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Research Article

Quantitative Phytochemical Analysis of *Annona muricata* and *Artocarpus heterophyllus* Leaves Using Gas Chromatography-flame Ionization Detector

¹C.L. Onuah, ¹C.C. Chukwuma, ¹R. Ohanador, ¹C.N. Chukwu and ²J. Iruolagbe

¹Department of Biochemistry, Faculty of Science, University of Port Harcourt, P. M. B 5323, Port Harcourt, Nigeria

²Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, P. M. B 5323, Port Harcourt, Nigeria

Abstract

Background and Objective: Phytochemicals are bioactive non-nutritive secondary metabolites found in different parts of plants. They have been effective in the treatment of diseases and infections. There has been an increase in the use of natural products due to their little or no side effects. *Annona muricata* (Soursop) and *Artocarpus heterophyllus* (Jackfruit) are tropical plants used in folk medicine in the treatment of different diseases and infection. Hence the quantitative phytochemical screening of the leaves of *Annona muricata* (Soursop) and *Artocarpus heterophyllus* (Jackfruit) were analyzed to ascertain the bioactive compounds present in them.

Materials and Methods: The phytochemical content of the leaves of *Annona muricata* (Soursop) and *Artocarpus heterophyllus* (Jackfruit) were quantified using gas chromatography-flame ionization detector. **Results:** The result showed the presence of Sparteine, Anthocyanin, Sapogenin, Morphine, Phenol, Quinine, Ribalinidine, Ephedrine, Resveratrol, Catechin, Saponin, Oxalate and Quercetin in *Annona muricata* leaves while *Artocarpus heterophyllus* leaves contained Sparteine, Anthocyanin, Sapogenin, Morphine, Phenol, Glycitein, Quinine, Ribalinidine, Ephedrine, Resveratrol and Catechin at different concentrations. **Conclusion:** The quantitative phytochemical screening of the leaves of *A. muricata* and *A. heterophyllus* showed that both plants are rich in both alkaloids and flavonoids (phenolic compounds).

Key words: Phytochemical, phenolic, alkaloid, flavonoid, anti-oxidant, *Annona muricata*, *Artocarpus heterophyllus*

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Corresponding Author: C.L. Onuah, Department of Biochemistry, Faculty of Science, University of Port Harcourt, P. M. B 5323, Port Harcourt, Nigeria

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Phytochemicals are non-nutritive chemicals secreted by plants, which possess bioactive properties¹, they also serve as defence systems against pathogens and animals². Different classes of phytochemicals exist, they include carbohydrates, lipids, phenolics, terpenoids and alkaloids and other nitrogen-containing compounds³.

Phytochemicals play different roles in plant, they protect plants from harmful insects, microbes, ultraviolet (UV) rays and extreme temperatures. They also attract birds and insects that promote pollination, germination and seed dispersal⁴. Besides they provide colour to plants and also serve as a source of flavor to plants⁵. Besides the roles phytochemicals play in plants, they also play important roles in man and animals. Most phytochemicals possess antioxidant activities hence they help in cleaning up free radicals and also prevent diseases that manifest from reactive oxygen species (ROS)⁶⁻⁸. Phytochemicals prevent and ameliorate diseases such as diabetes, cancer, hyperlipidaemia, cardiovascular diseases, liver toxicity, Alzheimer, cataract, age related function decline, stroke and others.

Different phytochemicals that have been isolated from plants include alkaloids, saponins, glycosides, flavonoids, kaempferol, phytol, gallic acid, kolaviron and essential oils⁹.

The *A. muricata* generally called graviola, guanabana¹⁰ is a green leafy plant which belong to annonaceae family¹¹. It is a fruit tree found mostly in the tropics and commonly cultivated in South America, Central America, Africa and Asia¹².

Every part of *A. muricata* has rich deposit of plant chemicals which have healing effects. These phytochemicals include; alkaloids, tannin, megastigmanes, flavonol, triglycerides, flavonoids, alkaloids, steroids, triterpenoid¹³ and cyclopeptides¹⁴. Phytochemicals such as tannins, coumarins, stearic acid, myristique acid and ellagic acid have been identified in *A. muricata* leaves and stems¹¹.

