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Evaluation of The Phytonutrients and Vitamins Content of Citrus Fruits

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Abstract: Phhytochemical studies of five varieties of citrus species; sweet orange (*Citrus sinensis)*, tangerine (*Citrus reticulata)*, lemon (*Citrus limonum*), lime (*Citrus aurantifolia)* and grape (*Citrus grandis)* revealed the presence of bioactive compounds comprising alkaloids (0.33-0.04 mg 100 g^{-1}), flavonoids (0.19-0.57 mg 100 g^{-1}), phenols (0.01-0.42 mg 100 g^{-1}) and tannins (0.01-0.04 mg 100 g^{-1}). These citrus fruits are good sources of water-soluble vitamins: ascorbic acids (19.36-61.60 mg 100 g^{-1}), riboflavin (0.01-0.11 mg 100 g^{-1}), thiamin (0.06-0.12 mg 100 g^{-1}) and niacin (0.03-0.43 mg 100 g^{-1}). These phytonutrients and vitamins may be responsible for the antioxidants, anticancer, anti-inflammatory properties of the citrus species. The citrus spices have their popular use in herbal medicine and as fruits in Nigeria.

Key words: Phytonutrients, vitamins, citrus, antioxidants, anti-inflammatory, herbal medicine

INTRODUCTION

Citrus fruits, which belong to the family of rutaceae, are one of the main fruit tree crops grown through out the world. Although sweet orange (*Citrus sinensis*) is the major fruit in this group, accounting for about 70% of citrus output. The group also includes small citrus fruits such as tangerine tree (*Citrus reticulata*), grapefruit tree (*Citrus grandis*), lime tree (*Citrus aurantifulia*) and lemon tree (*Citrus limonum*).Citrus fruits are well known to be rich in certain phytonutrients especially flavonoids that protects humans against cancer and cardiovascular diseases[1]. They are also rich in minerals that help in lowering blood pressure levels and substantially reduce the risk of stroke.

Citrus fruits are well endowed with a variety of phytonutrients that are vital in both health promotion and disease prevention. These phytonutrients may act as antioxidant. They may stimulate the immune system, induce protective enzymes in the liver or block damage to genetic materials[2]. Citrus fruits are good sources of vitamin C because they also provide organic acids such as citric acids. The juice provides phytonutrients, vitamins and minerals, which acts as antioxidants[3].

All citrus fruits share in common their sweet and sour flavor. They possess refreshing juice and are available almost all-round the year.[4]. Citrus fruits and citrus juice have several beneficial, health promoting and nutritive properties.

Apart from being rich in ascorbic and folic acids, citrus fruits are good sources of fiber. They are fat free, low in sodium content and without cholesterol[6]. They may help to reduce the risk of heart disease and certain types of cancer. Citrus fruits are also helpful to reduce the risk of pregnant women to have children with birth diseases[6]. Citrus fruits are consumed as fresh fruits or utilized for processed citrus products and citrus by-products. Approximately, one third of the total citrus production is utilized for processing. This proportion is higher in the case of sweet oranges (*Citrus sinensis*) as more than 40% of globally produced oranges are utilized for processing. In addition, the utilization of oranges apply to their juice which are used as flavoring in beverages[7]. Research has shown that the most important processed citrus fruit product is orange juice[3]. Orange juice is the freshly squeezed orange juice and frozen concentrated orange juice. The juice is squeezed from ripped fresh fruits and packaged in paper cartons, glass or plastic containers without being pasteurized. The product is clearly labeled and located in the produce of dairy section with a shelf life of only a few days. It is also sometimes prepared at homes. Traditionally, freshly squeezed orange juice is prepared at homes. In frozen concentrated orange juice, it is obtained by removing through evaporation the water from the orange juice or fresh, ripe oranges that have been graded, sorted, washed and strength-ration to normal single strength orange juice[8].

