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RESEARCH ARTICLE

Flavonoid Content of *Citrus* Species Grown in Awka, Anambra State, Southeastern Nigeria

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ABSTRACT

The flavonoid contents of different parts of *Citrus aurantifolia* (Christm.) Swingle (Lime), *C*itrus *grandis* Osbeck (Shaddock/Pummelo), *C*itrus *limon* (L.) Burm. f. (Lemon), *Citrus paradisii* Macf. (Grapefruit), *Citrus reticulata* Blanco (Mandarin/Tangerine) and *Citrus sinensis* (L.) Osbeck (Sweet orange) commonly grown in Southeastern Nigeria were examined. The parts evaluated were roots, stems, stem barks, leaves and peels. The homogenous sample of each of the samples of the roots, stems, stem barks, leaves, and peels of the six species of *Citrus* was subjected to phytochemical analyses for qualitative and quantitative determinations of flavonoid. All the parts of *Citrus* plants were found to contain flavonoid, with the highest level found in the leaves of *C. grandis* and *C. paradisii* respectively. It was also observed that the parts of *C. paradisii* contained the highest level of flavonoid when compared with others. These indicated that parts of *Citrus* plants contained high level of flavonoid and could be regarded as possible sources of it; thus, their usefulness in ethnomedicine as foods and herbal drugs is suggested.

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INTRODUCTION

Citrus L. is a dicotyledonous genus belonging to the family Rutaceae (Pandey, 1981; Singh, 2004; Manner *et al*., 2006; Nyananyo, 2006). *Citrus* has 12 species (Nyananyo, 2006), with six species common in Southeastern Nigeria. They include: *Citrus aurantifolia* (Christm.) Swingle (Lime), *C*itrus *grandis* Osbeck (Shaddock/Pummelo), *C*itrus *limon* (L.) Burm. f. (Lemon), *Citrus paradisii* Macf. (Grapefruit), *Citrus reticulata* Blanco (Mandarin/Tangerine) and *Citrus sinensis* (L.) Osbeck (Sweet orange).

Katz and Weaver (2003) reported that all commercial *Citrus* cultivation uses trees produced not by seeds but by grafting the desired fruiting cultivars onto root stocks selected for disease resistance and hardiness. Although *Citrus* can be grown from seed, there are such disadvantages as the fact that seedling trees do not bear fruit until nearly a decade old, seeds and young trees are vulnerable to disease and unfavorable soil conditions, and, because *Citrus* trees hybridize very readily, sometimes trees produced by seeds are not true-to-type with the mother tree (Katz and Weaver 2003; Manner *et al*., 2006).Thus, most *Citrus* are produced by budding. The trees do best in a consistently sunny, humid environment, with fertile soil and adequate rainfall or irrigation (Pandey, 1981). In addition, the fruits ripen throughout the year. Many genera of fungi, bacteria and viruses have been reported as causative microorganisms of diseases of *Citrus* trees (IITA, 2003; NIHORT, 2003; Ezeibekwe, 2011). Manner *et al.* (2006) noted that *Citrus* plants are very liable to infestation by aphids, whitefly, and scale insects. In addition, these ectoparasites serve as vectors to some of the viral infections (Manner *et al.*, 2006).

Flavonoids are class of plant secondary metabolites that are abundant components of fruit and vegetables (Crozier *et al*., 2006). They are divided into five major subclasses: flavonols, flavan-3-ols (monomers and proanthocyanidins), flavones, flavanones and anthocyanins (Manach *et al.* 2004; Crozier *et al*., 2006). They are the most numerous of the phenolics and are found throughout the plant kingdom (Harborne, 1993; Crozier *et al*., 2006). In plants, flavonoids are involved in such diverse processes as UV protection, pigmentation, stimulation of nitrogen-fixing nodules and disease resistance (Koes *et al*., 1994; Pierpoint, 2000). The metabolism and pharmacokinetics of flavonoids has been an area of active research in the last decade (Crozier *et al*., 2006).

