



Research Article

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

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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 consequential 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 signaling (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

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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*, *Palythoacari baeorum*, *Averrhoa carambola* L. 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 round 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

Dragendroff test

A few drops of Dragendroff'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 (Salkowstis 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 (BSL) assay

Hatching of the brine shrimp

Artemia salinacysts 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* in 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:

$$\% \text{ DPPH scavenging activity} = \frac{(\text{absorbance of control} - \text{absorbance of sample})100}{\text{absorbance of control}}$$

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:

$$\% \text{ Nitric oxide scavenging activity} = \frac{(\text{absorbance of control} - \text{absorbance of sample})100}{\text{absorbance of control}}$$

Cytotoxic activity of fruit of *A. muricata* using Brine Shrimp Lethality (BSL) assay

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BSL assay of fruit of *Amuricata*

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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 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 (Bribi, 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 (Moghadamtousi *et al.*, 2015; Usunobun *et al.*, 2015).

Despite the ability of water to extract $25.45 \pm 2.04\%$ 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-Muniri *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 ($79.05 \pm 0.58 \mu\text{g/mL}$) revealed a substantial

lethality on 50% population of newly hatched nauplii. Other fractions showed LC_{50} which are mentioned as followed: hexane ($1015.00 \pm 31.36 \mu\text{g/mL}$), acetone ($104.73 \pm 0.04 \mu\text{g/mL}$), acetic acid ($146.74 \pm 16.11 \mu\text{g/mL}$), aqueous ($330.21 \pm 24.99 \mu\text{g/mL}$), and methanol ($886.05 \pm 23.88 \mu\text{g/mL}$) (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 $1000 \mu\text{g/mL}$ as non-toxic, LC_{50} of 500 to $1000 \mu\text{g/mL}$ (low toxic), LC_{50} of 100 to 500 $\mu\text{g/mL}$ (medium toxic), LC_{50} of 0 to 100 $\mu\text{g/mL}$ (highly toxic) (Clarkson *et al.*, 2004). Cytotoxicity of fruit of *A. muricata* has been previously reported by (Kuethe *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*.

Phytochemical	Crude aqueous extract
Alkaloids	+
Glycosides	-
Flavonoids	+
Phenolics	+
Tannins	+
Steroids	+
Saponins	+
Carbohydrates	+

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*

Crude aqueous extract or fractions	Percentage yields (%)
Crude aqueous extract	25.45 ± 2.04
Hexane	$1.80 \pm 0.05^*$
Ethyl acetate	$0.72 \pm 0.03^*$
Acetone	$0.87 \pm 0.05^*$
Acetic acid	$0.22 \pm 0.02^*$
Methanol	$0.93 \pm 0.04^*$
Aqueous	$2.02 \pm 0.09^*$



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

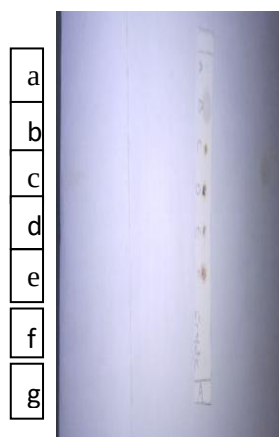


Figure 2a. (Absence of DPPH)

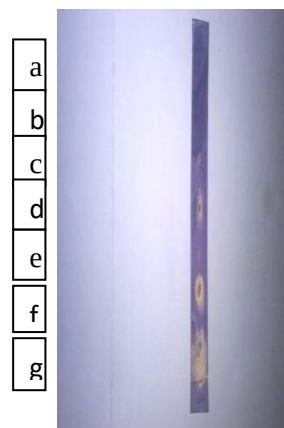


Figure 2b. (Presence of DPPH)

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.

