*A*ntioxidative potentials of *Annona muricata* pulp and *Allium cepa* bulb juices

ABSTRACT

Aim: To establish the *invitro* antioxidative potentials of juice samples of*Annonamuricata*(soursop) fruit pulp and*Alliumcepa*(onion) bulb.

Study design: The quasi experimental design was adopted in this study.

Place and duration of study: Springboard Laboratories, Awka, Nigeria, in June 2022.

Methodology: The parameters assayed for were; ferric reducing antioxidant power (FRAP), nitric oxide, ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid), superoxide, hydrogen peroxide (H_2O_2), DPPH (2,2-diphenyl-1-picrylhydrazyl), hydroxyl radical scavenging activities, total phenol content, total antioxidant capacity, and total flavonoids content using standard laboratory techniques at 100, 200 and 300 mg/ml concentrations of the sample in triplicates.Appropriate standards were set up alongside the tests to determine antioxidant properties of the test sample.

Results: The results showed that *Annona muricata*pulp and *Alliumcepa* bulbjuices, at the concentrations studied, possesses these antioxidant properties studied in varying quantities. It was also observed that values for total antioxidant capacity for soursop juice at 300 mg/ml concentration, and ABTS scavenging activity for onion juice at all concentrations were significantly higher than the reference. The value obtained for DPPH scavenging activity for onion juice at 300 mg/ml concentration was statistically similar to the reference.

Conclusion: Both plants' parts are natural sources of exogenous antioxidants which can be exploited to benefit humans.

KEYWORDS: *Allium cepa* bulb, *Annona muricata* pulp, Antioxidant properties, *In-vitro* ~~study~~, Juice

1. INTRODUCTION

Reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, singlet oxygen, hydrogen peroxide, are by-products of metabolism in living organisms [1][2]. The ROS play some physiologic roles in the body, and the body has its own mechanisms of keeping them at low levels within cells. However, environmental stressors like heavy metals, pollution, UV radiations, and xenobiotics increase the production of ROS in the body. An imbalance between production ~~and~~ accumulation of ROS and their detoxification in biological systems result in oxidative stress [3].

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Research has shown that oxidative stress is responsible for the onset and/or progression several diseases such as cancer, cardiovascular diseases, metabolic disorders, atherosclerosis, and diabetes [4]. The body is equipped with endogenous substances synthesized by the body that help in the detoxification of these ROS, while also relying on other exogenous substances supplied through diets [5]. These substances are known as antioxidants, and also referred to as free radical scavengers. They constitute a defense mechanism of the body, protecting it from the damaging effects of reactive oxygen species (ROS) exerted on the cells [6].Plants (fruits, vegetables and grains) are great sources of exogenous antioxidants [7].

Allium cepa Linn (Onion), a member of the genus *Allium*, is the most cultivated species of the genus. It is the second most important horticultural crop after tomatoes, and found in Africa, Asia, Europe and North America [8]. Most dishes made today include onion as an ingredient, due to their flavour and health benefits. Flavonoids and the alk(en)yl cysteine sulphoxides (ACSOs) are found in abundance in onions and are perceived to be beneficial to human health. Anthocyanins and flavanols, such as quercetin and its derivatives, are flavonoids subgroups found in onion. Onions, due to the presence of organo-sulfur and phenolic compounds [9], have ~~been~~ demonstrated to have anticarcinogenic properties, antiplatelet activity, antithrombotic activity, antiasthmatic and antibiotic effects [8].

Annona muricata L. (sour sop or graviola) is an evergreen, terrestrial plant whose parts are widely used in traditional medicine. It is a member of the Annonaceae family [10], native to the warmest tropical areas in South and North America and now widely distributed throughout tropical and subtropical parts of the world, including India, Malaysia and Nigeria [11]. *A. muricata* is an erect tree that can reach 5-8 m in height, and its edible fruits are large, heart shaped and green in colour, and the diameter varies between 15-20 cm [12]. The fruit is used as a natural medicine for neuralgia, arthritis, diarrhoea, dysentery, fever, malaria, parasites, rheumatism, skin rashes. It is also eaten to enhance milk production in lactating mothers. The leaves are employed in the treatment of cystitis, diabetes, headaches and insomnia, the administration of the leaves decoction is believed to exhibit anti-rheumatic and neuralgic effects [11]. The seeds are believed to possess antihelmentic potentials against worms and parasites.