Another is the refrigerated orange juice that have been processed to obtain the frozen concentrate and reconstituted by adding back the water that has been initially removed. Reconstituted single strength juice is

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Table 1: Review of the various medicinal uses of the study plants

Common names	Scientific names	Traditional use	Reference
Sweet orange	Citrus sinensis	Used as appetizer, as a sedative, preventing scurvy, thrombosis, arteriosclerosis and circulation dysfunction.	Roger[4] Oliver-Bever[10]
Lemon	Citrus limonum	It is used against insomnia, sedative, asthma and scurvy, dissolve kidney stones and act as a buffer it renders good results in cases of gout and arthritls	Roger[4] Oliver-Bever[10]
Lime	Citrus aurantifolia	Acts against menstrual disorder, chronic diarrhea and dysentery. Lowers cholesterol level of the blood and cure ulcer.	Roger[4]
Grape	Citrus graudis	Used against stomach upset, cure gout, arthritis and kidney stones, cure ulcer	Roger[4]
Tangerine	Citrus reticulate	Used as a veno-tonic and in capillary protection. Used as antiseptic. Cure ulcer and wounds, hydrates the skin.	Roger[4]

normally reconditioned by the beverage industry and sold as a ready-to-serve product[8].

Furthermore, citrus fruits can be processed to obtain other food products such as dehydrated citrus products like jams, jellies or marmalades, which are very much-appreciated[4,8]. Both the sweet and the bitter orange trees have the same properties. However, in phytotherapy, the flowers, leaves, and barks of bitter oranges are preferred due to their higher concentration of active component[4]. Citrus essential oil is another by product of citrus. Essential oils are derived from the citrus fruits peels' sacks. Citrus essential oils are used by the food industry to give flavor to drinks and foods[8]. The essential oils from citrus are also used in pharmaceutical industry for the preparation of drugs and in the cosmetic industry for the preparation of soaps and perfumes and for home cleaning products.

The dietetic and therapeutic properties of all citrus fruits are similar due to their phytonutrient content. Their various uses in traditional medicine are reviewed in Table 1. This study investigates the fundamental scientific bases for the use of citrus fruits in herbal medicine by declining and quantifying the percentage of crude phytonutrients and vitamin constituents present in the citrus fruits.

MATERIALS AND METHODS

Sample collection: The experimental citrus fruits comprising sweet orange (*Citrus sinensis*), lime (*Citrus aurantifolia*), grape (*Citrus grandis*), tangerine (*Citrus reticulata*) and lemon *(Citrus limonum)* were harvested from the National Roots Crops Research Institute (NRCRI) orchard Umudike, Ikwuano Local Government Area, Abia State, Nigeria on 25th April 2005. The citrus species were identified by Dr. A. Nmeregini of Taxonomy Station, Forestry Department, Michael Okpara University of Agriculture Umudike, Nigeria.

Sample preparation: The epicarps of the five different species were peeled out and disposed. The mesocarps and endocarps were cut into smaller particles with a sharp clean knife. This helps in reducing the surface area and thus enhances drying. They were air-dried for 6 days. The dried materials were pulverized into powdery form using a Thomas Wiley machine and stored in airtight bottles till required for analysis.

Also the juices from the five citrus fruit samples were pressed out from the fruit preserved and stored in airtight bottles in a refrigerator till required for analysis.

Determination of vitamins: The B-complex vitamins comprising thiamin, riboflavin and niacin were determined using Skalar Analyzer 918 solar model.

Determination of thiamin: 5g of the juice extract were homogenized with ethanolic sodium hydroxide (50 mL). It was filtered into a 100 mL flask. 10 mL of the filtrate was pipette and the color developed by addition of 10 mL of potassium dichromate and read at 360 nm. A blank sample was prepared and the color also developed and read at the same wavelength. A standard solution was prepared using thiamin acid to get 100 ppm and serial dilutions of 0.0,0.2,0.4,0.6 and 0.8 ppm were made. This was used to plot the calibration curve.

Determination of riboflavin: 5g the fruit juice were extracted with 100 mL of 50% ethanol solution and shaken for 1 h. This was filtered into a 100 mL flask. 10 mL of the extract was pipette into 50-ml volumetric flask. 10 mL of 5% potassium permanganate and 10 mL of 30% H_2O_2 were added and allowed to stand over a hot water both for about 30 minutes. 2 mL of 40% sodium sulphate solution was added and a yellowish pale color was formed. This was made up to 50 mL mark and the absorbency measured at 510 nm in a spectrophotometer.

