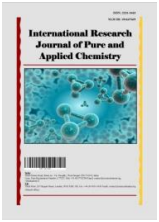

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# Comparative Phytochemical and *in vitro* Antioxidant Screening of the Root and Stem Bark of *Annona muricata* Linn

**C. E. Anarado[1*], F. M. Chukwubueze[1], C. J. O. Anarado[1], N. L. Umedum[1] and C. B. Nwanya[1]**

*[1]Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To compare the phytochemicals and antioxidant activities of stem bark and root extracts of *Annona muricata.*
**Methodology:** The stem bark and root of *Annona muricata* were collected, washed, air-dried, ground and each extracted with methanol, ethyl acetate and n-hexane. The extracts were analysed for the presence of phytochemicals. Antioxidant screening was also carried out on the samples.
**Results:** Cardiac glycosides were present in all the extracts of both root and stem bark. Alkaloids were present in moderate abundance in all the extracts except the ethyl acetate stem bark extract. Saponins and tannins were found in methanol extracts of both parts and also in very high abundance but the stem contained higher amount of saponins while alkaloids and tannins were found more in the root. Flavonoids were only found in the ethyl acetate stem bark extract. Steroids were absent in all the extracts except n-hexane root extract. The root showed greater enzymatic antioxidant activities than the stem bark. The solvent polarity affected the phytochemical found in each extract. The antioxidant activities of the catalase, superoxidase dismutase and glutathione

**Corresponding author: E-mail: ce.anarado@unizik.edu.ng;*

peroxidase were significantly higher in the root of *Annona muricata* than in the stem. Conversely, peroxidase showed a significantly higher activity in the stem than in the root.
**Conclusion:** The stem bark and root exhibited good antioxidant properties, so there is need to isolate the compounds responsible for antioxidant property exhibited by the plant parts.

*Keywords: Annona muricata; stem; root; antioxidant; methanol; ethyl acetate; N-hexane; phytochemicals.*

## 1. INTRODUCTION

Plants have been used to help mankind sustain its health since the dawn of medicine [1].They are the richest bioresources of both traditional and modern drugs and have been the basis of health preservation and care. According to WHO, about 21,000 plant species have potentials of being used as medicinal plants, where as more than 30% of the entire plant species are already in use and out of the 250,000 to 500,000 species of existing plants on earth, only about 300 species are utilized in the food, pharmaceutical, cosmetics and perfume industries [2,3,4,5]. About 80% of the world's population still depend solely on herbal medicine for treatment of diseases especially in Africa and other developing countries. The developed world has also witnessed an ascending trend in the utilization of medicinal plants within the past decades. This is because of its effectiveness in disease treatment and zero toxicity or adverse effect when used [6,7,1]. Plants typically contain mixtures of different phytochemicals known as Secondary Metabolites. The phytochemicals in plants have been a pivotal pipeline for pharmaceutical discovery. They act individually, additively or in synergy to improve health [8]. The importance of these active ingredients of plants in agriculture and medicine has stimulated significant scientific interest in the biological activities of these substances. The combined actions of the substances tend to increase the activity of the main medicinal constituents by speeding up or slowing down its assimilation in the body. It also increases the stability of the active compounds and reduces adverse side effects [7]. Plants with a long history of use in ethno medicine are considered rich sources of active phytoconstituents that provide medicinal benefits against various diseases. One of such plants with extensive traditional use is *Annona muricata*. However, phytochemical analyses are conducted on medicinal plants to ascertain the presence of the constituents that are known to exhibit medicinal factors as well as physiological activities [9]. The analysis is also essential to promote the proper use of herbal medicine in order to determine their potential as a source for the new drugs [10]. *Annonaceae* is a very homogenous family producing edible fruits and oils. It comprises of about 130 genera and more than 2300 species with *Annona squamosa, Annona muricata, Annona reticulata,* and *Annona cherimoya* being the most predominant [11,12,8]. *Annona muricata* L. also known as soursop, graviola and guanabana, is a species of *Annonaceae* family that has been widely studied in the last decades due to its therapeutic potentials [13]. It is an evergreen plant that is mostly distributed in tropical and subtropical regions of the world. *Annona muricata* is a slender, small, and cold-intolerant tree, generally reaching heights of 4-6 meters. It is adapted to areas of high humidity and relatively warm winters; temperatures below 5°C (41°F) causes damage to leaves and small branches, and temperatures below 3°C (37°F) can be fatal. A wide array of ethnomedicinal activities is attributed to different parts of *A. muricata*, and indigenous communities in Africa and South America extensively use this plant in their folk medicine. The fresh leaves when crushed are applied on skin eruption for quick healing. A poultice of young *A*. *muricata* leaf is applied on the skin to alleviate rheumatism and other skins like eczema. When applied during the healing of wounds it results in less or no skin scars. The decoction can also be used as wet compress on swollen feet and other inflammations. The juice of the fruits is taken orally as herb remedy of arthritis, haematuria and liver ailments. The leaf tea is used for catarrh in the Peruvian Andes. The seed extracts are used to kill external parasites, head lice, and worms. In the Peruvian Amazon, the bark, root and leaves are used for the treatment of diabetes as well as sedative and antispasmodic drugs. The leaf or bark tea or combination of both is used as a sedative and heart tonic by the indigenes of Guyana. In the Brazilian Amazon, a leaf tea is used for liver problems and the oil of the leaves and unripe fruit is mixed with olive oil and used externally for neuralgia, rheumatism, and arthritic pain [14,12,15]. *Annona muricata* is also known to has a wide potent anti cancerous agents coined as Acetogenins which play a key role towards inhibiting cancer cells [8]. The plant possesses