This study reports the *invitro* antioxidant properties of juice samples of *Allium cepa* bulb and *Annona muricata* pulp.

2. METHODOLOGY

2.1 Sample Collection, Identification and Preparation

Fresh ripe fruits of soursop and bulbs of common onion were bought at Relief market, Owerri, Nigeria. The plant samples were identified bya taxonomist in the Department of Biology, Federal University of Technology, Owerri, Nigeria, ~~Dr. C. M. Duru~~, the authentication numbers were; FHI 110177 (*Annonamuricata* L.) and FHI 107561 (*Alliumcepa* L.). The samples were washed clean under running tap water, and were handled separately. The onion was cut into small pieces. The soursop fruit was cut open to collect the pulp, while the seeds were removed. The onion and soursop pulp were blended, filtered and the filtrates were used for tests.

2.2 Antioxidant Activity Assay

The following tests were conducted to determine the antioxidant properties of the juice samples of *Annona muricata* pulp and *Allium cepa* bulb:
The FRAP of the samples was determined by the method described by Pulido *et al.* [12]. Estimation of nitric oxide scavenging activity of the samples *in vitro* was measured using the method reported by Gupta *et al.* [13]. The total phenols in the samples were determined by the method reported by Mundhe *et al*. [14]. Total flavonoids content in the samples was determined by the method described by Mythri *et al*. [15].

Total antioxidant capacity of the samples was estimated by the phosphomolybdate method according to Jayaprakasha *et al*. [16]. Superoxide scavenging property of the samples was measured by the method reported by Sivakrishnan and Muthu [17]. The hydrogen peroxide scavenging activity of the samples was assayed by the method reported by Gulcin *et al*.[18].

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The extent of hydroxyl radical scavenging by the samples from Fenton reaction was quantified using 2'-deoxyribose oxidative degradation as reported by Gupta *et al*. [13]. The ability of the natural antioxidants of the samples towards scavenging the stable free DPPH radical was measured by the method of Mensor *et al*. [19]. The ABTS radical cation decolourisation assay was carried out according to the method of Shirwaikar *et al*. [20].

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2.3 Statistical Analysis

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The quasi experimental design was adopted for this study. The experiments were carried out in triplicates and results expressed as mean ± standard deviation. Data was analyzed using analysis of variance (ANOVA). Statistical significance was accepted at $p<0.05$.

3. RESULTS AND DISCUSSION

Table 1: Ferric reducing antioxidant property of juice samples of *Annona muricata* pulp and *Allium cepa* bulb

Sample concentration (mg/ml)	Antioxidant activity of Soursop juice (µg/ml)	Antioxidant activity of Onion juice (µg/ml)
100	1.67 ± 0.04^{a}	2.63 ± 0.12^{a}
200	1.80 ± 0.12^{b}	2.71 ± 0.04^{a}
300	2.26 ± 0.00^{c}	2.76 ± 0.03^{a}
Reference (gallic acid)	3.59 ± 0.02^{d}	3.59 ± 0.02^{b}

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different ($p<0.05$).

Table 2: Nitric oxide scavenging property of juice samples of *Annona muricata* pulp and *Allium cepa* bulb

Sample concentration (mg/ml)	Antioxidant activity of Soursop juice (µg/ml)	Antioxidant activity of Onion juice (µg/ml)
100	2.39 ± 0.02^{a}	2.29 ± 0.00^{a}
200	2.52 ± 0.03^{a}	2.32 ± 0.01^{a}
300	2.53 ± 0.41^{a}	2.46 ± 0.31^{a}
Reference (gallic acid)	3.44 ± 0.03^{b}	3.44 ± 0.03^{b}

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different ($p<0.05$).

Table 3: Total phenol content of juice samples of *Annona muricata* pulp and *Allium cepa* bulb

Sample concentration (mg/ml)	Concentration in Soursop juice (µg/ml)	Concentration in Onion juice (µg/ml)
100	2.99 ± 0.01^{a}	1.99 ± 0.11^{a}
200	4.87 ± 0.17^{b}	2.65 ± 0.04^{b}
300	6.31 ± 0.02^{c}	3.52 ± 0.09^{c}
Reference (gallic acid)	9.67 ± 0.08^{d}	9.67 ± 0.08^{d}

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different ($p<0.05$).

