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Phytochemical Analysis and Nutrient Content of Soursop Leaves from Iganga District in Eastern Uganda

Ssempala Muhammad[1], Omer Ishag[2]

[1]Department of chemistry, school of natural sciences, Kampala International University (KIU)
[2]Department of applied and industrial chemistry, faculty of pure and applied sciences, International University of Africa (IUA), Khartoum Sudan.

ABSTRACT:
Standard procedures were used to perform phytochemical and approximate composition analysis on Annona muricata leaves. The leaves had 88.98% dry matter, 11.02% moisture, 25% crude protein, 14.86% ash, 22.20% crude fiber, 21.21% fat, and 16.61% carbohydrate contents, according to the results of the proximate composition analysis. The phytochemicals flavonoids, alkaloids, cardiac glycoside, tannins, triterpenoid, saponin, and reducing sugar were found in the ethanolic leaf extracts. According to the research, Annona muricata leaves have the potential to be a very nutritious feed ingredient and a phytomedicine. With the variety of ethnopharmacological applications of the plant across many regions of the world, they are relevant to nutrition, medicine, and animal care.

KEYWORDS: leaves, phytochemicals, Soursop, proximate, ethanol,

INTRODUCTION

The usage of medicinal plants has gradually come back into favor in developing nations in recent years due to reports that they are safe and have no negative side effects, especially when compared to manufactured pharmaceuticals. Therefore, it makes sense to look for novel medications that have more effective and affordable plant-based alternatives. These plants' therapeutic significance is derived from a few chemical compounds that have specific physiological effects on humans (Usunobun U., *et al*., 2014). *Annona muricata* commonly called Soursop is commonly found in southern part of Uganda. It is mostly eaten as fresh fruits. It is cultivated mainly in homegardens. The tree yields up to 10 tons per *ha* and each fruit weighs 0.5 to 2 kg (Oyenuga, 1978). Soursop has found its uses in many areas. It is consumed as a desert fruit. It is made into a fruit jelly with the addition of some gelatin or used in the preparation of beverages, ice creams and syrups. Its white edible pulp has moderate levels of vitamins B1 and B2, 1% protein, 18% carbohydrate, and 80% water. The firm, flat seeds provide everything needed to make paint or pesticide (Rice *et al.*, 1991). The soursop's leaves and juice are said to have several therapeutic benefits. The seed makes up around 4% of the entire fruit, and there hasn't been much information recently released regarding the nutritional and chemical makeup of the leaves. The purpose of this study is to identify specific nutritional and phytochemical characteristics of Annona muricata leaves.

MATERIALS AND METHODS

COLLECTION, IDENTIFICATION AND PREPARATION OF ANNONA MURICATA LEAVES

Annona muricata leaves that were still fresh were taken from the farm garden. After being cut off from the stalk, the Annona muricata leaves were cleaned, allowed to air dry at room temperature (24°C), and then ground into a fine powder before being weighed. For proximate analysis, aliquot quantities of the powdered leaves were weighed.

EXTRACTION OF THE PLANT LEAVES

To create an ethanolic extract, 100g of dry powdered plant leaves were soaked in 1000ml of 100% ethanol for 48 hours at room temperature (to ensure a thorough extraction). Next, cotton wool and Whatmann filter paper No. 42 (125 mm) were used to filter the extract. After that, the extract was freeze-dried after being concentrated using a rotary evaporator in a water bath set at 60°C to a tenth of its initial volume. Then, 4ºC was used to preserve the dried residue, or crude extract. For phytochemical screening, aliquot quantities of the crude plant extract residue were weighed.

TECHNIQUES FOR PHYSICAL CHEMISTRY SCREENING

Phytochemical screening was performed using standard procedures (Sofowora, 1993, Trease andEvans, 1989, Ayoola *et al.*, 2008).

TEST FOR SAPONINS

0.5g of extract was added to 5ml of distilled water in a test tube and the solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

TEST FOR TRITERPENOIDS

One milliliter of chloroform was added to 0.5 grams of the extract. First, 1 milliliter of acetic anhydride and then 2 milliliters of pure H2SO4 were added. The presence of triterpenoids is indicated by the formation of a reddish violet color.

