A STUDY OF CYTOTOXIC POTENTIAL AND ANTIOXIDANT ACTIVITY OF FRUIT OF ANNONA MURICATA

Oyinlade C. Ogundare, Ghemisola M. Saibu, Oluwatosin B. Adu, Omotayo M.A., Temitone V. Akinyemi and Adewale O. Adepoju

Department of Chemical Science, School of Pure and Applied Sciences, Lagos State Polytechnic, Ikorodu, Lagos State
Department of Biochemistry, Lagos State University, Ojo. P.M.B 0001, LASU Post office, Lagos State

ABSTRACT

The use of various parts of Annona muricata (Sour Sop) in the management of diseases cannot be over emphasized. This study considered the cytotoxic potential and antioxidant activity of A. muricata fruit. The crude aqueous extract of A. muricata fruit was screened for bioactive compounds and successively partitioned with n-hexane, ethyl acetate, acetone, acetic acid and methanol. The resulting fractions were evaluated for cytotoxicity using brine shrimp lethality assay (BSLA) and their antioxidant activity was determined using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) scavenging ability. Alkaloids, glycosides, flavonoids, phenolics, saponins, steroids and carbohydrates were detected in the crude aqueous extract. Cytotoxicity of the ethyl acetate fraction was significantly ($p<0.05$) higher than the crude extract and all other fractions. However, the antiradical activity of the ethyl acetate fraction in the presence of DPPH or nitric oxide was comparable to acetone fraction of fruit of A. muricata, but significantly ($p>0.05$) lower than ascorbic acid. These results substantiated the presence of intermediate polar compound(s) with potential biological ability resulting from antiradical and consequent cytotoxic activity.

Keywords: Annona muricata, Brine Shrimp Lethality, Cytotoxicity, Antiradical activity, Ascorbic acid.

INTRODUCTION

Application of plants for food and agents for the management or treatment of illness is as old as humanity. Most importantly, plants are used as essential components of drugs and medicines for the management and treatment of different health conditions (Hasan, 2015). Whole plants, fruits, leaves, bark, stem, roots and related parts of medicinal plants are used directly for treatment of diseases or indirectly, for a biochemical prototype for the development of conventional medicines (Falodun, 2010). Moreover, medicinal plants have economic benefits, convenient for use and possess little or no toxicity. Thus, a search for therapeutic compounds from plants allows the application of such plants in the prevention and/ or treatment of diseases (Chugh et al., 2018).

Therapeutic activities of a medicinal or natural product are subject to the miscellany and quantity of its chemical constituents such as alkaloids, tannins, flavonoids, saponins, steroids, terpenoids and many more. Lavilla and Villazorda (2015) explained that phytochemicals are gigantic collection of biologically active compounds with various chemical configurations and possess protective characteristics on plants and their consumers. Recently, many attentions are given to the investigation of therapeutics including cytotoxicity as well as antioxidant activities of chemicals obtained from plant materials (Nanyonga et al., 2013). Antioxidants which are chemical substances with a tendency to defend the body from oxidative damages resulting from attack by free radicals, and are also important in cell signalling (Alencar et al., 2019). Free radicals are obtained from oxidation of exogenous chemicals and a variety of endogenous metabolic pathway of biological molecules resulting into cell death and tissue damage (Moualek et al., 2016). Aside from this, is the exposure to certain drugs, operational chemicals and electrical radiations from microwave and mobile phones (De Iuliis et al., 2009). Studies have
revealed the presence of natural antioxidants from different species of plant (Rahman et al., 2013) through the evaluation of effect of such substances on radicals like 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide. These plants include Cordia africana, Cassia fistula, Paltyicoacari baerorum, Averrhoa carambolaL, and A.muricata (Gavamukulya et al., 2014; Jothy et al., 2011; Muthu et al., 2015).

Cytotoxicity is a measure of pharmacological activities of bioactive compounds, and is commonly determined by use of BSLA (Ogundare et al., 2017) or the use of tetrazolium bromide assay. The parameter has been found relevant in investigation of anti-tumour activity. Scientists employ BSLA at the preliminary stage of investigation of anti-tumour or anti-cancer agents due to its simplicity, consistency and expediency, and a correlation between the zoological model (Artemia salina) and human cell lines (Ramachandran et al., 2011).