Table 4: Total flavonoids content of juice samples of *Annona muricata* pulp and *Allium cepa* bulb

Sample concentration (mg/ml)	Concentration in Soursop juice (µg/ml)	Concentration in Onion juice (µg/ml)
100	58.36 ± 0.12^{a}	58.83 ± 0.07^{a}
200	58.21 ± 0.02^{a}	58.88 ± 0.02^{a}
300	58.17 ± 0.02^{a}	59.19 ± 0.58^{a}
Reference (gallic acid)	59.33 ± 0.19^{b}	59.33 ± 0.19^{a}

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different ($p<0.05$).

Table 5: Total antioxidant capacity of juice samples of *Annona muricata* pulp and *Allium cepa* bulb

Sample concentration (mg/ml)	Antioxidant activity of Soursop juice (µg/ml)	Antioxidant activity of Onion juice (µg/ml)
100	3.98 ± 0.33[a]	2.60 ± 0.15[a]
200	4.53 ± 0.29[b]	3.88 ± 0.08[b]
300	13.09 ± 0.25[d]	8.24 ± 0.06[c]
Reference (gallic acid)	8.83 ± 0.05[c]	8.83 ± 0.05[d]

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

Table 6:Superoxide scavenging property of juice samples of *Annona muricata* pulp and *Allium cepa* bulb

Sample concentration (mg/ml)	Percentage inhibition for Soursop juice (%)	Percentage inhibition for Onion juice (%)
100	54.69 ± 0.93[a]	52.85 ± 0.69[a]
200	57.66 ± 0.62[b]	58.13 ± 0.67[b]
300	61.83 ± 0.55[c]	63.72 ± 0.87[c]
Reference (gallic acid)	70.31 ± 0.52[d]	70.31 ± 0.52[d]

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

Table 7: Hydrogen peroxide scavenging property of juice samples of *Annona muricata* pulp and *Allium cepa* bulb

Sample concentration (mg/ml)	Percentage inhibition for Soursop juice (%)	Percentage inhibition for Onion juice (%)
100	47.72 ± 0.00[a]	47.24 ± 0.03[c]
200	47.54 ± 0.03[ab]	46.54 ± 0.03[b]
300	47.44 ± 0.03[b]	46.21 ± 0.05[a]
Reference (gallic acid)	60.76 ± 0.20[c]	60.70 ± 0.23[d]

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

Table 8: Hydroxyl radical scavenging property of juice samples of *Annona muricata* pulp and *Allium cepa* bulb

Sample concentration (mg/ml)	Percentage inhibition by Soursop juice (%)	Percentage inhibition by Onion juice (%)
100	30.46 ± 1.36[c]	65.94 ± 0.64[c]
200	18.33 ± 0.46[b]	42.44 ± 0.32[b]
300	14.55 ± 1.21[a]	41.16 ± 0.32[a]
Standard (gallic acid)	80.45 ± 0.76[d]	80.45 ± 0.76[d]

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

Table 9: DPPH scavenging activity of juice samples of *Annona muricata* pulp and *Allium cepa* bulb

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Sample concentration (mg/ml)	Percentage scavenging activity by Soursop juice (%)	Percentage scavenging activity by Onion juice (%)
100	85.87 ± 0.14[a]	85.41 ± 0.72[a]
200	87.21 ± 0.17[b]	93.09 ± 0.86[b]
300	95.23 ± 0.53[c]	97.77 ± 0.25[c]
Reference (BHT)	98.24 ± 0.00[d]	98.24 ± 0.00[c]

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

Table 10: ABTS scavenging activity of juice samples of *Annona muricata* pulp and *Allium cepa* bulb

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Sample concentration (mg/ml)	Percentage scavenging activity by Soursop juice (%)	Percentage scavenging activity by Onion juice (%)
100	55.29 ± 0.11^{a}	67.13 ± 0.11^{b}
200	57.80 ± 0.29^{b}	68.18 ± 0.11^{c}
300	59.62 ± 0.21^{c}	71.14 ± 0.11^{d}
Reference (gallic acid)	61.31 ± 0.00^{d}	61.31 ± 0.00^{a}

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<0.05).