major pharmacological activities which include cytotoxic, antileishmanial, treatment of hypertension, skin diseases, Fever, pain, diabetes, respiratory illness, internal and external parasites wound healing, antimicrobial activity, anticarcinogenic, anti malaria, anti ulcer and genotoxic effect. Most of the beneficial activities in human health are attributed to the presence of bioactive compounds (BC) with several *in vitro* and in vivo biological activities, including anti-inflammatory, antitumoral, and analgesic effects [16,17,18]. The bark, leaves, and roots are considered sedatives, antispasmodic, hypoglycemic, hypotensive, diuretics, neuralgia The fruits of *A. muricata* are extensively used to prepare syrups, candies, beverages, ice creams. The fruit and the fruit juice are taken for worms and parasites, to cool fevers, increase mother's milk after childbirth, Galactogogue, Obesity, gastritis, dyspepsia, diabetes, inflammation, cancer, Stomach pain, hypertension, Kidney disorders, liver diseases and leprosy and as an astringent for diarrhea and dysentery [8,19,20,21]. Studies have linked *A. muricata*-derived compounds to a variety of anticancer effects including cytotoxicity, induction of apoptosis, necrosis, and inhibition of proliferation on a variety of cancer cell lines, including breast, prostate, colorectal, lung, leukemia renal, pancreatic, hepatic, oral, melanoma, cervical, and ovarian cancers and some compounds isolated from defatted seeds from ripe soursop have been reported to be responsible for these anti cancer effects [22,23]. Another study on effects of methanol extracts of *Annona muricata* on biochemical indices of alloxan induced diabetic rats has reported that methanol leaf extract of *A. muricata* could offer some benefits in the management of diabetic complications especially with regard to cardiac and renal health [24]. The thrombolytic activity and Antidiarrheal activity of fruit extract of *Annona muricata* was assessed and the result revealed that the methanolic extracts of *Annona muricata* fruits showed 8.98 ±4.08, 11.87 ±7.32, 12.72±3.92, 17.67± 6.086 and 18.33±3.87% of clot lysis at a concentration of 2,4,6,8, and 10 mg/ml, respectively and all of the aliquot of extracts of *Annona muricata* fruits were found to be effective in a dose dependent manner against castor oil induced diarrhea on experimental mice [25]. Some minerals have been reported to be found in different parts of *Annona muricata* plant like K, Ca, Na, Cu, Fe and Mg [26]. Phytochemical analysis and antioxidant activity of *A. muricata* showed that the ethanol leaf extract contained steroid, alkaloid, flavonoid, phenolic and saponin and also has antioxidant activity by scavenging DPPH radical [27]. *In vitro* study conducted on the leaf of *A. muricata* revealed that *A. muricata* leaf extract possessed anti-inflammatory activity as it inhibited the inflammatory mediators, TNF-a, IL-1b, IL-6 and nitric oxide (NO) [28].

