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Nutrient and Bioactive Components of *Annona muricata* and *Fagara zanthoxyloide* of South-Southern Nigeria

O. U. Ekere[1*], C. C. Monago-Ighorodje[1] and C. U. Ogunka-Nnoka[1]

[1]Department of Biochemistry, University of Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author OUE conceived of the study participated in its design and carried out sample collection, processing and analysis and performed the statistical analysis. Authors CUON and CCMI participated in the design of the study assisted in drafting contents of the study and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed at investigating the nutrient and bioactive components of *Annona muricata* and *Fagara zanthxoyloide* from south-southern Nigeria. The roots and leaves of these plants were collected from communities within this region and an analysis of the phytochemical, mineral and vitamin components of these plant parts were carried out using standard methods. The results of the investigation revealed the a high presence of alkaloids (27.34 ± 0.15 and 12.98 ± 0.98), flavonoids (19.66 ± 0.04 and 3.71 ± 0.46) and phenols (15.10 ± 0.11 and 0.07 ± 0.42) in the leaves and roots of *Annona muricata* while alkaloids (35.55 ± 0.95 and 50.90 ± 0.83), tannins (28.70 ± 0.19 and 55.37 ± 0.47) and terpenoids (18.23 ± 0.08 and 41.21 ± 0.16) were observed in leaves and roots of *Fagara zanthoxyloide*. Mineral analysis revealed the presence of iron (20.23 ± 0.01 and 5.21 ± 0.02), calcium (3.67 ± 0.06 and 1.59 ± 0.01), copper (2.17 ± 0.011 and 0.16 ± 0.01) and magnesium (3.04 ± 0.01 and 2.18 ± 0.005) in leaves and roots of *Annona muricata* and iron, copper (2.53 ± 0.011 and 7.38 ± 0.017) and zinc (5.16 ± 0.02 and 5.32 ± 0.011) in leaves and roots of *Fagara zanthoxyloide.* The leaves and roots of both plants also showed the presence of folate (26.82±0.48 and 23.47±0.03 for *A. muricata* and 15.82±0.18 and 20.63±0.91 for *F. zanthoxyloide*) and ascorbate (31.97±0.03 and

**Corresponding author: E-mail: oghenekarouche@gmail.com;*

26.89±0.19 for *A. muricata* and 13.86±0.13 and 30.21±0.01 for *F. zanthoxyloide*) in appreciable quantities while vitamins D, E and K were also observed in minute concentrations in both plant samples. These results may thus suggest that these plants from this region as a result of their rich nutrients and bioactive compositions may play a large role in alleviating the salient nutritional, physiological and medical challenges observed among people within this region.

Keywords: Annona muricata; Fagara zanthoxyloide; minerals; vitamins; phytochemicals.

1. INTRODUCTION

Humans depend on the availability of nutrients for the sustenance of life and well being. These nutrients are derived majorly from plant sources with the exception of a few which are available from animal sources. Several plants have been used by rural dwellers within Nigeria as a source of medicine, fibers, and food or to maintain nutritional nourishments in periods of famine, drought, and civil unrest [1]. Most of these have remained far underutilized in our urban areas with only a minimal proportion of the population with deep roots to their ancestral background carrying on this practice in urban areas in Nigeria.

With the increased interest in alternative medicines and healthy feeding which has been observed in the past decades, urban dwellers have widened their scope to embrace the possible nutritional and medicinal value attached to several plants observed around cities and towns as well as villages [1,2,3]. The growing concern of the alternatives has spurred research into several plants to further broaden the genetic diversity and suggested varieties in nutritionally viable alternatives to the increasing food and malnutrition problems [4] especially in south-southern Nigeria.

The south-southern region of Nigeria as a highly explorative and industrialized region may result in an unusual change in the environmental conditions of the ecosystem in this region. Changes in the environmental conditions such as UV light, soil salts, drought *etc* have been known to result in changes in the bioactive components of plant species. These bioactive compounds are what elicit several physiological responses which cause alleviation from the symptoms of certain illness [5]. The level of these compounds varies depending on plant and location.

