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# Determination of phytochemicals and some selected antioxidants from the juice of sweet orange

**JC Iwuji[1], VC Okonkwo[1], EC Ogbodo[2,*], C Amaifeobu[2], SBN Ajah[2], AO Okezie[3], IJ Onuora[4], AK Amah[5]**

[1]*Dept. of Science Laboratory, Federal University of Technology, Owerri, Nigeria*
[2]*Dept. of Medical Laboratory Science, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Awka, Nigeria*
[3]*Dept. of Chemical Pathology, Medical Laboratory Services, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria*
[4]*Dept. of Chemical Pathology, Faculty of Medicine, Nnamdi Azikiwe University, Awka, Nigeria*
[5]*Dept. of Physiology, College of Medicine, Imo State University, Owerri, Nigeria*

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A B S T R A C T

**Background of study:** *Citrus sinensis,* sometimes known as sweet orange, is a fruit native to Southeast Asia that is now consumed all over the world as a rich source of vitamin C, a potent natural anti-oxidant that aids in optimal body function. The phytochemicals and selected antioxidants in sweet orange juice were determined in this study.

**Materials and Methods:** The concentrations of phytochemicals in sweet orange juice were determined by titrimetrically measuring phytic acid and oxalate, gravimetrically measuring cardiac glycosides, and spectrophotometrically measuring saponins, flavonoids, phenols, terpenoids, tannins, alkaloids, and steroids against the absorbance of the control. Vitamin C (ascorbic acid) was used as a standard control for measuring antioxidant and free radical scavenging activity against DPPH, NO, Hydroxyl value, $H_2O_2$, and Reducing power ability.

**Results:** The results showed the presence of flavonoid (17.64±0.00), saponin (1.227±0.0028), terpenoids (1.64±0.74), phenol (0.50±0.0014), alkaloid (15.855±0.02), cardiac glycosides (6.3±1.40), steroid (7.68±0.00), oxalate (0.031±0.00), phytate (0.29±0.042) with tannin (282.925±0.30) being the most abundant. Antioxidants studies of the $H_2O_2$ on the orange juice showed that as the concentration of the test sample increases, the scavenging activity increases. DPPH radical scavenging activity being an accepted mechanism for screening the anti-oxidants activity of plants extracts when compared with Ascorbic acid, the result showed that the Ascorbic acid has more scavenging property than the DPPH. With an increasing level of the Ascorbic acid absorbance, the result showed strong reducing power ability. The hydroxyl radical scavenging activity of the sweet orange juice extract when compared with the ascorbic acid showed that the Ascorbic acid has more scavenging activity.

**Conclusion:** sweet orange may be recommended for individuals due to its rich antioxidants and phytochemical properties.



## 1. Introduction

Food's significance to humans cannot be overstated. Food is extremely important in sustaining good health and in the prevention and treatment of disorders.[1] The

* *Corresponding author*.
*E-mail address*: augustinee442@gmail.com (E. C. Ogbodo).

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International Covenant on Economic, Social, and Cultural Rights (ICESCR) established the "right to an acceptable quality of living, including adequate food," as well as the "basic right to be free from hunger," in recognition of the importance of food to man. Carbohydrate, protein, lipids, water, vitamins, and minerals are the different types of foods.[2] Fruits are not only colorful and flavorful additions to our diet, but they also provide a significant amount of energy, vitamins, minerals, and dietary fiber.[3] Fruits are commonly ingested as fresh juices, salads, or fruit-based drinks and are acknowledged to be an important element of the diet. Fruits and vegetables are known to lessen the risk of various chronic diseases.[4–6]

Sweet oranges (*Citrus sinensis*), tangerines/mandarins (*Citrus reticulata*), lemons (*Citrus limon*), limes (many species), and grape fruits (*Citrus paradis*) are all members of the citrus family.[7] Sweet orange is currently grown in tropical and subtropical climates, with the United States, Brazil, Spain, Italy, India, South Africa, and Egypt leading in production.[8] It has been reported to contain several minerals and vitamins such as Calcium, sodium, potassium, magnesium, Zinc, copper, manganese, vitamin C as well as protein, carbohydrate and fibre.[9,10] These substances are important for optimal growth, development, and general health.[11] Sweet orange has been shown to ameliorate blood glucose, lipid profile and liver, kidney functions in diabetic andhypercholesterolemic rats.[12,13] Herbs and plants have been utilized as a treatment for many diseases and to maintain health in various cultures around the world since ancient times. The citrus peel is a rich source of flavonone and numerous poly methoxylated flavones which are uncommon in other plants[14] and it also produces an essential oil that has significant economic importance in the pharmaceutical and food industries as a flavoring agent to cover bad taste.[15] Synephrine, flavanones, limonin, and auraptene are the most dominant alkaloids, flavonoids, limonoids groups, and coumarins found in citrus fruit.[16] Citrus fruits are the primary source of phytochemicals and antioxidants which play pivotal roles in the prevention and management of chronic conditions. As a result, the current study is necessary.

