Phytochemical, Antioxidant, Proximate, and Selected Mineral Status of Egyptian Citrus paradisi Fruit obtained from Wamakko Local Government Area of Sokoto State, North-western Nigeria

Bandiikilima Ibrahim, Ogbiko Cyril*

ABSTRACT
The search for new antioxidant compounds and minerals to combat the nutritional needs of the populace of Nigeria is on the increase. This study investigates the phytochemical composition, antioxidant, proximate, and mineral status of Egyptian Citrus paradisi. A popular citrus fruit consumed in Wamakko Local Government Area of Sokoto State using standard procedures. The result revealed that the presence of important phytoconstituents, namely, flavonoids, terpenoids, saponins, phenolics, cardiac glycosides, and reducing sugars with alkaloid was reported to be absent. The juice concentrates exhibit an appreciable but significantly lower (P < 0.05) 1,1-diphenyl-1-picryl-hydrazyl radical free screening activity compared to the reference antioxidant. The result of the vitamin, proximate, and mineral analysis confirms the concentrate to be a rich source of Vitamin C, minerals such as sodium, magnesium, potassium, calcium, as well as moderately to poor source of carbohydrate, lipid, protein, fiber, and potassium. These findings will greatly help nutritionist in properly making nutrition recommendations to meet the nutritional priorities of the populace.

Keywords: 1, 1-Diphenyl-1-picryl-hydrazyl radical, Concentrate, Mineral, Nutritional, Phytochemical

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INTRODUCTION
Health and nutritional problems continue to be of increasing concern worldwide. Malnutrition-related disease conditions constitute a serious global health problem in developing countries like Nigeria, especially among its rural populace, even though seasonal fruits are available among all aged peoples.[1] Fruits have been known to form an integral composition of human diet since prehistoric times and have become a culture in developed and developing to incorporate fruits to meals.[2] Fruits are frequently consumed in rural communities where they serve not only as a suitable alternative sources of fresh enzymes, coenzymes, vitamins, and essential nutrients to the body but are also relatively cheap to procure.[3,4] Minerals are inorganic substances required by an organism in small amount required for the maintenance of vital processes essential for life.[5] Fruits have been established to be an excellent source of nutrients like minerals as they play a physiological role, particularly in the body metabolism where they play a key role in the general well-being of the body as well as in the cure of several diseases.[6]

Free radicals are often generated in vivo from normal physiological processes where they are implicated in different disease conditions ranging from aging, atherosclerosis, and neurodegenerative diseases.[7,8] Natural antioxidants from plant sources such as food and fruits are potent source of antioxidants and effective in the protection from free radical related oxidative stress.[9,10]

An estimated 100 million tons of citrus fruits are produced annually, thereby making the citrus family the largest contributor to the world’s fruit production.[11] Citrus is one of the largest plant species known, consisting of 40 species that are distributed around the world.[12] In recent years, scientists and nutritionists have started believing in the therapeutic role of valuable vitamins and essential nutrients.[13,14] Some plants contain significant amount of minerals of which the presence and quantity depend on the plant family, history, and phytoconstituents inherent in the plant.[15]

Juices from citrus fruits are consumed majorly because of their nutritional value, bioactive compounds such as phenolics, flavonoids, and vitamins among others as they are required for the maintenance of good health and prevention of diseases.[16] They are believed to be responsible for a wide range of protective health benefits including antioxidative, anti-inflammatory, antitumor, and antimicrobial activities.[12,16] This study was, therefore, aimed at evaluating the phytochemical composition, nutritive values by analyzing quantitatively the carbohydrate, Vitamin C, and essential minerals of Egyptian Citrus paradisi fruit popularly consumed in Wamakko Local Government Area of Sokoto State.

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Materials and Methods

Collection and Identification of Fruit
Fresh fruits were purchased from street vendors in Wamakko Local Government Area of Sokoto State, Nigeria. The fruits were identified by a plant botanist in the Botany unit of the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria. All the analyses were presented as mean of triplicate analysis was applicable.

Preparation of Fruit Juice Concentrates
The fruits were rinsed thoroughly with distilled water and were cut into halves. The juice was extracted from the fruits using a juice extractor. The fruit juices were then lyophilized and the concentrates obtained were preserved at 4°C in airtight containers until subsequent use.