TESTING FOR TANNINS

To check for tannins, two techniques were employed:
(a) 0.5g of extract was added to 10ml of newly made 10% KOH in a beaker, and the mixture was agitated to dissolve. Tannin was present as evidenced by the unclean precipitate that was seen.
(b) In a test tube, around 0.5g of the extract was heated in 10ml of water and subsequently filtered. After adding a few drops of 0.1% ferric chloride, the solution's color was checked for blue-black or brownish-green.

TESTING FOR REDUCING SUGAR (FEHLING'S TEST)

After dissolving 0.5g of the extract in 5ml of distilled water, it was filtered. The filtrate was heated using Ferling's A and B solutions, neutralized with NaOH, then hydrolyzed with diluted HCl. The presence of reducing sugars was detected by the formation of red precipitate.

TESTING FOR ANTHRAQUINONES

0.5g of the extract was boiled with 10ml of H_2SO_4 and filtered while hot. The filtrate was shaken with 5ml of chloroform, the chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

TESTING FOR STEROIDS

After dissolving 0.5g of the extract in 10ml of chloroform, an equivalent volume of concentrated H_2SO_4 was added to the test tubes' sidewalls. Steroids are indicated by a reddish top layer and a yellowish sulphuric acid layer with green fluorescence.

TESTING FOR CARDIAC GLYCOSIDES (KELLER-KILLIANI TEST)

A solution of glacial acetic acid containing one drop of ferric chloride solution was added to 0.5g of extract dissolved in 5ml of water, along with 2ml of the mixture. One milliliter of pure H_2SO_4 was applied underneath this. A dark ring near the contact suggested that cardenolides have deoxysugar properties. In the acetic acid layer, a greenish ring may emerge slightly above the brown ring and progressively expand across the layer, while a violet ring may appear below the brown ring.

TESTING FOR FLAVONOIDS

Two methods were used to test for flavonoids:

- A portion of the extract was heated with 10ml of ethyl acetate over a steam bath for 3 minutes, the mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids.
- Dilute ammonia (5ml) was added to a portion of an acqueous filtrate of the extract. Then, concentrated sulphuric acid (1ml) was added. A yellow colouration indicated the presence of flavonoids.

TESTING FOR ALKALOIDS

Each extract was separately dissolved in diluted HCl before being filtered.

- Potassium mercuric iodide, also known as Mayer's reagent, was applied to filtrates. When an ammonia-colored precipitate forms, alkaloids are present.
- A solution of potassium bismuth iodide, known as Dragendroff's reagent, was applied to the filtrate. The presence of alkaloid is shown by the formation of red precipitate.

Hager's reagent (saturated picric acid solution) was applied to the filtrate. The presence of an alkaloid is verified by the appearance of a yellow precipitate.

METHODS FOR PROXIMATE ANALYSIS

The leaves that had been powdered were taken for close examination. The Association of Official Analytical Chemists' standard procedures were used to assess the quantities of dry matter, moisture, ash, crude fat, crude protein (nitrogen x 6.25) and crude fiber (AOAC, 2000). The net difference between the percentage composition of all the nutrients and the amount of carbohydrates was used to determine the content of carbohydrates.

RESULTS

Following an ethanolic leaf extract of Annona muricata, a phytochemical screening revealed the presence of a number of secondary metabolites, including reducing sugars, cardiac glycosides, flavonoids, alkaloids, saponins, tannins, and triterpenoids (Table 1). These results imply that this plant may be a valuable natural antioxidant source with anti-inflammatory, anti-analgesic, anti-hyperlipidemic, and therapeutic properties.

Table 1: Phytochemical screening of Annona muricata ethanolic leaf extract

Pytochemicals	*Annona muricata*
Flavonoids	Present
Saponins	Present
Tannins	Present
Steroids	Absent
Alkaloids	Present
Triterpenoids	Present
Anthraquinone	Absent
Reducing sugar	Present
Cardiac glycosides	Present

The result in Table 2 indicate that the leaves of *Annona muricata* in percentage (%) are rich in carbohydrates (16.61 ± 0.09), proteins (25.03±0.06), fibers (22.20 ± 0.05), ash (14.96 ± 0.05), fats/oil (21.12 ± 1.01), dry matter (88.98±0.74) and moisture content (11.02 ± 0.82).

Table 2: Proximate analysis of *Annona muricata* powdered leaves

Proximate composition	*Annona muricata* (%)
Dry matter	88.98 ± 0.74
Moisture Content	11.02 ± 0.82
Crude protein	25.03 ± 0.06
Crude fibre	22.21 ± 0.05
Ash content	14.86 ± 0.05
Crude fat/oil	21.12 ± 1.01
Carbohydrate	16.61 ± 0.09

Values are means ± SD for 3 determinations.