## 2. Materials and Methods

### 2.1. Source of material

Fresh ripe sweet oranges were purchased from relief market in Imo State, Nigeria. The Sweet orange fruits were washed with tap water, peeled off and hand squeezed to obtain the juice from the fruit.

### 2.2. Quantitative phytochemical analysis

Quantitative phytochemical analysis was carried out by Measuring titrimetrically for the phytic acid and oxalate, gravimetrically for the cardiac glycosides and by measuring the absorbance of the sample against the absorbance of control spectrophotometrically for the saponins, flavonoids, phenols, terpenoids, tannins, alkaloids and steroids.

### 2.3. Qualitative phytochemical screening

Qualitative phytochemical screening was carried out to analyse the presence of various phytoconstituents such as steroids, alkaloids, flavonoids, saponins, terpenoids, phenol, cardiac glycosides and tannins. General reactions in this analysis revealed the presence or absence of these compounds.

### 2.4. Antioxidants analysis

The antioxidants analysis and free radicals scavenging activity was assessed against 1,1-diphenyl-2-picrylhydrazyl (DPPH), Nitric oxide (NO), Hydroxyl value, Hydrogen peroxide ($H_2O_2$) and Reducing power ability using Vitamin C (Ascorbic acid) as standard control.

## 3. Results

Table 1 gives the tabular presentation of the phytochemical present or below detection limit in the plant extract. Qualitatively, flavonoid,saponin, terpenoids, phenol, alkaloid cardiac glycosides, steroid, oxalate, and tannin were found to be present in the sweet orange juice while phytate was below detection limit.

The result of each analyte was calculated averages of two (2) analytical values. Statistical values were obtained using IBM-MS Excel Software version 22, 2010 edition and presented as mean±SD. The results showed the presence of flavonoid (17.64±0.00), saponin (1.227±0.0028), terpenoids (1.64±0.74), phenol (0.50±0.0014), alkaloid (15.855±0.02), cardiac glycosides (6.3±1.40), steroid (7.68±0.00), oxalate (0.031±0.00), phytate (0.29±0.042) with tannin (282.925±0.30) being the most abundant. Table 2.

The scavenging activity of $H_2O_2$ in orange juice increased as the concentration of the test sample increased. The result showed that the Ascorbic acid has more scavenging property than the DPPH. With an increasing level of the Ascorbic acid absorbance, the result showed strong reducing power ability. The hydroxyl radical scavenging activity of the sweet orange juice extract when compared with the ascorbic acid showed that the Ascorbic acid has more scavenging activity. Tables 3, 4, 5 and 6 respectively.

## 4. Discussion

In this study, the amount of alkaloids found in orange juice was 15.855±0.02$\mu$g/g. This figure is very low when compared to orange juice assay of Mbaebie and colleagues, which found 9.80±0.01g/100g.[17] Again, the amount of

**Table 1:** Qualitativeraw sample of phytochemicals present in an orange juice

| Parameter | Inference |
|---|---|
| Tannins | + |
| Saponins | + |
| Alkaloids | + |
| Phenols | + |
| Cardiac Glycoside | + |
| Steriods | + |
| Favonoid | + |
| Terponoids | + |
| Oxalates | + |
| Phytates | - |

+= Present, - = below detection limit

**Table 2:** Quantitative phytochemical composition of Orange juice (mean±SD; n=2)

| Parameters | Results |
|---|---|
| Flavonoids ($\mu$g/g) | 17.64±0.00 |
| Saponins (%) | 1.227±0.0028 |
| Terpenoids (mg/g) | 1.64±0.0.74 |
| Phenol (mg/g) | 0.50±0.0014 |
| Alkaloids ($\mu$g/ml) | 15.855±0.02 |
| Cardiac Glycoside (%) | 6.30±1.40 |
| Tannins (%) | 282.925±0.30 |
| Steroids (mg/100g) | 7.68±0.00 |
| Oxalate (%) | 0.031±0.00 |
| Phytates (%) | 0.29±0.042 |