## 1.1 Antioxidants

Antioxidant means "against oxidation". When oxidation occurs in biological cell system, it causes damage or death to the cell. Therefore, any substance at low concentrations compared to that of an oxidizable substrate that significantly delays or prevents oxidation of that substrate is called as antioxidant. They are compounds responsible for hindering autoxidation reactions in food system and reducing oxidative stress in human body. In human body, about 5% of the inhaled oxygen is converted into reactive oxygen species which encompasses the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage. As a defense mechanism against reactive oxygen species, addition of antioxidants is required to food system. Antioxidants play vital role in preserving the quality of food and maintaining health of human being.

Natural antioxidants are extracted, usually in a mixture of several compounds, from variable sources e.g plants. The composition of the mixture containing active substance(s) and other compounds, which may be inactive or possessing negligible activities, depend on the plant variety, agro-technology, climatic conditions, degree of ripeness, and many other factors.

There are five major types of antioxidants namely: Primary antioxidants, Oxygen scavengers, Secondary antioxidants, Chelating agents and enzymatic antioxidants.

Enzymatic antioxidants are those enzymes which function either by removing dissolved or head space oxygen, e.g., glucose oxidase, or by removing highly oxidative species, e.g., superoxide dismutase, glutathione peroxidase, catalase (CAT) and glutathione reductase, which catalyze free radical quenching reactions [29,30,31,32]. Recent studies show that the phenolic compounds are the major

phytochemicals responsible for the antioxidant potential of soursop leaves and fruits [17,32]. Among them, the phenolic acids (mainly hydroxycinnamic acids), flavonoids, and tannins (including procyanidin dimers) were determined in *A. muricata* L. leaves, pulp, and seeds [32]. In the course of this study, we will be comparing the phytochemical and antioxidant properties of the Stem barks and Roots of *Annona muricata*.

## 2. METHODOLOGY

The roots and stem barks of *Annona muricata* were collected from Onitsha, Anambra State and were identified in Department of Botany, Nnamdi Azikiwe University, Awka. Both were air-dried for 3 weeks and ground to powder using wood land electric grinding machine. The powdered samples were stored to be used for analysis.

### 2.1 Extraction of the Phytochemicals

In 100 ml of each of the three solvents – methanol, ethyl acetate and n-hexane, 10 g each of the powdered roots and stem barks were soaked for 48 hours with intermittent stirring. The mixtures were filtered, concentrated using rotary evaporator. The concentrated extracts were then used for the analysis. The solvents were selected based on the differences in their polarities-methanol (polar), ethyl acetate (semi polar), n-hexane (non polar).

### 2.2 Qualitative Phytochemical Analysis of the Plant Samples

Qualitative analyses were carried out using the standard methods described by Edeoga et al. (2005), Raaman (2006) and Harborne (1998) to ascertain the presence of phytochemicals [33,34,35].

### 2.3 Test for Presence of Alkaloids

**Wagner's reagent test:** Reagent: Wagner's reagent (2 g of iodide and 3 g of potassium iodide are weighed, mixed and dissolved in 30 ml distilled water and made up to 100 ml with distilled water).

**Procedure:** In a test tube, 1 ml of the filtrate was added, followed by addition of 1 ml of wagner's reagent. The solution was mixed properly and the colour change was observed. A reddish brown precipitate indicated presence of alkaloids.