A. muricata which belong to the family Annonaceae, is commonly referred to as Soursop in English, or Graviol in Portuguese. It is originally native to Central America but now found all around the world. It is a shrubby plant located majorly in the rain forest regions of Nigeria, where it is used locally for several ethno-medicinal purposes; as a laxative and purgative, wound healing, and many more. The health benefits of this plant have been attributed to its unique phytochemical components such as acetogenins, essential oils and numerous phytochemicals, some of which serve as an antioxidant (Agu et al., 2017; Okolie et al., 2013). All parts of the plant are used as natural medicine for numerous diseases including cancer. The fruit and fruit juice of the plant are used to eliminate worms and parasites, fever, increase mother’s milk after childbirth, hypertension, inflammation, diabetes mellitus, bacterial infections, cancer and as astringent for diarrhea and dysentery (Mao et al., 2011; Moghadamtousi et al., 2015; Patel et al., 2016). Thus, this research was designed with a bid to establish the biological activity of fruit of A. muricata through the studies of cytotoxic and antiradical activities of soluble compounds obtained from solvents of different polarities.

MATERIAL AND METHOD

Plant Materials

The fruits of A. muricata were obtained from a farm land at Igbesa, Ogun State, Nigeria in November 2017 and taken to the Herbarium at the University of Lagos for authentication with number LUH 6952. Preceding the extraction, the sampled fruits were washed painstakingly using tap water and stored in the refrigerator.

Preparation of crude aqueous fruit extract of A. muricata

A measure of two hundred and seventy grams (270 g) of peeled A. muricata was taken and 200 mL of ice-cold distilled water was added and blended to paste at a low speed. The resulted paste was filtered by means of Whatmann filter paper No. 42 and centrifuged at 1000 revolution per minute for 15 minutes to remove the debris from the soluble components of the fruit (aqueous fruit extract of A. muricata). A portion of the aqueous fruit extract was subjected to phytochemicals screening while the other portion was taken, freeze dried and stored at -20 °C until analysis.

Solvent - solvent partitioning of crude aqueous fruit extract of A. muricata

The crude aqueous fruit extract was partitioned with the use of a modified method of (Abu et al., 2017). The crude extract was dissolved in distilled water in the ratio 1: 10 and allowed to partition in different solvents (hexane, ethyl acetate, acetone, methanol and acetic acid) according to their polarities in a 500mL separating funnel. The resulting fractions from each solvent and the insoluble aqueous fraction (that remained in the separating funnel) was concentrated in a water bath at 40°C to 55°C to obtain a relatively pure fraction of the fruit of A. muricata. The percentage extraction yield for each concentrated fraction was calculated and the fractions were stored in the refrigerator until analysis.

Phytochemical screening of crude aqueous fruit extract of A. muricata

The crude aqueous fruit extracts of A. muricata were tested for the presence of phytochemicals such as saponins, flavonoids, alkaloids, steroids, glycosides, carbohydrates and tannins using the standard procedures described by Harbone (1973), Sofowora (1993) and Evans (2002).

Tests for alkaloids

Dragendorff test

A few drops of Dragendorff’s reagent (potassium bismuth iodide solution) were mixed with 2 mL of the extract. The formation of orange red precipitate showed the existence of alkaloids.

Mayer’s test

A measure of 2 mL of the extract was acidified with dilute HCl and a few drops of Mayer’s reagents were added. The formation of white precipitate showed the existence of alkaloids.

Test for glycosides (Froth test)

A measure (2 mL) of the crude extract was mixed with 2 mL of water in a test tube. It was shaken vigorously for few minutes. Froth appeared which indicated the presence of glycosides.

Test for flavonoids (Shinoda test)

A measure of 2 mL of the extract was mixed with few magnesium turnings and boiled for 5 minutes in a water bath. The appearance of orange or red colour indicated the presence of flavonoids.
Alkaline test
A measure of 2 mL of the extract was mixed with 0.5 mL of lead acetate solution and observed after 5 min for the appearance of yellow precipitates which indicate the presence of flavonoids.

Test for phenolic (Nitric acid test)
A measure of 2mL of the extract was mixed with few drops of dilute trioxonitrate (V) acid (HNO₃). Formation of reddish to yellowish colour indicated the presence of phenolic.

Test for tannin (Wohler’s Test)
A few drops of basic lead acetate solution were mixed to 2 mL of the extract. The appearance of a white precipitate indicated the presence of tannin.

Test for carbohydrates (Benedict test)
A measure of 2 mL of the extract was mixed with 1mL of Benedict’s solution in a test tube. The mixture was boiled for 15 minutes in a water bath. The appearance of red colour indicated the presence of reducing sugar.

