.1 ml and 0.2 ml of sample extract in two other test tubes. Make up the volume to 1ml in all the test tubes, a tube with 1 ml of water serves as blank. Added 5ml of Folin-ciocalteau reagent to the each test tube including the blank. Mixed well and allowed to stand for 10 min. Then added 0.5ml of Alkaline copper solution mixed well and incubated at room temperature, in the dark for 30min. Then absorbance was read at 660nm and standard graph was plotted. From this standard graph, calculate the amount of protein in the sample.

2.3.2 Estimation of Carbohydrates By DNS Method

Weighed 100mg of sample into a boiling tube. Hydrolysed by keeping it in a boiling water bath for 3 hours, with 5ml of 2.5N HCl and cooled at room temperature. Neutralized it with solid sodium carbonate until the effervescence ceases and centrifuged at 10000rpm for 10 minutes at room temperature. Collected the supernatant and take 0.5 ml in the aliquotes for analysis. Prepared the standards by taking 0, 0.2, 0.4, 0.6, 0.8, and 1ml of the working standard(glucose). 0 serves as blank. Make up the volume to 1ml in all the tubes including the sample tubes by adding distilled water. Then added 4ml of DNS reagent to the tubes. Boiled for 10 minutes in a boiling water bath and cooled rapidly. Read the intensity of the green to dark red at 630nm.The absorbance was read to plot the standard graph and used to find the unknown concentration. From the graph calculated the amount of carbohydrate present in the sample.

2.3.3 Estimation of Free -Fatty Acids

Dissolved 100mg of extract in 2ml of neutral solvent in 100 ml conical flask. Then centrifuged the sample extract at 6000 rpm for 10 min. Collected the supernatant in another flask. Added few drops of phenolphthalein. Titrated the contents against 0.1N KOH. Shaked constantly until a pink colour which persists for 15 min.

2.3.4 Determination of Cholesterol

Pipetted out 0.1 ml of sample and 9.9ml of Ferric chloride- Acetic acid reagent into a centrifuge and it was covered with a piece of parafilm and mixed well by inversion . Let stand for 10 to 15 minutes for the proteins to flocculate and centrifuged it at 6000rpm. Transfered 5ml of the clear supernatant fluid to a dry conical flask and marked as Test(T). Another dry conical flask marked as standard(S) and pipetted out 5ml of cholesterol as standard .As blank, take 5ml of Ferric chloride –acetic acid reagent in a dry test tube and marked as B. Added 3ml of Sulphuric acid to each test tube and mixed well by swirling .Let stand for 20-30 minutes and read the optical density values using a green yellow filter (560nm) [11].

***2.4 In Vitro* Antibacterial Activity**

In vitro Antibacterial analysis by well diffusion method . Different concentrations (10 µl, 20 µl , 30 µl , 40 µl and 50 µl) of extract were tested against *Escherichia coli- DH5,* and *GST* strain ,*Staphylococcus saprophyticus* These plates were incubated at 37°C for 24 hours. After incubation, the diameter of inhibition zones was measured [12].

2.5 Estimation of Antioxidant Activity By DPPH Method

Take a 100ul of extracts, added 3ml of free radical solution (DPPH) and incubated for 15 minutes in dark at room temperature. The absorbance was read at 550nm and methanol was taken as a blank

3.Results And Discussion

3.1Ethanol Extraction by soxhlet method (*C.sinensis, C. limon ,C. limetta*)

Extraction of *C.sinensis, C.limon, C.limetta* was successfully carried out by Soxhlet using Ethanol solvent and it was allowed for condensation for the evaporation of solvent

Fig :1 Soxhlet extraction

Fig :2 condensation of extract

3.2phytochemical Screening of Bioactive Compounds

Phytochemicals such as phenols, carbohydrates ,phytosterols, Alkaloids, proteins, flavonoids, saponins, Terpenoids and their presence were reported from the above analysis in various citrus peels(*C.sinensis,C.limon,C.limetta).*
Johann *et al*., (2007) and Ghasemi *et al*., (2009) that citrus varieties are considered and containing a rich source of secondary metabolites with the ability to produce a broad spectrum of biological activities.[13, 14].

Tab:1 Phytochemical screening of bio active compounds in citrus peels

1.	Alkaloids	Mayer's Test	Positive
2.	**C**arbohydrates	Molisch's Test	Positive
3.	Saponins	Foam's Test	Positive
4.	Phytosterols	Salkowski's Test	Positive
5.	Phenols	Ferric chloride Test	Positive
6.	Tannins	Gelatin Test	Negative
7.	Flavonoids	Lead acetate Test	Positive
8.	Glycosides	Fehling's Test	Positive
9.	Diterpenes	Copper acetate Test	Positive

3.3 Estimation of Biomolecules

3.3.1 Estimation of Total Carbohydrate By DNS Method

The amount of carbohydrates present in the extracts of Citrus peel was estimated using DNS method. The amount of carbohydrate present in 100ml of sample of *C.sinensis*-24mg, *C.limon*-28mg, *C.limetta*-20mg. Among these ,*C.limon* have high amount of sugar.