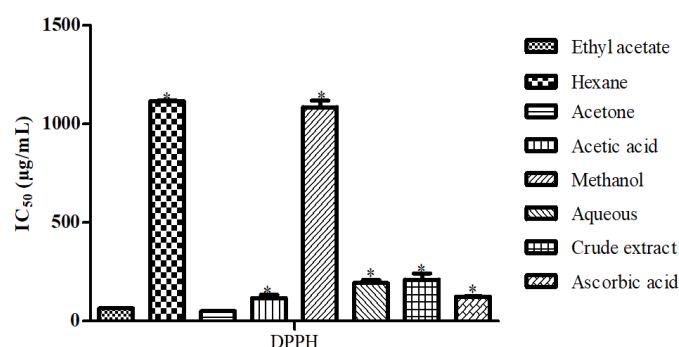


Figure 3. DPPH radical scavenging activities of fruit of *A. muricata* fruit. Data are represented in terms of mean $\pm$ standard deviation of triplicate readings. The (*) implies a significant difference at ($p < 0.05$).

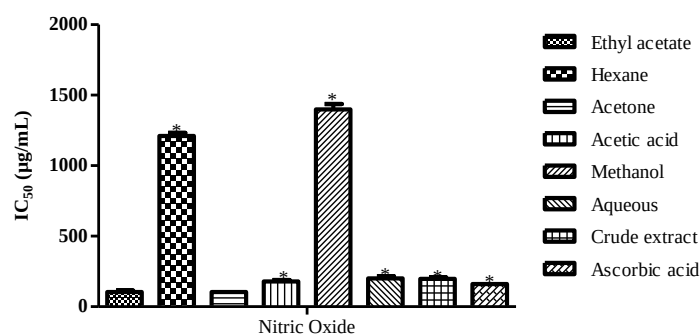


Figure 4. Nitric oxide radical scavenging activities of fruit of *A. muricata*. Data are represented in terms of mean $\pm$ standard deviation of triplicate readings. The (*) implies a significant difference at ($p < 0.05$).

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 anti-oxidant of some medicinal plants was reported by Rached *et al.*, 2010, Guerrini *et al.*, 2016 and Belaqziz *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 IC₅₀ (124.52 ± 0.73 µg/mL) compare to the IC₅₀ 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 (NO₂) production, and calculated as nitric oxide scavenging activity given as the inhibitory concentration (IC₅₀) 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* contends 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-Suarez *et al.*, 2016); and acetogenins (Moghadamtousi *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.

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REFERENCES

- Abu, F., Taib, M., Norma, C., Moklas, M., Aris, M. and Mohd Akhir, S. (2017). Antioxidant Properties of Crude Extract, Partition Extract, and Fermented Medium of Dendrobium sabin Flower. *Evidence-Based Complementary and Alternative Medicine*, 2017:1-9.
- Agu, K.C., Okolie, N.P., Falodun, A., Erharuyi, O., Igbe, I., Elekofehinti, O.O. and Oghagbon, S.E. (2017). Isolation and Elucidation of 15-Acetylguanacone from Soursop (*Annona muricata* Linn) Fruit and Molecular Docking Experiments. *Journal of Applied Sciences and Environmental Management*, 21(2), 236-243.
- Ahmed, J., Mulla, M.Z. and Arfat, Y.A. (2016). Thermo-mechanical, structural characterization and antibacterial performance of solvent casted polylactide/cinnamon oil composite films. *Food Control*, 69, 196-204.
- Ajayi, G., Olagunju, J., Ademuyiwa, O. and Martins, O. (2011). Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of *Plumbago zeylanica*, Linn. *Journal of Medicinal Plants Research*, 5(9), 1756-1761.
- Ajuru, M.G., Williams, L.F. and Ajuru, G. (2017). Qualitative and quantitative phytochemical screening of some plants used in Ethnomedicine in the Niger delta region of Nigeria. *Journal of Food and Nutrition Sciences*, 5(5), 198-205.
- Al Muniri, R. M.S. and Hossain, M.A. (2017). Evaluation of antioxidant and cytotoxic activities of different extracts of folk medicinal plant *Hapllophyllum*