Determination of niacin: 5g of the citrus juice were treated with I N sulphuric acid and shaken for 30 minutes. 3 drops of ammonia solution was added to the sample and filtered. 10 mL of the filtrate was pipette into a 50 mL volumetric flask and 5 mL of potassium cyanide was

Table 2: Phytonutrient content of Citrus species on dry weight basis expressed as mg/100g

Species	Alkaloids	Flavonoids	Tannins	Phenols	Saponins
C. reticulata	0.38±0.10	0.26±0.11	0.02±0.10	0.03±0.20	0.03±0.11
C. aurantifolia	0.33±0.11	0.29±0.20	0.04±0.11	0.02±0.10	0.22±0.30
C. limonum	0.54±0.20	0.57±0.10	0.01±0.10	0.05±0.11	0.42±0.10
C. grandis	0.64±0.11	0.26±0.11	0.03±0.20	0.0;8±0.01	0.21±0.11
C. sinensis	0.62±0.10	0.19±0.20	0.04±0.11	0.01±0.10	0.08±0.10

Data are means ± standard deviations of triplicate determinations

added. This was acidified with 5 mL of 0.02N H_2SO_4 and absorbency measured in the spectrophotometer at 470 nm wavelength. This was used to plot the calibration curve.

Determination of ascorbic acid (Vitamin C): 5g of the citrus juice were weighed into a extraction tube and 100 mL of EDTA/TCA (2.1) extracting solution were mixed and the mixture shaken for 30 min. This was transferred into a centrifuge tube and centrifuged at 3000 rpm for about 20 minutes. It was transferred into a 100 mL volumetric flask and made up to 100 mL mark, with the extracting solution. 20 mL of the extract was pipette into a volumetric flask and 1% starch indicator, was added and titrated with 20% $CuSO_4$ solution to get a dark end point[10].

QUANTITATIVE DETERMINATION OF THE PHYTOCHEMICAL CONSTITUENT OF THE CITRUS FRUITS

Preparation of a fat free sample: 2g of the powdery sample were defated with 100 mL of diethyl ether using a Soxhlet apparatus for 2 h.

Alkaloid determination: 5g of the sample were weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide solution and then filtered. The residue is the crude alkaloid which was weighed[11].

Determination of total phenols by spectrophotometric method: The fat free sample was boiled with 50 mL of ether for the extraction of the phenolic component for 15 minutes. 5 mL of the extract were pipette with a 50 mL flask, than 10 mL of distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were also added. The samples were made up to the mark and left to read for 30 min. for color development. The absorbency of the solution were read at 505 nm wave lengths using a spectrophotometer. Standard solutions of phenol were prepared at 0.0, 2.00, 4.00, 6.00, 8.00 and 10.00ppm receptively with the same treatment.

Calibration curves of the absorbency values versus concentration of the standard were constructed and the value of phenol in the sample was calculated[11].

Saponin determination: The samples were ground. 20g of each plant sample were dispersed in 200 mL of 20% ethanol. The suspensions were heated over a hot water bath for 4 hrs with continuous stirring at about 55°c. The mixture was filtered and the residue re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°c. The concentrate was transferred into a 250ml-separator funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated[12].

Flavonoid determination: 10g of the plant sample were extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighted[13].

Tannin determination: 500 mg of the sample was weighted into 100 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 h. in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipette out into a tube and mixed with 3 mL of 0.1m $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbency was measured in a spectrophotometer at 120 nm wavelength within 10 minutes. A blank sample was prepared and the color also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured[14].