In another, *A. muricata* pulp contains 6.44 mg/100 g anthocyanin and 1.90 mg/100 g of alkaloid¹⁵ while methanol and aqueous extract of *A. muricata* showed the presence of cardiac glycosides, anthraquinones, phlobatannin and reducing sugars, respectively¹⁶. Compounds isolated from the leaves, fruits, seeds, pericarp, roots and stem of *A. muricata* have shown toxicity against cancer, they include annopentocin, cis-Annomuricin-D-one and Annocatalin¹⁴.

The *A. heterophyllus* known as Jackfruit in English and Kanthal in Bangladesh is a fruit in the mulberry family known as Moraceae. It grows wildly in the tropics especially in India and Malaysia¹⁷. It is grown in the Central and eastern Africa and also in the Caribbean¹⁸. The leaves, fruits, seeds, barks, roots and the stem of *A. heterophyllus* are rich in isoflavons,

niacin, saponin, lignans, flavonoids, alkaloids, glycosides, tannins, triterpenes¹⁹, phenolic compounds²⁰, 'morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, cyloartocarpin, artocarpesin, artocarpetin, norartocarpetin, cycloartinone and artocarpanone²¹. Recently, a lot of interest has been focused on natural products especially those from plants. Plants are known to be rich in a wide range of bioactive chemicals which are used in the treatment of different kinds of diseases and infections. The *A. muricata* (Soursop) and *A. heterophyllus* (Jackfruit) leaves are one of such plants which are used especially in folk and Ayurveda medicine in the treatment of diseases. The *A. muricata* leaves have been effectively used locally in the treatment of diabetes while *A. heterophyllus* has been used in the treatment of diabetes mellitus, convulsion/epilepsy and microbial infections¹⁹. The effectiveness of these plants in the treatment of different kinds of disease and infections can be attributed to their phytochemical contents, hence the knowledge of different bioactive compounds present in these plants may stand as a yardstick in pharmaceutical production of drugs.

MATERIALS AND METHODS

Gas chromatography-flame ionization detector (BUCK M910) was used for the quantification of the phytochemicals present in the plants.

Collection and identification of plants: The leaves of *A. muricata* were obtained from Abuja park of University of Port Harcourt while the leaves of *A. heterophyllus* were obtained from Ozuoba Obior/Akpo Local Government Area of Rivers state.

Plant material: *Artocarpus heterophyllus* and *Annona muricata* were identified and confirmed botanically by Dr. Ekeke Chimezie of the Department of Plant Science and Biotechnology, University of Port Harcourt, Choba, Nigeria and the voucher specimen deposited at the herbarium of Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

Determination of quantitative phytochemical content of the plants

Extraction of the phytochemicals: About 1 g of each of the plant samples (*A. muricata* and *A. heterophyllus*) were added 15 mL of ethanol and 10 mL of 50% m/v potassium hydroxide. This was kept in a water bath for 60 min at a temperature of 60°C. The different extracts obtained were washed three times with 10 mL of 10% v/v ethanol aqueous solution, dried with anhydrous sodium sulphate and the

solvent was evaporated. Each of the sample extracts were solubilized in 1000 μL of pyridine, 200 μL was transferred to a vial for analysis.

Quantification by GC-FID: The quantification of the phytochemicals (plant chemical) present in the plants (*A. muricata* and *A. heterophyllum*) were done using BUCK M910 GC equipped with a flame ionization detector. The injector temperature was up to 280°C with split less injection of 2 μL of sample and a linear velocity of 30 cm^{-1} , the carrier gas used is Helium 5.0 pa. s with a flow rate of 40 mL min^{-1} . The oven operated from a temperature of 200°C until it heated to 330°C at a rate of 3°C min^{-1} . This temperature was maintained for 5 min and the detector operated at a temperature of 320°C. The concentration of the different phytochemicals was expressed in $\mu\text{g g}^{-1}$.

