Comparative Studies on *In vitro* Antioxidant Properties and Free Radical Scavenging Capacity of Methanolic Extracts of *Annona muricata* and *Citrus maxima* Leaves

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ABSTRACT

Studies on the bioactive contents, antioxidant minerals and *in vitro* antioxidant properties of *Annona muricata* and *Citrus maxima* were carried out following standard method of analysis. The results of the bioactive content of *C. maxima* and *A. muricata* extract revealed that β -carotene, lycopene and ascorbic acid content of *C. maxima* (62.23 ± 2.04^a $\mu\text{g/g}$, 52.48 ± 1.03^a $\mu\text{g/g}$ and 55.73 ± 1.56^a mg/kg respectively) were significantly higher than the values in *A. muricata* (6.4 ± 2.67^b $\mu\text{g/g}$, 2.4 ± 2.76^b $\mu\text{g/g}$ and 33.00 ± 0.96^b mg/kg respectively) while Vitamin E level in both *A. muricata* and *C. maxima* (21.26 ± 0.89^a $\mu\text{g/g}$ and 25.74 ± 0.89^a $\mu\text{g/g}$ respectively) did not differ significantly. The mineral content analysis showed that *A. muricata* has high concentration of iron (69.96 ± 15.79^a mg/kg) followed by copper (15.21 ± 2.98^b mg/kg), zinc (7.99 ± 2.90^a mg/kg) and selenium (3.72 ± 2.09^b mg/kg) while *C. maxima* contains iron (57.00 ± 12.90^b mg/kg), copper (27.00 ± 19.27^a mg/kg), zinc (3.27 ± 2.09^b mg/kg) and selenium (7.30 ± 0.89^a mg/kg). The iron content of *A. muricata* (69.96 ± 15.79^a mg/kg) was significantly higher ($P < 0.05$) when compared to *C. maxima* (57.00 ± 12.90^b mg/kg) while the Copper content (27.00 ± 19.27^a mg/kg) of *C. maxima* was significantly higher than that of *A. muricata* (15.21 ± 2.98^b mg/kg). DPPH radical scavenging assay showed that *C. maxima* leave extract has a higher free radical scavenging capacity compared to *A. muricata*. The % RSA of the samples were in the order of BHA standard $>$ *C. maxima* $>$ *A. muricata*. This therefore suggest that *C. maxima* has a higher antioxidant properties and free radical scavenging capacity compared to *A. muricata* leaves.

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1. INTRODUCTION

The cultivation of tropical plant has bloomed since the past few years due to the attractive sensory properties and antioxidants content of their fruits. Antioxidant in tropical fruit includes polyphenols, carotenoids, and vitamins that impart health benefits beyond basic nutrition (Yahia, 2010). Several tropical fruits such as *Citrus maxima* and *Annona muricata* are native to Southeast Asia and these fruits have high antioxidant activity (Leong and Shui, 2002). Among these, *C. maxima* has been reported to possess high antioxidant property (Isabelle *et al.*, 2010). It is a large citrus fruit with a common name of pomelo or shaddock that belongs to the family of Rutaceae (Scora, 1975; Morton, 1987). It is a perennial plants predominantly found in India. The tree is about 5-15 m tall and the leaves are large evergreen elliptic 10-20 cm long (Vijaylakshmi and Radha, 2015). Like other citrus plant, pomelo fruits are rich in vitamin C, the bark and root are rich in β -sitosterol, acridine alkaloids, the leaf contain essential oil while the unripe fruits contains limonin, geraniol and nerolol (Xu *et al.*, 2008). *C. maxima* fruits also contain high amount of polyphenolic compounds like naringin, hesperidin, caffeic acid (Xu *et*

al., 2008). The uses of *C. maxima* includes treatment of cough, convulsion, hemorrhagic diseases, epilepsy (Arias and Remon-laca, 2005).