Annona muricata; a plant with widely acclaimed historical benefits to human beings [6] and commonly referred to as sour soup in Nigeria. Its fruit is the main portion consumed by most south-southerner Nigerians who term it a luxury fruit due to its high purchase cost. The plant have been acclaimed to possess hepatoprotective, antihypertensive and antiplasmodic potentials; as well as been used in the treatment of neuralgia, palpitation, parturition, rashes, rheumatism, ringworm, skin disorders, spasms, tumors and ulcers [7] with little mention of its nutritional potential.

Fagara zanthoxyloide is another ethnomedicinal plant which belongs to the family *Rutaceae*. It is an indigenous plant that is widely used as a chewing stick for tooth cleaning in West Africa, as well as various other purposes. *Fagara zanthoxyloide* has been acclaimed to possess antiplasmodial activity and have been used in the treatment of elephantiasis, impotence, sexually transmitted infections such as gonorrhea, abdominal pain and malaria [8,9,10].

2. MATERIALS AND METHODS

2.1 Plant Materials

The leaves and roots of *Annona muricata* (AM) was obtained for Alakahia community (3.92°N, 7.80°E) in Obio/Akpor Local government area of Rivers state; while leaves and roots/stems of *Fagara zanthoxyloide* (FZ) was obtained from Opoo community (8.28°N, 3.67°E) in Itesiwaju Local Government Area of Oyo State. The plant materials alongside pictures were identified by the experts in the Department of Plant Science and Biotechnology, University of Port-Harcourt and the Chief Technologist, University of Ibadan Herbarium (UIH). A voucher copy was placed in the herbarium for reference.

The leaves (after separation from the stalk) and the roots were washed and air dried at room temperature (25°C) until constant weight was obtained and were then milled with a Thomas-Willey (model 3383L40) mechanical grinder into a uniform coarse powder. Three hundred grams of each of the dried powdered samples were placed in a conical flask and extraction was performed using 3 litres of absolute ethanol for a

period of 1 week. The mixture was agitated at an interval of 48 hours on a rotary shaker. The extracts were centrifuged twice at 1500rpm for 15 min in a Wilten-bioteknika Microspin-12 LCM-3000 centrifuge, filtered with Whatman No 1 filter paper, evaporated to dryness at 40°C with a rotary evaporator and lyophilized to recover the residue as sticky pastes which were stored at 4°C in a refrigerator for further to use.

2.2 Phytochemical Analysis

The powdered extract samples were screened for phytochemical constituents according to methods described by [11,12,13].

Spectrophotometric quantification of the tannin concentration in sample was performed by the method described in [14] and [15] using a Spectrum Lab 23A spectrophotometer. The method employed in [14] and [16] were used in the quantitative estimation of flavonoids content in samples. Phenols, alkaloid and saponin content were determined spectrophotometrically by the method described in [14]. Saponin content was assayed by the method of [14] and [17].

2.3 Mineral Analysis

The atomic absorption spectrophotometric method (GF-AAS, Analytik Jena Vario-6) was employed in the evaluation of the potassium, calcium, magnesium, copper and iron concentration according to procedures set aside in AOAC, 2010. The sodium and potassium concentrations were measured by flame photometry as described in [18].

2.4 Vitamin Analysis

The concentrations of retinol and α-tocopherol were assayed by the method stipulated in [19] and [20]. Thiamine and niacin were assayed by the method of [21] while riboflavin, Vitamin K and folate were assayed by [22] prescribed methods.

3. RESULTS

Phytochemical analysis of the plants revealed the presence of alkaloids, flavonoid, tannins, and steroids in the leaves and roots of both plants used in the study. Saponin was however not observed in the roots of *Annona muricata* while present in *Fagara zanthoxyloide* as seen in Table 1 below. Quantitative examination showed a high presence of phytochemicals in the leaves of *Annona muricata* than in the roots with the exception of anthraquinone content which was observed to be significantly higher ($p \leq 0.05$) in the roots when compared with the leaves.