RESULTS AND DISCUSSION

Quantitative phytochemical content of the leaves of *A. muricata*: The quantitative phytochemical content of the leaves of *A. muricata* is presented in Fig. 1.

Quantitative phytochemical content of the leaves of *A. heterophyllum*: The quantitative phytochemical screening of the leaves of *A. heterophyllum* is presented in Fig. 2.

The quantitative phytochemical screening of the plants using GC-FID showed that the leaves of *A. muricata* and *A. heterophyllum* are rich mainly in alkaloids and flavonoid (phenolic compounds).

Alkaloids are a group of naturally occurring plant secondary metabolites which contains basic nitrogen atoms in the heterocyclic ring and are derived from amino acids^{22,23}. Alkaloids are known for their biological activities which include anti-oxidant activity, muscle relaxant property, anti-microbial, amoebicidal, anti-cancer and anti-diabetic activities²³.

Phenolic compounds are chemical substance that possess aromatic ring, they may contain one (phenol) or more (polyphenol) hydroxyl substituents⁸. Phenolic compounds are known for their anti-cancer, anti-bacterial and anti-fungal activities^{8,24}. The presence of glycitein a phytoestrogen in the leaves of *A. heterophyllum*, in Table 1 shows that the plant may possess estrogenic activities²⁵.

The result of the present study shows that *A. muricata* and *A. heterophyllum* contains high concentration of catechin as shown in Table 1 and 2. Catechin is a natural occurring phenolic compound, it is known for its anti-oxidant activities. Besides having anti-oxidant activities, catechin have the potential to reduce cardiovascular disease, stroke, obesity

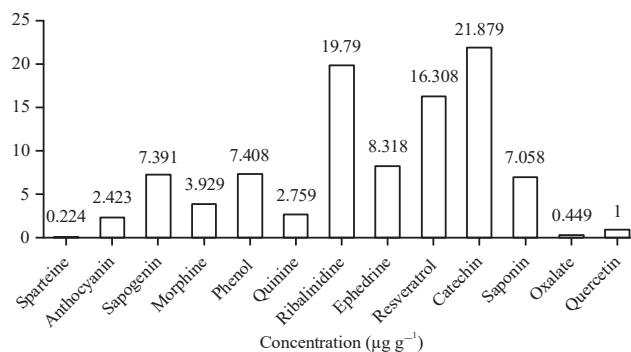


Fig. 1: Quantitative phytochemical content of *A. muricata* leaves

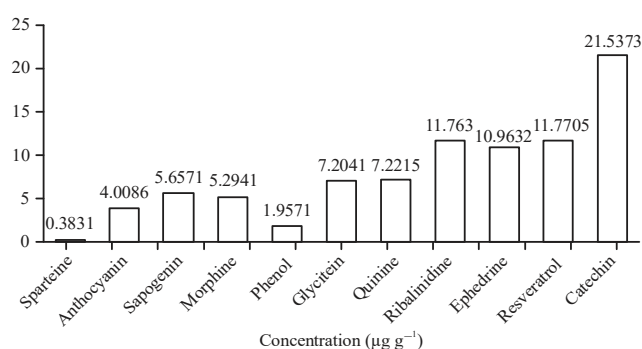


Fig. 2: Quantitative phytochemical content of the leaves of *A. heterophyllum*

Table 1: Phytochemical content of the leaves of *A. muricata*

Components	Class of phytochemical	Concentration ($\mu\text{g g}^{-1}$)
Sparteine	Alkaloid	0.224
Anthocyanin	Flavonoid	2.423
Sapogenin	Steroids or triterpenes	7.391
Morphine	Alkaloid	3.929
Phenol	Flavonoid	7.408
Quinine	Alkaloid	2.759
Ribalinidine	Alkaloid	19.79
Ephedrine	Alkaloid	8.318
Resveratrol	Flavonoid	16.308
Catechin	Flavonoid	21.879
Saponin	Steroids or triterpenes	7.058
Oxalate		0.449
Quercetin	Flavonoid	1.000