**Meyer's reagent test:** Reagents: Dissolve Meyer's reagent (1.4 of mercuric chloride in 60 ml distilled water and 4.5g of potassium iodide in 20 ml distilled water. The two solutions are mixed and diluted to a 100 ml with distilled water).

**Procedure:** In a test tube, 1 ml of filtrate was added, followed by addition of 1 ml of Meyer's reagent in the test tube. The solution was mixed properly and the colour change was observed. A cream colour /precipitate indicated presence of alkaloid.

### 2.4 Test for the Presence of Steroids

Liebermann-Burchard's test was used to test for steroids. 1 ml of each extract was treated with 0.5 ml of acetic anhydride and cooled, mixed with 0.5 mL of chloroform and 1 ml of concentrated sulphuric acid was carefully added using a pipette. There was the formation of a reddish brown ring which indicated the presence of steroids.

### 2.5 Test for the Presence of Flavonoids

**Ammonium Test:** In ammonium test, 4 ml of filtrate was shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellow colour in the ammonical layer indicated the presence of flavonoids.

**Aluminum Chloride Test:** In aluminum chloride test, 4ml of the filtrate was shaken with 1 ml of 1% aluminum chloride solution. The layers were allowed to separate and the yellow colour in the aluminum chloride layer indicated presence of flavonoids.

### 2.6 Test for the Presence of Terpenoids

Salkowski's test was used to test for the presence of terpenoids. 5 ml of each extract was mixed with 2 ml of chloroform followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate was produced immediately indicating the presence of terpenoids.

### 2.7 Test for the Presence of Saponins

To determine the presence of saponins, 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, an emulsion formation indicated the presence of saponins.

### 2.8 Test for the Presence of Tannin

**Ferric chloride test:** To 3 ml of the filtrate in the test tube, few drops of ferric chloride were added.

A greenish black precipitate indicated the presence of Tannins.

**Lead acetate test:** Few drops of lead acetate were added to 3 ml of the filtrate in a test tube. A cream precipitate appeared showing presence of Tannins.

## 2.9 Test for Cardiac glycosides (Keller Killani Test)

In Keller Killani test, 5ml of the filtrate was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

## 2.10 Quantitative Determination of the Phytochemical Constituents of the Plant Samples

**Alkaloid Determination:** The determination of the concentration of alkaloids in the roots and stem barks of *Annona muricata* was carried out using the alkaline precipitation gravimetric method described by Harborne (1973) and Edeoga et al. [36,33]. 5 g of the powdered samples was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. The mixture was allowed to stand for four hours at room temperature (25°C). The mixture was filtered through Whatmann filter paper No. 42. The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloids, concentrated ammonia hydroxide solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the residue on the filter paper is the alkaloid, which is dried in the oven at 80°C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed.

$$\%\ \text{weight of Alkaloid} = \frac{W_2 - W_1}{\text{Weight of sample}} \text{x} \frac{10\ 0}{1}$$

Where:-

$W_1$ = weight of filter paper
$W_2$ = weight of filter paper + alkaloid precipitate (residue)

**Flavonoids Determination:** Quantity of flavonoids was determined by extracting 10 g of the plant samples with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath.

$$\%\ \text{Flavonoid} = \frac{W_2 - W_1}{\text{Weight of sample}} \text{x} \frac{100}{1}$$

Where:

$W_1$= Weight of crucible
$W_2$= Weight of crucible + Flavonoid extract (residue)

**Determination of Saponins:** In a conical flask, 20 g of the samples and 100cm$^3$ of 20% aqueous ethanol were added. The mixture was heated over a hot water bath for 4 hours with continuous sliming at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at 90°C. The concentrate was transferred into 250ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added and washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in water bath and dried in an oven to a constant weight. The saponin content was calculated in percentage.

$$\%\ \text{Saponin} = \frac{W_2 - W_1}{\text{Weight of sample}} \text{x} \frac{100}{1}$$

