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PHYTOCHEMICAL SCREENING, QUANTITATIVE ANALYSIS OF FLAVONOIDS AND MINERALS IN ETHANOLIC EXTRACT OF *CITRUS PARADISI*

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ABSTRACT: Like most other plants *Citrus paradisi* contain various secondary metabolites with great potentials. The aim of this paper is to evaluate the phytochemicals by using quantitative and qualitative analysis of ethyl acetate, ethanol, *n*-hexane and aqueous extracts with the help of standard techniques. The findings from quantification and phytochemical screening showed the presence of alkaloids, flavonoids, reducing sugars, Phenols, proteins, amino acids, saponins, tannins, terpenoids, and glycosides. Further, the study findings revealed that ethanolic extract of fruit extract was found to have more constituents when compared with other extracts by quantitative method. Elemental analysis showed the presence of selenium (1.56 mg); manganese (4.95 mg); iron (5.12 mg); magnesium (34.5 mg) and zinc (9.85 mg). Chromatogram of flavonoid standards such as rutin, quercetin, gallic acid, hesperidin and ethanolic extract of *Citrus paradisi* showed the high amount of naringin. Hence the ethanolic extract of *Citrus paradisi* shows many compounds and may have been used in traditional medicine for prevention of several diseases.

INTRODUCTION: Citrus fruits are highly nutritious, medicinal and are found to be commonly in cultivation throughout the tropics which belongs to the genus citrus, subgenus papeda, and related genera. The endocarp is the palatable portion, partitioned into 10 - 14 sections segregated by thin septa, containing up to 8 seeds / septa, but appeared regularly as one. Each segment consists of juice vesicles ("pulp"), with long stalks attached to the juice containing outer wall, citrus fruits include oranges, lemons, limes in addition to tangerines and pomelos. Citrus fruits constitute only 0.9% of total daily calories [1].

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Though there are many studies on antioxidant and antibacterial effect of juice and edible parts, but there is meager literature on the citrus fruit. The citrus fruit extract is rich in nutrients and contain many phytochemicals. The citrus fruits are a rich source of flavonoids, glycosides, coumarins, β and γ sitosterol, and volatile oils and these can be efficiently used as drugs or as food supplements which are believed to be responsible for a range of protective health benefits including antioxidative, anti-inflammatory, antitumor, and antimicrobial activities [2, 3]. Most of the phytochemicals though non-nutritive are known to have some disease preventive properties. Thus they offer protection against pathogens [4] and these peels and pomace are a source of sugars, minerals and organic acids, dietary fibers and phenolics which have a wide range of actions which includes antioxidants, antimutagenic, cardio preventive, antibacterials and antiviral activities [5].

Polyphenols and antioxidants may have economic benefit for food processors. Moreover the wastes and by-products of fruits are an abundant source of antioxidant polyphenols and the vegetable processing in India generates substantial quantities of waste, income and employment [6]. The present investigation is aimed to investigate and characterize the peel pulp of citrus paradisi for phytochemical analysis, by qualitative and quantitative analysis.

MATERIALS AND METHODS:

Plant Material Collection: The raw *Citrus paradisi* fruit was collected from the cultivator and was authenticated by Dr. P. Jayaraman, Director of National Institute of Herbal Science, Plant Anatomy Research Centre, Tambaram. The voucher number of the specimen is PARC/2017/ 3380.

Plant Extract Preparation: The skin of fruits *Citrus paradisi* was peeled off and seeds were removed. The pulp of the fruit was taken and cut into small pieces, dried and later made as powder.

Preparation of Extract: The *Citrus paradisi* fruit powder was extracted with varies solvents. 10 gm of dried powder of fruit was suspended in 200 ml of water, ethanol and ethyl acetate solvents. Extraction was done using Soxhlet apparatus for 5h at a specific temperature for each solvents but not exceeding the boiling point. Further, the extract was preserved in refrigerator in glass bottle throughout the experiment (*i.e.* for both quantitative and qualitative analysis).

Qualitative Phytochemical Screening:

Test for Carbohydrates: The presence of carbohydrates was confirmed when 2 ml of extract was treated with 1 ml of Molisch's reagent and few drops of concentrated sulphuric acid which resulted in the formation of purple or reddish color.

Test for Tannins: To 1 ml of extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Saponins: 2 ml of extract, 2 ml of distilled water were added and shaken in a graduated cylinder for 15 min lengthwise. It resulted in the formation of 1 cm layer of foam that indicated the presence of saponins.

