Phytoconstitution and Antioxidant Evaluation of the Ethanolic Leaf and Stem Bark Extracts of Soursop (*Annona muricata*)

[1]Edet O. Odokwo and [2]Ngozi M. Uzoekwe
[1]Department Of Chemistry, Federal University Otuoke, Bayelsa State, Nigeria
[2]Department Of Physical Sciences, Benson Idahosa University, Edo State, Nigeria
[1]Email: odokwoee@fuotuoke.edu.ng

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Abstract

The qualitative and quantitative phytoconstitution and the quantitative antioxidant activity evaluation of the leaves and stem of soursop (Annona muricata) have been carried out. The plant parts were collected from Otuoke community, Ogbia Local Government Area of Bayelsa state, Nigeria. The parts were chopped separately, dried, extracted with absolute ethanol and then concentrated under vacuum. The leaf extract (IDL) and stem extract (IDS) were evaluated for their phytoconstitution using standard procedures and the in vitro antioxidant activity using 1,1-diphenyl picrylhydrazine (DPPH) technique. Phytochemicals such as alkaloids, phenols, flavonoids, glycosides, tannins, saponins and terpenoids were detected and quantified in comparative levels in mg as: (alkaloids, 13.0), (phenols, 0.440), (flavonoids, 8.3 $\times 10^{-5}$), (cardiac glycosides, 486.0), (tannins, 10.6) and (terpenoids, 322.0) in the leaves and (alkaloids, 20), (phenols, 0.70), (flavonoids, 8.2 $\times 10^{-5}$), (cynogenic glycosides, 300.0), (tannins, 6.20) and (terpenoids, 215.0) in the stem with no significant different at $p < 0.05$ using t-test statistical analysis. The DPPH assay revealed that both IDL and IDS exhibited antioxidant activity in a dose gradient concentration of 1.0 mg/ml to 0.625 mg/ml. the present study has established that both the leaves and stem bark of soursop have phytochemicals and do exhibit antioxidant activity.

Keywords: *Soursop, phytochemicals, antioxidant*

Introduction

The distribution of secondary metabolites or phytochemicals in plant tissues is not even distributed across the ecosystem due to ecological factors and biosynthetic consequence (Kucharikova *et al*., 2016). Phytochemicals such as the alkaloids, flavonoids, steroids, glycosides and saponins are nature's bio-organic molecules known for their therapeutical signatures. Soursop with the biological name '*Annona muricata*' has the ethnomedicinal claims of being used as laxative and purgative (Agu and Okolie, 2017), antihypertension recipe and other bioactivities such as antioxidant (Baskar *et al*., 2007; Bryan-Thomas, 2016; Gavamukulya *et al*., 2014), cytotoxicity, antitumoral, antiparasitic (Gleye, 1999) and antihyperglycemic, antihypolipidemic (Ahadya *et al*.,

2014). Bioactive compounds and essential oil constituents of *A. muricata* have been reported in literatures (Agu *et al*., 2017; Gleye, 1999).

Previous studies have reported the present of flavonoids, steroids, alkaloids, glycosides and tannins in the leaf of soursop (Qorina *et al*., 2019), tannins, flavonoids, saponins, terpenoids, carbohydrates, cardiac glycosides, reducing sugar, monosaccharides, pentoses, ketoses, starch, protein, arginin, cysteine, aromatic amino acids, phenolic amino acids, alkaloids, steroids and phenolic in the fruit, leaf, stem bark and root bark of soursop (Agu and Okolie, 2017, Agu *et al*., 2017, Edeoga *et al*., 2005). IC_{50} value of 35.51 ppm from the antioxidant activity data was observed for the ethanolic leaf extract of soursop (Qorina *et al*., 2019) and IC_{50} of 32.6 µg/ml was observed for the vitamin C being used as standard for the DPPH analysis carried out with the fruit showing a better antioxidant potential (Agu and Okolie, 2017). The present study is designed to validate and add to existing phytochemical and antioxidant data on the leaves and stem bark of *Annona muricata*.

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 identifieid 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, 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.

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 colouration 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).
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 phytoconstitution 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.

*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, 2-diphenyl-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:

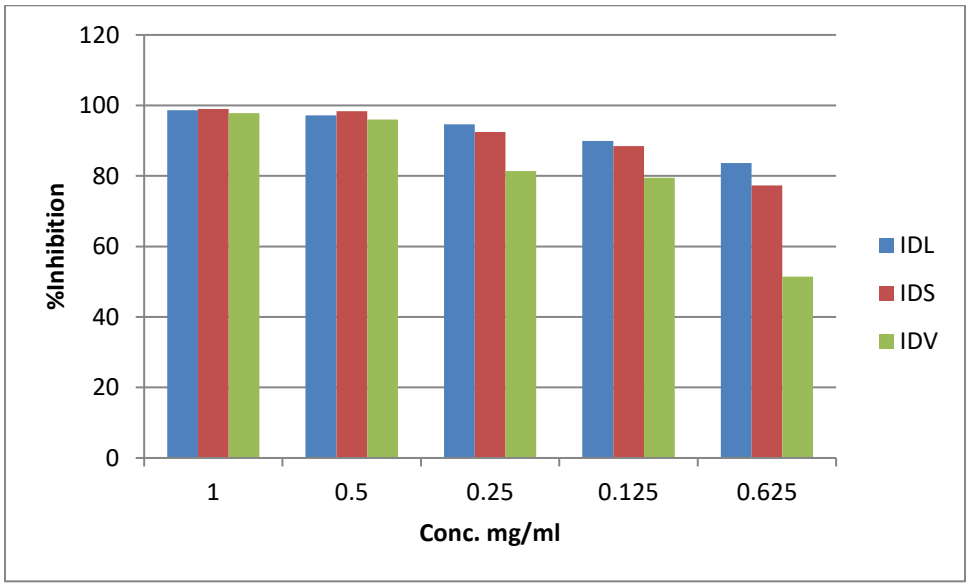
$$\%I = \frac{A_{BLANK} - A_{EXTRACT}}{A_{BLANK}}$$

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).



*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 were 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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