A. muricata is a tropical plant species of the genus *annona* of the *annonaceae* family. It bears a large edible fruit called Soursop. The tree of *A. muricata* could reach a height of 6-8 m (Arias and Remon-laca, 2005). The fruits has some medicinal values such as reducing fever and improving mother's milk secretion (Arias and Remon-laca, 2005). These plants are mainly used in ethnomedicine because of their medicinal properties.

According to Becker *et al.* (2004), antioxidants are class of molecules that are capable of inhibiting the oxidation of other molecules. Oxidation reactions produces free radicals. Ananya (2016) defined free radicals as atoms or group of atoms with unpaired number of electrons formed by interaction of oxygen molecules with some other molecules. Free radicals initiate a chain reaction which causes damage to cells while the antioxidants terminate these chain reaction by removing free radical intermediates and inhibit other oxidation reaction (Abeysinghe *et al.*, 2017). Although oxidation reactions are crucial for life, they can also be damaging; hence plants and animals maintain complex systems of multiple types of antioxidants such as glutathione, vitamin c and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. This work was aimed at comparing the *in vitro* antioxidant properties and free radical scavenging capacity of *A. muricata* and *C. maxima* leaves in order to know the leave with a better medicinal value and to scientifically support the ethnomedicinal uses of the plants.

2. MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Sample Collection

The plant leaves of *A. muricata* and *C. maxima* were collected from Doutimiye Garden Tombia Town, Bayelsa State, Nigeria during the month of June. The leaves were identified and authenticated by Dr. C.G. Ukpaka of Biological Science Department, Chukwuemeka Odumegwu Ojukwu University, Uli campus, Nigeria. The leaves were dried at room temperature for seven (7) days and pulverized using manual grinder. The samples were stored in an air tight container until further analysis.

2.1.2 Sample preparation

Twenty grams (20 g) of the powdered samples were extracted using 250 mL of methanol for 48 hrs. These were filtered using Whatman paper No. 4 and the filtrate were concentrated over a water bath at 60 °C. The concentrated extract was weighed and redissolved in methanol at a concentration of 10 mg/mL and evaporated to dryness. The dried methanolic extract were then stored at 4 °C till further analysis.

2.2 METHODS

2.2.1 Determination of β-Carotene and Lycopene

The concentration of β-carotene in the samples was analyzed spectrophotometrically as described by Arabshahi *et al.* (2007). The dried methanolic extract (100 µg) was vigorously shaken with acetone/hexane mixture (4:6) and filtered. The absorbance of the filtrate was measured at 453 nm, 505 nm and 663 nm. Concentrations of β-carotene and lycopene were calculated and the results expressed in mg/100 mL.

$$\beta\text{-carotene (mg/100 mL)} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}.$$

$$\text{Lycopene (mg/100 mL)} = 0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}.$$

The result were expressed as µg/g of carotenoid/g of extract.

2.2.2 Determination of Ascorbic Acid Content

The Ascorbic Acid (Vitamin C) content was determined by titrimetric method as describe by Barros *et al.* (2007). Half a gram (0.5g) of sample was extracted three times with 5 mL of 5 % HCl and filtered into a 100 mL graduated flask using funnel with cotton wool. The graduated flask was made up to 100 mL with distilled water and mixed properly. Ten milliliter (10 mL) of the extracted solution was titrated with 2, 6 – dichlorophenolidophenol until the last drop that caused a persistent pink coloration for about 30 seconds.

$$\text{Vitamin C (mg/kg)} = \frac{0.088 \times \text{Titre volume} \times 100 \times 1000}{10 \times \text{Sample weight}}$$

2.2.3 Determination of Vitamin E

The extract (0.33g) and 20 mL of ethanol were mixed and filtered. To 1.0 mL of the filtrate was added 2.0 mL of 0.2 % ferric Chloride (FeCl_3) in ethanol and diluted to 5.0 mL using distilled water. Vitamin E content was estimated by reading the absorbance at 520 nm using spectrophotometer.