Test for steroids (Salkowsit test)
A measure of 2 mL of chloroform was added to 2 mL of the extract and conc. H₂SO₄ was carefully added to form the bottom layer. The presence of a reddish brown coloured interface indicated the presence of steroids.

Cytotoxic activity of fruit of A. muricata using Brine Shrimp Lethality (BSLA) assay
Hatching of the brine shrimp
Artemia salina cysts were hatched in a clean transparent rectangular plastic container containing clean fresh sea water. The tip of a tiny hose from an air pump was sited into the bottom of the container to ensure accurate freshening. The hatching container was lightened up using a Gallemp lamp for the 48 hours and the newly hatched brine shrimps (nauplii) were attracted to one side of the container according to the light source.

Brine shrimp lethality assay (BSLA) of fruit of Amuricata
Protocol of Meyer et al. (Meyer et al., 1982) was adopted with slight modifications. Active nauplii (10 numbers) were picked by use of Pasteur pipette and separately exposed to 1mL of different concentrations (0 µg/mL to 1000 µg/mL) of crude and fractions of A. muricata DMSO for 24 hours in test tubes. The volume was made up to 10 mL with clean sea water. The active nauplii were counted after 24 hours and the percentage mortality was obtained. The assay was performed in triplicate for each concentration. Lethal concentration that killed 50% of the population of the nauplii (LC₅₀) was determined using Probit analysis by means of the software IBM SPSS Statistics version 20.

Antioxidant activity of fruit of A. muricata
DPPH radical scavenging assay
The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging radical activity was determined by the modified method of (Joshi et al., 2015). Stock solutions (1000 µg/mL) of crude and fractions of fruit of A. muricata, and standard (ascorbic acid) were separately prepared and diluted to 10 µg/mL to 1000 µg/mL. A portion (2.0 mL) of various concentrations of test samples or standard was mixed vigorously with 1.0 mL of 20 µM of DPPH in methanol. The solution was allowed to react for 30 minutes at 27°C in a cupboard, and the absorbance was measured using Thermo Scientific Genesys 10S UV-Vis Spectrometer at λ, 517 nm against reagent blank (2.0 mL of test sample in 1.0 mL methanol). All the reactions were carried out in triplicates.

The Percentage scavenging activity of the fruit was calculated using the below listed equation:

\[
\% \text{DPPH scavenging activity} = \left( \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \right) \times 100
\]

Nitric oxide scavenging assay
Ability of fruit of A. muricata to scavenge the nitric oxide obtained from sodium nitroprusside was performed according to the method of (Nanyonga et al., 2013) with slight modification. A stock solution with concentration 1000µg/mL was prepared and diluted successively to get concentrations of 10µg/mL to 1000µg/mL of test and standard sample. Sodium nitroprusside was used to generate nitric oxide (Rao, 1997) and measured through nitrite formed from the reaction between oxygen and nitric oxide in the presence of by using Griess reagent . The dilute test sample or ascorbic acid (0.5mL) was separately mixed with 2.5mL of 0.01M sodium nitroprusside in phosphate buffer (pH 7.4) and left at room temperature for 2 hours 30mins. An aliquot of the reaction mixture was combined with equal volume of Griess reagent and its absorbance taken at 540nm against a related solution of blank (dilute the test sample or ascorbic acid and equal volume of distilled water). The control contained the same reaction mixture in the absence of the extract but distilled water. The mixture was mixed thoroughly and allowed to react at room temperature for 2 hours 30mins. Absorbance was measured using Thermo Scientific Genesys 10S UV-Vis Spectrometer at 540 nm. All the reactions were carried out in triplicate. The percentage scavenging activity was calculated as follows:

\[
\% \text{Nitric oxide scavenging activity} = \left( \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \right) \times 100
\]
Cytotoxic activity of fruit of A. muricata using Brine Shrimp Lethality (BSL) assay

Hatching of the brine shrimp

Artemia salina cysts were hatched in a clean transparent rectangular plastic container containing clean fresh sea water. The tip of a tiny hose from an air pump was sited into the bottom of the container to ensure accurate freshening. The hatching container was lightened up using a Gallenkamp lamp for the 48 hours and the newly hatched brine shrimps (nauplii) were attracted to one side of the container according to the light source.