Flavonoids are widely distributed in plant-based foods at varying levels (Robards and Antolovich, 1997; Escarpa and Gonzalez, 2001; Kyle and Duthie, 2006). They are found in fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers (Middleton *et al*., 2000). They are of particular importance in the human diet as there is

evidence that they act as free radical scavengers, antioxidants, diuretic, antiviral, antibacterial, antimicrobial, anti-inflammatory, anti-tumor, anti-platelets agents (Hertog *et al*., 1993; Middleton and Kandaswami, 1992; Kandaswami and Middleton,1994; Corkan *et al*., 1998; Pourmorad *et al*., 2006; Soetan, 2008; Omale, 2009; Crozier *et al*., 2006; Enwerem *et al*., 2001, Njoku and Obi, 2009; Monache *et al*., 1996; Rao *et al*., 1996; Sofowora, 1993); and are associated with reduced risk of cancer and cardiovascular diseases (Knekt *et al*., 1996; Del-Rio *et al*., 1997; Middleton *et al*., 2000; Okwu, 2004; Omale, 2009). Furthermore, different bioactive compounds isolated from *Citrus* were reported to improve bone in orchidectomized rats especially flavonoids and limonoids (Mandadi *et al*., 2009; Horcajada – Molteni *et al*., 2008). These have made *Citrus* plants to receive a huge attention.

Ezeibekwe (2011), reported that total world population of *Citrus* was estimated at 36 metric tons with Nigeria producing 0.3 metric tons of the world's production. In spite of the production of *Citrus* plants in Nigeria, there is a paucity of information on the phytochemicals in their different parts. This necessitated the investigation of different parts of *Citrus* species for flavonoid content. The objective of this work, therefore, was to investigate different parts of *Citrus* species for presence of flavonoid and to note their levels. The information obtained will determine weather they could serve as possible sources of it and suggest their usefulness in ethnomedicine as foods and herbal drugs.

MATERIALS AND METHODS

Sources of Materials

The roots, stems, stem barks, leaves and fruits of *Citrus aurantifolia*, *C. grandis*, *C. limon*, C. *paradisii*, *C. reticulata* and *C. sinensis* were collected in the months of November – December at optimum maturity, from Agricultural and Natural Resources Department Market Garden, Amawbia, Awka South Local Government Area, Anambra State.

The *Citrus* species were authenticated by Prof. C.U. Okeke, a Plant Taxonomist, in Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State. The voucher specimens were deposited at Department of Botany Herbarium, Nnamdi Azikiwe University, Awka.

Preparation of Plant Materials for Flavonoid Determination

The rinds of healthy ripe fruits of the six *Citrus* species were peeled off with a knife. The roots, stems, stem barks and peels were sun dried for seven days whereas the leaves were air dried in the laboratory at room temperature for ten days. The dried samples were then crushed with mortar and pestle before grinding into fine powder using a manual grinder (Corona, USA.).

Qualitative Determination of Flavonoid

The homogenous sample of each of the samples of the roots, stem, stem barks, leaves, and peels of the six species of *Citrus* was subjected to phytochemical analysis for qualitative determinations of flavonoid according to the methods described by Harborne (1973), Trease and Evans (1989), Sofowora (1993), Adamu *et al*. (2007) and Nyam *et al*. (2009). The performed qualitative tests were briefly described as:

Two grams (2g) of the extract was completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone on a water bath (GFL, Germany). The mixture was filtered while hot and then cooled; 5 mls of 20% sodium hydroxide was added to an equal volume of the detanned extract. A yellow solution indicates the presence of flavonoid.

The following rankings were used:

+ = Present
++ = Deeply present
+++ = Very deeply present

Quantitative Determination of Flavonoid

The flavonoid content of the samples of the plants was determined by the gravimetric method as was described by Harborne (1973).

