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Phytochemistry, micronutrient composition, and antioxidant potentials of *Citrus maxima* (Shaddock) fruit juice

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Abstract

Citrus maxima fruit, the largest of all *Citrus* varieties has been observed to be less consumed compared to other *Citrus* fruits. Paucity of information/awareness about its nutritive/medicinal properties could be a reason for that hence an increased research on its nutritive potentials could boost its consumption. This study was designed to evaluate its phytochemistry, micronutrient composition and *in vitro* antioxidant potentials. *Citrus maxima* juice was obtained from its fruit using a manual screw juice extractor. Analysis was carried out using standard methods. Phytochemical analysis of fresh juice of *C maxima* fruit showed the presence of phenols, flavonoids, tannins, alkaloids, glycosides, steroids and terpenoids. Zinc, copper, iron, magnesium, Vitamins C and E were present in appreciable quantities. *Citrus maxima* juice scavenged 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and superoxide radical (O_2). This study showed that *Citrus maxima* fruit juice possesses considerable amounts of phytochemicals, minerals, vitamins C and E, and relative antioxidant potentials.

Keywords: Antioxidants, *Citrus maxima,* micronutrients, phytochemistry

1. Introduction

Citrus fruit species usually consumed as fresh produce or juice are one of the most popularly consumed fruits in the world today [1]. *Citrus* is a common term and genus of flowering plants belonging to the family of *Rutaceae*, which originated in tropical and subtropical Southeast Asia [2]. *Citrus* fruit and its juices have long been considered a valuable part of a healthy and nutritious diet and the nutrient and non-nutrient bioactive compounds contained in *Citrus* fruits are known to promote health and provide protection against chronic disease [3]. *Citrus maxima* fruit commonly known as pomelo or shaddock is the largest of all *Citrus* varieties. The pulp appears as white or pinkish red with spindle-shaped juice sacks that separate easily from one another and it has a sweetish-acidic flavour. It has been reported to possess antibacterial, antifungal, antiplatelet, antidiabetic, anti-inflammatory, antihyperlipidemic and antitumour properties in experimental studies, and with traditional use [4, 5].

The interest in *Citrus* fruits has increased for their different important roles like nutritive, antimicrobial, antioxidant and many other properties. These protective roles could be mainly attributed to various nutrient and non-nutrient bioactive compounds such as the vitamins, minerals and phytochemicals present in them.

Citrus maxima fruit intake has been observed to be low compared to other *Citrus* fruits. One of the factors associated with the limited use of some plants for both nutritive and medicinal purposes is the paucity of information and/or awareness of its nutritive and/or medicinal potentials. Therefore, the assessment of such properties is imperative so as to provide more information about varieties of plant sources in which nutrients and phytoconstituents could be obtained, and also to find promising natural products for functional foods and/or nutraceuticals. This study was therefore designed to evaluate the micronutrients and phytochemical composition, and antioxidant potential of *Citrus maxima* fruit juice.

2. Material and methods

2.1 Materials

Plant Material

Citrus maxima (Shaddock) fruits (white variety) were obtained from Ngwo town in Enugu State and were identified by Mr. Onyeukwu, C.J. of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Chemicals/Reagents
All chemicals used in this study were of analytical grade and products of Sigma Aldrich (USA), British Drug House (BDH) (England), Burgoyne (India), Hopkins and Williams (England), Qualikems (India), Fluka (Germany) and May and Baker (England).

2.2 Methods
2.2.1 Preparation of plant material
Citrus maxima (Shaddock) fruits were washed with clean water, peeled and the juice extracted with a manual screw juice extractor. Juice obtained was called 100% *Citrus maxima* juice (CMJ).

2.2.2 Phytochemical analyses
Qualitative and Quantitative phytochemical analyses were carried out by the methods described by Harbone [6] and Trease and Evans [7].

2.2.3 Micronutrients composition of *Citrus maxima* fruit juice
Mineral contents of the CMJ were carried out using atomic absorption spectrophotometer (AAS). Vitamins C and E contents were determined using the method of Pearson [8].