Where:

$W_1$= Weight of filter paper
$W_2$= Weight of filter paper + Saponin extract (residue)

**Tannin Determination:** To a 50 ml plastic bottle, 500 mg of the samples was added. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml of volumetric flask and and made up to the mark. 5 ml of the filtrate was added in a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.00 8M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

$$\% \text{ Tannin } = \frac{An}{As} x\ C\ x \frac{100}{W} x \frac{Vf}{Vn}$$

An= Absorbance of test sample
As=Absorbance of standard solution
C= Concentration of standard solution
W= Weight of sample
Vf= Total volume of extract
Vn= Volume of extract analyzed

## 2.11 Enzymatic Antioxidant Analysis

**Assay of Peroxidase(POD):** The method proposed by Reddy et al. (1995) and Hadwan (2018) was adopted for assaying the activity of peroxidase [37,38].

**Procedure**

**Preparation of enzyme sample:** A 20% homogenate was prepared in 0.1M phosphate buffer (pH 6.5) from the various parts of the sample, clarified by centrifugation and the supernatant was used for the assay.

**Assay:** To 3.0ml of pyrogallol solution, 0.1M of the enzyme sample was added and the UV spectrophotometer was adjusted to read zero at 430 nm. To the test cuvette, 0.5 ml of $H_2O_2$ was added and mixed. The change in absorbance was recorded every 30seconds up to 3minutes in a spectrophotometer (Genesys 10-S, USA). One unit of peroxidase is defined as the change in absorbance per minute at 430nm.

$$\text{Concentration} = \frac{\text{Absorbance x Total Reaction Volume}}{\text{Sample Volume x Extinction co} - \text{efficient}}$$

**Assay of glutathione peroxidase(GST):** Glutathione Peroxidase was assessed by the methods described by Weydert and Cullen, 2010; Habig et al. [39,40].

**Procedure**

**Preparation of enzyme sample:** The sample (0.5 g) were homogenized with 5.0 ml of phosphate buffer. The homogenates were centrifuged at 5000 rpm for 10 minutes and the supernatants were used for the assay.

**Assay:** The activity of the enzyme was determined by observing the change in absorbance at 340 nm. The reaction mixture contained 0.1 ml of GSH, 0.1 ml of CDNB and phosphate buffer in a total volume of 2.9 ml. The reaction was initiated by the addition of 0.1 ml of the enzyme sample. The readings were recorded every 15 seconds at 340 nm against distilled water blank for a minimum of three minutes in UV spectrophotometer (Genesys 10-S, USA). The assay mixture without the sample served as the control to monitor nonspecific binding of the substrates. GST activity was calculated using the extinction co-efficient of the product formed and was expressed as n moles of CDNB conjugated per minute.

$$\text{Concentration} = \frac{\text{Absorbance x Total Reaction Volume}}{\text{Sample Volume x Extinction co} - \text{efficient}}$$

**Assay of superoxide dismutase (SOD):** SOD was assayed according to the method of Weydert and Cullen, 2010 [39].

**Procedure**

**Preparation of enzyme sample:** The sample of 0.5 g were ground with 3.0 ml of potassium phosphate buffer, centrifuged at 2000 g for 10 minutes and the supernatants were used for the assay.

**Assay:** The assay mixture contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS, 0.3 ml of NBT, 0.1 ml of the enzyme preparation and water in a total volume of 2.8 ml. The reaction was initiated by the addition of 0.2 ml of NADH. The mixture was incubated at 30°C for 90 seconds and arrested by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was then shaken with 4.0 ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560 nm in spectrophotometer (Genesys 10-S, USA). One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

**Assay of Catalase:** Catalase activity was assayed following the method of Iwase et al. and Luck [41,42].

**Procedure**

**Preparation of enzyme sample:** A 20% homogenate of different parts of the sample was prepared in phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay.