Five grams (5g) of the powdered sample was placed into a conical flask, 50 ml of water and 2 ml HCl solution were added. The solution was allowed to boil for 30 min. The boiled mixture was allowed to cool before it was filtered through Whatman filter paper (No 42). Ten milliliters (10ml) of ethyl acetate extract which contained flavonoid was recovered, while the aqueous layer was discarded. A pre weighed Watman filter paper was used to filter the second (ethyl-acetate layer), the residue was then placed in an oven to dry at 60 ^{0}C. It was cooled in a desiccator and weighed. The quantity of flavonoid was determined using the formula:

$$\text{Percentage (\%) Flavonoid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

Where:-

W_1=weight of empty filter paper

W_2 =Weight of paper + Flavonoid extract

Statistical Analysis

The quantitative data obtained were statistically analyzed by calculating the mean of three replicates followed by calculation of the Sum of Square (ss), Variance (s^2) Standard Deviation (s) and Standard error ($\bar{s}x$). The results were presented as mean $\pm$ standard error.

RESULTS AND DISCUSSION

The investigation revealed that the highest level of flavonoid was contained in the leaves of *C. grandis* [0.88$\pm$0.01] and *C. paradisii* [0.81$\pm$0.01] respectively (Tables 1 and 2). In recent years, *C. grandis* has received

Table 1: Qualitative Flavonoid content of roots, stem, stem bark, leaves and peels of *Citrus* species (%).

Species	Root	Stem	Stem bark	Leaves	Peels
Citrus aurantifolia	++	+	+	+	++
C. grandis	++	+	++	+++	+
C. limon	++	+	++	++	+
C. paradisii	++	++	++	+++	+
C. reticulata	++	+	+	+	+
C. sinensis	++	+	+	++	+

Table 2: Quantitative Flavonoid content of roots, stem, stem bark, leaves and peels of *Citrus* species (%).

Species	Root	Stem	Stem bark	Leaves	Peels
Citrus aurantifolia	0.64±0.40	0.33±0.01	0.42±0.01	0.06±0.07	0.51±0.02
C. grandis	0.54±0.01	0.30±0.01	0.48±0.01	0.88±0.01	0.17±0.01
C. limon	0.60±0.01	0.34±0.02	0.47±0.01	0.65±0.03	0.48±0.01
C. paradisii	0.50±0.05	0.63±0.01	0.53±0.01	0.81±0.01	0.19±0.01
C. reticulate	0.65±0.03	0.27±0.03	0.36±0.01	0.04±0.02	0.08±0.04
C. sinensis	0.63±0.01	0.29±0.01	0.38±0.05	0.63±0.03	0.35±0.01

Data are means of triplicate determinations ± Standard error.

much attention because of its nutritional value and antioxidant properties, especially flavonoids in Asia (Wattanasiritham *et al*.,2005:Xu *et al*.,2008;Wang *et al*., 2007; Nagata *et al*., 2006; Chaiwong and Theppakorn, 2010). Morton (1987) reported the medicinal uses of *C. grandis*; in Philippines and Southeast Asia, decoctions of the leaves, flowers, and rind are given for their sedative effect in cases of epilepsy, chorea and convulsive coughing; the hot leaf decoction is applied on swelling and ulcers. The levels of flavonoid in the leaves of *C. limon* [0.65±0.03] *and C. sinensis* [0.63±0.03] respectively, were relatively high when compared with those of *C. aurantifolia and C. reticulata*. Crozier *et al*. (2006) reported that flavonoids are present in high concentrations in the epidermis of leaves and have important and varied roles as secondary metabolites. Considerable high level of flavonoid was contained in the peels of *C. aurantifolia.* High level of flavonoid was observed in the roots of all the *Citrus* species (Tables 1 and 2). The stem and stem bark of *C. paradisii* contained highest level of flavonoid when compare with others; this indicated that the highest level of flavonoid was contained in parts of *C. paradisii* (Tables 1 and 2).This might contribute to the deeper yellow colour of ripened fruits of *C. paradisii* when compared with others. Crozier *et al*. (2006) noted that flavonoids are present in high concentrations in the skin of fruits. In addition, it has been reported by several workers that the flavonoids subclass of phenolics are primarily recognized as the pigments responsible for the autumnal burst of hues and the many shades of yellow, orange, and red in flowers and food (Dillard and German, 2000; Middleton *et al*., 2000; Onyeike *et al*., 2010).