2.2.4 *In vitro* evaluation of antioxidant activity of *Citrus maxima* fruit juice
Scavenging activity of DPPH free radicals by the sample was assessed according to the method reported by Gyamfi *et al.* [9]. Superoxide radical scavenging activity was performed according to the methods of Beauchamp and Fridovich [10]. Nitric oxide (NO^-) generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci *et al.* [11]

2.3 Statistical analysis
Experimental results were expressed as mean ± SD. Statistical analysis was done using Statistical Product and Service Solutions (SPSS). The EC_{50} values were calculated from linear regression analysis. Values were considered statistically significant at $p<0.05$.

3. Results
3.1 Qualitative phytochemical composition of *Citrus maxima* fruit juice
The qualitative phytochemical analyses of *Citrus maxima* fruit juice (Table 1) showed the presence of phenols, flavonoids, tannins, terpenoids, steroids, glycosides and alkaloids. Saponins were not detected.

Table 1: Qualitative phytochemical composition of *Citrus maxima* fruit juice

Phytochemicals	Bioavailability
Phenols	+
Flavonoids	+
Tannins	+
Terpenoids	+
Steroids	+
Glycosides	+
Saponins	ND
Alkaloids	+

Key: + (Present), ND (Not detected)

3.2 Quantitative phytochemical composition of *Citrus maxima* fruit juice
The quantitative phytochemical analyses of CMJ (Table 2) showed appreciable amount of phenols, flavonoids, tannins, and glycosides.

Table 2: Quantitative phytochemical composition of *Citrus maxima* juice

Phytochemicals	Concentration (Mean ± SD) n=3
Phenols	2.51 ± 0.02 mgGAE/ml
Flavonoids	2.25 ± 0.55 mg/ml
Tannins	4.00 ± 0.15 mg/ml
Glycosides	0.64 ± 0.05 mg/ml

3.3 Mineral content of *Citrus maxima* fruit juice
The mineral analyses of CMJ (Table 3) showed the presence of zinc, copper, iron and magnesium in moderate quantities.

Table 3: Mineral contents of *Citrus maxima* juice

S/N	Minerals	Concentration (ppm) (Mean ± SD)
1	Zinc	0.07 ± 0.01
2	Copper	0.01 ± 0.01
3	Iron	0.08 ± 0.02
4	Magnesium	1.20 ± 0.00

3.4 Quantitative determination of vitamin C and E content of *Citrus maxima* juice
Table 4 shows vitamins C and vitamin E concentration in CMJ with vitamin C higher than vitamin E.

Table 4: Vitamin C and E content of *Citrus maxima* juice

S/N	Vitamins	Concentration (mg/dl) (Mean ± SD)
1	Vitamin C	2.23 ± 0.14
2	Vitamin E	1.19 ± 0.04

3.5 *In vitro* antioxidant potential of *Citrus maxima* juice
3.5.1 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of *Citrus* maxima juice
Citrus maxima juice showed a concentration dependent inhibitory effect on DPPH radical with a strong correlation.

Table 5: Percentage inhibition of DPPH radical by *Citrus maxima* juice

Concentration(%/ml)	% Inhibition
50	65.35
25	76.26
12.5	84.9
6.25	85.66
3.13	80.00
1.56	89.24

3.5.2 Superoxide anion radical scavenging activity of *Citrus* maxima juice
Citrus maxima juice of CMJ showed a concentration dependent inhibitory effect on superoxide anion.

Table 6: Percentage inhibition of superoxide anion radical by *Citrus maxima* juice

Concentration (%/ml)	% Inhibition
50	80.01
25	58.06
12.5	8.02
6.25	12.1
3.13	13.86

3.5.3 Antiradical activity of *Citrus* maxima juice
Data obtained from the graph of percentage inhibition against concentration, for the DPPH and superoxide tests were used to explain the antiradical activity of the juice for the radicals (Table 6).

