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Research Article

PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF SOURSOP (ANNONA MURICATA) LEAVES AND STEM BARK

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Abstract
The qualitative and quantitative phytochemical composition and antioxidant activity of the leaves and stem bark of *Annona muricata* (soursop) were investigated. Plant samples were collected from Otuoke community, Ogbia Local Government Area, Bayelsa State, Nigeria. The collected plant parts were chopped, dried, and extracted using absolute ethanol, followed by concentration under vacuum. Both the leaf extract (IDL) and stem extract (IDS) were analyzed for their phytochemical constituents using standard procedures. The analysis revealed the presence of several phytochemicals including alkaloids, phenols, flavonoids, glycosides, tannins, saponins, and terpenoids. The leaf extract contained alkaloids (13.0 mg), phenols (0.440 mg), flavonoids (8.3 × 10^-5 mg), cardiac glycosides (486.0 mg), tannins (10.6 mg), and terpenoids (322.0 mg), while the stem extract had alkaloids (20.0 mg), phenols (0.70 mg), flavonoids (8.2 × 10^-5 mg), cyanogenic glycosides (300.0 mg), tannins (6.2 mg), and terpenoids (215.0 mg). Statistical analysis using the t-test showed no significant difference in the concentrations of these phytochemicals between the leaf and stem extracts at $p < 0.05$. The antioxidant activity of both extracts was evaluated using the 1,1-diphenylpicrylhydrazine (DPPH) method, with both extracts showing significant antioxidant potential across a dose gradient range of 1.0 mg/ml to 0.625 mg/ml. This study confirms that both the leaves and stem bark of *Annona muricata* are rich in phytochemicals and exhibit noteworthy antioxidant activity, thereby validating the traditional ethnomedicinal uses of the plant.
Keywords: Soursop, phytochemicals, antioxidant, *Annona muricata*, DPPH

Introduction

The distribution of secondary metabolites or phytochemicals in plant tissues exhibits a notable variability across ecosystems, driven by a combination of ecological factors and the biosynthetic pathways inherent to each species (Kucharikova et al., 2016). These bio-organic molecules, also known as phytochemicals, serve diverse roles in plants, from deterring herbivores and pathogens to aiding in reproduction. Among the many types of secondary metabolites, alkaloids, flavonoids, steroids, glycosides, and saponins stand out due to their significant pharmacological properties. These compounds have attracted considerable attention for their therapeutic potential, offering a vast array of bioactive benefits for human health. These compounds, in addition to serving

ecological functions in plants, have been found to exhibit various biological activities such as antioxidant, anti-inflammatory, anticancer, antimicrobial, and antihypertensive properties, making them crucial in the development of pharmaceutical and nutraceutical products.

One such plant, *Annona muricata* (commonly known as soursop), has long been lauded in traditional medicine for its various health benefits. Soursop, belonging to the Annonaceae family, has been used extensively in folk medicine for its claimed therapeutic effects, including as a laxative, purgative, and treatment for hypertension (Agu and Okolie, 2017). Soursop is also renowned for its potential to combat a wide array of conditions, with studies documenting its antioxidant, cytotoxic, antitumoral, antiparasitic, and antihyperglycemic properties (Baskar et al., 2007; Bryan-Thomas, 2016; Gavamukulya et al., 2014; Gleye, 1999; Ahadya et al., 2014). The plant's leaves, fruits, seeds, and bark are rich in bioactive compounds, contributing to its reputation as a valuable medicinal plant. These properties have piqued the interest of researchers in understanding the underlying bioactive molecules that mediate such therapeutic effects, and in validating traditional uses with scientific evidence.

The phytochemical composition of *Annona muricata* has been the subject of numerous studies, which have identified a range of bioactive compounds across different plant parts. According to earlier research, the leaves of soursop contain significant quantities of flavonoids, steroids, alkaloids, glycosides, and tannins (Qorina et al., 2019). Similarly, comprehensive phytochemical analyses of the fruit, leaf, stem bark, and root bark have revealed the presence of tannins, flavonoids, saponins, terpenoids, carbohydrates, cardiac glycosides, reducing sugars, monosaccharides, pentoses, ketoses, starch, proteins, and amino acids including arginine, cysteine, and phenolic amino acids (Agu and Okolie, 2017; Agu et al., 2017; Edeoga et al., 2005). These compounds play pivotal roles in the plant's bioactivity, contributing to its medicinal potential in various therapeutic domains.