DISCUSSION

Numerous typical foods and herbs made from plants include potent phytochemical compounds that can enhance our overall health. We are protected against numerous diet-related disorders by phytochemicals. The results of the phytochemical screening of the ethanolic leaf extract of Annona muricata revealed the presence of cardiac glycosides, flavonoids, saponins, tannins, alkaloids, triterpenoids, reducing sugar, and the absence of steroids and anthraquinone.

Animal consumption of these phytochemicals results in a variety of pharmacological and metabolic

effects. Bioactive principles with biological activity are claimed to be present in plants that are used to treat illnesses. Some of these principles are responsible for the distinctive color, scent, and texture of the plant, while others confer its culinary, medicinal, or toxic qualities. (Evans, 2002). The qualitative phytochemical screening of *Annona muricata* was in agreement with the works of Foong and Hamid, (2012), Falodun *et al*, (2011), and Vijayameena *et al* (2013).It has been reported that flavonoids and phenolics are free radical scavengers that preventoxidative cell damage, and have strong anticancer activities (Pourmorad *et al*., 2006; Ugwu *et al*., 2013) and they might induce mechanism that affect cancer cells and inhibit tumor invasion (Rafat *et al.*, 2008). These activities could be attributed to their ability to neutralize and quench free radicals (Ugwu *et al*., 2013; Pourmorad *et al*., 2006; Omale and Okafor, 2008). It can also be due to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Rice-Evans *et al*., 1995).

Alkaloids are good compounds for plants that act as a deterrent to parasites and predators. these most likely gives these class of drugs its antibacterial properties. Early humans are said to have employed a number of medical plants that contain alkaloids as stimulants for recreation, pain relief, or to induce a trance during religious rituals in order to communicate with God or ancestors. (Heinrich *et al*., 2005; Gurib-Fakin, 2005).

It is thought that saponins interact with the cholesterol-rich membranes of cancer cells to inhibit their capacity to proliferate and remain viable. (Roa *et al.*, 1995). Saponins have the property of precipitating and coagulating red blood cells (Yadav and Agarwala, 2011). Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al.*, 2000; Okwu, 2004). Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation (Just *et al.*, 1998; Okwu and Emineke, 2006, Liu and Henkel, 2002).

Cardiac glycosides are important class of naturally occurring drugs whose actions helps in thetreatment of congestive heart failure (Yukari *et al*., 1995). *Annona muricata* is used for thetreatment of cardiac infections along with other ailments such as cough, and chest pain inJamaica, Haiti, and the West Indies (Technical Data Report for Graviola, 2005; Taylor, 2002) Though several works reporting compositional evaluation and functional properties of varioustypes of edible wild plants in use in the developing countries abound in literature, much still needto be done. Dietary fiber, polyunsaturated fatty acids (PUFA), proteins, amino acids, minerals,vitamins and other bioactive compounds are considered as beneficial nutrient components(Andlauer and Fürst, 2002). The nutrient composition in this study revealed that *Annonamuricata* leaves contained protein, fiber, ash, fats/oil as well as carbohydrate as shown in Table 2. Our result suggests that *Annona muricata* leaves could serve as better sources of dietary carbohydrate, protein and lipids. Hence *Annona muricata* leaves add to the calorific value of food and possess odour and flavor carrying ability thereby enhancing the palatability of food. There is scarce data on proximate analysis in *Annona muricata* leaves.

The leaves of *Annona muricata* contained crude protein value of 25% which is higher than protein content of *Momordica foecide* (4.6%) leaves consumed in Nigeria and Swaziland, *Lesianthera africanas* (13.1%) (Hassan and Umar, 2006; Ogle and Grivetti, 1985; Isong and Idiong, 1997), *Amaranthus candatus* (20.5% DW), but lower than *Piper guineeses* (29.78% DW)and *T. triangulare* (31.00% DW) (Akindahunsi and Salawu, 2005; Antia *et al*., 2006; Etuk *et al*., 1998). High amount of protein is essential for animal growth and increased milk production (Tangka, 2003). Plant proteins are a source of food nutrient especially for the less privileged population in developing countries including Uganda. Proteins are one of the

macromolecule and it is an alternate energy source when other energy sources are in short supply. They are building block units and food protein is needed to make vital hormones, important brain chemicals, antibodies, digestive enzymes, and necessary elements for the manufacture of DNA. Someproteins are involved in structural support, while others are involved in bodily movement, or in defense against germs (Bailey, 2008). *Annona muricata* leaves can thus be considered a good source of protein because they provide more than 12% of caloric value from protein. Therefore, the protein content of the *Annona muricata* leaves will go a long way in meeting the protein requirement of the local people.