- tuberculatum*. *Egyptian Journal of Basic and Applied Sciences*, 4(2), 101-106.
- Al Saeedi, A.H., Al-Ghafri, M.T.H. and Hossain, M.A. (2017). Brine shrimp toxicity of various polarities leaves and fruits crude fractions of *Ziziphus jujuba* native to Oman and their antimicrobial potency. *Sustainable Chemistry and Pharmacy*, 5, 122-126.
- Alencar, P.O.C., Lima, G.C., Barros, F.C.N., Costa, L. E.C., Ribeiro, C. V. P.E., Sousa, W. M., Sombra, V.G., Abreu, C.M.W.S., Abreu, E.S., Pontes, E.O.B., Oliveira, A.C., de Paula, R.C.M. and Freitas, A.L.P. (2019). A novel antioxidant sulfated polysaccharide from the algae *Gracilaria caudata*: In vitro and in vivo activities. *Food Hydrocolloids*, 90, 28-34.
- Anderson, J., Goetz, C., McLaughlin, J. and Suffness, M. (1991). A blind comparison of simple bench-top bioassays and human tumour cell cytotoxicities as antitumor prescreens. *Phytochemical Analysis*, 2(3), 107-111.
- Antignani, P.L. and Caliumi, C. (2007). Medical treatment of chronic venous insufficiency. *Vascular Disease Prevention*, 4(2), 117-124.
- Awah, F.M., Uzoegwu, P.N., Ifeonu, P., Oyugi, J.O., Rutherford, J., Yao, X., Fehrmann, F., Fowke, K.R. and Eze, M.O. (2012). Free radical scavenging activity, phenolic contents and cytotoxicity of selected Nigerian medicinal plants. *Food Chemistry*, 131(4), 1279-1286.
- Belaqziz, M., Tan, S.P., El-Abbassi, A., Kiai, H., Hafidi, A., O'Donovan, O. and McLoughlin, P. (2017). Assessment of the antioxidant and antibacterial activities of different olive processing wastewaters. *PloS One*, 12(9), e0182622.
- Braguini, W.L., Pires, N.V. and Alves, B.B. (2018). Phytochemical Analysis, Antioxidant Properties and Brine Shrimp Lethality of Unripe Fruits of *Solanum viarum*. *Journal of Young Pharmacists*, 10(2).
- Bribi, N. (2018). Pharmacological activity of Alkaloids: A Review. *Asian Journal of Botany*, 1, 1-6.
- Choudhary, M., Kumar, V., Malhotra, H. and Singh, S. (2015). Medicinal plants with potential anti-arthritis activity. *Journal of Intercultural Ethnopharmacology*, 4(2), 147.
- Chugh, N. A., Bali, S. and Koul, A. (2018). Integration of botanicals in contemporary medicine: road blocks, checkpoints and go-ahead signals. *Integrative Medicine Research*, 7(2), 109-125.
- Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O.M., Pillay, P., Matsabisa, M. G., Bhagwandin, N. Smith, P.J. and Folb, P.I. (2004). In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of Ethnopharmacology*, 92(2-3), 177-191.
- Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology*, 23(2), 174-181.
- De Iuliis, G.N., Newey, R.J., King, B.V. and Aitken, R.J. (2009). Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. *PLoS One*, 4(7), e6446.
- Di Meo, S., Reed, T.T., Venditti, P. and Victor, V.M. (2016). Role of ROS and RNS sources in physiological and pathological conditions. *Oxidative Medicine and Cellular Longevity*, 2016:1245049.
- Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxicologic Pathology*, 35(4), 495-516.
- Evans, W.C. (2002). Trease and Evans. *Pharmacognosy*, 553-557.
- Falodun, A. (2010). Herbal medicine in Africa-distribution, standardization and prospects. *Research Journal of Phytochemistry*, 4(3), 154-161.
- Farzaneh, V. (2016). Development of the nutraceutical and pharmaceutical applications of plants selected from Portugal and Iran with presumptive health potentials. 1-290.
- Fernández, V., Bahamonde, H. A., Javier Peguero Pina, J., Gil-Pelegrín, E., Sancho-Knapik, D., Gil, L., Heiner, E.G. and Eichert, T. (2017). Physico-chemical properties of plant cuticles and their functional and ecological significance. *Journal of Experimental Botany*, 68(19), 5293-5306.
- Gavamukulya, Y., Abou-Ellella, F., Wamunyokoli, F. and AEl-Shemy, H. (2014). Phytochemical screening, antioxidant activity and in vitro anticancer potential of ethanolic and water leaves extracts of *Annona muricata* (Graviola). *Asian Pacific Journal of Tropical Medicine*, 7, S355-S363.
- Guerrini, A., Sacchetti, G., Grandini, A., Spagnoletti, A., Asanza, M. and Scalvenzi, L. (2016). Cytotoxic effect and TLC bioautography-guided approach to detect health properties of amazonian *hedyosmum Sprucei* essential oil. *Evidence-Based Complementary and Alternative Medicine*, 1-8.
- Han, J., Weng, X. and Bi, K. (2008). Antioxidants from a Chinese medicinal herb—*Lithospermum erythrorhizon*. *Food Chemistry*, 106(1), 2-10.
- Harbone, J. (1973). Method of plant analysis. *Phytochemical Methods*, 1-32.
- Hasan, A.U. (2015). *Cytotoxic Activity of Curcumin, Melissa, and Cloves Extracts on Colon, Lung, and Breast Cancer Cell Lines*. Tennessee State University. 1-14.
- Hayat, S., Ali, B. and Ahmad, A. (2007). Salicylic acid: biosynthesis, metabolism and physiological role in plants *Salicylic acid: A plant Hormone*, 1-14.
- Jimenez-Suarez, V., Nieto-Camacho, A., Jiménez-Estrada, M. and Alvarado Sanchez, B. (2016). Anti-inflammatory, free radical scavenging and alpha-glucosidase inhibitory activities of *Hamelia patens* and

