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Phytochemical Content and Antioxidant Potential of *Citrus reticulata* Collected in the Thanjavur District, South India

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Abstract

This study was designed to investigate the phytochemical and the antioxidant activities of fruits, peels and leaves extracts of *Citrus reticulata*. The total alkaloids, saponin, phenolic acid and flavonoids were determined spectrophotometrically while antioxidant potentials were evaluated with *2,2'-diphenyl-1-picrylhydrazyl* (DPPH), hydrogen peroxide (H_2O_2) protocols. The fruit extract shows the highest phenolic acid content of 33.94 mg/GAE/g. Flavonoids content was highest in the peels extract 14.30 mg/QE/g. All the extracts showed significant antioxidant activities H_2O_2, DPPH along a concentration gradient. Antioxidant capacity is also high in the test plant suggesting its biomedicinal value to human health.

Key words: Citrus reticulata, antioxidant, phytochemicals, total phenols, DPPH

INTRODUCTION

Phytochemicals are a large group of secondary metabolites found in vegetables and fruits, and they can be classified as carotenoids, phenolics, alkaloids, and organosulfur compounds, among others, depending on the variations of their chemical structures [1]. Numerous studies have demonstrated that consumption of a large amount of plant food rich in phytochemicals was negatively associated with the risks of chronic and degenerative diseases [2].

Citrus reticulate is a tropical or subtropical fruit widely distributed around the world. As one of the most consumed fruits it also has great economic importance. Besides its value as a delicious fruit, its nutritional values are also important. Previous studies have reported a variety of bioactivities of citrus fruit, like antioxidant [3], anticancer [4,5], anti-inflammation [6], anti-fat [7] and anti-diabetes properties [8,9]. Many of the bioactivities are attributed to the phenolics and flavonoids that are abundant in citrus fruit [10,11]. Citrus can be classified in several types, including mandarins, tangerines, oranges, pummelos, hybrids, lemons, limes, etc. [12]. The present study aimed to carry out a comprehensive investigation on the phenol, flavonoid composition and antioxidant capacity of some botanicals parts of *citrus reticulata* from South India.

MATERIALS AND METHODS

Plant Material
The plant material *Citrus reticulata* was collected from Thanjavur, Tamil Nadu, India.

Phytochemical screening analysis
Qualitative phytochemical studies analysis was done in the ethanolic extract of various botanical parts of *C.reticulata* using the procedure of Chandra Mohan *et al*., [13] , Sofowara [14] and Brain *et. al.,* [15].

Total phenolic content
The total phenolic content (TPC) of *C. reticulata* extracts was estimated spectrophotometrically using the Folin-Ciocalteau method [16]. For each sample, 0.3 mL (80 µg/mL) was mixed with Folin-Ciocalteau reagent (1.5 mL; diluted 10 times) and sodium carbonate (1.2 mL; 7.5% w/v). After incubation of the mixture for 30 min at room temperature, the absorbance was measured at 765 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg per g of dry material (mg GAE/g), using a standard calibration curve.

Total flavonoid content
The total flavonoid content (TFC) of *C.reticulata* extracts was determined according to *Loganayaki et al.* [17]. First, an 0.25 mL aliquot of the extract (80 µg/mL) was mixed with distilled water (1 mL), followed by the addition of 5% $NaNO_2$ solution (0.075 mL). After 5 min, 10% $AlCl_3$ solution (0.15 mL) was added to the mixture, which was then incubated for 6 min. Finally, 4% NaOH (0.5 mL) was added and the volume was adjusted to 5 mL with distilled water. After incubation for 15 min, the absorbance was determined at 415 nm. The total flavonoid content was expressed as quercetin equivalents in mg per g of dry material (mg quercetin/g), using a standard calibration curve.

Antioxidant Assay
DPPH (1, 1-diphenyl-2- picrylhydrazyl) radical scavenging assay
After varying the sample concentrations (10µl, 20µl, 30µl, 40µl, and 50µl), 50µl of 0.659 mM DPPH dissolved in methanol solution was added to bring the final concentration with double distilled water. For twenty minutes, the tubes were incubated at 25 °C. Using a Shimadzu UV 1800 spectrophotometer, the absorbance value was measured at 510 nm. For the control without a sample, the same process was used. % inhibition determined using the formula:

$$\text{DPPH inhibition activity (I \%)} = \frac{OD\ control - OD\ sample}{OD\ sample}\ X\ 100\%$$

Hydrogen peroxide radical scavenging assay
0.6ml of 40mM of Hydrogen peroxide was prepared using 50mM phosphate buffer (pH 7.4). Different concentrations (10µl, 20µl, 30µl, 40µl & 50µl) of sample was added to hydrogen peroxide solution. The tubes were incubated for 10 minutes. The absorbance values were recorded at 230nm using shimadzu UV 1800 spectrophotometer

RESULTS AND DISCUSSION

The phytochemical analysis conducted on *C. reticulata* botanical parts revealed the presence of flavonoids, saponins, phenols and alkaloids.