Test for Alkaloids: To 2 ml of extract, 2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for Flavonoids: To 2 ml of extract, 1 ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for Glycosides: To 2 ml of extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for Quinones: To 1 ml of extract, 1 ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Test for Phenols: 2 ml of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the extract. Formation of blue or green color indicates presence of phenols.

Test for Terpenoids: 0.5 ml of the extract was treated with 2 ml of chloroform and conc. sulphuric acid. Formation of red brown colour at the interface indicates the presence of terpenoids.

Test for Cardiac Glycosides: To 0.5 ml of the extract, 2 ml of glacial acetic acid and few drops of ferric chloride were added. This was under layered with 1 ml of conc. sulphuric acid. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

Ninhydrin Test: To 2 ml of the fruit extract few drops of 0.2% ninhydrin reagent was added and heated for 5 min. Formation of blue colour indicates the presence of amino acids.

Test for Coumarins: 1 ml of 10% sodium hydroxide was added to 1ml of the extract. Formation of yellow colour indicates the presence of coumarins.

Anthraquinones: To 1 ml of fruit extract few drops of 10% ammonia solution was added, appearance of pink color precipitate indicates the presence of anthraquinones

Steroids: To 1 ml of fruit extract equal volume of chloroform is added and a few drops of

concentrated sulphuric acid added appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Test for Phlobatannins: Few drops of 2% hydrochloric acid were added to 1ml of the extract. Appearance of red colour precipitate indicates the presence of phlobatannins.

Anthracyanine: To 1 ml of the extract was added 1 mL 2N sodium hydroxide and heated for 5 min at 100 °C. Formation of bluish green color indicates the presence of anthocyanin.

Quantitative Determination of Secondary Metabolites:

Estimation of Alkaloids: Alkaloids were determined using Harborne method [7]. Five grams of the sample was weighed into a 250 ml beaker, 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Flavonoids: The total flavonoid content in the sample was estimated by the method of Chang [8]. A volume of 0.25 ml of the sample was diluted to 1.25 ml with distilled water. 75 µl of 5% sodium nitrite was added and after six minutes 0.1 5 ml of aluminium chloride solution was added. 0.5 ml of 0.1M NaOH was added after 5 min and made up to 2.5 ml with distilled water. The solution was mixed well and the absorbance was read at 510 nm along with standard quercetin at 5 - 25 µg concentration. The results are expressed as mg of flavonoids as quercetin equivalent / gm of dried sample.

Total Phenolic Content (TPC): Total phenolic content of extract was determined according to the Folin-Ciocalteau method of Slinkard and Singleton [9] with some modifications. Briefly, 0.1 ml of extract (200, 600 and 1000 µg/ml), 1.9 ml distilled water and 1 ml of Folin-Ciocalteau's reagent were seeded in a tube, and then 1 ml of sodium carbonate was added. The reaction mixture was incubated at 25 °C for 2 h and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate and a calibration curve with six data points for catechol was obtained. The results were compared with catechol calibration curve and the total phenolic content of sample was expressed as mg of catechol equivalents per gram of extract.

Total Tannins Content (TTC): Tannins - phenolics were determined by the method of Peri and Pompei [10]. 1 ml of the sample extracts of concentration 1mg/ml was taken in a test tube. The volume was made up to 1ml with distilled water and 1 ml of water serves as the blank. To this 0.5 ml of Folin's phenol reagent (1:2) followed by 5ml of 35% sodium carbonate was added and kept at room temperature for 5 min. Blue colour was formed and the colour intensity was read at 640 nm. A standard graph (gallic acid - 1 mg/ml) was plotted, from which the tannin content of the extract was determined. The total tannin content was expressed in mg/g of extract.

Total Saponins: The fruit extract was ground and 20 g of extract put into a conical flask and 100 ml of 20% ethanol is added to the sample [11]. The sample is heated over a hot water bath for 4 h with continuous stirring at about 55 ºC. The mixture is then filtered and the residue re-extracted with another 200 ml of 20% ethyl alcohol. The combined extracts are reduced to 40 ml over a water bath at about 90 ºC. The concentrate is then transferred into a 250 ml separating funnel and 20 ml of diethyl ether is added to the extract and vigorously shaken. The aqueous layer is recovered while the diethyl ether layer is discarded and the purification process is repeated. 60 ml of *n*-butanol is added and the combined *n*-butanol extracts is washed twice with 10 ml of 5% sodium chloride. The remaining solution is then heated in a water bath and after evaporation; the samples are dried in the oven to a constant weight and values are expressed as mg/g of extract.