Tab:2 Estimation of total carbohydrate by DNS method

Sample	*C.sinensis*	*C.limon*	*C.limetta*
Absrbance of sample	0.153	0.180	0.100
Amount of protein	24mg	28mg	20mg

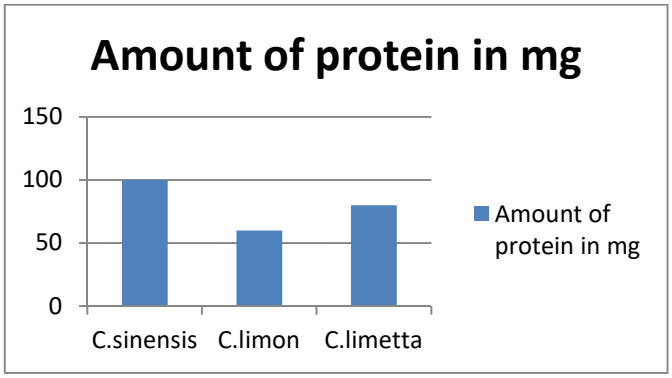
3.3.2 Estimation of Protein By Lowry'method

The amount of protein present in the extracts of citrus peel was estimated using Lowry's method. The appearence of blue colour in the above test tubes indicates the presence of protein content in the citrus fruit peel . The amount of protein present in 100ml of sample of *C.sinensis*-100mg, *C.limon*-60mg, *C.limetta*-80mg.Among these *C.sinensis* have high content of protein. The results were tabulated and the standard graph was plotted. From the graph the amount of protein present in the sample was calculated

Tab:3 Estimation of Protein By Lowry'method

Sample	*C.sinensis*	*C.limon*	*C.limetta*
Absorbance of sample	0.074	0.039	0.059
Amount of protein	100mg	60mg	80mg

Fig : 1 Protein estimation by Lowry's method



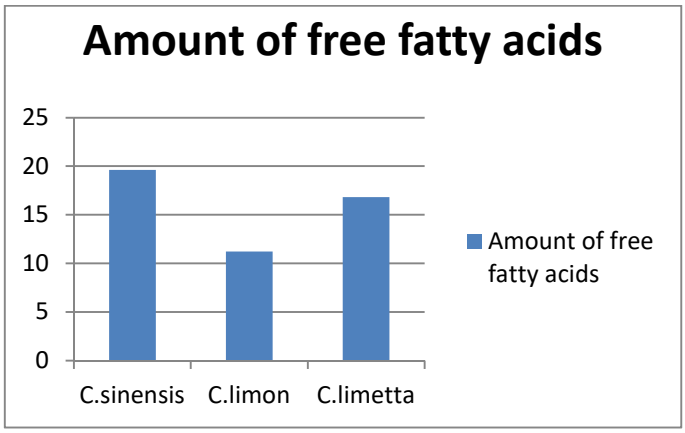
3.3.3 Estimation of Free Fatty Acids

The amount of Free fatty acids present in the extracts of citrus peel was estimated. The amount of free fatty acid present in sample of *C.sinensis*-19.63,*C.limon*-11.22, *C.limetta*-16.83.Among these *C.sinensis* have high content of free fatty acids.

Tab:4 Estimation of Free Fatty Acids

Sample	*C.sinensis*	*C.limon*	*C.limetta*
Amount of free fatty acids	19.63	11.22	16.83

Fig : 2 Estimation of Free Fatty Acids



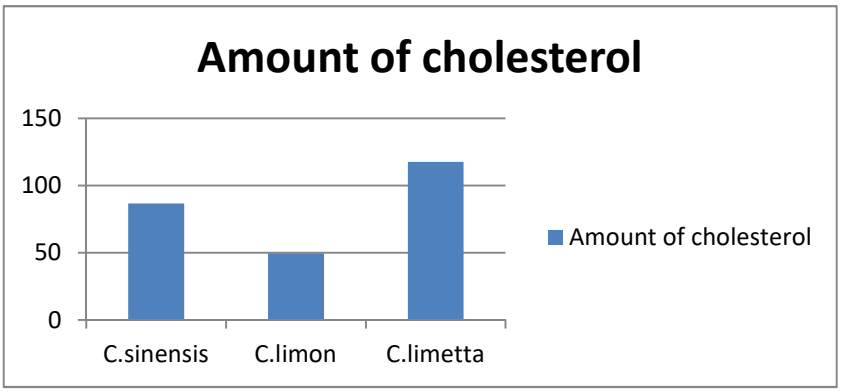
3.3.4 Estimation of Cholesterol

The amount of cholesterol present in the extracts of citrus peel was estimated. The amount of cholesterol present in100ml of sample of *C.sinensis*-86.72mg,*C.limon*-49.55mg, *C.limetta*-117.5mg.Among these *C.limetta* have high content of cholesterol

