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Studies on the Phytochemical and Proximate Properties of the Extract of Unripe *Annona muricata* (Linn.) Fruit

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Authors' contributions

This work was carried out in collaboration between both authors. Author EOD designed the study, identified the species of plant and edited the manuscript while author OSF wrote the protocol, managed literature searches, performed the experimental analyses and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Study was carried out to investigate the phytochemical and proximate properties of unripe *A. muricata* fruit extract.
Place and Duration of Study: It was carried out at the Federal University of Technology, Akure Central Laboratory between June 2014 - August 2014.
Methodology: Matured unripe fruits of *A. muricata* were collected, washed, dried, powdered and extracted using 70% ethanol. After which the proximate and phytochemical properties were determined using standard methods.
Results: The result of the qualitative phytochemical screening revealed the presence of saponin, tannin, terpernoid, flavonoid, anthraquinone and cardiac glycoside but alkaloid and phlobatanin were absent while the quantitative screening showed that cardiac glycoside (27.19 mg/g) was the highest occurring phytochemical in the extract followed by terpenoid (19.31mg/g), tannin (13.12 mg/g), flavonoids (9.09mg/g), saponin (4.63 mg/g), and anthraquinone (1.12 mg/g). Meanwhile, the results of the proximate composition (%) of unripe *A. muricata* fruit extract showed that

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carbohydrate was the highest occurring nutrient with 48.7, followed by moisture content (24.51), fat (17.13), crude protein (9.09) and ash (0.58). However, crude fibre was not detected.

Conclusion: The results of this present study indicate that *Annona muricata* fruit extract is potentially useful in phytomedicine and also provide information on the nutritive importance to body health.

Keywords: Phytochemical; proximate; Annona muricata; unripe fruit; extract.

1. INTRODUCTION

Phytomedicines have been existing since the beginning of modern age [1]. Plants with one or more of their tissues containing substances that can be utilized for curative purposes or which can serve as constituents of conventional drugs are known as medicinal plants. They are used around the world for different purposes. The better understanding of medicine derived from plants depends on two major factors that go hand in hand. One of them entails the proof to show that the constituted drug does what it is claimed to do while the other is the identification of the active components using various chemical analyses [2]. Plants possess endless ability to produce chemical substances referred to as secondary metabolites, of which less than 10% have been synthesized. Chemicals present in plants can be grouped as primary and secondary metabolites [3]. Primary metabolites include amino acids, proteins, purines, sugars, chlorophylls and pyrimidines of nucleic acids while secondary components ranged from alkaloids to terpenoids and acetogenins to different phenols [3]. The quality and quantity of these metabolites differ from plant to plant, part to part and from one location to the other [4]. These active compound are used by plants for different purposes such as defense against predation by insects, microorganisms and herbivores [4]. In addition, some of them are responsible for plant odour (terpenoids), pigmentation (tannins and quinines), and flavour (capsacin) [5]. The defensive metabolites in these plants confer on them various medicinal properties which are employed by humans because of their great importance in health care of individuals and communities [6].

Annona muricata (L.) also called soursop has been named as popular fruit grown throughout the tropics [2]. The fruit is more or less oval or heart-shaped, sometimes irregular, lopsided or curved, due to improper carper development or insect injury. A number of medicinal properties are attributed to the leaves and fruit of soursop [6]. Fruit and fruit juice are used for treating infections caused by worms and parasites. In the Brazilian Amazon, the oil of the leaves and unripe fruit is mixed with olive oil and used externally for neuralgia, rheumatism and arthritis [7]. Pulverized immature fruits, which are very astringent, are decocted as a dysentery and diarrhoea remedy. In addition, the flesh of the unripe fruit is applied as a poultice and left unchanged for 3 days to speed up wound healing [8].

Various researches on phytochemical and proximate screenings of the extracts of *A. muricata* have been carried out. Usunobun et al. [6] and Solomon-Wisdom et al.[9] carried out findings on the phytochemical and proximate properties of *A. muriata* leaf extract. Similarly, Neela and Alexander [10] reported the proximate constituents of unripe soursop mesocarp and epicarp powder. However, much has not been done on the chemical and nutritional constituents of the unripe fruits. Therefore, the present study was focused on studying the phytochemical and proximate properties of the ethanol extract of unripe *Annona muricata* fruit.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Fresh unripe fruits of *Annona muricata* (Linn) were collected from a garden in Arimoro area of Ilesha, Osun State, Nigeria. The fruits were identified and authenticated by using the herbarium specimens of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Ondo State, Nigeria.