The antioxidant activity of *Annona muricata* has also been thoroughly investigated, as oxidative stress is a key contributor to numerous chronic diseases, including cardiovascular diseases, diabetes, and cancer. In one study, the ethanolic extract of soursop leaves exhibited an IC50 value of 35.51 ppm in antioxidant activity assays (Qorina et al., 2019), indicating significant free radical scavenging potential. Additionally, the fruit of *Annona muricata* has demonstrated even stronger antioxidant properties with an IC50 value of 32.6 µg/ml, as compared to vitamin C, which was used as the standard for the DPPH analysis (Agu and Okolie, 2017). These findings emphasize the importance of *Annona muricata* as a natural antioxidant source, and the potential therapeutic applications of its leaves and fruit in managing oxidative stress-related conditions.

Beyond antioxidant activity, the cytotoxic and antitumoral properties of soursop have been subjects of considerable research. Studies have shown that extracts from *Annona muricata* exhibit selective cytotoxicity against cancer cell lines, suggesting its potential as a natural chemotherapeutic agent (Gavamukulya et al., 2014). These anticancer properties are largely attributed to the presence of acetogenins, a class of bioactive compounds unique to the Annonaceae family, which have been shown to inhibit cancer cell proliferation and induce apoptosis in tumor cells (Bryan-Thomas, 2016). Similarly, *Annona muricata* extracts have demonstrated antiparasitic activity, particularly against protozoan and helminthic infections, further validating its ethnomedicinal applications (Gleye, 1999).

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In addition to its effects on oxidative stress and cancer, *Annona muricata* has shown promising results in the management of hyperglycemia and dyslipidemia. Several studies have explored its antihyperglycemic effects, finding that soursop extracts can help regulate blood sugar levels, making it a potential complementary treatment for diabetes (Ahadya et al., 2014). Moreover, the antihypolipidemic effects of *Annona muricata* have been demonstrated in animal models, with evidence suggesting that it can reduce elevated cholesterol and triglyceride levels, thereby promoting cardiovascular health (Baskar et al., 2007). These pharmacological properties contribute to the growing interest in soursop as a multi-faceted medicinal plant.

Despite the extensive body of research on *Annona muricata*, there remains a need for further studies to validate and expand on existing data. Previous studies have predominantly focused on the phytochemical composition and antioxidant properties of soursop, but more comprehensive investigations are necessary to explore the full extent of its bioactive potential. Additionally, much of the research to date has been based on the fruit and leaves of the plant, with less attention given to the stem bark, which may also harbor valuable phytochemicals with unique therapeutic properties. In this context, the present study seeks to validate and build upon existing phytochemical and antioxidant data, focusing specifically on the leaves and stem bark of *Annona muricata*. By examining the phytochemical profile and assessing the antioxidant activity of these plant parts, this study aims to contribute to the growing body of evidence supporting the therapeutic potential of soursop in modern medicine.

This study will employ a range of analytical techniques to identify and quantify the bioactive compounds present in the leaves and stem bark of *Annona muricata*. Additionally, it will evaluate the antioxidant potential of these extracts using established in vitro assays, contributing valuable data to the understanding of the plant's therapeutic properties. Given the promising results reported in previous studies, this research will not only help validate the medicinal claims associated with soursop but also provide insights into the broader applicability of this plant in the development of natural health products.

In summary, *Annona muricata* stands out as a plant of significant pharmacological interest due to its diverse array of bioactive compounds and therapeutic effects. The leaves, stem bark, and other parts of the plant have been shown to possess a wide range of medicinal properties, including antioxidant, anticancer, antiparasitic, antihyperglycemic, and antihypertensive activities. However, more research is needed to fully elucidate the phytochemical composition of these plant parts, particularly the stem bark, and to explore their potential therapeutic applications. The present study aims to address this gap in knowledge by investigating the phytochemical and antioxidant profiles of *Annona muricata*, contributing to a deeper understanding of the plant's medicinal value and its potential role in the development of new health interventions.