Tab:5 Estimation of Cholesterol

Sample	*C.sinensis*	*C.limon*	*C.limetta*
Amount of cholesterol(mg)	86.72	49.55	117.5

Fig :3 Estimation of Cholesterol



3.4 Antibacterial Assay

The microorganism *E.coli*, which is already known to be multi-resistant to drugs, was also resistant to the plant extracts tested. It was susceptible only to the juice of *C. limon.* Similar result was noted with *S. epidermidis* with vulnerability only to the extract of dry *C. limon*, *S. agalactiae* and *C. albicans* to the juice of *C. limon*.[15]. Ethanolic extracts of *C.limon,C.limetta,C.sinensis* against *DH5* , *GST* Strains of *E.coli and Staphylococcus saprophyticus showed the significant antibacterial activity . The results were shown in the table 6*

Table:6 Antibacterial activity of various extracts of citrus peels

Organism	Concentration of extract (Ethanol)	Diameter of the inhibition zone		
		Mosambi	Lemon	Orange
E.coli GST	10µl	-	-	-
	20 µl	-	-	-
	30 µl	+	-	++
	40 µl	+	+	++
	50 µl	++	++	+++
E.coli DH5	10µl	-	-	-
	20 µl	-	-	-
	30 µl	+	+	+
	40 µl	+	++	+
	50 µl	++	++	++
Staphylococcus saprophyticus	10µl	-	+	+
	20 µl	-	+	+
	30 µl	+	++	+
	40 µl	+	++	+
	50 µl	++	+++	++

(Growth analysis: **+++** = abundant; **++** = normal; **+** = less; **–** = no) (Parekh et al.,2005)

3.5 Antioxidant Analysis

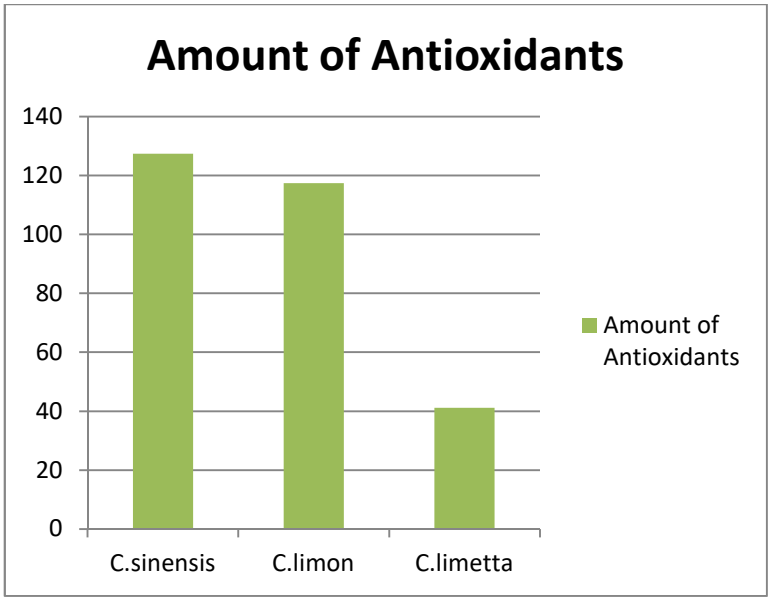
Antioxidant analysis by DPPH

The percentage of antioxidants activity of ethanol extract of *C.sinensis* peel have high antioxidant content(127.4) when compared to *C.limon* and *C.limetta.*

Table:7 Estimation of Antioxidant by DPPH Method

Sample	*C.sinensis*	*C.limon*	*C.limetta*
Absrbance of sample	0.225	0.286	0.750
Percentage of Antioxidant	127.4	117.4	41.11

Fig : 4 Estimation of Antioxidant by DPPH Method



The above graph represents the graphical representation of percentage of antioxidants of ethanolic extract of various citrus peel. Among these, *C.sinensis* have high antioxidant content(127.4) when compared to *C.limon* and *C.limetta.*

4. Conclusion

Citrus fruit peel extract have a promising antibacterial activity. Finding new naturally active compounds from plants or plant-based agricultural products have a great attention to many researchers. Hence, a huge deal of attraction has been paid to the antibacterial activity of citrus as a potential and promising source of pharmaceutical agents . This study to give a big attention in bringing useful products from citrus waste materials and also waste minimization in citrus fruit juice processing industry.

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