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A Comparative Evaluation Of Ascorbic Acid, Crude Fibre And Antioxidant Properties Of Selected Citrus Species Collected From Kanyakumari District

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Abstract
Citrus fruits belong to the "Rutaceae" family, and are cultivated globally in tropical and subtropical regions with an approximate production of 102 million tons per year. They contain large quantities of vitamin C as well as appreciable amounts of several other nutrients such as minerals, vitamins, micronutrients, and dietary fibres. Citrus fruits are considered as one of the natural resources of antioxidants, which contain an appreciable amount of ascorbic acid, flavonoids, and phenolic compounds. The regular consumption of citrus fruits by humans has been associated with lower incidence of chronic-degenerative diseases, especially those mediated by free radicals. Most of the health-promoting properties of citrus fruits derive from their antioxidant content of carotenoids and ascorbic acid (ASC). In the present study, we have chosen three Citrus species viz., Citrus sinensis, Citrus japonica and Citrus depressa prevalently available in Kanyakumari District. Generally Citrus species consists of higher Ascorbic acid content so here we have selected three species to compare its ascorbic acid, crude fibre and antioxidant property. In the present study, the ethanolic extract of the whole fruit of these selected Citrus species were studied to find out the total ascorbic acid and crude fibre present in the selected Citrus species. The antioxidant activities were assessed and compared by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging effect. The results reported that compared to the three Citrus species, Citrus japonica revealed the higher Vitamin C, Crude fibre and Antioxidant property.
Key words: *Citrus sinensis, Citrus japonica and Citrus depressa, Ascorbic Acid, Crude fibre, antioxidant property*

INTRODUCTION

Citrus fruits and their juices are probably one of the most accessible and relevant sources of key antioxidants for humans worldwide (Liu et al., 2012). Apart from their significant content of ascorbic acid and flavonoids, the antioxidant properties of citrus fruits strongly depend on the carotenoid composition in different species such as oranges, mandarins and grapefruits (Zacarías-García et al., 2021). The red pigmentation of citrus fruit pulp is an attractive trait for consumers and several red-fleshed sweet orange varieties have been selected in breeding programs and characterized, such as the recently described 'Kirkwook Navel' and 'Ruby' Valencia, both of South African origin (Grosso et al., 2013). Fruit peels can be used in the food sector, the cosmetics industry, and as a source for making useful medications. These compounds, which were obtained from peels, shown notable antibacterial activity against the pathogenic bacteria that causes food poisoning (Hussain et al., 2022). Citrus fruit peels are used in traditional Chinese medicine to treat high blood pressure, cough, stomachaches, and muscle soreness (Ju Mi Hyun et al., 2017). Citrus fruits, due to the presence of many nutrients, such as vitamins A and E, B vitamins (thiamine, riboflavin and niacin), minerals, and antioxidants, i.e., flavonoids, vitamin C, phenolic compounds, and carotenoids, as well as dietary fiber, have a positive stimulatory effect on the immune, cardiovascular, and digestive systems (Rafiq et al., 2018). They also have anti-inflammatory, anti-sclerotic, antiviral, antibacterial, and anti-cancer properties (Gorinstein et al., 2004: Anagnostopoulou et al., 2004). The citron's pericarp (Its entire envelope of the fruit including the mesocarp or albedo and the epicarp or zest) Lacks both fats (lipids) and sugars (carbohydrates) but is high in fibre. The zest is rich in volatile flavonoid compounds (rutin and neohesperidin), limonene and gamma-terpinene (Venturini et al., 2014). In addition, the peel of citrus fruit, like the pulp, contains many natural prenyloxycoumarin compounds,

such as auraptene, bergamottin, imperatorin, and heraclenin, as well as macro- and micro-minerals, whose presence increases its dietary and therapeutic value (Genovese et al., 2014). Therefore, the low cost and availability of the peel, which is a waste product of citrus fruit, can be considered a potential source of nutraceuticals (Rafiq et al., 2018). The aim of the study was to compare the ascorbic acid, crude fibre content and antioxidant property of whole citrus fruits of Citrus sinensis, Citrus japonica and Citrus depressa.

MATERIALS AND METHODS

• Collection and Processing of Citrus Fruit samples

The selected Citrus fruit species viz., C. japonica, C. sinensis and C. depressa used in the present study were harvested at commercial maturity from adult trees growing under standard agronomical and environmental conditions from the wild gardens Marthandam, Arumanai and Villukuri, Kanyakumari District, Tamilnadu, India. The collected samples were processed by cleaning with tap water to make samples free from foreign materials. After washing the Citrus fruits were cute in to small slices and allowed to shade dry for a particular period of time and then it was grind to make it into powder form. The sample were packed in glass container and stored in room temperature for further analysis.