The result of *Fagara zanthoxyloide* showed a high concentration of phytochemicals in the roots on comparison with the leaves. The roots were observed to be rich in alkaloids, tannins and terpenoid which were also significantly higher ($p \leq 0.05$) in roots than in the leaves (Table 2).

Mineral analysis revealed significantly high ($p \leq 0.05$) concentrations of iron, copper, zinc and calcium in the leaves of *Annona muricata* when compared with the roots which had a moderate concentration of phosphorous, calcium, zinc and sodium. The roots and leaves of *Fagara zanthoxyloide* were observed to be also rich in the following minerals iron, copper, zinc, and calcium.

Analysis of vitamins revealed varying concentrations of vitamins with significantly high ($p \leq 0.05$) concentrations of folate and ascorbate observed in both *Annona muricata* and *Fagara zanthoxyloide*. Leaves of *Annona muricata* were observed to contain significantly higher ($p \leq 0.05$) concentrations of vitamins D, E and K.

Table 1. Phytochemicals in leaves and roots of *Annona muricata* and *Fagara zanthoxyloide*

Phytochemical	*Annona muricata*		*Fagara zanthoxyloide*	
	Leaves	**Roots**	**Leaves**	**Roots**
Alkaloids	++	++	++	++
Anthraquinones	-	+	+	+
Flavonoids	++	+	+	++
Tannins	++	+	+	++
Terpernoids	+	++	+	++
Saponins	+	-	+	++
Steroids	++	+	+	+
Phenols	++	-	+	++

Highly present: ++, Present: +, Absent: -

Table 2. Quantitative phytochemicals in the leaves and roots of *Annona muricata* and *Fagara zanthoxyloide*

Phytochemical (mg/100g)	*Annona muricata*		*Fagara zanthoxyloide*	
	Leaves	**Roots**	**Leaves**	**Roots**
Alkaloids	27.34 ±0.15 ^{a}	12.98 ± 0.98^{a,b}	35.55 ± 0.95^{a,c}	50.90 ± 0.83^{b,c}
Anthraquinones	2.35 ± 0.05^{b,c}	12.50 ± 0.06^{b}	6.41 ± 0.43^{a,b}	7.24 ± 0.07^{c,d}
Flavonoids	19.66 ± 0.04^{c,d,e}	3.71 ± 0.46^{b,c}	3.27 ± 0.34^{c,e}	8.63 ±0.27^{a,c}
Tannins	11.24 ± 0.05^{a,c}	3.86 ± 0.22^{c,d}	28.70 ± 0.19^{a,e}	55.37 ± 0.47^{b,c,e}
Terpernoids	8.19 ± 0.11^{b,d}	5.21 ± 0.19b,^{c}	18.23 ± 0.08^{c,d,e}	41.21 ± 0.16^{c}
Saponins	6.32 ± 0.14 ^{a,e}	1.25 ± 0.07^{a,d,e}	7.43 ± 0.41^{a,d}	19.44 ± 0.59^{a,e}
Steroids	7.34 ± 0.06 ^{e}	3.69 ± 0.14^{c,e}	5.38 ± 0.48^{c,d,e}	6.37 ± 0.11^{c,e}
Phenols	15.10±0.11^{a,c}	0.07±0.42^{b,c}	2.17±0.2^{a,d}	13.23±0.17^{c,d}

Result expressed as Mean ± Standard error of Mean of triplicate determinants. Values with different superscript are statistically significant (p≤0.05)

Table 3. Mineral content of the leaves and roots of *Annona muricata* and *Fagara zanthoxyloide*