Table 3: Vitamin composition of *Citrus* juice expressed as mg/100g

Species	Ascorbic acid (Vitamin C)	Thiamin (Vitamin B_1)	Riboflavin Vitamin B_2	Niacin (Nicotinic acid)
C. reticulata	31.66±0.20	0.12±0.10	0.01±0.11	0.43±0.10
C. aurantifolia	22.88±0.10	0.11±0.20	0.04±0.22	0.03±0.22
C. limonum	61.60±0.11	0.08±0.22	0.02±0.10	0.14±0.33
C. grandis	36.08±0.20	0.09±0.11	0.06±0.11	0.36±0.20
C. sinensis	19.36±0.10	0.06±0.20	0.11±0.20	0.38±0.10

Data are means ± standard deviations of triplicate determinations

RESULTS AND DISCUSSION

The phytonutrient content of the citrus fruits is shown in Table 2. The flavonoids content was very high on *C. limonum* (0.57 mg $100g^{-1}$), followed by *C. aurantifolia* which contained (0.29 mg $100g^{+}$) of flavonoid while *C. reticulata* and *C. grandis* contained 0.26 mg/100g of flavonoids, respectively.

The biological functions of flavoniods include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors[2,7,15].

As a result of the availability of flavonoids in these citrus fruits, they prevent platelet stickiness and hence platelet aggregation. Moreover, the citrus fruits protect the vascular system and strengthen the tiny capillaries that carry oxygen and essential nutrients to all cells. As antioxidant, flavonoid from citrus helps in the digestion and assimilation of food to the body system. The presence of flavonoids in citrus fruits may be the reason for their use in herbal medicine for the treatment of capillary and vascular weakness (edema, varicose veins, blood clotting, dysfunction)[4,9] . Citrus flavonoids are a good digestive tonic, with an appetizer effect and it aids in digestion. It also has a slight sedative function. This also supported the popular use of lemon, lime or grape juice for people suffering from upset stomach or indigestion by the natives[4].

In relation to the flavonoids content, citrus juice reinforces the stability of the capillary vessels and improves venous blood flow. They are useful in cases of swollen legs, edema, varicose veins, hemorrhoids, thrombosis and emboli[4,9]. They are also recommended for people suffering from high blood pressure.

Apart from flavonoids, other secondary metabolite constituents of citrus fruits detected include the alkaloids. Enormous quantity of alkaloids were found on *C. grandis* (0.64 mg/100g), followed by *C. sinesis* which contained 0.62 mg/100g of alkaloids while *C. limonum* contained 0.54 mg/100g of alkaloids. Pure isolated plant alkaloids and their synthetic derivatives are used as a basic medicinal agent for its analgesic, antispasmodic and bactericidal effects[2,16,17]. They exhibit marked physiological activity when administered to animals. Most of the plant parts used in the cure of diseases have been reported to contain traces of alkaloids. For instance *Azadirachta indica* used in the cure of malaria contain alkaloids[18]. Quinine, isolated from *Cinchona* bark is the oldest known effective anti-malarial agent[19]. The presence of alkaloids in the citrus fruits investigated therefore suggests that the citrus fruits have medicinal properties.

The total content of phenols was 0.08 mg $100g^{-1}$ in *C. grandis,* 0.05 mg $100g^{-1}$ in *C. limonum,* 0.03 mg $100g^{-1}$ in *C. reticulata* and 0.02 mg $100g^{-1}$ in *C. aurantifolia* while *C. sinensis* contained 0.01 mg $100g^{-1}$ of phenol. The presence of phenol indicates that the citrus fruits could act as anti-inflammatory, anti-clotting, antioxidant, immune enhancers and hormone modulators[15]. Phenols have been the subjects of extensive research as disease preventives[20]. Phenols have been responsible in having the ability to block specific enzymes that causes inflammation. They also modify the prostaglandin pathways and thereby protect platelets from clumping[15,20].

Tannin content was more in *C. aurantifolia* and *C. sinensis:* having 0.04 mg $100g^{-1}$ of tannin respectively followed by *C. grandis* (0.03 mg $100g^{-1}$) while *C. reticulata and C. limonun* have 0.02 mg $100g^{-1}$ and 0.01 mg/100g of tannin respectively. The presence of tannin could be responsible for the bitter principle and sour taste of some citrus species. Tannins have astringent properties, hasten the healing of wounds, and inflamed mucous membranes[2]. This also supported the use of lemon juice in herbal medicine for the treatment of varicose ulcers, hemorrhoids, frostbite and burns by the natives[2].