Materials and Methods

Collection of Plant Material: The plant material was collected from Otuoke in Ogbia Local Government Area of Bayelsa state, Nigeria. The plant was identified at the Department of Biology, Federal University Otuoke, Otuoke, Bayesal state, Nigeria

Chemicals and Reagents: Chemicals and reagents used to carry out the experimental of this work were of analytical grade (products of BDH and Sigma-Aldrich). They include: pH 4.7 phosphate buffer, BCG solution,

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chloroform, aluminum trichloride, atropine, triple distilled water (TDW), Folin-Ciocalteu reagent, Na_2CO_3, ethanol, methanol, gallic acid, acetic acid, rutin, vanillin, sulphuric acid, NaOH, NH_4OH, tannic acid, 5% KI, $AgNO_3$, diosgenin, distilled water, $FeCl_3$, HCl, potassium ferrocyanide, DPPH.

Methods

Extraction of Phytochemicals: The plant material air dried for two (2) weeks to a constant weight under a shade to avoid direct sunlight effect on the phytochemicals. The plant material was then grinded for an increase surface area for better extraction. 500 g of the ground leaves sample was macerated using absolute ethanol for 72 hrs. The diluted form of the extract was then decanted and then concentrated with the aid of a rotator evaporator (Rotor 250) to yield a dried crude extract, IDL. Similar procedure was repeated for the stem bark material, yielding ethanolic crude extract of the stem bark, IDS.

Qualitative Phytochemical Screening: The qualitative phytochemical screening procedures of both IDL and IDS are presented in table 1.

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Table 1. Procedures for qualitative phytochemical screening

S/N.	Class of Phytochemicals	Procedures
1.	**Alkaloids**	Few drops of Hager's reagent (saturated solution of picric acid) to 2 ml extract. The presence of a yellow coloration was an indication of alkaloids (Hager's test).
2.	**Phenols**	5 drops of iron (iii) chloride to ethanolic fraction of the extract. A yellow-green precipitate indicates the presence of phenols (Firdouse and Alam, 2011).
3.	**Flavonoids:**	1 ml extract added to 10% lead acetate $(Pb(OAc)_4$. The presence of a yellow colouration was an indication of the presence of flavonoids (Legal's test).
4.	**Glycosides**	Dried chloroform fraction of the filterate of the aqueous alcoholic lead actetate and 2 ml acetic acid was dissolved in alkaline solution of 2 ml pyridine solvent and 2 ml of sodium nitropruside. The formation of a pink colouration was an indication of the presence of cardiac glycosides (Firdouse and Alam, 2011).
5.	**Tannins**	3 mg of blended extract was boiled in 5 ml water and then filtered and to the filterate was added 3 drops of 0.1% $FeCl_3$. The presence of a brownish green or a blue-black was an indication of tannins (Braymer's test) (Gul *et al*., 2017).
6.	**Saponins**	5.0 ml of distilled water was mixed with 200 mg aqueous extract in a test tube. The mixture was mixed thoroughly and then was added few drops of olive oil, shaken further. The appearance of foam showed the presence of saponins (Froth test) (Gul *et al*., 2017).

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7.	**Terpenoids**	2 ml of acetic acid anhydride and 2-3 drops of concentrated H_2SO_4 was added 2 ml of extract. The appearance of a deep red colouration was an indication of the presence of terpeniods (Liebermann-burchard's test) (Firdouse and Alam, 2011).

Quantitative Phytochemical Evaluation: The quantitative evaluation of the Phyto constitution of IDL and IDS were based on standard procedures reported by: Mythili *et al*., 2014; Akbari *et al*., 2019; Ejikeme *et* al., 2014; Amadi *et al*., 2004 and summarized in table 2.