Phytochemical Screening
Simple chemical tests to detect the presence of secondary metabolites were performed using standard procedures.[19-21]

Determination of 1, 1-Diphenyl-1-picryl-hydrazylradical (DPPH) Radical Scavenging Activity
Radical scavenging activity of the fruit juice concentrate against the DPPH was performed in accordance with the procedures outlined by Jain et al.[22] A 0.01–0.2 µg/mL of the concentrate was prepared in methanol and used for the assay. A 0.1 mM DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 3.0 mL of different concentrations ranging from 0.01 to 0.2 mg/mL of the concentrate. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as standard with the same concentrations of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as standard with the same concentrations as aid in the biological actions for which they are identified in folklore medicine.[23] The phytochemical composition of the fruit juice concentrates revealed that the presence of important phytoconstituents, namely, flavonoids, terpenoids, saponins, phenolics, cardiac glycosides, and reducing sugars with alkaloid was reported to be absent [Table 1]. These results agree partly with the findings of Ehigbai et al.[29] Rauf et al.[30] and Mathew et al.[31] who also reported the presence flavonoids, terpenoids, phenolics, and reducing sugars as well as the absence of alkaloids.

Vitamin C, Proximate, and Mineral Analyses
Ascorbic acid (Vitamin C) content was estimated by the 2,4-dinitrophenylhydrazine method.[24] A 5.0 g of the edible portion of the fruit was weighed into a crucible and the moisture content was determined by weight loss on drying at 105°C for 6 h. The nitrogen content (N) was determined by the Kjeldahl procedure.[25] Using N × 6.25, the total protein content was determined. Lipid, ash, and fiber contents were determined according to the procedures outlined by AOAC.[26] The total carbohydrate was determined by simple difference as follows: Carbohydrate = 100 – (%Ash + %Crude protein + %Crude lipid + %Crude fiber) g/100 g.[26] For the mineral analyses, 5.0 g of the edible portion was wet-ashed using a mixture of 18 M sulfuric acid, 12 M perchloric acid, and 16 M nitric acid [0.5:1.0:0.5 v/v/v].[26] Na, K, Ca, Mg, and P were analyzed using the procedures outlined by Walinga et al.[27]

Statistical Analysis
The data were applicable which were expressed as mean ± standard deviation of three replicates. The data from the antioxidative assay were subjected to one-way analysis of variance.

Results
The phytochemical screening of the fruit juice concentrates showed the presence of important secondary metabolites which is presented in Table 1.

The DPPH radical scavenging assay is a commonly used tool for accessing the antioxidant capacity of natural products because it is relatively cheap to perform and possesses a high speed of completion. The results of the antioxidative screening are presented in Figure 1 with the 50% inhibitory concentration of the fruit juice concentrates in comparison to ascorbic acid standard which is presented in Table 2.

The Vitamin C, proximate, and selected mineral compositions of the Egyptian citrus fruit are presented in Tables 3 and 4, respectively.

Discussion
Phytochemicals are the invaluable chemicals present in plant from which they possess varying degrees of pharmacological activities. They serve as a major source of raw materials as well as aid in the biological actions for which they are identified in folklore medicine.[22] The phytochemical composition of the fruit juice concentrates revealed that the presence of important phytoconstituents, namely, flavonoids, terpenoids, saponins, phenolics, cardiac glycosides, and reducing sugars with alkaloid was reported to be absent [Table 1]. These results agree partly with the findings of Ehigbai et al.[29] Rauf et al.[30] and Mathew et al.[31] who also reported the presence flavonoids, terpenoids, phenolics, and reducing sugars as well as the absence of alkaloids.

The findings from this study differ in the reported presence of saponins and glycosides. These differences may be attributed to differences in species as well as the geographical location which is known to affect both the morphology and expression of phytochemicals.[32,33]

Figure 1: 1, 1-Diphenyl-1-picryl-hydrazylradical radical scavenging activity of the citric fruit concentrate and the synthetic antioxidant (ascorbic acid) at different concentrations. Each point is a mean from triplicate measurement. ECPC: Egyptian Citrus paradisi concentrate.
Table 1: Phytochemical composition of the Egyptian Citrus paradisi fruit juice concentrates