Table 7: Antiradical activity of *Citrus maxima* juice

	DPPH	Superoxide anion
R^2	0.86	0.88
P value	0.01	0.02
EC_{50} (% v/v)	86.90	29.08
EC_{50} (g/ml)	0.87	0.29
Antiradical power (ARP)	1.15	3.45

3.5.4 Nitric oxide radical (NO˙) scavenging activity of *Citrus* maxima juice
Figure 1 shows the nitric oxide scavenging activity of *Citrus maxima* juice. There was a dose dependent increase in nitrite ion concentration in the reaction test tube containing CMJ. At the 180th minute, a significant decrease in this concentration was observed. A decrease in nitrite ion concentration in the reaction test tube containing ascorbic acid (standard) was observed.

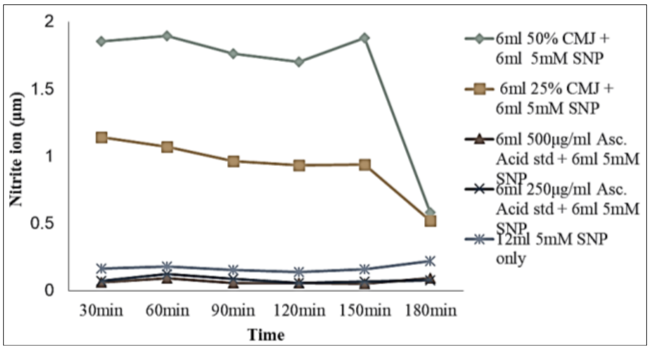

Fig 3: Nitric oxide radical (NO˙) scavenging activity of *Citrus maxima* juice

4. Discussion
The study showed that *Citrus maxima* juice possesses an array of phytochemicals. Phytochemicals are secondary metabolites of plants known to exhibit varied pharmacological and biochemical effects on living organisms [7]. These phytochemicals found to be present in the CMJ have been associated with antioxidant activity, protection against coronary heart diseases, anti-inflammatory, anticancer and antimicrobial activities, anti-malarial, inhibition of cholesterol synthesis and anti-viral activities in various studies. The presence of these phytochemicals in *C. maxima* could be responsible for the various reported biological activities of the plant with traditional use. Khanam *et al*. [12] also reported the presence of phenols, alkaloids, flavonoids, terpenoid, and the absence of saponins in *Citrus maxima* juice.
The minerals zinc, iron, copper and magnesium were found to be present in minute quantities, with magnesium highest and copper lowest in quantity. These minerals are known to play various important biochemical roles in the body. Zinc is needed in various aspects of cellular metabolism such as in protein and nucleic acid synthesis, immune function, wound healing, testosterone production, normal functioning of the brain and central nervous system, and is vital during periods of rapid growth such as infancy, adolescence and during recovery from illness [13]. It is also required for the catalytic activity of more than 200 enzymes. Magnesium (Mg) is needed for over 300 of our bodies' important biological processes such as in most reactions involving phosphate transfer believed to be essential in the structural stability of nucleic acid and intestinal absorption, adenosine triphosphate (ATP) energy production and muscular contractions [14]. Iron is an important cofactor of some enzymes and macromolecules such as hemoglobin which is required for the transport of oxygen throughout the body. Copper is essential for maintaining the strength of the skin, blood vessels, epithelial and connective tissue throughout the body [12].
Vitamin C and E were also found to be present with C higher than E. These vitamins play varied important biochemical roles in the body especially their function as antioxidants, scavenging free radicals in the aqueous phase (vitamin C) and lipid phase (vitamin E) of cells. The antioxidant mechanisms of vitamin C (ascorbic acid) are based on donation of hydrogen atom to lipid radicals, quenching of singlet oxygen, and removal of molecular oxygen. Scavenging aqueous radicals and regeneration of α–tocopherol from the tocopheroxyl radical species are also well known antioxidant mechanisms of ascorbic acid [15].
The *in vitro* free radical scavenging activity of *Citrus maxima* juice was estimated using three different methods.
Citrus maxima juice showed a positive scavenging activity for DPPH and superoxide radical as indicated by the percentage inhibitions of radical for the different concentrations of the juice (Table 5and 6 respectively). DPPH is a stable nitrogen centered free radical which on receiving a hydrogen atom from an antioxidant gets reduced, bringing about a color changes from violet to yellow. Substances which are able to perform this action can be considered as antioxidants and therefore radical scavengers [16]. Various authors also reported the DPPH scavenging activity of CMJ [5, 17]. Superoxide radical is the most common free radical generated *in vivo*. It is involved in the formation of more reactive oxygen species such as hydrogen peroxide and hydroxyl radical, which results in oxidative damage of biomolecules such as DNA, lipids and proteins [5]. By scavenging superoxide anion radical, it prevents the increased production of some reactive species which could cause the initiation of deleterious free radical-mediated chain reactions. The result of the study showed that the juice contains substances that are free radical inhibitors and primary antioxidants that could scavenge radicals by donating hydrogen atom or electrons to stabilize them.
A graph of percentage inhibition against concentration was plotted and data obtained from it was used to explain the antiradical activity of the juice for the radicals tested (Table 6). The high R^2 values obtained indicated a strong positive correlation, while the p values less than 0.05 indicated that the correlations were statistically significant. This implies that the observed radical scavenging activity/percentage inhibition of radicals significantly depended on the antioxidant potential of *C. maxima* juice. The antioxidant potential of the juice was defined by the amount of antioxidant required to decrease the initial radical concentration by 50% (EC_{50}) obtained from the equation of the graph. The antiradical power (ARP), which is the reciprocal of the EC_{50} was calculated. The lower the EC_{50} value, the higher the ARP, the more efficient the antioxidant. From the result obtained, we could infer that *C. maxima* juice showed good antioxidant activity for both radicals but was more potent in scavenging superoxide anion radical. The phytochemical analysis of the juice showed the presence of phenols, flavonoids and tannins which are known to possess radical scavenging activity and hence could be partly