Table 2: Phytochemical content of the leaves of *A. heterophyllum*

Components	Class of phytochemical	Concentration ($\mu\text{g g}^{-1}$)
Sparteine	Alkaloid	0.3831
Anthocyanin	Flavonoid	4.0086
Sapogenin	Steroids or triterpenes	5.6571
Morphine	Alkaloid	5.2941
Phenol	Flavonoid	1.9571
Glycitein	Isoflavone (phytoestrogens)	7.2041
Quinine	Alkaloid	7.2215
Ribalinidine	Alkaloid compound	11.7630
Ephedrine	Alkaloid	10.9632
Resveratrol	Flavonoid	11.7705
Catechin	Flavonoid	21.5373

Table 3: Biological activities of the phytochemicals characterised in the leaves of *A. muricata* and *A. heterophyllum*

Components	Class of phytochemical	Uses
Sparteine	Alkaloid	Anti-arrhythmic, anti-convulsant, reduces locomotor activity ²⁶
Anthocyanin	Flavonoid	Anti-diabetic, delays cataracts in rats, improves vision ²⁷
Sapogenin	steroids or triterpenes	Anti-methanogenic potentials, modulate rumen fermentation ²⁸
Morphine	Alkaloid	Controls the brain, pain killer ²⁹
Phenol	Flavonoid	Acts as anti-oxidant ³⁰
Quinine	Alkaloid	Anti-malarial ^{31,32}
Ribalinidine	Quinoline Alkaloid	Free radical scavenging activity ³³
Ephedrine	Alkaloid	Temporary relief of shortness of breath, chest tightness and wheezing due to bronchial asthma, enhance mental function ^{34,35}
Resveratrol	Flavonoid	Cardioprotective, anti-arrhythmic agent, anti-obesity effect ^{36,37}
Catechin	Flavonoid	Antimicrobial, anti-cancer, prevents cardiovascular disease, improves blood pressure ³⁸
Saponin	Steroids or triterpenes	Antimicrobial, anti-fungal, anti-parasitic, insecticidal properties, lowering of serum cholesterol levels, cytotoxic agent ³⁹
Oxalate		No biological activity reported
Quercetin	Flavonoid	Anti-carcinogenic, anti-inflammatory and antiviral, reduces lipid peroxidation, protects the skin against UVB induced oxidative damage ^{40,41}
Glycitein	Isoflavone (phytoestrogens)	Atherosclerotic cardiovascular diseases, estrogenic activity, protects against beta amyloid-induced toxicity and oxidative stress ⁴²

and cancer³⁸. This research also revealed the presence of morphine in both plants, morphine is in the class of drugs known as opioid. Morphine serve as analgesics and can easily be abused due to psychological dependence of it when taken for too long²⁹. GC-FID analysis of the plants also revealed the presence of quinine, a type of alkaloid. A lot research has shown that quinine is an effective antimalarial drug³².

Table 3 shows a brief summary of the biological activities of the phytochemicals present in the leaves of *A. muricata* and *A. heterophyllum*.

CONCLUSION

The role of phytochemicals cannot be over emphasised, various research on the activities of plants have shown that most plants possess one or more biological activities which has been attributed to their rich phytochemical contents. As shown in the Table 3, the leaves of *A. muricata* and *A. heterophyllum* possess mainly alkaloids and flavonoids (phenolic compounds) which have the potential to inhibit the activities of α -amylase and α -glucosidase enzyme. This could be exploited in the pharmacological production of antidiabetic drugs. The presence of glycitein an estrogenic compound in the leaves of *A. heterophyllum* may also serve as a potent drug to boost fertility while the presence of quinine in both plants shows that the plants can also be optimized in the pharmacological production of anti-malarial drugs.

SIGNIFICANCE STATEMENT

This study showed the quantitative phytochemical content of the leaves of *A. muricata* and *A. heterophyllum*. *A. muricata* and *A. heterophyllum* are used locally in the

treatment of different diseases and infections. This research revealed that the leaves of these plants are rich in both alkaloids and flavonoids. The presence of quinine, morphine (alkaloids) and flavonoids (phenolic compounds) suggest that the plants may rightly be used locally as anti-malarial, pain relief and possibly anti-diabetic.

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