Determination of Flavonoids by HPLC Method: HPLC was carried out by the method of Hertog *et al*., [12] For the purpose of determining flavonoids. The coloumn used is C18 equipped with pump (LC-10AT VP1), SIL-6A automatic injector, and

detector (SPD-10AVP) set as 370 nm. The sample extract was injected into the loop and the temperature was maintained at 40 ºC and mobile phase consist of 50ml of methanol, 1ml of water, and 50ml of phosphoric acid (100:100:1) with the flow rate of about 1.5 mL/ min. The flavonoids were expressed as mg/g of fresh weight.

Determination of Minerals Elements: The minerals were determined by the dry ash extraction method using atomic spectrometry.

RESULTS AND DISCUSSION:

Phytochemical Screening: The phytochemical analysis of various extracts of *Citrus paradisi* is shown in the **Table 1**. From the qualitative findings presented in **Table 1**, it is observed that the *Citrus paradisi* of different extracts confirmed the presence of alkaloids, flavonoids, reducing sugars, flavanoids, phenols, proteins, amino acids, saponins, tannins, terpenoids and glycosides. Surprisingly, anthraquinone and steroids was not observed in any of the extracts. However, alkaloids, flavonoids, reducing sugars, phenols, amino acids and terpenoids components are present in three extracts of ethanol, aqueous and ethyl acetate.

While, carbohydrates, saponins, tannins and glycosides was only found in two extracts namely ethanol and aqueous extract. Proteins are found in all three extracts. The study revealed that ethanolic fruit extract was found to have more constituents when compared with other extracts.

TABLE 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS

S. no.	Phytochemical constituent	Ethanol extract	Aqueous extract	Ethyl acetate extract	*n*-hexane extract
1	Alkaloids	++	+	+	-
2	Carbohydrates	++	++	-	-
3	Reducing sugars	++	+	+	+
4	Flavanoids	++	+	+	-
5	Phenols	+++	++	+	+
6	Proteins	+	-	+	+
7	Amino acids	+	+	+	+
8	Saponins	+	+	-	-
9	Tannins	+	+	-	-
10	Steroids	-	-	-	-
11	Terpenoids	+	+	+	+
12	Anthraquinone	-	-	-	-
13	Glycosides	+	++	-	-

Note: ++ present in moderate; +++ present in more quantity; - Absent

The presence of phytochemicals in *Citrus paradisi* possesses varying degrees of disease preventive antioxidants and antimicrobial molecules. Hence, it is termed as health protective agent. In addition, these phytochemicals acts as best antioxidants and protect the cells against free radical damage, e.g. carotenoids, polyphenols *etc*.[13] or in decreasing the cancer risk by inhibiting the production of tumour [14] or hormonal stimulation and antibacterial activity [15]. The studies of Chede [16] and Lawal *et al*., [17] examined the nutrients and numerous phyto-chemicals with a potential for drug production and supplements for food. The study of Sharma and Sharma [18] reported the phytochemical analysis of extract of leaves of *Citrus paradisi* confirmed the presence of amino acids and tannins, the absence of alkaloids, flavonoids, glycosides and saponins in chloroform and petroleum ether extracts.

TABLE 2: QUANTITATIVE ASSAY RESULTS OF PHYTOCHEMICALS OF *CITRUS PARADISI*

S. no.	Phytochemical constituents (mg/g)	Concentration in mg/g of fruit extract		
		Ethanolic extract	Aqueous extract	Ethyl acetate extract
1	Total flavonoids	3.83 ± 0.05	1.88 ± 0.01	1.11 ± 0.05
2	Total phenols	7.44 ± 0.01	3.93 ± 0.58	4.23 ± 0.05
3	Total Tannins	3.22 ± 0.05	2.22 ± 0.05	1.11 ± 0.02
4	Total Alkaloids	8.42 ± 0.02	3.97 ± 0.58	2.42 ± 0.05
5	Total Saponins	1.50 ± 0.02	0.67 ± 0.01	0.41 ± 0.02

Quantitative analysis *Citrus paradise* was found to possess flavonoids (3.83 ± 0.05); tannins (3.22±0.05); alkaloids (8.42 ± 0.02); saponins (1.50 ± 0.02) and phenols (7.34 ± 0.01) when compared to other two extracts and is represented in **Table 2**.

HPLC Analysis: HPLC analysis of ethanolic extract of *Citrus paradisi* showed RT equal to 12.36 and the peak area detected at wavelength 150 - 180 nm. All standard flavonoids were taken to construct a calibration curve. These RT correlated with standard flavonoids. From the calibration graph it was compared with ethanolic extract of *Citrus paradisi* and it was observed that presence of naringin with (12.370%), hesperidin (8.030 %), gallic acid (87.033%), quercitin with (3.277%) and rutin (2.847%). The flavonoid content in citrus paradisi is given in **Table 3**.