BSL assay of fruit of Amuricata

Protocol of Meyer et al. (Meyer et al., 1982) was adopted with slight modifications. Active nauplii (10 numbers) were picked by use of Pasteur pipette and separately exposed to 1mL of different concentrations of crude and fractions of A. muricata in DMSO for 24 hours in test tubes. The volume was made up to 10mL with clean sea water. The active nauplii were counted after 24 hours and the percentage mortality was obtained. The assay was performed in triplicate for each concentration. Lethal concentration that killed 50% of the population of the nauplii (LC50) was determined using Probit analysis by means of the software IBM SPSS Statistics 20.

Thin layer bioautography of fruit of A. muricata

The determination of antioxidant and cytotoxic characters involves the use thin layer chromatography (TLC) techniques such as TLC bioautography using the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as derivatization agent. This is a screening where the antioxidant in the test sample is determined qualitatively using the simple TLC bioautography assay. The compound with antioxidant character is established by the formation of pale yellow coloured picryl group accredited to the reduction of the purple coloured DPPH radicals as these receive an electron or hydrogen ion from the test sample (Molyneux, 2004)

Statistical analysis

All data of an analysis, graphs and the significant difference among the treatment were analyzed or obtained by means Dunnet’s multiple range test by means of Graph Prism version 5.

RESULTS AND DISCUSSION

Analysis of medicinal plants for biologically active compounds is relevant in the determination of pharmacological importance of such plants. Qualitative screening of crude extract of fruit of A. muricata revealed the detection of compounds such as alkaloids, flavonoids, phenolics, tannins, steroids, saponins and carbohydrates with the exclusion of cyanogenic glycosides (Table 1). These compounds are pharmacologically active substances that protect the plant and animal health (Ajuru et al., 2017). Alkaloids are known for cytotoxic, anti-carcinogenic, anti-fungal, anti-viral, anti-bacterial abilities (Bibri, 2018). Detection of flavonoids in the fruit suggests the medicinal value of the plant as in the anti-radical, anti-oxidative, anti-microbial, anti-inflammatory, anti-mutagenic and anti-carcinogenic agents (Ahmed et al., 2016; Choudhary et al., 2015; Farzaneh, 2016; Pietta, 2000). Moreover, since flavonoids are sparingly soluble in water and retained for a relatively long time in the stomach, they are used as candidate for drug production, and tool to prevent cardiovascular diseases (Hayat et al., 2007). Most phenolic are known for anti-oxidant, anti-microbial, anti-inflammatory, anti-viral, anti-tumour and cytotoxic activities (Tyagia et al., 2017; Wijesinghe et al., 2012; Zhang et al., 2013). Saponins are steroidal glycosides with foaming characteristics and bitter taste that have beneficial effect on the blood cholesterol levels, fight cancer and help in health and stimulation of the immune system (Saxena et al., 2013), and known for venotonic, anti-oedematous and anti-inflammatory activities (Antignani and Caliumi, 2007; Vinha et al., 2012; Yuan et al., 2006). Tannins on the other hand, are plant chemicals that have a harsh bitter taste, interact with organic compounds including proteins, thereby shrinking and precipitating the protein molecules. Tannins are used as anti-cancer, anti-viral, anti-tumor, and anti-inflammatory agents and possesses healing properties on wounds, kidney and some related organs or tissues (Ajayi et al., 2011; Suryakumar et al., 2011; Uraku et al., 2015). Previous reports also disclosed that parts of A. muricata contained some of these plant chemicals such as alkaloids (Moghdamtousi et al., 2015; Usunobun et al., 2015).

Despite the ability of water to extract 25.45 ± 2.04% of fresh fruit of A. muricata, the solvent- solvent partitioning of the extracted fruit of A. muricata offered a significant ($p<0.05$) difference in the extraction yields of the fractions obtained from selected solvents in the following order: acetic acid< ethyl acetate< acetone< methanol<hexane< aqueous fractions (Table 2). The variation in the extraction yields explains the presence of different soluble components ranging from polar, mid-polar and non-polar compounds of the fruit extract. Al-Munirri et al. (2017) and Al-Saeedi et al. (2017) also reported that the soluble components of an extract is largely dependent on the selected solvents and their polarities.