The leaves of *Annona muricata* contained crude fibre value of 22.20% which is high when compared to *Ipomea batatas* (7.20%), *T. triangulare* (6.20%) *P. guineensis* (6.40%), and *Corchorus olitorius* (7.0%), (Akindahunsi and Salawu, 2005; Antia *et al.,* 2006). Fibre cleanses the digestive tract by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Fibre also adds bulk to the diet and prevents the intake of excess starchy food (Mensah *et al.*, 2008) and may therefore guard against metabolic conditions such ashypercholesterolemia and diabetes mellitus (Henry, 2004). Dietary fiber has a positive effect in the management of diabetes by controlling post-prandial hyperglycemia. It delays gastric emptying or increase the viscosity of gastro-intestinal tract content thereby suppressing digestionof carbohydrate and delays its absorption. The substantial amount of fibre in *Annona muricata* leaves shows that they can help in keeping the digestive system healthy and functioning properly. Fibre aids and speeds up the excretion of waste and toxins from the body, preventing them from sitting in the intestine or bowel for too long, which could cause a build-up and lead to several diseases (Hunt *et al*., 1980). Adequate intake of dietary fibre can lower the serumcholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Rao and Newmark, 1998; Ishida *et al*., 2000). The RDA of fibre for children, adults, pregnant and lactating mothers are 19 – 25, 21-38, 28 and 29 g, respectively (Jimoh *et al*.,2011).

Annona muricata had ash value of 14.96% and the ash content is a reflection of the mineral contents preserved in the plants leaf. The ash content of *Annona muricata* leaves compare favorably with the values reported for *Vernonia colorate* (15.86%) and *Moringa oleifera* (15.09%) (Lockeett *et al*., 2000; Antia *et al*., 2006) and lower than that of some leafy vegetables commonly consumed in Nigeria such as *Talinum triangulare* (20.05%) but higher than some other vegetables such as *Occimum graticimum* (8.00%) and *Hibiscus esculentus* (8.00%) (Akindahunsi and Salawu, 2005). The result therefore suggests a high deposit of mineral elements in *Annona muricata* leaves.

Annona muricata had carbohydrate value of 16.62%, which is lower than reported values for *Corchorus tridens* (75.0% DW) and sweet potatoes leaves (82.8%) (Asibey-Berko and Taiye, 1999). Therefore, Annona muricata's energy value is influenced by its carbohydrate content. For plants and animals to continue to exist, carbohydrates are necessary. They are also provide raw material for many industries (Ebun-Oluwa and Alade, 2007). Plant-based carbohydrates are one of the three primary energy sources in food, along with fat and protein. Animals that consume plants release energy that has been stored as carbohydrates through respiration, a chemical reaction that results in the production of water, carbon dioxide, and energy from glucose and oxygen. Animal cells also use glucose to produce other compounds required for growth. (Westman, 2002). With a crude fat content of 21.22%, Annona muricata adds to the plant's energy value. By absorbing and holding onto flavors, dietary fat improves the palatability of food (Antia et al., 2006). The leaves of Annona muricata contain relatively little moisture. Therefore, by inhibiting the growth of rotting bacteria, the lower moisture level would increase shelf life. (Ruberto and Baratta, 2000).

According to this study, Annona muricata leaves are a rich source of phytochemicals, and it is highly advised to use them for overall health. The leaves of Annona muricata are stores of compounds that scavenge free radicals, including flavonoids, alkaloids, tannins, terpenoids, phenolic acids, and vitamins. These metabolites are essentially abundant in antioxidant properties. The fact that these plant metabolites can have their output increased through genetic manipulation is highly intriguing. To make other edible crops easier for consumers to consume, the DNA copy or gene(s) causing the expression of these metabolites may also be cloned and inserted. This suggests that, in this case, purchasing synthetic medications from over-the-counter pharmacies might not be necessary.

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