Table 2. Procedures for quantitative phytochemical evaluation

S/N.	Phytochemicals	Procedures
1.	**Estimation of Alkaloids**	1 ml extract + 5 ml phosphate Buffer (pH 4.7) + 5 ml BCG solution+ 4 ml of chloroform. The absorbance of the complex was read at 470 nm with atropine as standard
2.	**Total Phenolics**	1ml (100 mg extract + 100 ml of triple distilled water (TDW)) in a test tube + 0.5 ml 2N Folin-Ciocalteu reagent + 1.5 ml 20% of Na_2CO_3 solution + volume made up to 8 ml with TDW + vigorous shaking + standing for 2 hours after which the absorbance was taken at 765 nm with gallic acid used as standard.
3.	**Total Flavonoids**	100 µl extract in methano + 100 µl of 20 % alcoholic $AlCl_3$ + A drop of acetic acid + 5 ml using methanol and left for 40 min. The absorbance of the complex was read at 415 nm against the blank with rutin solution (0.5 mg/ml) used as standard.
4.	**Total Cyanogenic Glycosides**	Part A: Aqueous extract (allowed to stand for 2 hrs) + tannic acid (anti-foaming agent) + 25% NaOH+ simple distillation. Part B: Distillate (100 cm^3) (from part A) + 8 cm^3 NH_4OH + 2 cm^3 5% KI + titration against 0.02M $AgNO_3$ over a black background. Cyanogenic glycoside (mg/100 g) = $\frac{Titre\ value \times 1.08 \times exact\ volume}{Aliquot\ volume \times mass\ of\ sample(g)} \times 100$
	Total Saponins	80% methanolic solution of extract + 2ml ethanolic Vanilin + 2ml of 72% sulphuric acid solution + mixed well + heating on a water bath at 60 ^{0}C for 10 min The absorbance was measured at 544 nm against the blank with diosgenin used as the standard.
5.	**Total Tannins**	500 mg extract in a 50 ml plastic bottle + 50 ml of distilled water + shaking for 1 hr (mechanical shaker) + filtered into a 50 ml volumetric flask and made up to the mark. 5 ml of the filterate + 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl + 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes.

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Blank: same as each of the procedures above without the extract*

Antioxidant Profiling: The antioxidant profiling of IDL and IDS was carried out using the 2, 2diphenyl-1-picrylhydrazyl, DPPH method (Odokwo and Onifade, 2018) with absorbance recorded at 517 nm (UV-Visible spectrophotometer (Labomed spectro UV-2505) and vitamin C (ascorbic acid) was used as natural standard, in a triplicate concentration dose range.

Preparation of DPPH Solution: 3.94 mg of DPPH was measured with the aid of an analytical weighing balance and then it was dissolved in 100 ml of methanol and labeled as the stock solution of DPPH.

Preparation of the Ethanolic Crude Extracts for Antioxidant Analysis: 1.0 mg of the crude ethanolic extract of the leaves of *Annona muricata* was dissolved in 1 ml of methanol to yield 1.0 mg per ml of sample solution and a serial dilution was made from it to a concentration 0.625 mg per ml. The same method was applicable to IDS and the standard, vitamin C (IDV).

To each of the extracts (IDL and IDS) and standard (IDV) solutions prepared through serial dilution was added 3 ml of the DPPH stock solution and allowed to stand for 10 min before they were read at 517 nm. The absorbance of 3ml of stock solution without the sample material was read at the same wavelength as the blank solution.

The percentage inhibition, $\%I$ was calculated using the relationship:

$$\%I = \frac{A_{BLANK} - A_{EXTRACT}}{A_{BLANK}}$$

Where: $\%I$ – percentage inhibition,

A_{BLANK} – Blank absorbance,

A_{EMP} – Extract absorbance

Statistical Analysis: Data obtained were expressed as mean of triplicates determinations ± standard deviation (SD). The Statistical Package for Social Scientists (SPSS version 20.0) was used for all data analysis. **Results and Discussion**

The qualitative and quantitative evaluations of both IDL and IDS have shown that phytochemicals such as: alkaloids, phenols, flavonoids, glycosides, tannins, saponins and terpenoids are present and distributed in both the leaves and stem bark of soursop. The presence of these profound secondary metabolites is a basis for the therapeutical confirmations of the ethnomedicinal claims associated with *Annona muricata*. Significant activities such as cytotoxicity, antileishmanial, wound healing and antimicrobial activity that have been associated with *Annona muricata* are as a result of the phytochemical constitution of the plant. The plant is also known to exhibit

anticancer and genotoxic effect, anti-inflammatory, anti-protozoan, antioxidant, insecticide, larvicide in *in vitro* studies (Coria-Tellez *et al*., 2018; Gajalakshmi *et al*., 2012). These activities would not have been possible if there were no phytochemicals present in the leaves and stem *Annona muricata*. Previous reports had also established the qualitative presence of the reported phytochemicals (Qorina *et al*., 2019; Agu and Okolie, 2017, Agu *et al*., 2017, Edeoga *et al*., 2005).