- its chemical constituents. *Pharmaceutical Biology*, 54(9), 1822-1830.
- Joshi, K. R., Devkota, H. P., Nakamura, T., Watanabe, T. and Yahara, S. (2015). Chemical constituents and their DPPH radical scavenging activity of Nepalese crude drug *Begonia picta*. *Records of Natural Products*, 9(3), 446.
- Jothy, S.L., Zuraini, Z. and Sasidharan, S. (2011). Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitory activities of *Cassia fistula* seeds extract. *Journal of Medicinal Plants Research*, 5(10), 1941-1947.
- Kanagavalli, U. and Priya, L. (2019). The comparative preliminary phytochemical investigation, tlc analysis and antioxidant activity of different solvent extracts of *boerhavia diffusa* linn. *International Journal of Research in Pharmaceutical Sciences*, 10(1), 245-256.
- Kuete, V., Mbaveng, A.T., Nono, E.C., Simo, C.C., Zeino, M., Nkengfack, A. E. and Efferth, T. (2016). Cytotoxicity of seven naturally occurring phenolic compounds towards multi-factorial drug-resistant cancer cells. *Phytomedicine*, 23(8), 856-863.
- Lavilla, J.R.C.A. and Villazorda, M.G.L. (2015). Phytochemical, brine shrimp lethality assay, antibacterial and antioxidant activity screening of *nypafruticans* (Nipa) leaf extracts. *International Journal of Chemical Science*, 13(3), 1522-1528.
- Mao, J.J., Palmer, C.S., Healy, K.E. Desai, K. and Amsterdam, J. (2011). Complementary and alternative medicine use among cancer survivors: a population-based study. *Journal of Cancer Survivorship*, 5(1), 8-17.
- Matkowski, A. and Piotrowska, M. (2006). Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. *Fitoterapia*, 77(5), 346-353.
- Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D.J. and McLaughlin, J.L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta medica*, 45(05), 31-34.
- Moghadamtousi, S., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. and Kadir, H. (2015). *Annona muricata* (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. *International journal of molecular sciences*, 16(7), 15625-15658.
- Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, 26(2), 211-219.
- Moualek, I., Aiche, G.I., Guechaoui, N.M., Lahcene, S. and Houali, K. (2016). Antioxidant and anti-inflammatory activities of *Arbutus unedo* aqueous extract. *Asian Pacific Journal of Tropical Biomedicine*, 6(11), 937-944.
- Muthu, S., & Durairaj, B. (2015). Evaluation of antioxidant and free radical scavenging activity of *Annona muricata*. *European Journal of Experimental Biology*, 5(3), 39-45.
- Nanyonga, S.K., Opoku, A., Lewu, F., Oyediji, A.O. and Singh, M. (2013). Chemical composition, antioxidant activity and cytotoxicity of the essential oils of the leaves and stem of *Tarchonanthus camphoratus*. *Journal of Biology, Agriculture and Healthcare*, 5(4), 150-160.
- Ogundare, O. C., Akoro, S. M., Ogunfowora, A. O. and Oladunni, A. (2017). Investigation of Antioxidant Activity and Cytotoxic Effect of Stem of *Anthocleista djalensis*. *European Journal of Medicinal Plants*, 1-8.
- Okolie, N., Agu, K. and Eze, G. (2013). Protective effect of ethanolic leaf extract of *Annona muricata* Linn on some early events in cycasinduced colorectal carcinogenesis in rats. *Journal of Pharmaceutical and Scientific Innovation*, 2(4), 14-21.
- Patel, M.S. and Patel, J.K. (2016). A review on a miracle fruits of *Annona muricata*. *Journal of Pharmacognosy and Phytochemistry*, 5(1), 137.
- Pietta, P.G. (2000). Flavonoids as antioxidants. *Journal of Natural Products*, 63(7), 1035-1042.
- Rached, W., Benamar, H., Bennaceur, M. and Marouf, A. (2010). Screening of the antioxidant potential of some Algerian indigenous plants. *Journal of Biological Science*, 10(4), 316-324.
- Rahman, M.A., Sultana, R., Emran, T.B., Islam, M.S., Rahman, M.A., Chakma, J.S. and Hasan, C.M.M. (2013). Effects of organic extracts of six Bangladeshi plants on in vitro thrombolysis and cytotoxicity. *BMC Complementary and Alternative Medicine*, 13(1), 25.
- Ramachandran, S., Vamsikrishna, M., Gowthami, K.V., Heera, B. and Dhanaraju, M.D. 2011. Assessment of Cytotoxic Activity of *Agave cantula* Using Brine Shrimp (*Artemia salina*) Lethality Bioassay. *Asian Journal of Scientific Research*, 4: 90-94.
- Rao, M. (1997). Nitric oxide scavenging by curcuminoids. *Journal of Pharmacy and Pharmacology*, 49(1), 105-107.
- Ray, G., & Husain, S. A. (2002). Oxidants, antioxidants and carcinogenesis. *Indian Journal of Experimental Biology*, 40(11), 1213-32.
- Saxena, M., Saxena, J., Nema, R., Singh, D., & Gupta, A. (2013). Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry*, 1(6).
- Sofowora, A. (1993). Screening plants for bioactive agents. *Medicinal Plants and Traditional Medicinal in Africa*, 2, 134-156.