The brine shrimp assay is a rapid, cheap and simple bioassay for testing of plant extracts for cytotoxicity on newly hatched shrimps (nauplii) (Elmore, 2007). The protocol has a substantial correlation with cytotoxic and anti-tumor properties (Anderson et al., 1991). In this study, fractions prepared through partitioning of crude extract of fruit of A. muricata in hexane, ethyl acetate, acetone, acetic acid and methanol extracted various compounds as reflected in varied degrees of biological activities (cytotoxicity and antioxidant activities). Similarly, the crude extract and all the fractions caused the death of newly hatched nauplii at different capacities (LC50). Ethyl acetate fraction (79.05 ± 0.58 µg/mL) revealed a substantial
lethality on 50% population of newly hatched nauplii. Other fractions showed LC$_{50}$ which are mentioned as followed: hexane (1015.00 ± 31.36 µg/mL), acetone (104.73 ± 0.04 µg/mL), acetic acid (146.74 ± 16.11 µg/mL), aqueous (330.21 ± 24.99 µg/mL), and methanol (886.05 ± 23.88 µg/mL) (Figure 1). This implies that the ethyl acetate of fruit of A. muricata had significantly ($p<0.05$) lessen LC$_{50}$ value than all the analyzed fractions. This was followed by acetone fraction while the highest value was obtained from the hexane fraction.

Usually the lower the LC$_{50}$, the higher the degree of cytotoxicity of the analyzed compound. Therefore, our results suggest that the ethyl acetate fraction is the most cytotoxic of all the analyzed soluble constituents of the understudied fruit. This is closely followed by the intermediate polar acetone (medium cytotoxic), while the hexane fraction; showed least cytotoxicity on the nauplii. Clarkson’s classification of toxicity of plant extracts defined extracts with LC$_{50}$ above 1000 µg/mL as non-toxic, LC$_{50}$ of 500 to 1000 µg/mL (low toxic), LC$_{50}$ of 100 to 500 µg/mL (medium toxic), LC$_{50}$ of 0 to 100 µg/mL (highly toxic) (Clarkson et al., 2004). Cytotoxicity of fruit of A. muricata has been previously reported by (Kuete et al., 2016), and expressed as a function of plant compounds such as flavonoids, saponins, tannins (Braguini et al., 2018; Daglia, 2012). Some of these chemicals reportedly have antioxidant activity; scavenge free radicals and suppress oxidative stress during cellular respiration which results to anti-tumour and cytotoxic characteristics (Ray et al., 2002).

Table 1. The qualitative phytochemical screening of crude extract fruit of A. muricata.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Crude aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
</tbody>
</table>

Where (+ve) indicates that the compound is detected and (-ve) indicates that the compound is not detected.

Table 2. The percentage extraction yields of crude extracts and fractions of fruits of A. muricata

<table>
<thead>
<tr>
<th>Crude aqueous extract or fractions</th>
<th>Percentage yields (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude aqueous extract</td>
<td>25.45±2.04</td>
</tr>
<tr>
<td>Hexane</td>
<td>1.80±0.05*</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.72±0.03*</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.87±0.05*</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.22±0.02*</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.93±0.04*</td>
</tr>
<tr>
<td>Aqueous</td>
<td>2.02±0.09*</td>
</tr>
</tbody>
</table>

Figure 1. Cytotoxic activities of fruit of A. muricata fruit. Data are represented in terms of mean± standard deviation of triplicate readings. The (*) implies a significant difference at ($p<0.05$).
Figure 2a. (Absence of DPPH)

Figure 2b. (Presence of DPPH)

Figure 2a and 2b. Thin layer chromatograms of crude aqueous extract (a), hexane (b), methanol (c), acetic acid (d), aqueous (e) acetone (f) and ethyl acetate (g) fractions A. muricata fruit respectively.

Figure 3. DPPH radical scavenging activities of fruit of A. muricata fruit. Data are represented in terms of mean± standard deviation of triplicate readings. The (*) implies a significant difference at (p<0.05).

Figure 4. Nitric oxide radical scavenging activities of fruit of A. muricata. Data are represented in terms of mean± standard deviation of triplicate readings. The (*) implies a significant difference at (p<0.05).

DPPH scavenging assay is a popular, stable, easy and fast procedure for determination of antioxidant ability of biologically active compounds. When the DPPH radical is mopped by an antioxidant, the evaluated antioxidant compounds change purple coloured DPPH to yellow colour DPPH (picryl) derivative (Awah et al., 2012). The assay which was conducted using TLC bio-autography at the preliminary stage of this study to establish the antiradical ability of the crude and partitioned fractions of the fruit extract of A. muricata has revealed by the yellow colour bands produced by reaction of crude extract, acetone, acetic acid, methanol, or aqueous fractions to DPPH radicals (Figure 2). The case of hexane or ethyl acetate fractions was different as there was no visible yellow colouration from its interaction with DPPH; this indicates a lack of anti-radical activity. Moreover, the selected solvents might
have solubilized compounds according to their polarities since polar solvents have ability to extract polar compounds while non-polar solvents extract non-polar compounds. Assessment of anti-radical activity by TLC bio-autography at the preliminary stages of investigation of antioxidant of some medicinal plants was reported by Rached et al., 2010, Guerrini et al., 2016 and Belagitz et al., 2017.