**Assay:** $H_2O_2$ phosphate buffer (3.0 ml) was taken in experimental cuvette, followed by the rapid addition of 0.1ml of enzyme sample and

mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240 nm in a spectrophotometer (Genesys 10-S, USA). The enzyme solution containing $H_2O_2$ free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

$$\text{Concentration} = \frac{\text{Absorbance x Total Reaction Volume}}{\text{Sample Volume x Extinction co} - \text{efficient}}$$

## 3. RESULTS AND DISCUSSION

The result of the qualitative phytochemical analysis in Tables 1 & 2 showed that alkaloids were present in moderate abundance in all the extracts except the ethyl acetate bark extract. The high abundance of alkaloids in most of the extracts confirmed the therapeutic use of the plant as local anesthetics, analgesics, antioxidant and anti inflammatory agent [43,16,44,45,46, 47,48,49,50]. Sapaonins and tannins were both present in methanol extracts of both the root and stem. It is not surprising that the methanol extracts contained both saponins and tannins since it has been reported that methanol can dissolve polar compounds like tannins and saponins [51]. Also saponins have reported to have anti inflammatory, anti parasitic, ant diabetics, anti tumor activities and tannins have been reported to show antimicrobial, antioxidant, anti cancer, cytotoxic and astringent properties at low concentration All these properties validate the pharmacological use of the plant as anti inflammatory, antioxidant, anti diabetic, anti tumor, and antiparasitic agents [52,16,17,53,54, 55,56,57, 58,59,60,61,62,63,64]. The validation of antioxidant property of the plant by the methanol root and stem extracts of *A.* muricatais in line with the report of Truong et al. 2019 that methanol extract was the most potent extract with free- radical scavenging activities [65]. Flavonoids were only present in the ethyl acetate extract of the stem bark. Flavonoids have antioxidant properties, can act antibiotics [17] and also have anti cancer property, and so confirmed the therapeutic use of the plant as ant cancer agent [66,22,23]. Cardiac glycosides were present in all the extracts of both root and stem bark. Cardiac Glycosides have been reported to be highly toxic at high concentration, but has some pharmacological uses like antibacterial, antifungal, analgesic, anti inflammation, antihypertensive, muscle relaxation and anticancer activity, as heart tonic, diuretics and emetics, in the treatment of congestive heart failure and cardiac arrhythmia and these confirms some of the therapeutic uses of the plant [67,68,69,70]. Steroids were absent in all the extracts of stem bark samples but present only in moderate abundance in the n-hexane extract of the root samples, the presence in only n-hexane extract is understandable since steroid being non polar can only be dissolved in non polar solvent like n-hexane [51]. Terpenoids were present in all the extracts of both the root and stem bark samples. Some terpenoids exhibit biological activities such as anti cancer, antimalaria, anti inflammatory, antiseptic, anthelminthic, diuretic, antiplasmodial, anti-oxidant, insecticidal and antibiotics and could validate the plant's therapeutic uses [71,72]. Tannins were only found in the methanol extracts of the root and stem. This is understandable, because tannins being phenolic compounds would be soluble in polar solvents, which is also in line with the report of Ghasemzadeh et al. 2011 that total phenolic compounds increased with increase in polarity of solvents [73].

The result of the quantitative phytochemical analysis (Tables 3 & 4) showed that Flavonoids were present in the stem bark extracts (9.07%) but absent in the root extracts, which is in line with the result obtained from qualitative phytochemical analysis (Tables 1 and 2). There was greater percentage of Alkaloids in the root samples (5.48%) than in the stem bark (1.96%).