	Annona muricata		*Fagara zanthoxyloide*	
	Leaves	**Roots**	**Leaves**	**Roots**
Phosphorus (mg/100g)	0.60 ± 0.01^{c}	1.81 ± 0.02^{a,b}	0.35 ± 0.03^{a,d}	0.72 ± 0.011^{a}
Calcium (%)	3.67 ± 0.06^{a,c}	1.59 ± 0.01^{c,d}	0.19 ± 0.020^{a}	1.03 ± 0.015^{b,d}
Magnesium (mg/100g)	3.04 ± 0.01^{b,d}	2.18 ± 0.005^{b,d}	0.27 ± 0.01^{c,d}	0.47 ± 0.040^{a,c}
Sodium (%)	0.36 ± 0.38^{a,d}	1.08 ± 0.015^{a}	0.27 ± 0.350^{b,c}	0.167 ± 0.011^{b,d}
Potassium (%)	0.47 ± 0.021^{c,e}	1.68 ± 0.040^{c}	0.28 ± 0.005^{a,e}	0.57 ± 0.012^{c,d,e}
Zinc(mg/100g)	0.34 ± 0.040^{c}	1.35 ± 0.010^{d,e}	5.16 ± 0.02^{d}	5.32 ± 0.011^{d,e}
Iron (mg/100g)	20.23 ± 0.01^{b,d}	5.21 ± 0.02^{a,d,e}	10.01 ± 0.01^{b,c,d}	15.02 ± 0.02 ^{c,e}
Manganese (%)	0.33 ± 0.040^{a,c}	0.21 ± 0.01^{c,d}	0.03 ± 0.001^{d,e}	BDL
Copper (mg/kg)	2.17 ± 0.011^{a}	0.16 ± 0.01^{a,b}	2.53 ± 0.011^{b,c}	7.38 ± 0.017 ^{a,c}

Result expressed as Mean ± Standard error of Mean of triplicate determinants. Values with different superscript are statistically significant (p≤0.05)

Table 4. Concentration of Vitamins in the leaves and roots of *Annona muricata* and *Fagara zanthoxyloide*

	Annona muricata		*Fagara zanthoxyloide*	
	Leaves	**Roots**	**Leaves**	**Roots**
Retinol (µg/100g)	3.81±0.14^{a,c}	1.97±0.09^{a,c,d}	0.16±0.17^{b}	-
Thiamine (mg/Kg)	5.86±0.02^{c,d}	5.10±0.38^{a,b}	4.27±0.92^{a,c}	5.62±0.03 ^{a,}
Riboflavin (mg/kg)	9.72±0.29^{c,e}	7.89±0.11^{a,d,e}	6.28±0.02^{c,e}	10.21±0.27^{c,d}
Niacin (mg/Kg)	4.86±0.19 ^{a}	4.23±0.32^{c,d}	9.18±0.19^{d,e}	8.23±0.81 ^{d,e}
Pyridoxine (mg/Kg)	7.91±0.10^{b,c}	5.84±0.81^{c,e}	10.63±0.02^{b,c}	9.02±0.73^{b,d}
Folate (mg/Kg)	26.82±0.48^{a,b}	23.47±0.03^{b,c}	15.82±0.18^{a,c}	20.63±0.91^{,d,e}
Ascorbate (mg/Kg)	31.97±0.03^{a,b}	26.89±0.19^{d,e}	13.86±0.13^{c,e}	30.21±0.01^{c,d}
Vitamin D (mg/Kg)	4.21±0.21^{c,d,e}	0.91±0.16^{c,e}	1.11±0.26^{b,d}	3.21±0.49^{b,d,e}
Vitamin E (mg/Kg)	5.82±0.01^{a,d}	0.18±0.19^{c,d}	0.27±0.48^{a,c,e}	5.08±0.04^{c}
Vitamin K (mg/Kg)	7.10±0.24 ^{a}	1.92±0.03^{b}	0.18±0.39^{,c}	4.61±0.85^{,e}

Result expressed as Mean ± Standard error of Mean of triplicate determinants. Values with different superscript are statistically significant (p≤0.05)

4. DISCUSSION

Environmental stress has been implicated as a route to the adaptive and defensive mechanisms exhibited by plants which result in the bioaccumulation of an array of phytochemical compounds more in plants in some environment than in others [23,24]. Plants growing in different environments could accumulate various levels of several metabolites which elicit several physiological responses in humans *e.g* high temperature has been seen to induce phytochemical accumulation, affecting the concentration of antioxidants.