2.2 Extraction of the Bioactive Constituents of Unripe *A. muricata* Fruit

The methods of Jothy et al. [11], Nweke and Ibiam [12] were employed. Unripe *A. muricata* fruits were washed with sterile water. The peels and seeds were separated from the pulp and then cut into smaller pieces. The fruits were oven dried at 50ºC for 4 days. The dried fruits were

then pulverized into fine powder by blending in a high-speed Philips Model blender. The fine powder was kept in an airtight container to avoid the absorption of moisture. Three hundred grams (300 g) of the powdered sample was soaked in two thousand, five hundred millimeters (2500 ml) of 70% ethanol as solvents to extract the bioactive compounds. The container was labelled appropriately and left for 72 hours (3 days). After which it was sieved using muslin cloth and then filtered using 0.45 µm micropore filter. The filtrates were vaporized to dryness using rotary evaporator and subsequently lyophilized to remove the extracting solvent. The ethanol extract was preserved in a sterile bottle at $4^{O}C$ until use.

2.3 Phytochemical Screening of Ethanolic Extract of *A. Muricata*

The qualitative and quantitative phytochemical analyses of ethanol extract of *A. muricata* were carried out according to standard procedures reported by AOAC [13], Dawang and Datup [14].

2.3.1 Qualitative Determination of Phytochemical Constituents of Unripe *A. muricata* Extract

2.3.1.1 Alkaloid

About 0.5 g of the extract was stirred with 5 ml of 1% aqueous HCl on a steam water bath, 1ml of the filtrate was treated with a few drops of Dragendorf reagent. Blue black turbidity was taken as preliminary evidence for the presence of alkaloid.

2.3.1.2 Saponin

The ability of saponin to produce frothing in aqueous solution was used as screening test for saponin. About 0.5 g of extract was shaken with distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for the presence of saponin.

2.3.1.3 Tannin

About 0.5 g of the extract was stirred with 100 ml of distilled water, filtered and ferric chloride reagent was added to the filtrate. A blue black green precipitate was taken as evidence for presence of tannin.

2.3.1.4 Phlobatannin

Deposition of red precipitate when 0.5 g of the extract was boiled with 1% aqueous HCl was taken as evidence for the presence of phlobatannin.

2.3.1.5 Anthraquinone

About 0.5 g of the extract was shaken with 10 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presences of pink red or violet colour in the ammonia layer indicated the presence of free anthraquinone.

2.3.1.6 Flavonoid

About 0.5 g of the extract was stirred with 20 ml of dilute ammonia solution and a yellow colouration was observed. The disappearance of the yellow colour after the addition of 1 ml Conc. H_2SO_4 indicates the presence of flavonoid.

2.3.1.7 Terpenoid

About 0.5 g of the extract was mixed with 20 ml of chloroform, filtered and 3 ml of conc. H_2SO_4 was added to the filtrate to form a layer. A reddish brown colour at the interface was observed which indicate the presence of terpenoid.

2.3.1.8 Cardiac glycosides

About 0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1 ml of Conc. H_2SO_4, a brown colouration obtained at the interface indicated the presence of a deoxy-sugar characteristic of cardenolides.

2.3.2 Quantitative Determination of Phytochemical components of Unripe *A. muricata* extract

2.3.2.1 Tannin

About 0.2 g of finely ground sample was weighed into a 50 ml sample bottle. 10 ml of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2 hours at $30^{O}C$. Each solution was then centrifuged and the supernatant was stored in ice bath. About 0.2 ml of each solution was pipetted into the test tube and 0.8 ml of distilled water was added. Standard tannic acid solutions were prepared from a 0.5 mg/ml of the stock made up to 1 ml with distilled water. About 0.5 ml of Folin ciocateau reagent was added to both sample and standard followed by 2.5 ml of

20% Na_2CO_3 the solution were then vortexed and allowed to incubate for 40minutes at room temperature. Its absorbance was read at 725 nm against a reagent blank and the concentration was obtained from a standard tannic acid curve prepared [15].