TABLE 3: DETAILS OF STANDARD FLAVONOIDS

Component name	RT	Area	Area %
Rutin	2.647	547.878	2.4
Quercitin	3.377	6197.184	27.5
Gallic acid	7.033	235.106	1.0
Hespiridin	8.030	787.001	3.5
Naringin	12.370	14756.639	65.5

Naringin was the predominant compound and it is one of major flavonoid present in *Citrus paradisi* followed by other compounds. HPLC analysis of standard flavonoids is given in **Fig. 1**.

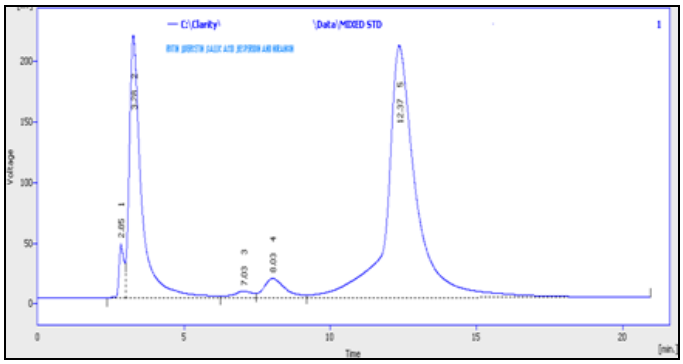

FIG. 1: HPLC ANALYSIS OF STANDARD FLAVONOIDS

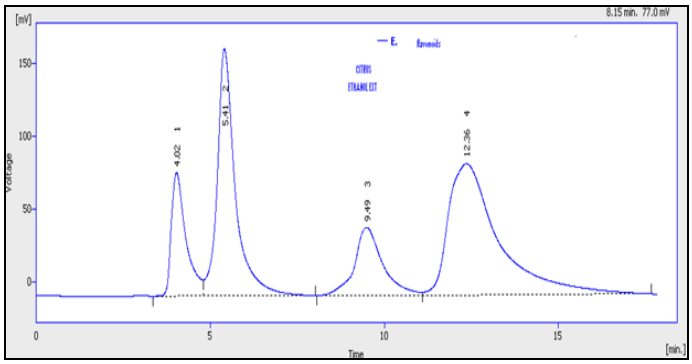

FIG. 2: HPLC ANALYSIS OF ETHANOLIC EXTRACT OF *CITRUS PARADISI*

Quercitin is a flavonoid which was most predominatly found in the form of glycosides. Quercitin is used to improve cardiovascular risk, cancer and also protects against osteoporosis, rutin inhibited leukemia and also had anticancerous effect [19]. Gallic acid had anti - inflammatory, antimutagenic, anticancer, and antioxidant activity and it prevent albuminuria. Naringin prevents hypercholesterolemia and also reduce LDL oxidation, hespiridin act as anticancer, anti-inflammatory agent and also helps to control blood sugar level.

HPLC Chromatogram of *Citrus Paradisi*, Peak Assignments of the Components Include Rutin, Quercetin, Gallic Acid, Hesperidin and Naringin: HPLC analysis of standards flavonoids compounds and extract. The chromatogram which was obtained from standard and fruit extract was compared, with RT value of 4.023, 5.407, 9.490, 12.363 was correlated with standards such as quercitin, gallic acid, hespiridin and naringin **Fig. 2**. It showed that ethanolic extract of *Citrus paradisi* contained quercitin, gallic acid, hespiridin, and naringin. From the peaks present in ethanolic extract of *Citrus paradisi*, it can be inferred that *citrus paradisi* contain high amounts of naringin **Table 4**.

TABLE 4: DETAILS OF MAJOR FLAVONOIDS IN ETHANOLIC EXTRACT OF *CITRUS PARADISI*

Component name	RT	Area	Area%
Quercitin	4.023	2481.66	12.3
Gallic acid	5.407	169.875	30.2
Hespiridin	9.490	46.705	42.9
Naringin	12.363	90.080	44.6

Elemental Composition of *Citrus paradisi* Fruit Extract:

TABLE 5: ELEMENTAL COMPOSITION OF *CITRUS PARADISI* FRUIT EXTRACT

S. no.	Minerals	Composition (mg/100 g)
1	Selenium	1.56 mg
2	Zinc	9.85 mg
3	Manganese	4.95 mg
4	Magnesium	34.5 mg
5	Iron	5.12 mg

Table 5 shows the trace elements which are present in *Citrus paradisi.* The minerals present were selenium (1.56 mg); manganese (4.95 mg); iron (5.12 mg); magnesium (34.5 mg) and zinc (9.85 mg). However, the most abundant minerals are magnesium (34.5 mg) and zinc (9.85 mg).

