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Evaluation of the Nutritional Value and Acute Toxicity of the Seeds of the Fruit of *Annona muricata* L. (Sursop) Consumed in Kinshasa

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Abstract

In this paper, the author aimed to determine the nutritional value of soursop (*Annona muricata* L. fruit) consumed in Kinshasa as well as the study of the acute toxicity of its seeds. The fruit of *Annona muricata* L. was sampled according to the ISO 7002 standard for agricultural and food products. The selected fruits were ripe, without physical damage. The usual analytical methods allowed the determination of the nutritional value of the fruit pulp of *Annona muricata* L. The water content was measured by the method of loss of mass on drying. The total amount of ash was determined by incineration in the oven at 550˚C. The mineral elements were determined by inductively coupled plasma spectrometry (ICP0). The determination of acute toxicity was carried out on 25 female mice of the NMRI SUISSE species according to OECD 425 guidelines. For 100 grams of fresh material from the fruit pulp, we noted a very high water content of 84% ± 6%. We also note a particularly high amount of carbohydrates with a rate of 12.2% ± 2%. Protein and lipid content were relatively low at 1% ± 0.01% and 0.7% ± 0.3% respectively. The dietary fiber content was 0.8% ± 0.2%. An energy value of 49.3 Kcal per 100 grams of pulp was determined. The LD 50 obtained was 3320 mg/kg, indicating slight toxicity of soursop seeds. The results of this study show that the white pulp of the fruit of *Annona muricata* L. consumed in Kinshasa is rich in carbohydrates when we compare it to others biomolecules. It also contains dietary fiber and mineral salts making soursop an excellent constituent of a weight loss diet with a low energy intake.

Keywords

Annona, Nutritional Value, Acute Toxicity

1. Introduction

Plants have always been used by people around the world for food and medicine. Today, the plant kingdom still represents the primary source of therapeutic substances in developing countries [1]. The World Health Organization (WHO) is resolutely committed to research and promotion of traditional pharmacopoeia. Discoveries in this area will help meet the primary health care and drug needs of African populations [2].

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of the seeds, berries, roots, leaves, bark, or flowers of a plant for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more and more common as improvements in analysis and quality control, as well as advances in clinical research, show the value of herbal medicine in the treatment and prevention of disease. Ancient Chinese and Egyptian papyrus writings describe the medicinal uses of plants as early as 3000 BC. Indigenous cultures (such as Africans and Native Americans) used herbs in their healing rituals, while others developed traditional medical systems (such as Ayurveda and Traditional Chinese Medicine) in which herbal therapies plants were used. Herbal medicine is used to treat many conditions, such as allergies, asthma, eczema, premenstrual syndrome, rheumatoid arthritis, fibromyalgia, migraine, menopausal symptoms, chronic fatigue, irritable bowel and cancer, among others [3].

The fruit of the *Annona muricata* L. plant or soursop is used in food because of its aromatic flavor. This fruit is also recognized as a source of essential nutrients, including minerals, which act on the metabolism of several functions in the human body [4]. The white pulp of the soursop can be eaten as it is, pressed into juice or incorporated into various desserts. The fruit is sometimes cooked like a vegetable, fried, grilled or cooked in soup as is the case in Indonesia. The fruits are fragile, their export is limited and their consumption depends on the local culture. However, it is observed globalization of the consumption of this fruit on the shelves of the whole world [5].

In the Democratic Republic of Congo, as everywhere in Africa, the population still resorts to the use of medicinal plants in self-medication to prevent or cure certain diseases that overwhelm it [6]. Thus all parts of *Annona muricata* L. are used in traditional medicine for their many therapeutic virtues. The plant is used against sleep disorders, heart disorders, parasitic infections, digestive disorders, etc [7]. Some authors report that the seeds and the pulp of the fruit have anti-arthritic, anticonvulsant, antidiabetic, hypolipidemic, analgesic, anti-inflammatory, antioxidant, hypotensive, anthelmintic, antimalarial, anti-diarrheal, hepatoprotective and antitumor properties [7].

However, this uncontrolled use of soursop exposes the Congolese population to a risk of toxicity. Unfortunately, few studies have been conducted to date to assess the nutritional value of this fruit and the toxicity of its seeds to ensure consumer safety [8].

To address this concern, we conducted this study to assess the nutritional value of the fruit pulp of *Annona muricata* L., as well as the acute toxicity of its seeds commonly used for therapeutic purposes in the Democratic Republic of Congo generaly and particularly in Kinshasa.

2. Material and Methods

The determination of the energy value, the chemical screening and the measurement of the biochemical parameters were carried out respectively in the Food Analysis Laboratory, the Pharmacognosy Laboratory and the Biochemistry and Hematology Laboratory, all of the Faculty of Pharmaceutical Sciences of the University of Kinshasa. The toxicity of the plant was determined in the animal facility of the National Institute for Biomedical Research (INRB).

The plant material used for our various tests consisted of the fruit of *Annona muricata* L. (Soursop), precisely the pulp and the seeds.