2.3.2.2 Saponin

The spectrophotometric method of Brunner [16] was used for saponin determination. Two grams of the finely ground sample was weighed into a 250 ml beaker, 100 ml of isobutyl alcohol or (But-2-ol) was added and the mixture was shaken for 5hours to ensure uniform mixing. The mixture was filtered with No 1 Whatman filter paper into 100 ml beaker containing 20 ml of 40% saturated solution of Magnesium carbonate ($MgCO_3$). The mixture obtained again was filtered through No. 1 Whatman filter paper to obtain a clean colourless solution. About 1 ml of the colourless solution was taken into 50ml volumetric flask using pipette, 2 ml of 5% Iron (III) chloride ($FeCl_3$) solution was added and made up to the mark with distill water. It was allowed to stand for 30 min for the colour to develop. The absorbance was read against the blank at 380 nm.

2.3.2.3 Cardiac glycosides

The procedure described by Sofowora [17] was used. Ten milliliters (10 ml) of the was extract pipetted into a 250 ml conical flask, then 50 ml chloroform was added and shaken on vortex mixer for 1 hour. The mixture was filtered into 100 ml conical flask after which 10 ml of pyridine and 2 ml of 29% of sodium nitroprusside were added and shaken thoroughly for 10 min. Then 3 ml of 20% NaOH was added to develop a brownish yellow colour and the absorbance was read at 510 nm. Standard curve of the glycosides standard (0-50 mg/ml) was plotted for calculation.

2.3.2.4 Terpenoid

The procedure described by Sofowora [17] was used. About 0.5 g of finely grounded sample was weighed into a 50 ml conical flask and 20 ml mixture of chloroform and methanol (2:1) was added. The mixture was shaken thoroughly and allowed to stand for 15 min at room temp. The suspension was centrifuged at 3000 rpm and the supernatant was discarded. The precipitate was re-washed with 20 ml mixture of chloroform and methanol (2:1), re-centrifuged and the precipitate was dissolve in 40 ml of 10% sodium dodecyl sulfate solution. About 1 ml of 0.01M ferric chloride was added and allowed to stand for 30 min before taken the absorbance at 510 nm. The standard terpenoid (alpa terpineol) concentration ranging from 0-5 mg/ml from the stock solution was used to obtain standard curve.

2.3.2.5 Flavonoid

The flavonoid content of the extract of *A. muricata* was determined using the aluminium chloride assay through colorimetry. An aliquot (0.5 ml) of the extract was taken in different test tubes then 2 ml of distilled water was added followed by the addition of 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) and allowed to stand for 6 min. Later 0.15 ml of Aluminium trichloride (10% $AlCl_3$) was added and incubated for 6 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4% w/v) and volume was made up to the 5ml with distilled water. After 15 min of incubation, the mixture turned to pink whose absorbance was measured at 510 nm using a colorimeter. Distilled water was used as blank. The flavonoid content was expressed in mg of catechin equivalents (CE) per gram of extract [18].

2.3.2.6 Anthraquinone

The anthraquinone content of the 70% ethanolic extract of *A. muricata* was determined using the method of Tekeshwar et al. [19].

2.4 Proximate Analysis of Ethanolic Extract of Unripe *Annona muricata* Fruit

The proximate analysis of the sample was carried out using the standard procedures of AOAC [13].

2.5 Statistical Analysis

Mean values of replicates were reported with their standard deviations using SPSS 16.0.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening of Unripe *A. muricata* Fruit Extract

The qualitative and quantitative phytochemical screenings of unripe ethanol fruit extract of *A. muricata* are shown on Fig. 1. Bioactive components such as tannin, saponin, flavonoid,

anthraquinone, cardiac glycoside and terpenoid were present while alkaloid, steroid and phlobatannin were not found. Cardiac glycoside (27.19 mg/g) was the highest occurring phytochemical in the extract followed by terpenoid (19.31mg/g), tannin (13.12 mg/g), flavonoids (9.09mg/g) and saponin (4.63 mg/g). However, anthraquinone (1.12 mg/g) was least present. Alkaloids are the most efficient therapeutically significant plant substances. They show marked physiological effects when administered to animals and are very important as they constitute most of the valuable drugs used in medicine and ethno-medicines [20]. Hence, the absence of alkaloid in the extract of unripe *A. muricata* fruit might impair its antimicrobial activity.