responsible for the observed antioxidant property of the *C. maxima* juice.

The increased nitrite ion concentration with *C. maxima* juice observed in the nitric oxide radical-scavenging test proposes that *C. maxima* juice might contain nitrate and/or nitrite molecules which could have contributed to the increase in nitrite ion concentration observed in the reaction system. Nitric oxide acts as a double-edged sword; with both deleterious and beneficial roles. Its toxicity increases when it reacts with superoxide anion radical to form peroxynitrite ion, a more dangerous radical specie. On the other hand, it plays a role some physiological process such as in endothelial function, immune system response and in neurotransmission [18, 19]. The roles of dietary nitrite and nitrate as an easy way of increasing nitric oxide availability have raised increasing attention with respect to their potential beneficial effects in vascular endothelial function, and in the prevention of lifestyle-related diseases such as cardiovascular diseases, insulin resistance, osteoporosis and cancer [20, 21]. The beneficial effects of these foods are attributed to the cyclic guanosine monophosphate (cGMP)-dependent actions of NO, including vasodilation and vascular endothelial protection from platelet aggregation and leukocyte adhesion, and protein *S*-nitrosylation-dependent intracellular signalling pathways [22, 23]. The result obtained here proposes C. maxima juice as a dietary source of nitrite and/or nitrate.

5. Conclusion

The study showed that *Citrus maxima* fruit juice possesses considerable amounts of phytochemicals, minerals (Zn, Fe, Cu, and Mg), vitamins C and E. The *in vitro* antioxidant tests showed that it possesses antioxidant potentials and hence could scavenge free radicals.

However, due to its purported role as an exogenous nitric oxide source, further studies could be done to elucidate its role of in managing disease conditions associated with endothelial dysfunction such as hypertension, dyslipidemia, diabetes, and angina pectoris.

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