Antioxidant activity of a plant depends on the presence of certain biologically active compounds, especially polyphenols, carotenoids, and vitamin E and C [21]. Reducing power is also widely used in evaluating antioxidant activity of plant polyphenols, generally associated with the presence of reductants, which exert antioxidant action by breaking free radical chains by donating a hydrogen atom [22]. Radical scavenging activities are very important to prevent the deleterious role of free radicals in different diseases, including cancer.

This study showed that both samples possessed all *invitro* antioxidant properties assayed for in varying concentrations. The FRAP values (Table 1) for both samples increased in a dose-dependent manner and were significantly lower than the reference. For Soursop juice, all values were significantly (p<0.05) different from each other. While for onion juice, all values were statistically similar. Onion juice showed higher values at each concentration. The nitric oxide scavenging capacity values (Table 2) for both samples were significantly lower than the reference, and increased as the concentration increased. Soursop juice showed higher values at each concentration. All values obtained for each of the samples were not significantly (p>0.05) different from each other.

The total phenol content (Table 3) of both samples were significantly (p<0.05) lower than the reference, but significantly increased with increase in concentration. All values obtained for soursop juice were observed to be higher than those of onion juice at the same concentrations. This implies that soursop juice has more phenolic content than onion juice. The total flavonoids content (Table 4) of soursop juice were significantly (p<0.05) lower than the reference. The values were statistically similar, although they decreased with increase in concentration. While for onion juice, the values obtained were not significantly different from each other and the reference, they also increased with increase in concentration. Onion juice showed higher values, showing flavonoids content similar to the reference.

Total antioxidant capacity (Table 5) for both juice samples also increased with increase in concentration. It was also observed that, while the total antioxidant capacity of onion juice at all the concentrations were significantly lower than the reference, soursop juice showed value significantly higher than the reference at 300 mg/ml. Soursop juice possessed stronger total antioxidant capacity than onion juice, and even the reference at the highest concentration. This property of soursop can be exploited to benefit humans, specifically, higher concentrations. The superoxide scavenging activity values (Table 6) for both samples were significantly lower than the reference, increasing with increase in concentration. The values were also not significantly different from each other. Onion juice showed higher values at 200 and 300 mg/ml concentrations.

The hydrogen peroxide scavenging activity results (Table 7) showed that values for both samples were significantly less than that of the reference, with the highest values obtained at 100 mg/ml concentration. These values decreased with increase in concentration, with Soursop juice showing higher values at each concentration. The hydroxyl radical scavenging activity values (Table 8) for both samples were significantly lower than the reference, with the highest values obtained at 100 mg/ml concentration. The values were significantly different from each other, with onion juice showing higher values at each concentration. For both samples, the values obtained were observed to decrease with increase in concentration.

The results for DPPH scavenging activity, as presented in Table 9, showed that values for both samples were significantly less than that of the reference, except for onion juice at 300 mg/ml concentration. The values for both samples differed from each other significantly. Onion juice also showed higher values at each concentration, implying that onion juice has prospects for good DPPH scavenging property. The ABTS scavenging activity results (Table 10) showed that values for soursop juice were significantly less than that of the reference, while those for onion juice were significantly higher than the reference. The values for both samples increased with increase in concentration. This

indicated that onion juice possesses good aqueous phase and lipid peroxyl radicals scavenging activity.

The findings of this study agreed with the reports of Agu & Okolie [23], Gupta *et al*. [24], Fredotovic *et al*. [25], and Santas *et al*. [26], who maintained that plants are natural sources of exogenous antioxidants which are due to the presence of certain phytochemicals.

4. CONCLUSION

The juices of *Annona muricata* (Soursop) pulp and *Allium cepa* (Onion) bulb possessed *in vitro* antioxidant potentials. This was with regards to the ferric reducing antioxidant property, nitric oxide scavenging property, superoxide scavenging activity, total phenol content, total antioxidant capacity, total flavonoids content, hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity, DPPH and ABTS scavenging activities. Specifically, soursop juice possesses strong total antioxidant capacity, while onion juice possessed promising DPPH scavenging activity and very strong ABTS scavenging activity. Hence, both soursop pulp and onion bulb juices were sources of natural antioxidants that can be exploited for the benefit of human health.

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