The presence of cardiac glycoside in this study is in agreement with the findings of Solomon-Wisdom et al. [9] who reported the presence of cardiac glycoside in the aqueous and methanolic leaf extracts of *A. muricata.* Cardiac glycosides are known to work by inhibiting the Na^{+}/K^{+} pump. This inhibition increases the amount of Ca^{2+} ions available for contraction of the heart muscles, which improves cardiac output and reduces distention of the heart. Thus, they are useful in the treatment of congestive heart failure and cardiac arrhythmia. They are also used to strengthen a weakened heart and all it parts to function more efficiently. Therefore, the presence of cardiac glycoside (27.19mg/g) in the extract of unripe *A. muricata* fruits justified the use of *A. muricata* plant in the treatment of various heart conditions as reported by Taylor [7]. It also possesses a mechanism for elimination of poisons from human and animal systems [21].

The quantity of flavonoid in this study (9.05 mg/g) compared favourably with 9.65 mg/g reported for leaf extract *Ocimum sanctum* but it is lower when compared to 22.80 and 17.90 mg/g reported for the methanolic leaf extract of *Gmelina arborea* and *Mentha spicata* respectively [3]. Flavonoids have been reported to be a potent water soluble anti-oxidant and free radical scavenger which prevent oxidative cell damage and also have strong anticancer activity. Therefore, the presence of flavonoid in the extract of *A. muricata* fruit confirmed the reported use of the fruits as an important anticancer agent [7].

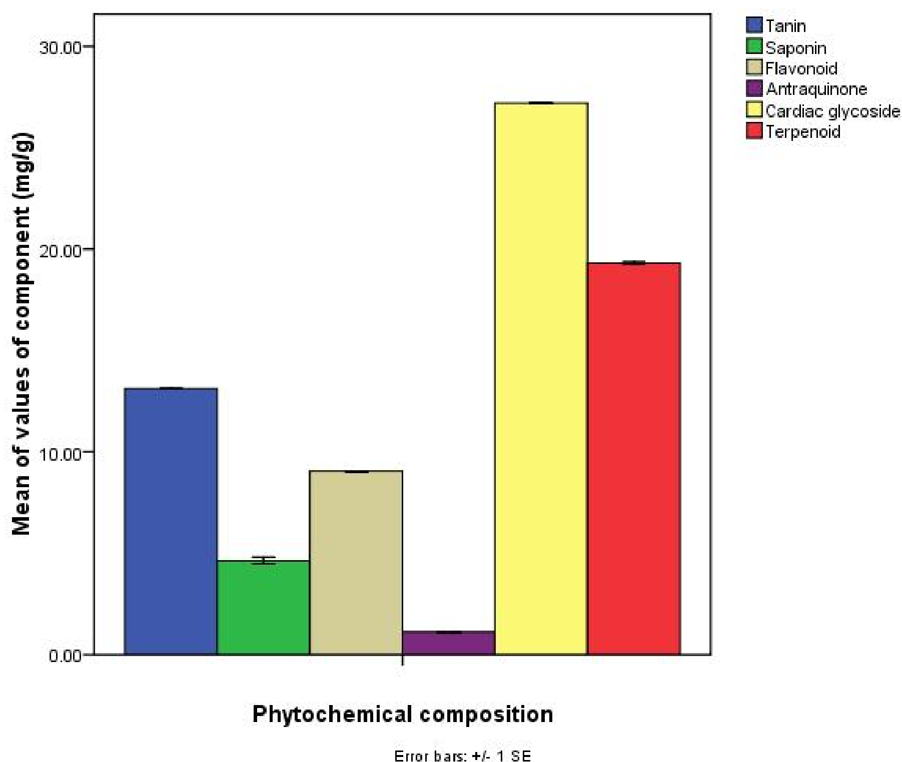

Fig. 1. Quantitative Phytochemical constituents of Unripe *A. muricata* fruit extract (g/100g)
Bars are presented as Mean ±S.D of replicates (n=3)

The quantity of tannin (13.12 mg/ml) observed in this study is higher than 4.60 mg/g and 6.25 mg/g reported in the methanolic extract of *P. argyreae* and *P. biaurita* respectively [22]. Tannin has been reported to possess astringent properties (faster the healing of wounds and inflamed mucous membrane). Their presence in plant extracts have also been attributed to analgesic, antiviral, antibacterial, anti-tumor and anti-inflammatory activities [23].