**Table 3:** Hydrogen peroxide scavenging activity of the orange juice.

| Concentration ($\mu$g/g) | Sample (%) | Absorbance | Ascorbic Acid (control %) | Absorbance |
|---|---|---|---|---|
| 0.2 | 77.19 | 0.013 | 68.42 | 0.018 |
| 0.4 | 70.18 | 0.017 | 71.93 | 0.016 |
| 0.6 | 61.40 | 0.022 | 70.18 | 0.017 |
| 0.8 | 77.19 | 0.013 | 54.39 | 0.026 |

**Table 4:** Percentage DPPH radical scavenging activity

| Concentration ($\mu$g/g) | Absorbance of Extract | Absorbance of Ascorbic Acid (control) | % DPPH |
|---|---|---|---|
| 0.2 | 0.151 | 0.450 | 66.44 |
| 0.4 | 0.291 | 0.390 | 25.38 |
| 0.6 | 0.207 | 0.460 | 55.00 |
| 0.8 | 0.159 | 0.440 | 63.86 |

**Table 5:** Nitrous oxide scavenging activity

| Concentration ($\mu$g/g) | Absorbance of Extract | Absorbance of Ascorbic Acid (ctrl) | % NO scavenging activity |
|---|---|---|---|
| 0.2 | 0.789 | 1.900 | 58.47 |
| 0.4 | 0.806 | 2.040 | 60.49 |
| 0.6 | 0.829 | 2.210 | 62.49 |
| 0.8 | 0.380 | 2.540 | 85.04 |

**Table 6:** Reducingpower ability

| Concentration ($\mu$g/ml) | Absorbance of extract | Absorbance of ascorbic acid control |
|---|---|---|
| 0.2 | 0.093 | 0.465 |
| 0.4 | 0.333 | 0.574 |
| 0.6 | 0.261 | 0.614 |
| 0.8 | 0.249 | 0.823 |

**Table 7:** Hydroxylvalue scavenging activity

| Concentration ($\mu$g/g) | Absorbance of Extract | Absorbance of Ascorbic Acid (ctrl) | % Hydroxyl Value scavenging Activity |
|---|---|---|---|
| 0.2 | 0.007 | 0.009 | 22.22 |
| 0.4 | 0.002 | 0.014 | 85.71 |
| 0.6 | 0.002 | 0.007 | 71.43 |
| 0.8 | 0.004 | 0.005 | 20.00 |
| 1.00 | 0.004 | 0.006 | 33.33 |

flavonoids in the orange juice was found to 17.64±0.00 $\mu$g/ml in the present study. This result is lower than that of some other previous studies.[18,19] Flavonoids are antioxidants and free radical scavengers that protect against oxidation and lessen the risk of heart disease in the intestine.[20] Glycosides found in the plant are known to boost immunity through improving bodily strength, making them useful as a dietary supplement.[21] Because they are found in practically every medicinal plant, cardiac Glycosides have a wide therapeutic efficacy.[21] The cardiac glycoside found in the orange juice was 6.3±1.40 (%). This is comparable with the analysis carried out by[22] which documented a value of 6.3±1.40 (%). Tannins were found to be 282.925±0.30/100g. When compared to the value of an analysis conducted on orange juice by.[23,24] which recorded a value of 11.40±0.02g/100g, this number is significantly higher. Tannins found in plants have been discovered to have a strict property that speeds wound and mucus membrane healing. However, saponins were discovered to have a value of 1.227±0.0048 percent in this study. This figure is comparable to the values found in,[25,26] which were 1.41±0.000g/100g and 1.61±0.005g, respectively. The phenol concentration was 0.50±0.014mg/g. This is lower than the value of 2.35±0.015g/100g reported by.[25] Phenol, which is found in plants, can be utilized to generate disinfectants and antiseptics that are used in mouthwash and surface cleaning. Steroids value was found to be 7.68±0.00mg/100g in the orange juice. Steroids are involved in a variety of processes including growth, development, energy consumption, homeostasis, and reproduction. Terpenoids were discovered to have a value of 1.64±0.74mg/100g. The value of Terpenoids reported by[27] was lower than the present result. Terpenoids are a natural flavour found in traditional herbal treatments.

