

International Journal of Biochemistry Research & Review

Volume 32, Issue 8, Page 28-38, 2023; Article no.IJBCRR.107521 ISSN: 2231-086X, NLM ID: 101654445

Antioxidative Potentials of *Annona muricata* **Pulp and** *Allium cepa* **Bulb Juices**

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Authors' contributions

This work was carried out in collaboration among all authors. Author IHA managed the analyses of the study, Performed the statistical analysis and wrote the first draft of the manuscript. Author ICO designed the study. Author UCO handled the literature searches. Author UDI wrote the protocol. All *authors read and approved the final manuscript.*

Article Information

DOI: 10.9734/IJBCRR/2023/v32i8832

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/107521

> *Received: 05/08/2023 Accepted: 12/10/2023 Published: 18/10/2023*

Original Research Article

ABSTRACT

Aim: To establish the *in vitro* antioxidative potentials of juice samples of *Annona muricata* (soursop) fruit pulp and *Allium cepa* (onion) bulb.

Study Design: The experimental research design was adopted in this study, but without intervention.

Place and Duration of Study: Springboard Laboratories, Awka, Nigeria, in June 2022. **Methodology:** Traditional method of juice preparation was used to prepare the juices of the soursop fruit pulp and onion bulb. The parameters assayed for were; ferric reducing antioxidant power (FRAP), nitric oxide, ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid), superoxide,

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hydrogen peroxide (H₂O₂), 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 samples in triplicates. Appropriate standards were set up alongside the tests to compare antioxidant properties of the test sample.

Results: The results showed that *Annona muricata* pulp and *Allium cepa* bulb juices, at the concentrations studied, possessed 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 and total flavonoids content for onion juice were statistically similar to the reference. **Conclusion:** Both juices 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]. 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], and *Allium cepa* and *Annona muricata* are not left out.

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]. *Allium cepa* bulb can be consumed raw, as a vegetable, such as in salads. It can also be cooked, with other vegetables and food ingredients, or processed as pickles, paste, powder and flakes [10]. Onion juice have also been used in hair maintenance.

Annona muricata L. (soursop or graviola) is an evergreen, terrestrial plant whose parts are widely used in traditional medicine. It is a member of the Annonaceae family [11], 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 [12]. "*A*. *muricata* is an erect tree that can reach 5-8m in height, and its edible fruits are large, heart shaped and green in colour, and the diameter varies between 15-20cm" [13]. "The leaves are employed in the treatment of cystitis, diabetes, headaches and insomnia, the administration of the leaves decoction is believed to exhibit antirheumatic and neuralgic effects" [12]. The seeds are believed to possess anthelmintic potentials against worms and parasites. The fruit is used as a natural medicine for neuralgia, arthritis, diarrhoea, dysentery, fever, malaria, parasites, rheumatism, skin rashes. The fresh ripe fruit is

also eaten to enhance milk production in lactating mothers [14], as food.

The consumption of raw onion and the fresh ripe fruits of soursop is to maximize their health benefits. Thus, assessing the juice samples of both plant parts provides information on the closest form in which they are mostly consumed (that is, raw/fresh) by humans. This study reports the *in vitro* 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 by a taxonomist in the Department of Biology, Federal University of Technology, Owerri, Nigeria, Dr. C. M. Duru, the authentication numbers were; FHI 110177 (*Annona muricata* L.) and FHI 107561 (*Allium cepa* L.). The plant materials used were selected by simple random sampling technique. They 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:

2.2.1 Ferric reducing antioxidant property determination

The FRAP of the samples was determined by the method described by Pulido et al. [15]. A volume of 0.25 ml each of the samples was mixed with 0.25 ml of 200 mM sodium phosphate buffer pH 6.6 and 0.25 ml of 1 % potassium ferrocyanide. The mixture was incubated at 50 \degree C for 20 minutes, thereafter 0.25 ml of 10 % trichloroacetic acid was added and centrifuged at 2000 rpm for 10 min. Then, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.2 ml of ferric chloride and the absorbance was measured at 700 nm.