**Table 1. Result of qualitative phytochemical analysis of the various extracts of roots of *Annona muricata***

| Phytochemical | Methanol extract | Ethyl acetate extract | N-hexane extract |
|---|---|---|---|
| Alkaloids | ++ | ++ | ++ |
| Saponins | + | - | - |
| Flavonoids | – | – | – |
| Tannins | + | – | – |
| Cardiac Glycosides | + | ++ | ++ |
| Steroids | - | - | ++ |
| Terpenoids | + | + | + |

**Table 2. Result of qualitative phytochemical analysis of the various extracts of stem barks of *Annona muricata***

| Phytochemical | Methanol extract | Ethyl acetate extract | N-hexane extract |
|---|---|---|---|
| Alkaloids | +++ | - | + |
| Saponins | +++ | - | - |
| Flavonoids | - | + | - |
| Tannins | + | _ | _ |
| Cardiac Glycosides | + | + | + |
| Steroids | - | - | - |
| Terpenoids | ++ | + | + |

*Key:+ = low abundance, ++ = moderate abundance, +++ = high abundance and – = absent.*

**Table 3. Result of quantitative phytochemical analysis of roots of *Annona muricata***

| Phytochemical | Percentage |
|---|---|
| Alkaloids | 5.48 |
| Saponins | 23.56 |
| Tannins | 30.37 |

**Table 4. Result of quantitative phytochemical analysis of stem barks of *Annona muricata***

| Phytochemical | Percentage |
|---|---|
| Flavonoids | 9.07 |
| Alkaloids | 1.96 |
| Saponins | 25.55 |
| Tannins | 30.32 |

**Table 5. Enzymatic antioxidant activity of roots and stem of *Annona muricata***

| Enzyme | Activity | | *P*-Value |
|---|---|---|---|
| | Root | Stem | |
| Catalase | 0.029 ± 0.001 | 0.020 ± 0.001 | 0.01 |
| Superoxidase Dismutase | 2.981 ± 0.089 | 2.334 ± 0.071 | < 0.01 |
| Peroxidase | 0.106 ± 0.001 | 0.695 ± 0.003 | < 0.01 |
| Glutathione Peroxidase | 0.042 ± 0.000 | 0.034 ± 0.000 | < 0.01 |

*Values are means of three replicate determination (n = 3) ± standard error.*

However, Saponins were more present in the stem bark extracts (25.55%) than the roots (23.56%). Tannins were greater in the root extracts (30.37%) than the stem bark (30.32%). This showed that Alkaloids and Tannins were more in the root extracts while Saponins and Flavonoids were more in the stem bark extracts. All these coincide with the result of the qualitative analysis.

The result of the antioxidant analysis (Table 5) showed that the Antioxidant enzymes had greater activities in the root extracts (Catalase = 0.029 ± 0.001, Superoxidase dismutase = 2.981 ± 0.089, Glutathione Peroxidase = 0.042 ± 0.000) than the stem extracts (Catalase = 0.020 ± 0.001, Superoxidase dismutase = 2.334 ± 0.071, Glutathione Peroxidase = 0.034 ± 0.000) except Peroxidase.

This could be as a result of the greater percentage of Alkaloids and Tannins present in the root. Alkaloids are found to have antitumor, and anti-inflammatory properties and tannins which are phenolic compounds are major group of compounds that acts as primary antioxidants or free radical scavengers [74,75]. Therefore, it is not surprising that the roots of *Annona muricata* are claimed to be useful in the treatment of breast cancer. However, the presence of other identified phytochemicals (in both the root and stem bark extracts) such as Terpenoids and Flavonoids which play a protective role as antioxidant, can be said to be a strong contributing factors to the application of the Annona muricata plant in the treatment of various diseases including cancer [76].