2.2.4 Mineral Analysis using Atomic Absorption Spectrophotometer (AAS)

Mixed acid digestion method of AOAC (1984) as reported by Ifemeje (2015) was used. Atomic absorption spectrophotometer (AAS) (bulk scientific atomic absorption/emission spectrophotometer model 210A system) was used. Two grams (2.0 g) of pulverized sample was weighed into a digestion flask and 15 mL of acid mixture (10 mL concentrated nitric acid + 5 mL perchloric acid) was added and was heated until a clear digest was obtained. The digest was cooled at room temperature, diluted with 50 mL deionized water, filtered and transferred into a 100 mL vial and the filtrate made up to mark with deionized water.

2.2.5 Determination of DPPH Scavenging Capacity

The stable 1, 1 diphenyl – 2 – picrylhydrazyl radical (DPPH) were used for the determination of the free radical scavenging activity of the methanolic extract of *A. muricata* and *C. maxima* leaves. This was assayed using the method of Ebrahimzadeh *et al.* (2009) and Ghasemi *et al.* (2009). Different concentration of the extract (100 μg) were mixed with an equal volume of methanolic solution of DPPH (100 μM) in test tubes in which butylated hydroxyanisole (BHA) solution was used as standard. The mixture was shaking and kept in dark for 30mins. The absorbance was taken at a wavelength of 517 nm using spectrophotometer. The percentage radical scavenging activities (RSA) was calculated using the formula:

$$\% \text{ RSA} = \frac{A_{\text{DPPH}} - A_1}{A_{\text{DPPH}}} \times 100$$

2.2.6 Statistical Analysis

Statistical Package for Social Sciences (SPSS) software version 18.0 were used for analysis. Significance were accepted at $P < 0.05$ and means were compared by independent student T- test. Data were expressed as mean $\pm$ standard deviation of triplicate determinations.

3. RESULTS AND DISCUSSION

Plants play an important role in human nutrition, apart from the fact that most of our recommended daily need of minerals and vitamins are derived from them, they also supply important bioactive compounds which are deficient in other food materials. Some of these bioactive compounds serve as antioxidants and free radical scavengers.

Bioactive compounds such as Ascorbic acid (vitamin C), α -Tocopherol (vitamin E), β -Carotene and Lycopene possess antioxidant potentials. In this study (Table 1), the levels of β -carotene, lycopene and ascorbic acid (6.4 ± 2.67^b $\mu\text{g/g}$, 2.4 ± 2.76^b $\mu\text{g/g}$ and 33.00 ± 0.96^b mg/kg respectively) in *A. muricata* were significantly lower ($P > 0.05$) compared to *C. maxima* (62.23 ± 2.04^a $\mu\text{g/g}$, 52.48 ± 1.03^a $\mu\text{g/g}$ and 55.73 ± 1.56^a mg/kg respectively). Vitamin E level in both *A. muricata* and *C. maxima* (21.26 ± 0.89^a $\mu\text{g/g}$ and 25.74 ± 0.89^a $\mu\text{g/g}$ respectively) did not differ significantly at $P < 0.05$. These bioactive compounds are found in fruits and vegetables; they quench the oxidation of some biochemical components and reduce oxidative stress in the physiological systems.

Table 1: Bioactive contents of *A. muricata* and *C. maxima* leave extracts

Bioactive content	<i>A. muricata</i>	<i>C. maxima</i>
β -carotene ($\mu\text{g/g}$)	6.4 ± 2.67^b	62.23 ± 2.04^a
Lycopene ($\mu\text{g/g}$)	2.4 ± 2.76^b	52.48 ± 1.03^a
Ascorbic acid (mg/kg)	33.00 ± 0.96^b	55.73 ± 1.56^a
Vitamin E ($\mu\text{g/g}$)	21.26 ± 0.89^a	25.74 ± 0.89^a

Values are mean $\pm$ SD of triplicate determinations. Values within the same row bearing the same superscript letters are significantly different at $P < 0.05$.