The extent of antioxidant activity of fruit of A. muricata was revealed by the spectrophotometric assay using DPPH (Figure. 3) and nitric oxide antiradical activities (Figure. 4). Ascorbic acid which was used as the reference for DPPH radical scavenging ability assay had the higher IC$_{50}$ (124.52 ± 0.73 µg/mL) compare to the IC$_{50}$ for ethyl acetate fraction (64.15 ± 0.32 µg/mL), this was equivalent with the acetone fraction (50.04 ± 0.06 µg/mL) of fruit of A. muricata. This result further supports the presence of antiradical agent(s) in the fruit of A. muricata and revealed that acetone extracted anti-radical agent in the same capacity as ethyl acetate; possible alternative extracting solvent for the plant’s antioxidant agent. Several reports have confirmed the antioxidant activity or capacity of medicinal plants by means of DPPH scavenging assay (Han et al., 2008; Kanagavalli et al., 2019; Matkowski et al., 2006; Moualek et al., 2016).

Nitric oxide (NO) or reactive nitrogen species react to cause alteration of structural and functional behaviour of cellular components resulting into diseases like diabetes mellitus, cardiovascular challenges and cancer (Di Meo et al., 2016). NO scavenging capacity is determined by a decrease in the absorbance of sodium nitroprusside in the presence of an antioxidant at 550 nm. In order to evaluate the antioxidant potency through NO scavenging activity by the A. muricata fruit, the change of optical density of NO was monitored (Figure 4). Incubation of sodium nitroprusside in presence of the extract or fractions of A. muricata at 25°C for 2 hours inhibited the production of nitrite. This results as determined by percentage inhibition of nitrite (NO$_3^-$) production, and calculated as nitric oxide scavenging activity given as the inhibitory concentration (IC$_{50}$) of the crude and the fractions of A. muricata. Acetone (103.89 ± 0.11 µg/mL) and ethyl acetate (104.23 ± 10.14 µg/mL) fractions demonstrated better ability to ward off oxidative challenges resulting from NO activity than ascorbic acid (160.11 ± 10.04 µg/mL). It follows that the A. muricata contents with oxygen to react with nitric oxide there by inhibiting the generation of nitrite. Hence, the ethyl acetate and acetone fractions significantly (p < 0.05) reduced the generation of nitrite than ascorbic acid. Moreover, inhibition of generation of nitric oxide radicals was substantially pronounced in the mid-polar to polar solvents (ethyl acetate, acetone, acetic acid, methanol and water). Therefore, crude extract and fractions of the tested fruit of A. muricata contained antioxidant character(s) which have relatively polar functional character(s) as expressed by their significant activity in selected extracting polar solvents.

Plant chemicals such as polyphenols (flavonoids and condensed tannins) are polar compounds containing hydroxyl functional group (-OH), which are indicted in the inhibition of nitrogen and intermediate radicals (Fernández et al., 2017). Previously, A. muricata fruit reportedly contained a substantial level of phenolic compounds including cinnamic acid analogues and p-coumaric acid (Fernández et al., 2017; Jimenez-Suárez et al., 2016) and acetogenins (Moghaddamoussi et al., 2015; Patel et al., 2016) which were isolated by use of solvents (ethyl acetate or acetone).

**CONCLUSION**

This study corroborates the fact that crude aqueous extract, ethyl acetate and acetone fractions of fruit of A. muricata possess favorable antioxidant and cytotoxic consequence, and may be applied in chemotherapy. It is suggested that consumption of A. muricata could be a good remedy for prevention of illness; especially cancer. However, further studies are recommended for the toxicological and pharmacological evaluation of the fruit of A. muricata in human cancer cell lines or model are recommended.

We wish appreciate the entire members of staff of Biochemistry Department of Lagos State University, Ojo and Lagos State Polytechnic, Ikorodu, Lagos State for their supports throughout the cause of study.

**REFERENCES**


Farzaneh, V. (2016). Development of the nutraceutical and pharmaceutical applications of plants selected from Portugal and Iran with presumptive health potentials. 1-290.


its chemical constituents. *Pharmaceutical Biology, 54*(9), 1822-1830.