Citrus plants could be regarded as medicinal due to the high level of flavonoid content in them. Flavonoids are of particular importance in the human diet as there is evidence that they act as free radical scavengers, antioxidants, diuretic, antiviral, antibacterial, antimicrobial, anti-inflammatory, anti-tumor, anti-platelets agents (Hertog *et al*., 1993; Middleton and Kandaswami 1992; Kandaswami and Middleton,1994; Corkan *et al*., 1998; Pourmorad *et al*., 2006; Soetan, 2008; Omale, 2009; Crozier *et al*., 2006; Enwerem *et al*., 2001; Njoku and Obi, 2009; Monache *et al*., 1996; Rao *et al*., 1996; Sofowora, 1993), and are associated with reduced risk of cancer and cardiovascular disease (Knekt *et al*. 1996;Del-Rio *et al*., 1997; Middleton *et al*., 2000;Okwu, 2004; Omale, 2009). In addition, Havsteen (2002) reported their low toxicity to animal cells. The uses of different parts of *Citrus* in medicine have been reported. Morton (1987) noted that the root decoction of *C. limon* is taken as a treatment for fever in Cuba; for gonorrhea in West Africa. The fresh peel of *C. sinensis* is rubbed on acne (Morton, 1987). This could be as a result of the antibacterial and antiviral activities of flavonoid. Recently, feeding of orange pulp was reported to improve bone quality in a rat model of male osteoporosis (Morrow *et al*., 2009). Nagwa *et al*. (2011) demonstrated that *C. aurantifolia* and *C. sinensis* extracts have the potential to develop a clinically useful anti-osteoporotic agent. Morton (1987) noted that a decoction of the dried leaves and flowers of *C. sinensis* is given as an anti-spamodic, cardiac sedative, digestive and remedy for flatulence .In addition, the juice of fresh *C. sinensis* leaves or a decoction of the dried leaves may be applied on sores or ulcers (Morton, 1987).These might be as a result of antibacterial, antimicrobial, anti-inflammatory activities of flavonoid. Havsteen (2002) reported that modern authorized physicians are increasing their use of pure flavonoids to treat many important common diseases, due to their proven ability to inhibit specific enzymes, to simulate some hormones and neurotransmitters, and to scavenge free radicals. Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Njoku and Obi, 2009; Edoga *et al*., 2005; Mann, 1978).

Flavonoid from *Citrus* plants could be used to fortify animal feeds. Makkar *et al*. (2007) reported that bioactive compounds from plants could also be used as feed additives for enhancing livestock productivity and reducing environment pollutants such as methane in the exhaled gas and nitrogen and phosphorus in urine. Parish (2006) noted that *Citrus* pulp, made by shredding, liming, pressing, and drying the peel, pulp, and seed residues from *Citrus* fruit are used as cattle feed. In addition, Morton (1987) reported that dehydrated *C. limon* peel are sold as a cattle feed.

Conclusion

Flavonoid was observed to be present in the parts of *Citrus* plants investigated. It was also observed to be high in some of the parts. *Citrus* plants have medicinal value, are cheaply available and easily accessible. They may be considered as excellent sources of flavonoid with potential health benefits. The use of these parts of *Citrus* plants in ethnomedicine, is thus, suggested. In addition, they can be added to animal feed to enhance livestock productivity. To ensure safe consumption of these parts of *Citrus* plants, a further investigation into their possible toxicity is required.

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