Fruit sampling was conducted at the main fruit and vegetable supply market in the capital, called "Zigida" located in the commune of Barumbu in Kinshasa in DR Congo. It was carried out according to the ISO 7002 standard on agricultural and food products. The selected fruits were ripe, without physical damage, and they were randomly sampled and transported to the laboratory in a clean, dry and waterproof container.

The identification of the plant material was carried out at the herbarium of the Faculty of Sciences of the University of Kinshasa. The pulp was used for the determination of nutritional value and the seeds were used for the identification of chemical groups and the determination of acute toxicity.

The nutritional value of the pulp was determined by the determination of the water content by the method of loss of mass on desiccation [9], the determination of the total ash content by incineration of the plant material in a muffle furnace at a temperature of 550˚C [10], the determination of mineral elements by inductively coupled plasma spectrometry [11], the determination of the protein content according to the Kjeldahl method [12], the determination of lipid content by centrifugation [8], determination of dietary fiber content by dissolution of organic compounds and determination of total carbohydrate content by the calculation method [13].

The chemical composition of the seeds of the fruit was determined by chemical reactions in solution based on coloration, precipitation or formation of mosses.

Annona muricata L. seeds were first air-dried and ground using a mechanical grinder. Then the extraction was carried out by maceration of 100 g of powder at room temperature in twice 500 mL of ethanol for 72 hours. After filtration, the filtrate was dried at 500C to constant weight. The content of substances extractable by ethanol is evaluated according to the formula:

$$T_E = \frac{(n' - n)}{PE} \times 100$$

The dissolution of the dry powder of the extract in different solvents allowed

the realization of the reactions of identification of the secondary metabolites. The groups were identified by precipitation with Dragendorff's reagent for alkaloids, by Stiasny's reagent for catechin tannins and FeCl3 for gallic tannins, by Shinoda's reagent for flavonoids and anthocyanins, by the foaming power of saponins, by Hirschson's reagent for steroids and terpenoids, by staining of cardiac glycosides in the presence of chloroform.

To assess the acute toxicity of extracts from the seeds of *Annona muricata* L., female mice of the NMRI Suisse species were used. The study was carried out on 25 mice weighing between 18 - 25 g. The mice were housed at 22˚C ± 3˚C in individual cages. They were fed with conventional preparations for laboratory rodents and had tap water available at all times.

The study of the acute oral toxicity of the ethanolic extract of the seeds of *Annona muricata* L. was carried out according to guidelines 425 of the OECD (Organisation for Economic Cooperation and Development). Acute toxicity was considered as induced toxicity, 24 hours after administration of a single dose of the ethanolic extract of soursop seeds. The LD100 was measured after performing the limit test. Then, the LD50 was calculated according to the approximate method of Karber and Behrens (April 1974) according to the formula:

$$DL50 = DL100 - \left(AB/N\right)$$

The toxicity of the extract was determined based on the LD50 according to the Hodger and Sterner chemical toxicity scale.

The measurement of the biochemical parameters was carried out at the end of the experiment, the mice were anesthetized by injection of ketamine for their sacrifices. Blood was collected from the heart in tubes without anticoagulant and centrifuged at 1500 rpm for 15 minutes to obtain serum. The measurement of the enzymatic activity of the transaminases ASAT and ALAT by the method of Reitman, made it possible to explore the liver function. The dosages of urea by the urease method and that of creatinine by the Jaffe method, made it possible to explore renal function.

The data is entered with a Microsoft Excel 2016 spreadsheet and the analyzes were carried out with the Microsoft SPSS software version 18. The results are presented in the form of mean ± standard deviation. The Student test was used to compare means. A significance level of 0.05 and a 95% confidence interval was set.

3. Results

Table 1 shows a quantity of water of 84 ± 6 g contained in 100 g of fresh materials. With a low total ash content of 2% ± 0.5%. Note also a quantity of total carbohydrates of 12.2% ± 2% against a lower quantity of proteins and lipids, for a total energy value of 49.3 Kcal per 100 grams of pulp.

Table 2 relating to the content of mineral elements shows the values of the various microelements and macroelements of the minerals present in the fruit, with a particularly high quantity of potassium with 275.4 mg/100g of fresh pulp.

Table 3 shows the phytochemical groups present in soursop seed after ethanolic extraction.

The yield of ethanol extraction is 10.1 g of extract per 100 g dry seed powder. The phytochemical screening of the ethanolic extract of the seeds revealed the absence of anthocyanins.

Table 4 shows the evolution of behavior of mice according to the dose of Annona extract administered and therefore the number of mice that died depending on the dose administered.

Table 1. Nutrient content of the fruit pulp of *Annona muricata* L.

Parameters	Content in g/100g	Energy value in Kcal
Water	84 ± 6	0
Total ash	2 ± 0.5	0
Lipids	0.7 ± 0.3	4,5
Proteins	1 ± 0.01	4
carbohydrates	12.2 ± 2	40,8
Diatery fiber	0.8 ± 0.2	0
Total 100		**49.3 Kcal**

Table 2. Content of mineral elements in the fruit pulp of *Annona muricata* L.