The amount of saponin (4.63mg/g) observed under this study is higher compared to 0.50mg/g detected in the extract of *Ocimum sanctum* [3] but lower than 8.05 and 7.10mg/g in methanloic extract of *P. argyreae* and *P. multiaurita* respectively [22]. Saponins found in the fruits are important dietary supplement and nutraceuticals. They are known to assist in combating bacterial, fungal and viral infections and have been shown to compliment the effectiveness of some vaccines. In traditional medicine preparation, they induce hydrolysis of glycoside from terpenoid to avert the toxicity associated with the intact molecule [24]. Duru et al. [25] reported the foaming and bitter nature of saponins. Hence, the presence of saponin in this study justified the foaming observed in the extract of unripe *A. muricata* fruit. Studies have also illustrated the beneficial effects of saponin on blood cholesterol levels, cancer, bone health and stimulation of immune system [21].

The presence of terpenoids (19.31mg/g) compared favourably with 18.41mg/g in the methanolic extract of *Spinacia oleracea* [3]. However, it is higher than 8.05mg/g and 7.10mg/g in methanolic extract of *P. argyreae* and *P. multiaurita* respectively [22]. Terpenoids are responsible for various flavours and fragrances of fruits. They are used as an alternative medicine due to their observed ability to reduce heart burn and gastric acid reflux. Similarly, they have been used for the treatment of human diseases such as cancer and malaria [26]. Also, anthraquinone (1.12mg/g) was detected in trace quantity in this study. Its presence agreed with the works of Gracelin et al. [22] who reported the presence of anthraquinone in the extract of *P. biaurita.* However, this result differed from a previous report that anthraquinone was not found in the extract of *P. confusa*, *P. vittata* and *P. multiaurita* [22]. Anthraquinone is a potent antioxidant and free radical scanvenger. It has been shown to protect cell membrane from damage [24].

3.2 Proximate Analysis of Unripe *A. muricata* Fruit Extract

The result of the proximate composition (%) of unripe *A. muricata* fruit extract is shown in Fig. 2. Carbohydrate was the highest occurring nutrient with 48.7, followed by moisture content (24.51), fat (17.13), crude protein (9.09) while ash (0.58) was least present. However, crude fibre was not detected. The carbohydrate content (48.70%) of the extract in this study compared favourably with 49.76% reported for leaves of *Loranthus micranthus* [27]. It is considerably higher than 19.41% and 18.92% reported for the leaf and root extract of *T. conophorum* [28] respectively. However, it is lower when compared to 75.00% and 82.20% reported for the leaves of sweet potato and *Corchorus tridens* respectively [29]. The presence of carbohydrate in a large amount indicates the vast potential of the plant to supply energy to cells, contribute to fat metabolism and act as mild laxative for human being [20,30].

Relatively high moisture content (24.51%) observed in this study compared favourably with 22.18% found in the leaves of *Launaea* taraxacifolia [31] while it is higher than 6.49% reported for the leaves of *L. micranthus* [27]. However, it is lower when compared to 41.02% and 43.10% in leaf and root extract of *T. conophorum* respectively [28]. The relatively high moisture content is the reason for the increased microbial spoilage and deterioration observed in the fruit of *A. muricata*. Hence, shortens the shelf life of the fruit. In addition, it gives an indication of water soluble vitamins present and the dry matter content of the fruit [30].

Also, the crude fat content (17.13%) of fruit extract of *A. muricata* reported in this study is higher than 11.04% in leaves of *L. micranthus* [27], 6.50% in leaves of *L. taraxacifolia* [31] and 1.74% in the fruit of *Nauclea latifolia* [20]. It is however lower when compared to 34.13% and 32.22% in the leaf and root extract of *T. conophorum* [28]. The presence of crude fat in this present study might be responsible for its reported auto-oxidation which can lead to rancidity that affects the flavour [30]. Hence, the short shelf life of *A. muricata* fruit observed with the fruit.