CONCLUSION: The present study showed that ethanolic extract of *Citrus paradisi* is rich in basic nutrients. Qualitative phytochemical screening showed that it is abundant in phytochemicals such as alkaloids, carbohydrates, saponins, reducing sugars, flavonoids, phenols, proteins, tannins, terpenoids and glycosides especially it was found in high amount in ethanolic extract than other extracts.

Quantitative analysis showed that ethanolic extract contains higher amounts than other two extracts (aqueous and ethyl acetate extract). From the findings of the study it may be concluded that the ethanolic extract of *Citrus paradisi* acts as the potential source of phytochemical which may be used traditional medicine for prevention of several diseases.

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CONFLICT OF INTEREST: Nil

REFERENCES:

1. Kumar KA, Narayani M, Subanthini A and Jayakumar M: Antimicrobial activity and phytochemical analysis of citrus fruit peels -Utilization of Fruit Waste. Int J Eng Sci Technol. 2011; 3(6): 5414-5421.
2. Aruoma OI, Landes B and Ramful-Baboolall D: Functional benefits of citrus fruits in the management of diabetes. Prev Med (Baltim). 2012; 54: S12-S16.
3. Karimi E, Oskoueian E, Hendra R, Oskoueian A and Jaafar HZE: Phenolic compounds characterization and biological activities of Citrus aurantium Bloom. Molecules 2012; 17(12): 1203-1218.
4. Kokate CK, Purohit AP and Gokhale SB: Pharmacognosy. Pune, India: NiraliPrakashan; 2008. https://www.amazon.com/pharmacognosy-Dr-C-KKokate/dp/8185790094:7-35.
5. John S, Priyadarshini S, Monica SJ and Arumugam P: Phytochemical profile and thin layer chromatographic studies of *Daucus Carota* peel extracts. Int J Food Sci Nutr. 2017; 2(1): 23-26.
6. Kodagoda K and Marapana R: Utilization of fruit processing by-products for industrial applications: A review. Int J Food Sci Nutr. 2017; 2(6): 24-30.
7. Harborne JB: Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 1st ed. Dordrecht: Springer Netherlands 1980: 1-25.
8. Chang CC, Yang MH, Wen HM and Chern JC: Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002; 10(3): 178-182.
9. Slinkard K and Singleton VL: Total Phenol Analysis: Automation and Comparison with Manual Methods. Am J Enol Vitic. 1977; 28: 49-55.
10. Peri C and Pompei C: Estimation of different phenolic groups in vegetable extracts. Phytochemistry. 1971; 10(9): 2187-2189.
11. Obadoni BO and Ochuko PO: Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in edo and delta states of Nigeria. Glob J Pure Appl Sci. 2002; 8(2): 203-208.
12. Hertog MGL, Hollman PCH and Venema DP: Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J Agric Food Chem. 1992; 40(9): 1591-1598.
13. Omoregie E and Osagie A: Antioxidant Properties of methanolic extracts of some Nigerian Plants on Nutritionally-Stressed Rats. Nig J Basic Appl Sci. 2012; 20(1): 7-20.
14. Devasagayam T, Tilak J, Boloor K, Sane KS, Ghaskadbi SS and Lele R: Free radicals and antioxidants in human health: current status and future prospects. J Assoc Physicians India. 2004; 52: 794-804.
15. Mathew BB, Jatawa SK and Tiwari A: Phytochemical Analysis Of Citrus Limonum Pulp And Peel. Int J Pharm Pharm Sci. 2012; 4(2): 269-371.
16. Chede PS: Phytochemical analysis of *Citrus sinensis* Peel. Int J Pharma Bio Sci. 2013; 4(1): 339-343.
17. Lawal D, Bala JA, Aliyu SY and Huguma MA: Phytochemical screening and in vitro anti-bacterial studies of the ethanolic extract of *Citrus senensis* (Linn.) Peel against some Clinical Bacterial Isolates. Int J Innov Appl Stud. 2013; 2(2): 138-145.
18. Sharma MC and Sharma S: Phytochemical screening and *in-vitro* antimicrobial activity of combined *Citrus paradisi* and *Ficus carica* Linn aqueous extracts. Int J Microbiol Res. 2010; 1(3): 162-165.
19. Lin JP, Yang JS and Lin JJ: Rutin inhibits human leukemia tumor growth in a murine xenograft model *in-vivo*. Environ Toxicol. 2012; 27(8): 480-484.

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