• Preparation of crude extract

40 g of each pulverized powder of the Citrus fruit viz., C. japonica, C. sinensis and C. depressa powders were steeped in 0.2 L of ethanol (80%), separately for 24 h while being occasionally stirred before performing, to ascertain analysis. The Citrus fruit powdered components that had been soaked in Ethanol were filtered using a filter paper (Whatman no. 42). To evaporate the solvent, a rotary evaporator was used. Under the chemical hood, the residues were air dried before their final collection in tiny microfuge tubes. Finally, a stock solution (5 mg/mL in Ethanol) of above different residues was prepared, and various quantities of the different Citrus samples were diluted subsequently.

• Estimation of Ascorbic Acid

Extraction 0.1g of the sample homogenate was mixed with 10 ml of 4% oxalic acid in a standard flask. Determination of ascorbic acid content Ascorbic acid content of the samples were determined by 2, 6-dichlorophenol indophenol (DCPIP) titration method. 5 ml of the ascorbic acid working standard (500 µg/5 ml) and 5 ml of 4% oxalic acid were pipetted out into a 100 ml conical flask. The contents in the flask were titrated against the dye solution (V1) until the appearance of a pale pink colour that persisted for a few min. 5 ml of the test samples C. japonica, C. sinensis and C. depressa were similarly titrated against the dye solution (V2).

Ascorbic acid content present in the test samples were determined using the formula: Amount of ascorbic acid content (mg/100 ml) = 500 x V2 x 10 x 100 mg /100 g V1×10×0.1×1000

Where, 500 = µg of standard ascorbic acid taken for titration V1 = Volume of dye consumed by 500µg of standard ascorbic acid 30 V2 = Volume of dye consumed by 5 ml of test sample 10 = Corresponds to total volume of the extract 100 = Ascorbic acid content/100ml of the sample 0.1 = weight of sample taken for extraction 10 = Volume of the test liquid sample taken for titration 1000 = conversion factor (µg to mg). Plate - 6 Standard Sample

• Determination of crude fibre content

1g of the test Citrus fruit viz., C. japonica, C. sinensis and C. depressa ethanol extracts was weighed and taken in a beaker. 25 ml of 10% v/v nitric acid was added and boiled with constant stirring up to 30 minutes. It was then filtered through a fine cotton cloth. The residues present on the cloth was washed with boiling water and then transferred to a fresh beaker. 25 ml of 2.5% v/v sodium hydroxide solution was added then and boiled for 30 minutes with constant stirring. Strained and washed with hot water as mentioned earlier. The residues were transferred to a cleaned and dried crucible or petriplate. The residues were dried in an oven and the weight was measured. The percentage weight of the crude fiber present was calculated with the formula. Crude fibre (before) Crude fibre (after).

• Determination of Antioxidant activity

DPPH free radical scavenging Assay

The free radical scavenging activity was calculated using ethanol solution of DPPH (Kazmi, et al., 2022). Methanolic solution (1.0 mL) of DPPH was mixed with 10 µL of ethanol extract of Citrus species viz., C.

japonica, C. sinensis and C. depressa extract that was previously dissolved in DMSO at a concentration of 4 mg/mL. After shaking vigorously, the solution were allowed to stay in the dark at room temperature for 30 min. The absorbance measurement was performed at 515 nm, whereas the scavenging activity was calculated by using the following equation.

%FRSA= [Absorbance control−Absorbance extract / Absorbance control] ×100

DMSO was used as a negative control, whereas Ascorbic acid was employed as a positive control. Ascorbic acid as well as extracts with DPPH ≥ 50% were investigated further at lower concentrations ranging from 100 to 1000 µg/mL (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 µg/mL) to determine their 50% inhibitory concentration (IC_{50}) in $\mu g.mL^{-1}$. IC_{50} was calculated by using GraphPad Prism 7.0 Software (Graph Pad Software Inc.) and all the analysis was performed in triplicate.