- Suryakumar, G., & Gupta, A. (2011). Medicinal and therapeutic potential of Sea buckthorn (*Hippophae rhamnoides* L.). *Journal of Ethnopharmacology*, 138(2), 268-278.
- Tyagia, T. and Argawak, M. (2017). Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. *Journal of Pharmacognosy and Phytochemistry*, 6(1), 195-206.
- Uraku, A. and Nwankwo, V. (2015). Phytochemical and nutritional composition analysis of murraya Koenigii Linn leaves. *British Journal of Pharmaceutical Research*, 6, 174-180.
- Usunobun, U., Okolie, N., Anyanwu, O., Adegbegi, A. and Egharevba, M. (2015). Phytochemical screening and proximate composition of *Annona muricata* leaves. *European Journal of Botany, Plant Sciences and Phytology*, 2(1), 18-28.
- Vinha, A. F., Soares, M. O., Castro, A., Santos, A., Oliveira, M. B. P. and Machado, M. (2012). Phytochemical characterization and radical scavenging activity of aqueous extracts of medicinal plants from Portugal. *European Journal of Medicinal Plants*, 2(4), 335-347.
- Wijesinghe, W., & Jeon, Y.J. (2012). Biological activities and potential industrial applications of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: A review. *Carbohydrate Polymers*, 88(1), 13-20.
- Yuan, G., Wahlqvist, M.L., He, G., Yang, M. and Li, D. (2006). Natural products and anti-inflammatory activity. *Asia Pacific Journal of Clinical Nutrition*, 15(2).
- Zhang, L., Ravipati, A.S., Koyyalamudi, S.R., Jeong, S.C., Reddy, N., Bartlett, J., Smith, P.T., de la Cruz, M., Monteiro, M.C., Melguizo, Á. and Melguizo, Á. (2013). Anti-fungal and anti-bacterial activities of ethanol extracts of selected traditional Chinese medicinal herbs. *Asian Pacific Journal of Tropical Medicine*, 6(9), 673-681.