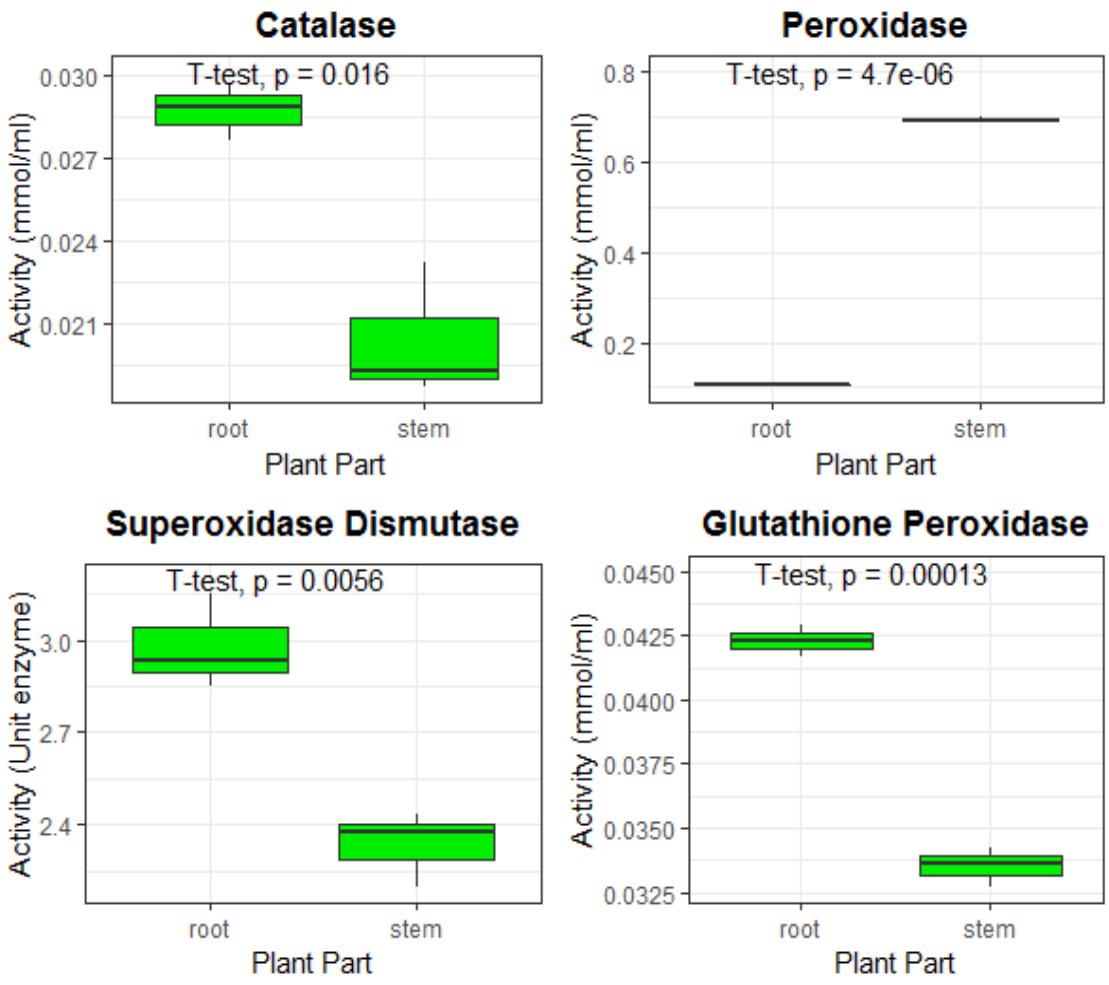


**Fig. 1. Boxplot of the enzymatic antioxidant activity of roots and stem of *Annona muricata***

### 3.1 Statistical Analysis

The student's t-test was used to compare the means of the enzymatic antioxidant activity of the root and stem of *Annona muricata.* Significant difference was determined at a probability levels of less than 5% ($P < 0.05$). The analysis was done using R, version 3.5.3.

### 3.2 Comparison of the Enzymatic Antioxidant Activity of the Root and Stem of *Annona muricate*

The antioxidant activities of the catalase, superoxidase dismutase, and glutathione peroxidase were significantly higher in the root of *Annona muricata* than in the stem. Conversely, peroxidase showed a significantly higher activity in the stem than in the root (Fig. 1 and Table 5).

## 4. CONCLUSION

The stem and root of *Annona muricata* have been shown to contain very important metabolites which also showed antioxidant properties. There is need to isolate the metabolites responsible for the antioxidant and other pharmacological properties exhibited by the plant parts and the development of the metabolites into drugs.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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