RESULT AND DISCUSSION

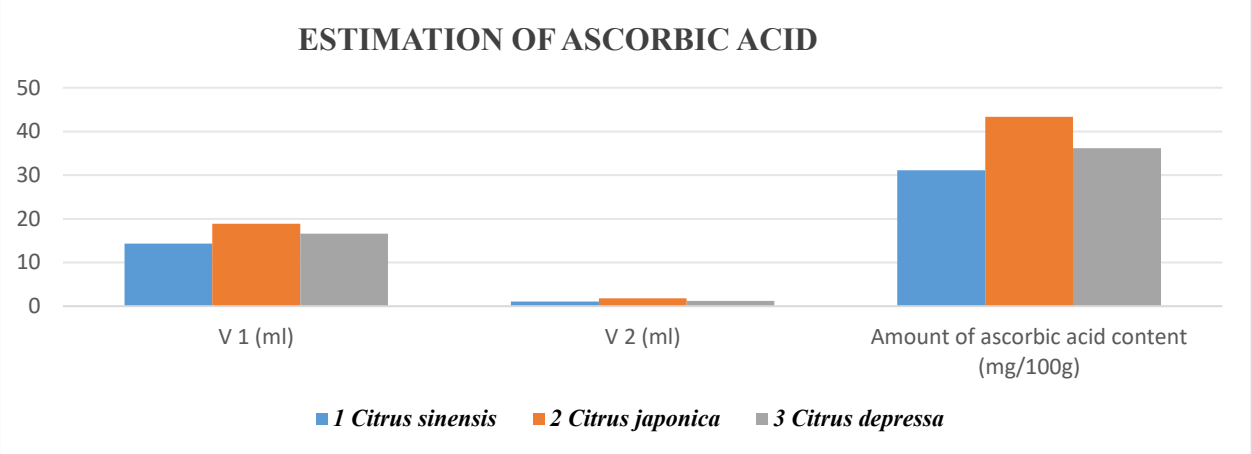
• Estimation of Ascorbic Acid:

Citrus fruits have long been considered as one of the best sources of micronutrients for human nutrition, especially because of their contents of ascorbic acid (ASC), carotenoids and polyphenols/flavonoids (Saini et al., 2022). Rabiatul Najwa et al., (2017) compared the vitamin C content of citrus fruits (orange, grapefruit, lemon, lime, kaffir lime and musk lime) using indophenol titration and HPLC-PDA methods. In the titration method, orange has the highest vitamin C content (58.30 mg/100g) In the present study, the Quantitative estimation of Vitamin C was carried out in the three different ethanol extract of Citrus species viz., C. japonica, C. sinensis and C. depressa extract. The result revealed that the Ethanol extract of C. japonica recorded the presence of higher Vitamin C content of 43.12 mg/100gm of the extract followed by C. depressa of 36.14mg/100 gm and the less amount of vitamin C content was denoted in C. sinensis. So comparatively C. japonica recorded higher Vitamin C Quantity compared to the other two Citrus species (Table 1: Figure: 1).

Table.1. Estimation of Ascorbic Acid in the Ethanol Extract of selected Citrus Species

SINO	Citrus Species	V 1 (ml)	V 2 (ml)	Amount of ascorbic acid content (mg/100g)
1	Citrus sinensis	14.3	1.1	31.12
2	Citrus japonica	18.9	1.8	43.32
3	Citrus depressa	16.6	1.2	36.14

Figure.1. Estimation of Ascorbic Acid in the Ethanol Extract of selected Citrus Species



• Estimation of Crude Fibre

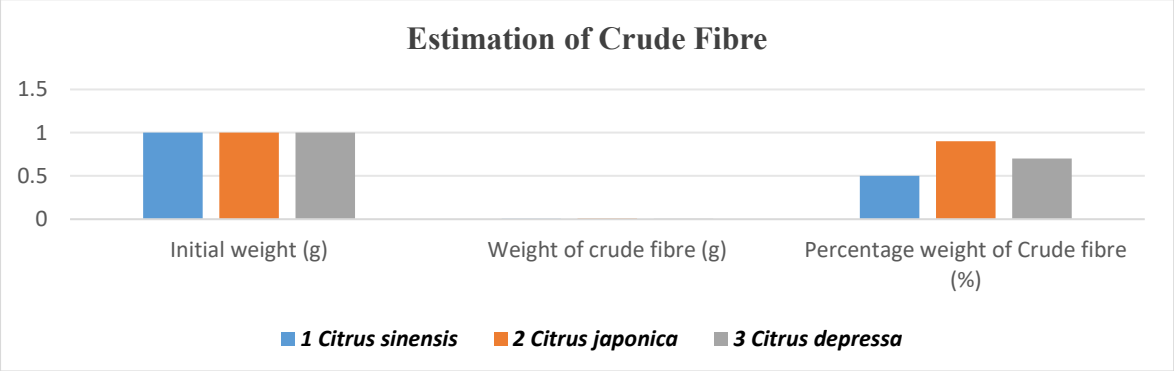
The functional properties of DF include the bulk volume, the hydration, hydrocolloidal and rheological properties, which contribute to application in food formula design and food manufacturing (Gómez-Ordóñez et al., 2010). Lei Wang et al., (2015) studied the dietary fibers (DFs) from five types of citrus fruit

peels (orange, grapefruit, lemon, gonggan and ponkan) were investigated. The contents of total, soluble and insoluble dietary fibers in citrus fruit peels had no significant difference ($p>0.05$) and the binding capacities of soluble dietary fibers (SDFs) of orange, lemon, gonggan and ponkan for sodium cholate and cholesterol were significantly ($p<0.05$) lower than those of grapefruit SDF. In the present study, the estimation of crude fibre was carried out in the three different ethanol extract of Citrus species viz., C. japonica, C. sinensis and C. depressa extract. The result revealed that the Ethanol extract of C. japonica recorded the presence of higher amount of crude fibre content of 0.9% of the extract followed by C. depressa of 0.7% and the less amount of Crude fibre content was indicated in C. sinensis (0.5%). So comparatively C. japonica recorded higher amount of crude fibre compared to the other two Citrus species (Table 2: Figure:2).