This is what gives orange juice its yellowish hue and flavor. The values for oxalate and phytate were 0.29±0.042 percent and 0.031±0.00 percent, respectively. The outcomes were equivalent to those obtained by Peter and colleagues.[24] Both oxalates and phytates bind to calcium, magnesium, zinc, iron, and other minerals and impede their absorption.[28] The present study observed a value of 15.855±0.02 $\mu$g/ml for alkaloids. Alkaloids are pharmacologically active and have been shown to have significant physiological effects.[25] The autonomic nervous system, blood vessels, diuresis promotion, respiratory system, gastrointestinal tract, uterus, malignant illnesses, and malaria are all affected by their effects. Pure separated plant alkaloids and their synthetic derivatives, on the other hand, are employed as basic therapeutic agents for analgesic, antispasmodic, and antibacterial properties.[25] Plants rich in carbohydrates, glycosides, and alkaloids are known to strengthen the immune system, making them useful as dietary supplements.[21]

The presence of flavonoids in orange juice shows that the plant has therapeutic benefit. Flavonoids and flavones are secondary metabolites having antioxidant and antiradical properties that are broadly dispersed.[26] As a result, flavonoids are antioxidants and free radical scavengers that prevent oxidation; they also have potent anticancer properties, protecting cells against all phases of carcinogenesis.[25] This shows that eating foods high in flavonoids lowers the risk of heart disease, which is significant in medicine, pharmacology, and nutrition.[26] Tannins have incredible binding capabilities. They have been reported to help wounds and inflamed mucous membranes heal faster. They have antibacterial properties, as suggested by.[26] This means that orange juice has chemicals that can be utilized to cure venereal infections and aid in skin regeneration.[25] These secondary metabolites have an important role in therapeutic plant biological activities such as hypoglycemia, anti-diabetic, antioxidant, anti-microbial, anti-inflammatory, anti-carcinogenic, anti-malarial, anti-cholinergic, and anti-leprosy.[29]

Hydrogen peroxide can cause lipid deoxidation by decomposing into oxygen and water, which produces hydroxyl ions and destroys DNA. Ascorbic Acid is frequently used to scavenge radicals generated by Hydrogen Peroxide. As a result, it is evident that the scavenging activity of $H_2O_2$ increases as the concentration of the test sample rises. This corresponds to the findings of[30] who investigated the antioxidant activity of *Clerodendrum minahasse* extract.

Because of the delocalization of the spare electrons, DPPH activity is classified as a stable free radical. As a result, the molecules of 1-1-diphenyl-2-picrylhydrazyl do not dimerize as they do in other free radicals. The ability of the orange juice extract to act as hydrogen atom or electron donors in DPPH-H transition was examined. Orange juice extract was discovered to be capable of converting the stable purple-colored radical DPPH into the yellow-colored DPPH-H. When compared to ascorbic acid, it was discovered that ascorbic acid had a higher scavenging ability than DPPH. The outcome is comparable to that obtained by.[30]

Nitrogen oxide is produced by vascular endothelial cells, phagocytes, and certain brain cells from the amino acid L-arginine. Because of its unpaired electron, NO is categorized as a free radical and exhibits significant reactivity with specific proteins and other free radicals. NO scavengers compete with oxygen, resulting in lower nitrite ion generation. Ascorbic acid scavenges the free radicals created by NO, according to the findings. With an increasing amount of ascorbic acid absorbance, the result demonstrated that ascorbic acid isolated from orange juice had high reducing power. This is consistent with the reducing power of methanolic acid, another well-known antioxidant.[30]

In biological systems, free radicals are constantly created, and they can cause substantial damage to tissue and biomolecules, leading to a variety of disease situations, particularly degenerative disorders. Hydroxyl ions are the most powerful reactive oxygen species, causing cell damage by reacting with cell membrane phospholipids made up of polyunsaturated fatty acid molecules. When comparing the hydroxyl radical scavenging activity of sweet orange juice to that of ascorbic acid, it was discovered that ascorbic acid has a higher scavenging property than the hydroxyl value. The existence of hydrogen donating ability of phenolic compounds in the extract may be responsible for the scavenging of hydroxyl radicals. This is consistent with the findings of the study.[31]

## 5. Conclusion

Flavonoids, steroids, Terpenoids, Phenol, Saponins, tannins, alkaloids, phenol, Oxalate, Cardiac glycoside, and Phytates were discovered in orange juice, with tannins being the most prevalent. Following the examination, the antioxidants detected in the orange juice included Hydrogen Peroxide, DPPH, Hydroxyl ions, NO, and Ascorbic acid. Due to its great nutritional value and antioxidant profile, sweet orange is one of the best fruits that could be recommended to individuals.

## 6. Conflict of Interest

The authors declare that they have no conflict of interest.

## 7. Source of Funding

None.

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## Author biography

**JC Iwuji,** Lecturer

**VC Okonkwo,** Graduate Assistant

**EC Ogbodo,** Medical Laboratory Scientist

**C Amaifeobu,** Intern Medical Laboratory Scientist

**SBN Ajah,** Medical Laboratory Scientist

**AO Okezie,** Principal Medical Laboratory Scientist

**IJ Onuora,** Lecturer

**AK Amah,** Associate Professor

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