Minerals play important role in maintaining proper function and good health in human (Bhat *et al.*, 2010). The mineral contents of *A. muricata* and *C. maxima* (Table 2) has shown that *A. muricata* has high concentration of antioxidant minerals in the order of iron > copper > zinc > selenium and *C. maxima* also maintained the same trend. However, the concentration of Fe and Zn (69.96 ± 15.79^a mg/kg and 7.99 ± 2.90^a mg/kg respectively) in *A. muricata* were significantly higher ($P < 0.05$) than in *C. maxima* (57.00 ± 12.90^b mg/kg and 3.27 ± 2.09^b mg/kg respectively) while Cu and Se levels in *A. muricata* (15.21 ± 2.98^b mg/kg and 3.27 ± 2.09^b mg/kg respectively) are lower compared *C. maxima* (27.00 ± 19.27^a mg/kg and 7.30 ± 0.89^a mg/kg).

Table 2: Mineral concentration (mg/kg) of *A. muricata* and *C. maxima* leave extracts

Mineral content (mg/kg)	<i>A. muricata</i>	<i>C. maxima</i>
Iron	69.96±15.79 ^a	57.00±12.90 ^b
Zinc	7.99±2.90 ^a	3.27±2.09 ^b
Copper	15.21±2.98 ^b	27.00±19.27 ^a
Selenium	3.72±2.09 ^b	7.30±0.89 ^a

Values are mean ± SD of triplicate determinations. Values within the same row bearing the same superscript letters are significantly different at P<0.05.

DPPH radical scavenging model is widely used method to evaluate antioxidant activity of natural compound and plant extracts. The study showed that both *Citrus maxima* and *A. muricata* have the potential of scavenging free radical in a dose dependent relationship in DPPH radical scavenging activity. In this study, % RSA of BHA (standard) was significantly higher (P<0.05) than *C. maxima* followed by *A. muricata*. This showed that *C. maxima* has higher antioxidant scavenging properties than *A. muricata*. Since % RSA is a measure of radical scavenging activity of the extract, it then follows that the extract with higher % RSA have greater antioxidant activity than the other (Table 3). This is supported by Bushra *et al.* (2013) that scavenging of the stable radical (DPPH) is considered as a valid and easy assay to evaluate scavenging activity of antioxidants. The involvement of free radicals especially their increased production may lead to development of cardiovascular diseases and cancer.

Table 3: Scavenging effect of *A. muricata* and *C. maxima* leave extracts on DPPH radical

Sample Concentration (µg/mL)	% RSA of <i>A. muricata</i>	% RSA of <i>C. maxima</i>	% RSA of BHA
20	6.36±0.99 ^c	6.49±1.89 ^b	18.74±0.56 ^a
40	12.44±1.34 ^c	12.65±1.45 ^b	32.92±0.79 ^a
60	16.62±0.90 ^c	16.75±0.91 ^b	39.87±1.99 ^a
80	22.46±2.50 ^c	23.79±2.03 ^b	53.56±0.33 ^a
100	29.06±1.25 ^b	29.12±4.31 ^b	56.14±0.45 ^a

Values are mean ± SD of triplicate determinations. Values within the same row bearing the same superscript letters are significantly different at P<0.05.

4. CONCLUSION

The study revealed that *Citrus maxima* and *Annona muricata* leave extract possesses antioxidant properties and free radical scavenging capacity. It also showed that *C. maxima* leave extract has profound reducing activity against stable free radical than *A. muricata*. This may be attributable to high content of bioactive compound and antioxidant minerals present. However, the consumption of *A. muricata* and *C. maxima* leave extracts may be beneficial in preventing cardiovascular diseases and also serve as a replacement therapy for oxidative stress.

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