Table.2. Estimation of Crude Fibre in the Ethanol Extract of selected Citrus Species

SINO	Citrus Species	Initial weight (g)	Weight of crude fibre (g)	Percentage weight of Crude fibre (%)
1	Citrus sinensis	1	0.005	0.5
2	Citrus japonica	1	0.009	0.9
3	Citrus depressa	1	0.007	0.7

Figure.2. Estimation of Crude Fibre in the Ethanol Extract of selected Citrus Species



• Antioxidant Property

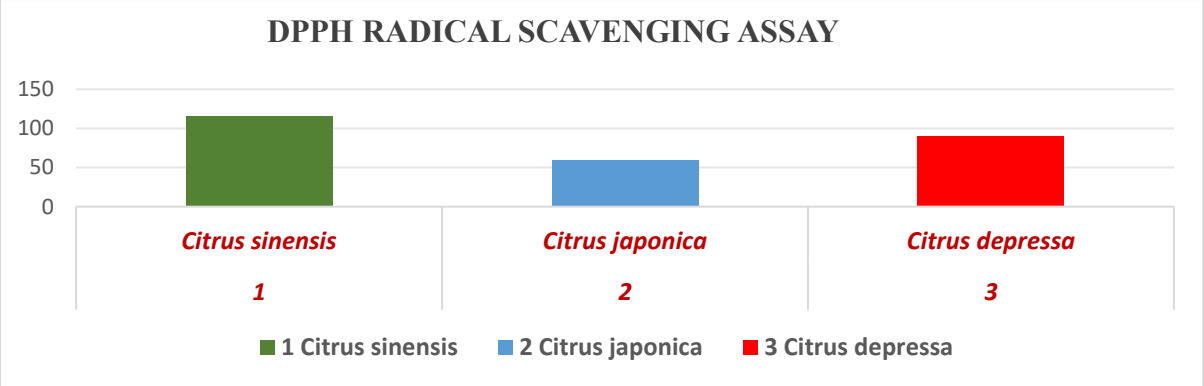
DPPH Radical Scavenging Activity

Muhammad Saleem et al., (2023) studied the DPPH Radical scavenging property in ethanolic (80%), methanolic and acetone extracts of the peel of local variants of orange (lemon, grape fruit, mousami, fruiter, and shikri malta). The highest free radical scavenging activity (93.1%) of DPPH was exhibited by lemon peels, whereas the least activity (78.6%) was shown by mousami peels. Ethanolic extract of orange peels demonstrated more reducing power while showing an absorption of 1.98, followed by methanolic (1.11) and acetone (0.81) extracts. In the present study, the antioxidant activity of the three different ethanol extract of Citrus species viz., C. japonica, C. sinensis and C. depressa extract was analysed by DPPH Assay and the results revealed that C. japonica recorded low IC50 value of 59.82 µg/ml which represents higher antioxidant activity compared to C. depressa (90.42 µg/ml) and higher IC 50 value of 115.9 µg/ml was denoted by C. sinensis. Thus, compared to the antioxidant property of the three Citrus species C. japonica represented higher DPPH reducing property compared to the other two Citrus species (Table. 3: Figure 3)

Table.3. DPPH Radical Scavenging property in the Ethanol Extract of selected Citrus Species

SINO	Citrus Species	IC 50 Value (µg/ml)
1	Citrus sinensis	115.9
2	Citrus japonica	59.82
3	Citrus depressa	90.42

Figure.3. DPPH Radical Scavenging Assay in the Ethanol Extract of selected Citrus Species



CONCLUSION

In the present research, the selected Citrus species C. japonica, C. sinensis and C. depressa had a rich vitamin, crude fibre and antioxidant property especially C. japonica recorded highest antioxidant property compared to the other two Citrus species. Both the pulp and the peel of citrus fruits are valuable sources of macro- and micronutrients. Their content in the peel of most of the fruits tested far exceeds their quantity in the pulp, and for this reason, special attention should be paid to its potential use as a component of a functional food (designer foods) or in the pharmaceutical industry. Citrus peels, being rich in bioactive compounds, can be used to produce functional foods or natural "dietary supplements", providing not only dietary fiber and antioxidant compounds, but also minerals.

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