2.2.2 Nitric oxide scavenging activity estimation

Estimation of nitric oxide scavenging activity of the samples *in vitro* was measured using the method reported by Green et al. [16]. The reaction was initiated by adding 2.0 ml of sodium nitroprusside (100 mM), 0.5 ml of phosphate buffered saline (PBS, pH 7.4), 0.5 ml of samples (50 mg) and incubated at 25 \degree C for 30 minutes. 0.5 ml Griess reagent (1 % sulphanilamide, 2 % H3PO⁴ and 0.1 % naphthylethylene diamine dihydrochloride) was added and incubated for another 30 minutes. Control tubes were prepared without the samples. The absorbance was read at 546 nm against the reagent blank, in a spectrophotometer (Turner® 390).

2.2.3 Total phenol content determination

The total phenols in the samples were determined by the method reported by Malik and Singh [17]. The each of the samples (0.5 ml) was thoroughly mixed in 10 times volume of 80 % ethanol, and centrifuged at 10,000 rpm for 20 minutes. The supernatant was preserved. Then, the sample was re-extracted with 2.5ml 80 % ethanol and centrifuged 10,000 rpm for 10 minutes. The supernatants were pooled and evaporated to dryness. Then, the residue was dissolved in 1.0 ml of distilled water. The different aliquots (100, 200 and 300 mg/ml) were pipetted out and the volume made up to 3.0 ml with distilled water in each tube. Then, 0.5 ml of 1 N Folin-Ciocalteau reagent and 2 ml of sodium carbonate were added, and the tubes were placed in boiling waterbath for one minute. The tubes were cooled and the absorbance read at 650 nm, against a reagent blank. Standard gallic solutions (20 %, 0.2-1 ml) corresponding to 2.0 to 10 μg concentrations were also treated as above.

2.2.4 Total flavonoids content determination

Total flavonoids content in the samples was determined by the method described by Cameron et al. [18]. Flavonoids were extracted using 0.5ml each of the samples first mixed with methanol:water mixture (2:1) and secondly, with the same mixture in the ratio of 1:1. The mixtures were shaken well and allowed to stand overnight. The supernatants were pooled, the volume measured, and then concentrated and used for the assay. Then, 0.5 ml of the aliquot was pipetted out and evaporated to dryness. 4.0 ml Vanillin reagent (1 % vanillin in 70 %

sulphuric acid) was added and the tubes heated in a boiling waterbath (Hilmedics H-420) for 15 minutes. Varying concentrations of the standard was also treated in the same manner. The optical density was read at 340 nm. A standard curve was constructed and the concentration of flavonoids in each sample was calculated.

2.2.5 Total antioxidant capacity estimation

Total antioxidant capacity of the samples was estimated by the phosphomolybdate method according to Jayaprakasha et al. [19]. An aliquot (30 ml) of different concentrations (100, 200 and 300 mg/ml) of the test samples was mixed with 3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) and taken in test tubes. The tubes were capped with aluminium foil and incubated in a boiling waterbath at 95°C for 90 minutes. The reaction mixture was allowed to cool to room temperature and the absorbance of the solution measured at 695 nm against a blank containing 3 ml of reagent solution and the appropriate volume of the dissolving solvents. The blank was incubated under the same conditions as the test samples.

2.2.6 Measurement of superoxide scavenging activity

Superoxide scavenging property of the samples was measured by the method reported by Winterbourn et al. [20]. Superoxide anions were generated in the samples that contained in it 3.0 ml of mixture of 0.02 ml of the samples (20 mg), 0.2 ml of EDTA (0.1 M containing 1.5 mg of NaCN), 0.1 ml of NBT (1.5 M), 0.05 ml of riboflavin (0.12 mM) and 2.63 ml of phosphate buffer (0.067M, pH 7.6). The control tubes were also set up where DMSO was added instead of the samples. All the tubes were vortexed and the initial optical density measured at 560 nm. The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560 nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity.

2.2.7 Estimation of hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of the samples was assayed by the method reported by Ruch et al. [21]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (0.1M, pH 7.4). The samples at the different concentrations (100, 200, and 300 mg/ml) were added to H_2O_2 solution (0.6 ml) and the total volume made up to 3 ml. The absorbance of the reaction mixture was recorded at 230 nm. A blank solution containing phosphate buffer, without H_2O_2 was prepared. The extent of H_2O_2 scavenging of the samples was calculated and presented as percentage inhibition of H_2O_2 .