Determination of TPC and TFC of *C. reticulata* leaves, peels and fruits extract
The present study was conducted to study the different botanical parts of *C.reticulata*. The fruit extract shows the highest phenolic acid content of 33.94 mg/GAE/g followed by peels 28.95 mg/GAE/g and the least was the leaves extract 18.24 mg/GAE/g. Flavonoids content was highest in the peels extract 14.30 mg/QE/g followed by fruits extract 12.05 mg/QE/g and the least was the leaves extract 1.81 mg/QE/g . Results are presented in Table 1.

Table 1 *C. reticulata* extracts, total phenolic and flavonoids content, and the IC_{50} values for DPPH, H_2O_2 scavenging activity

Extracts	Total phenolic content (mg GAE/g)	Total flavonoids content (mg quercetin/g)	IC_{50}			
			DPPH (µg/ml)	Standard (Ascorbic acid)	H_2O_2 (mg/ml)	Standard (Ascorbic acid)
Leaves	18.24	1.81	333.62	9.65	6.05	18.91
Peels	28.95	14.30	80.76		6.10	
Fruits	33.94	12.05	187.25		5.89	

DPPH antioxidant tests are commonly used in determining the primary antioxidant capacities. The DPPH radical scavenging mechanisms include two types: the electron transfer type when components dissolve in polar solutions and the hydrogen supply ability type in nonpolar solutions [18- 20]. In this study, the DPPH radical scavenging abilities were mainly based on the electron transfer ability of the antioxidant components since the citrus extracts were dissolved in water.

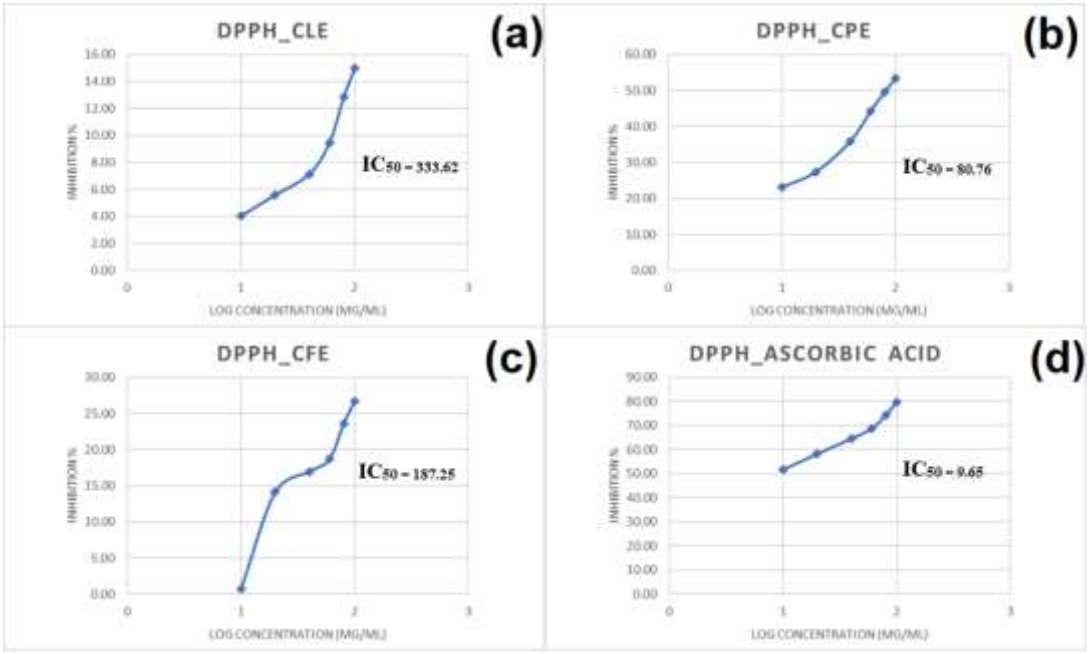

Fig.1: DPPH radical scavenging activity of *C. reticulata* (a) leaves extract (b) peels extract (c) fruits extract and (d) standard