The phytochemicals seen in *Annona muricata* and *Fagara zanthoxyloide* of south-southern Nigeria origin have been implicated in several studies to elicit several physiological properties. High phenol content in plants may induce physiological responses which have been implicated in treatment and management of haemolytic anaemia [25,26] this may be the reason for the antisickling properties of the leaves of *Annona muricata* and roots *Fagara zanthoxyloide* proposed by traditional medicine dispensers in this region. Phenols have also been shown to possess antioxidant properties [27,28] which according to the publication by [29] stern from the substitution of the hydroxyl groups in the aromatic rings of phenolics. With regards to the antisickling and anaemic curative potential, these plants may enhance the total antioxidant scavenging potential of human blood by binding to the membrane of red cells thus prolonging life span [30]. The presence of terpenoid has been known to elicit stimulation of the immune system [31]. Thus these plants may be applied to secondary immunodeficiency conditions such as; HIV/AIDs, graft vs host diseases, leukaemia and lymphoma.

Mineral deficiency is often observed in children of this region but with little prevalence. Plants with similar mineral constituents with that observed in *Annona muricata* and *Fagara* zanthoxyloide from this region through research have been known to control osmotic balance, reduce blood pressure as well as aid in bone formation. The presence of calcium in high amounts in the leaves of *Annona muricata* may be essential for blood clotting (hence coping with internal haemorrhage), bone formation and as a vital co-factor for the process of erythropoiesis [32]. Iron has been known to play a part in haemoglobin formation as well as aid in the oxidation of biomolecules [33]. In synergy with copper and cobalt, iron observed in *Moringa oleifera* was observed to stimulate bone marrow activity and enhance red blood cell production and maturation [34]. Thus, their presence in these plants studied may help in the use of these plants as blood boosters. Copper on the one hand, also aid proper absorption of iron from the gastrointestinal tract, thereby increase iron concentration as well as boosting iron stores. The minerals in these plants may thus be used to combat micronutrient deficiency. Zinc also observed in *Fagara zanthoxyloide* is known to play a pivotal role as essential components of several enzyme systems such as carbonic anhydrase, alanine peptidase, carboxypeptidase, carbonic anhydride [32]. Thus, it in these plants may imply the benefit of the plants to protein synthesis, cell differentiation and replication as well as increased immunity.

The vitamin components of these plants may prove their relevance in several nutritional deficiency disorders. The presence of vitamin E and riboflavin have been known to induce antioxidant properties when consumed and this protects cells of the body against free radical-induced oxidative damage [35]. The absence of these vitamins predisposes cell membrane to damages resulting in anaemia [36]. A diet rich in riboflavin has been linked to the proper maintenance of the connective tissues thus facilitating wound healing [37]. The concentration of niacin and riboflavin present may also aid co-enzyme formation leading to increased oxidative phosphorylation and thus energy production through the electron transport chain [36]. Retinol although observed in small quantities in the plants used in this study may in conjunction with ascorbic acid lead to an increase in iron absorption from the gastrointestinal tract and its release from iron stores [38]. Thus promoting the proliferation of the red blood cells in the bone marrow and reducing anaemic condition observed among young women and geriatric individuals in this region.

5. CONCLUSION

Plants are a great source of food and medicine for humans. The proposed acclaimed effect of these plants by traditional healers is due to the activity of several bio-molecules in them. An analysis of *Annona muricata* and *Fagara zanthoxyloide* has revealed that these plants from this region accumulate a high amount of phytochemicals and possess vitamins and minerals which can help in cases of micronutrient deficiency as well as alleviate symptoms observed in several physiological conditions observed within this region.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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