2.2.8 Measurement of hydroxyl radical scavenging activity

The extent of hydroxyl radical scavenging by the samples from Fenton reaction was quantified using 2'-deoxyribose oxidative degradation as reported by Kunchandy and Rao [22]. The reaction mixture containing 0.1 ml of deoxyribose (2.8 mM) , 0.1 ml of FeCl₃ (0.1 mM) , 0.1 ml of EDTA (0.1 mM), 0.1 ml of H_2O_2 , 0.1 ml of ascorbate (1.0 mM), 0.1 ml of KH2PO4-KOH buffer (20 mM, pH 7.4) and 20 µl of sample in a final volume of 1.0 ml was incubated at 37oC for 1 hour. After the incubation, 1.0 ml of 1% TBA was added and heated at 95°C for 20 minutes to develop colour. After cooling, the thiobarbituric acid reactive substance (TBARS) formed was measured spectrophotometrically at 532 nm against an appropriate blank. Results were expressed as percentage inhibition.

2.2.9 Measurement of DPPH scavenging activity

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. [23]. The samples (20 μl) were added to 0.5 ml of 0.1 mM methanolic solution of DPPH (0.3 mM in methanol) and 0.48 ml of methanol. The mixture left at room temperature for 30 minutes to react. Methanol served as the blank and DPPH in methanol, without the samples, served as the positive control while butylated hydroxytoluene (BHT) served as reference. After 30 minutes of incubation, the discoloration of the purple colour was measured at 518 nm. The radical scavenging activity was calculated as follows:

Scavenging activity $(%) = [100 - A518$ (sample) -A518 (blank)/A518 (blank)] × 100

2.2.10 Estimation of ABTS scavenging activity

The ABTS radical cation decolourisation assay was carried out according to the method of

Shirwaikar et al. [24]. The ABTS radical cations (ABTS+) were produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulphate. The mixture was allowed to stand in the dark at room temperature for 12 to 16 hours before use. Aliquots (0.5 ml) of the samples were added to 0.3 ml of ABTS solution and the final volume made up to 1 ml with ethanol. The absorbance was read at 745 nm and the per cent inhibition was calculated using the formula:

Inhibition (%) = $[(Control - test)/Control] \times$ 100

Gallic acid was used as reference to compare the activities of the samples, except for the DPPH scavenging activity assay.

2.3 Statistical Analysis

The experiments were carried out in triplicates and results expressed as mean ± standard deviation. Data was analyzed using analysis of variance (ANOVA) with repeated measures on Statistical Product and Service Solutions (SPSS) version 23. Duncan Multiple Range Test (DMRT) was used to test statistical significance at *p*<.05.

3. RESULTS AND DISCUSSION

Antioxidant activity of a plant depends on the presence of certain biologically active compounds, especially polyphenols, carotenoids, and vitamin E and C [25]. Reducing power is also widely used in evaluating antioxidant activity of plant polyphenols. It is generally associated with the presence of reductants, which exert antioxidant action by breaking free radical chains by donating a hydrogen atom [26]. Radical scavenging activities are very important to prevent the deleterious roles of free radicals in different diseases, including cancer.

This study showed that both samples possessed all the *in vitro* antioxidant properties assayed for in varying concentrations. The FRAP values (Table 1) for both samples increased in a dosedependent manner and were significantly lower than the reference. For Soursop juice, all values were significantly (*p*<.05) different from each other. While for onion juice, all values were statistically similar. Onion juice showed higher values at each concentration. There is a linear relationship between concentration of antioxidant compounds and assessment of FRAP of substances. The assay is also reproducible and

convenient. Compounds with the ability to convert Fe3+/ferricyanide complex to Fe2+/ferrous state have antioxidant potential [27]. 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*>.05) different from each other. Nitric oxide plays important roles in inflammation, but at high concentrations, they damage tissues and cause diseases. This toxic effects increase when they react with superoxide radical to yield peroxinitrite. Protonation of peroxinitrite forms peroxynitrous acid which is highly reactive and dangerous [28].

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*<.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 of total flavonoids content that compared well with the reference.