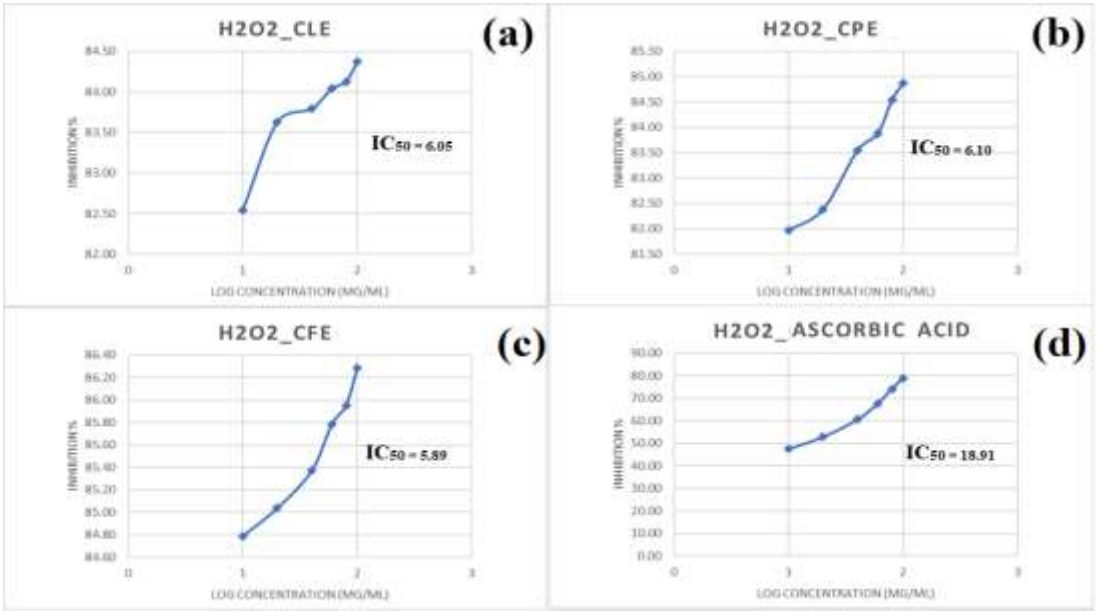

Fig.2: H_2O_2 radical scavenging activity of *C. reticulata* (a) leaves extract (b) peels extract (c) fruits extract and (d) standard

The antioxidant activity of *C. limon* leaf extracts cannot be evaluated by using only one method due to the complex nature of the phytochemical contents and its reaction mechanism dependent [21,22]. Therefore, it becomes relevant to employ the use of multiple antioxidant assay such as DPPH, H_2O_2 as applied in this study. Epidemiological studies have confirmed that the incidence of oxidative stress-related diseases can be reduced by the consumption of fruits and vegetables rich in compounds possessing high antioxidant activity [23]. The oxidative-stress linked diseases referred above are cancer, cardiovascular diseases, neural disorder, parkinson disease, alcohol induced liver diseases, ulcerative colitis and aging [24-30]. The high antioxidant activity of the studied plant extracts as observed in Figs. 1 and 2, which revealed that the *C. reticulata* extracts *in vitro* test demonstrated a powerful antioxidant activity against DPPH, H_2O_2 oxidative systems. The pharmacological properties of plants are linked to their phytochemicals such as phenolic acids, saponin and alkaloids contents [31]. The phytochemical content of plants has a relationship with bioactive capacities such as anti-inflammatory, antifungal and the antioxidative, which is influenced by their redox properties [32] and plays a relevant role in scavenging and absorbing free radicals [33]. The phytochemical analysis conducted on *C. reticulata* botanical parts revealed the presence of flavonoids, saponins, phenols and alkaloid. Flavonoids function as anti-oxidant by possessing free radical quenching properties as reported by [34]. A number of flavonoid compounds such as flavonols, isoflavonoids, anthocyanins and flavones have been found to be effective antifungal agents against a wide range of fungal pathogenic organisms. The mechanism of its anti-fungal activities involves enhancing the disruption of the plasma membrane, mitochondrial disfunctioning, inhibiting cell wall formation, cell division, protein synthesis and the efflux-mediated pumping system [34]. Furthermore, *C. reticulata* extracts revealed the presence of saponin that produces inhibitory effect on inflammation [35]. The result also revealed phenolic compounds as one of the major components of *C. reticulata*. In addition high antioxidant activity of *C. reticulata* in this study could justify the reason for the plant global usage in food and pharmaceutical industrial applications such as food flavouring, pharmaceutical aroma corrigents and cosmetic formulations [21]. It is also pertinent to note that the antioxidant properties of plants with the studied plant inclusive have linkage with phenolic contents [36] which could extend the shelf life of food, and exhibits antimicrobial activities [37].

CONCLUSION

Citrus fruits are consumed in large quantities worldwide due to their attractive aromas and taste. The composition and content of phytochemical and nutritional compounds in citrus vary dramatically among varieties, fruit parts, maturity, region of cultivation, and many other environmental factors. The result of this study shows that. *C. reticulata* exhibits high antioxidant properties to scavenge DPPH, H_2O_2 radicals. These antioxidant activities as well as the phytochemical content in terms of flavonoids, phenols, saponin and alkaloids may justify the prospective use of the plant for the treatment and management of cancer, oxidative stressed-related diseases and fungal skin infectious diseases, on the basis of further *in vivo* and *in vitro* cell line pharmacological testing models. In addition, the plant extract could also be investigated further for its usage as a biofungicides for improvement of shelf- life of fruits and vegetables in supermarkets. there are some challenges and issues that should be overcome in the future study of citrus. Under the premise of accurate profiling of phytochemical and nutritional substances in citrus, and given the tremendous differences in the chemical substances among different citrus varieties, fruit parts, maturity, and cultivation region, targeted utilization of citrus resources should be made in the future.

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