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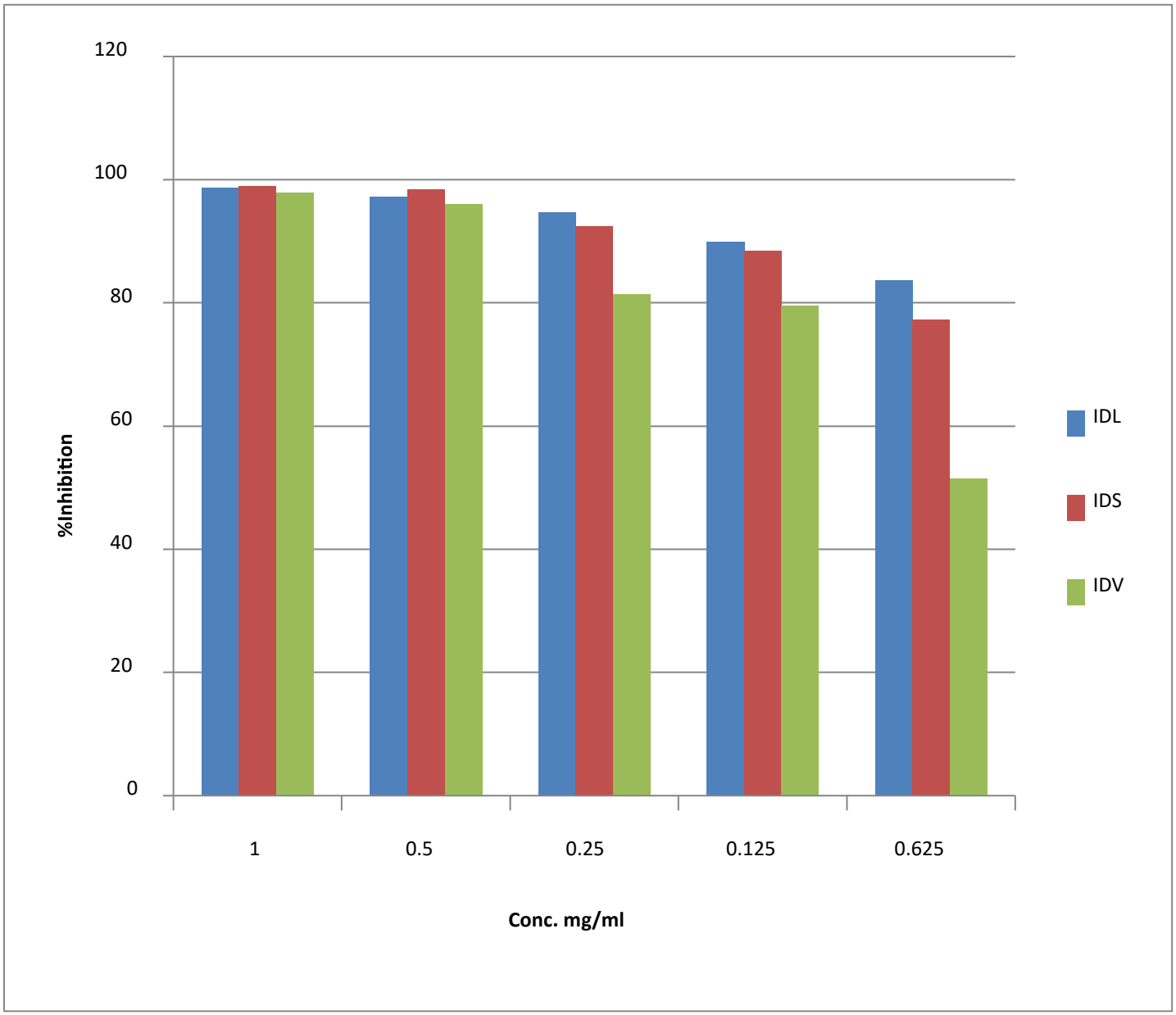

IDL*- ethanolic leaf extract, IDS- ethanolic stem bark extract, IDV-vitamin C standard

Figure 1. Antioxidant activity of the leaf, stem and vitamin acid (standard)

The antioxidant activity of the both the leaves and the stem increases as the concentration of both IDL and IDS increases. The antioxidant activity of the crude extracts was comparatively higher than those of the standard, IDV (figure 1.).

The plant has been shown to have exhibited antioxidant activity as obtained from the scholarly work of Correa-Gordillo *et al*. (2012). Antioxidant activity has gained tremendous attention because of the protective role against diseases such as cancer, diabetes, cardiovascular, arthritis and degenerative diseases such as Parkinson and Alzheimer (Almeida *et al*., 2011).

Conclusions

The present study has validated the presence and distribution of phytochemicals in both the leaf and stem bark of soursop. These phytochemicals are grounds for the observed *in vitro* antioxidant activity and other reported activities.

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