Saponin content was more in *C. limonum* (0.42 mg $100g^{-1}$) followed by *C. aurantifolia* which contain (0.22 mg/100g) of saponin while *C. reticulate* have the least saponin content of (0.03 mg $100g^{-1}$). Some of the general characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity and cholesterol binding properties and bitterness[21]. Saponins natural tendency to ward off microbes makes them good candidates for treating fungal and yeast infections. These compounds served as natural antibiotics, helping the body to fight infections and microbial invasion[22]. These compounds also appear to greatly enhance the effectiveness of certain vaccines. Plant saponins help humans to fight fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccine and knock out some kinds of tumor cells particularly lung and blood cancers[9]. They also lower blood cholesterol thereby reducing heart disease. The most outstanding and exciting prospects for saponins is how they inhibit or kill cancer cells. They may also be able to do it without killing normal cells on the process, as is the mode of some cancer fighting drugs. Cancer cells have more cholesterol-type compounds on their membranes than normal cells. Saponins therefore bind cholesterol and thus interfere with cell growth and division[9].

Table 3 shows the vitamin constituents of the citrus fruits using juice. The fruit juice contained ascorbic acid, niacin, riboflavin and thiamin in varying quantities. Ascorbic acid constituent varied markedly ranging from 19.36 mg $100g^{-1}$ in *C. sinensis* to 61.60 mg $100g^{-1}$ in *C. limonum.* The niacin constituents range from 0.03 mg $100g^{-1}$ in *C. aurantifolia* to 0.43 mg $100g^{-1}$ in *C. reticulata.* Thiamin constituents range from 0.06 mg $100g^{-1}$ in *C. sinensis* to 0.12 mg/100g in *C. reticulata* while riboflavin constituent range from 0.01 mg $100g^{-1}$ in *C. reticulata* to 0.11 mg $100g^{-1}$ in *C. sinensis.* Natural ascorbic acid is crucial for the body performance. It possesses anti-scorbutic activity. Citrus is the main source from which primates device vitamin C. Ascorbic acid in the body aids in iron absorption from the intestines. It is required for connective metabolism especially the scar tissue, bones and teeth[23]. It is necessary as an anti-stress and protector against cold, chills and damp. It prevents muscle fatigue and scurvy, which is characterized by skin hemorrhages, bleeding gums, fragile bones, anemia and pains in the joints and defects in skeletal calcification. This function of ascorbic acid also accounts for its requirement for normal wound healing[2,17]. It acts also as antioxidants in the skin by scavenging and quenching free radical generated by ultra violet (UV) radiation stabilization. The production of collagen is also dependent on vitamin C. It helps in the promotion and restoration of skin tone and improvement in fine wrinkles[5].

Ascorbic acid is necessary in the metabolism of cholesterol in the liver. It is required for hydroxylation of proline to hydroxyproline. Thus maintaining normal connective tissues. The reducing property is beneficial in some physiological processes such as the absorption of dietary iron where Fe is absorbed as Fe^{+2} rather than Fe^{+3}[23]. The role of vitamin C in bone mineralization involves hydroxylation reactions required for cross-linking in the bone matrix and amidation of calcitonin, a thyroid peptide hormone[2,23].

Other vitamins like niacin, thiamin and riboflavin are in trace amounts in the five citrus samples. Though they occur in trace amounts, they have very important biochemical roles to play in the body. Niacin is active in preventing pellagra, a disease resulting to diarrhea, dermatitis (inflammation of the skin) and dementic[5]. While thiamin prevents beriberi, a deficiency of riboflavin does not lead to any specific and identifiable disease. The symptoms of deficiency of riboflavin are inflammation of the tongue, lesions of the eyes and lips, congestion of conjunctival blood vessels and desquamation of the skin[17].

It is significant to note that soft drinks that are so-called lemonade or that they are made of lime not only lack medicinal properties but also are noxious for health due to their content of carbonic gas, artificial colorings and aromatizes and sugar or other flavors[4]. The herbal vitamins are dietary supplements and may be used safely as preventive health measure for nutritional values. Herbal vitamins are manufactured from parts of specific plants such as seeds, roots, stems, barks, leaves, berries or flowers and they are not necessarily processed as capsules or pills. The best way to take advantage of the many medicinal virtues of lemon, lime and other citrus juice is to consume them just after they have been squeezed from the fruits.

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