| Phytochemicals    | Test(s) conducted | Observation                                      | Inference |
|-------------------|-------------------|--------------------------------------------------|-----------|
| Flavonoid         | Shinoda           | Reddish color was observed                       | +         |
|                   | Alkaline reagents  | Intense yellow color was formed                  | +         |
| Terpenoid         | Salkowski’s       | Appearance of golden yellow ring at the interface| +         |
| Saponin           | Frothing          | Foam was formed                                  | +         |
| Alkaloid          | Mayer’s           | No precipitate formed                            | −         |
|                   | Wagner’s          | No precipitate formed                            | −         |
|                   | Drangendorf’s     | No precipitate formed                            | −         |
| Phenolics         | Ferric chloride   | Bluish-black color appeared                      | +         |
| Reducing sugars   | Molsch’s          | Violet ring was formed at the junction           | +         |
|                   | Fehling’s         | Red precipitate was formed                       | +         |
| Cardiac glycoside | Keller-Killiani’s | Violet ring at the interface of two liquid layers| +         |

+: Presence of phytochemical, −: Absence of phytochemical

Table 2: 50% inhibitory concentration of the citrus fruit concentrate and the ascorbic acid standard

| Parameter                  | IC_{50} |
|----------------------------|---------|
| Egyptian Citrus paradisi   | 13.42   |
| Concentrate                | 3.89    |

IC_{50}: 50% inhibitory concentration

Table 3: The Vitamin C and proximate composition of Egyptian Citrus paradisi fruit juice

| Parameters                  | %±SD    |
|-----------------------------|---------|
| Vitamin C (mg/100 g)        | 72.23±2.32|
| Moisture                    | 87.09±0.71|
| Crude ash                   | 4.54±0.11 |
| Crude lipid                 | 0.17±0.02 |
| Crude fiber                 | 0.23±0.01 |
| Crude protein               | 0.67±0.01 |
| Carbohydrate (g/100 g)      | 7.30±0.21 |

Table 4: Selected mineral analyses of Egyptian Citrus paradisi fruit juice

| Parameters | Content (mg/100 g) |
|------------|--------------------|
| Na         | 27.03±0.09         |
| K          | 100.73±1.02        |
| Ca         | 27.42±1.11         |
| Mg         | 17.17±1.42         |
| P          | 24.92±1.73         |

DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants because it is as table free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The fruit concentrates exhibit a reasonable antioxidant potential but not statistically significant (P < 0.05) to that of the ascorbic acid standard [Table 2]. This observed activity could be related to the presence of phenolic compounds such as flavonoids which is known to play a notable pharmacological and biochemical roles including radical scavenging properties as well as in the stabilization of lipid oxidation and may contribute directly to antioxidative action. The results showed that the juice concentrates are not as effective as ascorbic acid in scavenging free radicals probably because the latter contain more purified compounds than the former.

Vitamins are accessory factors which are required in diet to maintain good health and well-being of an individual of which fresh fruits are a veritable source. The fruit showed a rich source of Vitamin C [Table 3], moisture which could serve to partly quench thirst in dehydrating conditions, an appreciable source of minerals as revealed by its ash value and a poor source of carbohydrate, lipid, fiber, and protein. The composition of fruits nutrients and vitamins may vary due to climatic factors, nature of the cultivating soil, as well as the maturity of the fruit itself.

The studied minerals are the five (Na, K, Ca, Mg, and P) major macronutrients. These minerals are known to play significant functions like while sodium, phosphorus, and potassium play a key role in active transport across the cell membrane as well as in the maintenance of osmotic balance. Magnesium activates enzymatic systems while calcium is responsible for metabolisms in the bone and in the nerves. Fruits notably citrus fruits are known to be a rich source of the investigated minerals except phosphorus. The findings from the study revealed the concentration of 27.03 mg/100 g for sodium, 100.73 mg/100 g potassium, 72.42 mg/100 g calcium, and 24.92 mg/100 g magnesium. The concentration of 11.17 mg/100 g was reported for phosphorus. These findings compare favorably with findings of other researchers. Fruits mineral composition varies greatly with topographical factors as well the nature of cultivating soil, climatic factors such as the amount of rainfall as well as the maturity of fruits before harvest.

CONCLUSION

The information provided from this research shows that the Egyptian Citrus paradisi popularly consumed in Sokoto metropolis is a rich source of Vitamin C as well as important phytochemicals which have been established to play pivotal role in scavenging damaging free radicals. It is also an important source of minerals to human through dieting, especially Na, K, Ca, and Mg, as well as aid nutritionists to effectively make nutritional recommendation which will help greatly in the diet health assessment in Nigeria.

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