The protein content of the fruit extract was found to be 9.09% which is higher than 1.48% reported for leaves of *L. micranthus* [27]. However, it is lower when compared to 15.42% in the fruit of *N. latifolia* [20], 26.67% in

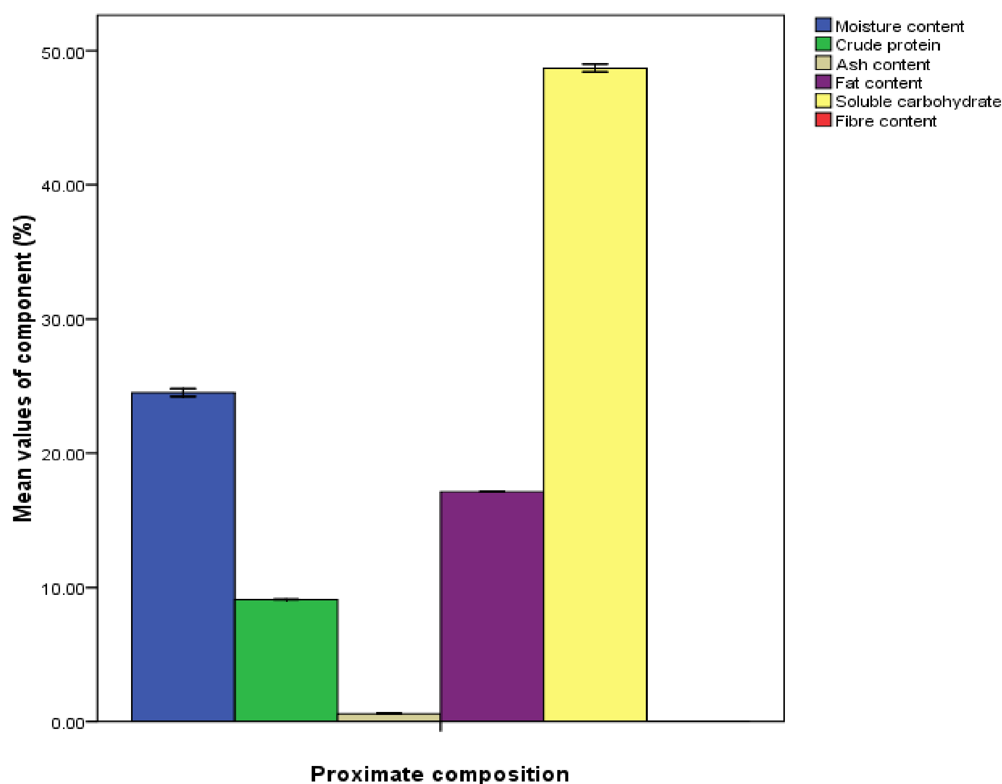

Fig. 2. Proximate composition of ethanolic extract of Unripe *A. muricata* fruit (%)
Bars are presented as Mean ±S.D of replicates (n=3)

the leaves of *L. taraxocifolia* [31] and 20.64% in the root extract of *T. conophorum* [28]. Consumption of proteinous fruit assist in growth, tissue repair and energy production of the body. The observed percentage of protein in this study could limit its usage for food as source of protein and feed formation [25].

The absence of crude fibre in this present study is in contrast to the findings of Duru et al. [25] who reported the presence of crude fibre in the unripe fruit of *Dacryodes edulis* to be 11.21%. The implication of the absence of the crude fibre is its effect on the slow digestibility of the fruit when taken. This is because crude fibre has been reported to aid digestibility of food/fruit [31,32]. Also, high fibre content in diets have been reported to result in increased removal of carcinogens, potential mutagens, steroids, bile acids and xenobiotics by binding or absorbing to dietary fibre components and be rapidly excreted. Hence, the health promoting benefits for the ruminants and non-ruminants [33].

The trace amount of the ash (0.58%) in this study is lower when compared to 11.67% detected in leaves of *L. micranthus* [27]. The ash content can provide an estimate of the mineral content which promote good body metabolism. Hence, the low presence of ash in this extract is indicative of low mineral content of the fruit.

4. CONCLUSION

It is concluded that the presence of bioactive compounds such as flavonoids, saponin, tannin and cardiac glycosides with pharmaco-therapeutic properties is suggestive of the medicinal potential of the extract and also confirm its nutritional importance to body health. However, the absence of alkaloid in this study might have an adverse effect on the antimicrobial properties of the extract of unripe *A. muricata* fruit because alkaloid was reported to be the most significantly therapeutic bioactive compound.

It is recommended that a comparative study of the phytochemical constituents of the unripe fruit of *A. muricata* should be carried out using other solvents apart from ethanol used in this study in order to determine which solvent will best isolate active components of the fruit extract.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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