Total antioxidant capacity (Table 5) for both juice samples also increased with increase in concentration. It was also observed that the total antioxidant capacity of onion juice at all the concentrations were significantly (*p*<.05) lower than the reference. While, soursop juice showed values significantly(*p*<.05) 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, especially, higher concentrations. Phenolic and flavonoid compounds are antioxidant compounds in plants that deactivate free radicals. This is because they are able to donate hydrogen atoms to free radicals, and their structures are ideal structural for scavenging free radicals [27]. Studies have reported that total phenolic and flavonoid content correlate linearly with antioxidant capacity [29].

The superoxide scavenging activity values (Table 6) for both samples were significantly (*p*<.05) 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. Superoxide anion is dangerous to the components of the cell, and are scavenged efficiently by action of flavonoids [28].

The hydrogen peroxide scavenging activity results (Table 7) showed that values for both samples were significantly (*p*<.05) less than that of the reference. The highest values were obtained at 100mg/ml concentration. These values decreased with increase in concentration, with soursop juice showing higher values at each concentration. Hydrogen peroxide (H_2O_2) is generated during the activities of many oxidizing enzymes in biological systems. It is a strong oxidizing agent produced as a signal which stimulates cellular metabolic responses [30].

However, abnormal H_2O_2 accumulation results in oxidative stress and inflammatory reactions causing diseases such as cardiovascular diseases, cancer, and diabetes [31, 32].
Hydrogen peroxide-rapidly decomposes. peroxide rapidly decomposes, generating hydroxyl radical which initiates lipid peroxidation and cellular damage [33]. The ability of antioxidants from plants to regulate the generation of H_2O_2 generation is of particular interest in scientific research.

The hydroxyl radical scavenging activity values (Table 8) for both samples were significantly (*p*<.05) lower than the reference. Highest values were recorded at 100 mg/ml concentration. The values were significantly (*p*<.05) 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. Lipid peroxidation and various biological damage are mainly caused by hydroxyl radicals.

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

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<.05)

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

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<.05)

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

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<.05)

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

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<.05)

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

Values are means \pm standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (*p*<.05)

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

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<.05)

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	$87.21 \pm 0.17^{\circ}$	$93.09 \pm 0.86^{\circ}$
300	95.23 ± 0.53 °	97.77 ± 0.25 ^c
Reference (BHT)	98.24 ± 0.00 ^d	98.24 ± 0.00 ^c

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

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<.05)

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

Values are means ± standard deviations of triplicate determinations. Values on the same column bearing different superscript letters are significantly different (p<.05)

DPPH is very useful in the determination of the antioxidant activity of plant and microbial products. The results for DPPH scavenging activity (Table 9) showed that values for both samples were significantly (*p*<.05) less than 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. The value recorded at 300 mg/ml was statistically similar to the reference. This implies that onion juice has prospects for exerting good DPPH scavenging property at high concentrations. At room temperature, DPPH is a stable free radical. It is converted to a stable diamagnetic molecule by accepting an electron or hydrogen radical. This reaction is regarded as a model lyophilic radical activity [28]. The ABTS scavenging activity results (Table 10) showed that values for soursop juice were significantly (*p*<.05) less than that of the reference, while those for onion juice were significantly (*p*<.05) higher than the reference. The values for both samples increased with increase in concentration. This indicated that onion juice possessed good aqueous phase and lipid peroxyl radicals scavenging activity.

The findings of this study agreed with the reports of Agu & Okolie [34], Gupta et al. [35], Fredotovic et al. [36], and Santas et al. [37], 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 FRAP, nitric oxide scavenging property, superoxide scavenging activity, total phenol content. And also, total antioxidant capacity, total flavonoids content, hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity, DPPH and ABTS scavenging activities. Specifically, soursop juice possessed strong total antioxidant capacity at high concentrations. While, onion juice possessed promising DPPH scavenging activity and very strong ABTS scavenging activity. These antioxidant activities exhibited by the plant samples were as a result of bioactive compounds they contain. Hence, both soursop pulp and onion bulb juices were sources of natural antioxidants that can be exploited for the benefit of human health.

ACKNOWLEDGEMENT

The Tertiary Education Trust Fund (TETFund, TEFT/DASTD/POLY/IMO

STAFF/TSAS/2020/VOL.1) of the Federal Government of Nigeria provided sponsorship for the Ph.D programme that led to the conduct of this research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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