Mineral elements	Content (mg/100g).
Aluminium	4.53
Arsenic	0.00
Calcium	31.43
Cobalt	Tracks
Cadmium	0.12
Chromium	0.20
Copper	2.10
Iron	10.15
Potassium	275.4
Magnesium	24.9
Manganese	2.40
Sodium	49.2
Sélénium	Tracks
Nickel	0.27
Lead	1.44
Zinc	6.37

Table 3. Phytochemical groups present in soursop seed extract.

Yield	**10.1%**
Alkaloids	+
Anthocyanins	−
Flavonoids	+
Tannins: • Catechics • Gallics	 + +
Saponins	+
Steroids and Terpenoids	+
Glycosides	+

Alk.: Alkaloids; Ant.: Anthocyanins; Flav.: Flavonoids; Sapo.: Saponins; Ster.and terp.: steroids and terpenoids; Tan.: Tannins, Cat.: Catechics; Gal.: Gallics. +: Presence; −: Absent.

Table 4. Evolution of behavior according to the dose administered.

Dose of extract administered in mg/kg	Number of mice tested	Number of dead mice
0	5	0
1090	5	0
1750	5	1
2600	5	2
5000	5	5

Table 5. Estimated LD_{50} for the ethanolic extract of *Annona muricata* L. seeds.

Parameters	LD100	LD50	Toxicity
Extract	5000 mg/kg	3320 mg/kg	Light

Table 5 shows the estimated LD_{50} for the ethanolic extract of Annona seeds. We can see that the ethanolic extract of the seeds of *Annona muricata* L. is slightly toxic.

Table 6 shows the evaluation of the effect of the extract of Annona seeds on biochemical parameters. For all the biochemical parameters, there is no significant difference between the groups of mice. Thus the seeds don't have a liver or kidney toxicity.

4. Discussion

This study consisted in determining the nutritional value of soursop and the acute toxicity of the seeds of this fruit. After analysis and dosage of different parameters, we noted that:

In the field of nutrition and food science, all natural foods that contain more

Table 6. Evaluation of the effect of the extract on biochemical parameters.

Parameters	Urea (g/L)	Creat (mg/L)	GOT(UI/L)	GPT(UI/L)
Control group/standard	41.88 ± 11.50	0.654 ± 0.10	189.93 ± 89.33	47.78 ± 34.44
Group of 1090 mg/kg	39.3 ± 4.81	0.612 ± 0.10	204.69 ± 121.28	34.51 ± 15.30
Group of 1750 mg/kg	45.85 ± 8.71	0.697 ± 0.08	260.3 ± 69.71	38.64 ± 69.29
Group of 2600 mg/kg	55.90 ± 30.81	0.72 ± 0.11	244.7 ± 101.46	34.74 ± 24.01
p-value	0.513	0.811	0.777	0.341
Decision	NS	NS	NS	NS

NS: non-significant difference.

than 50% water are considered to be water-rich. Fruits are among the most water-rich foods; they contain more than 80% water [14]. The results obtained with the pulp of soursop attest to this, since we found 84% ± 6% (for 100g of pulp) of water in this fruit.

Our results show that the total ash content of soursop is 2% ± 0.5%. In general, any natural food contains less than 5% ash, while some processed foods can have an ash content of more than 10% [15]. Our results show that soursop is an excellent source of minerals.

Soursop is one of the fruits with lower fat content; our results show that this fruit contains 0.7% ± 0.3% of lipids. In a study published by Fabrice Hard (2021), the lipid content of soursop was 0.3 g per 100 g or 0.3%, thus confirming the theory that this fruit is lower in fat. So for slimming diet, feeding soursop (low calorie fruits) is highly recommended.

Soursop is reputed to be very high in dietary fiber; USFDA data indicates a fraction of 3.3 g of dietary fiber per 100 g of pulp. A study conducted by Neela B. *et al.*, indicates a fiber content of 0.79 g, however our results indicate a dietary fiber content of 0.8 ± 0.2 g per 100 g of pulp [16]. The content found in the present study is lower than the USFDA soursop data. This difference is probably due to biological variations of a physiological nature, but also because of agronomic and environmental variations [17].

According to USFDA data, the protein content for 100 g of soursop is 1 g, we also found the same content during our analysis of the fruit. Our results also agree with those found by Neela B. *et al.* in a study on *Annona muricata* L. [16]. This confirms that the fruit is not rich in protein; it is nevertheless full of other essential nutrients for the body, including vitamin C, dietary fiber, minerals and antioxidants.

USFDA data indicates a value of 17 g of dietary fiber; the latter does not play an energy role. Our results indicate content of 12.2 ± 2 g of total carbohydrates per 100 g of pulp. In a study [16] already cited in this work, the total carbohydrate content was 14.63 g per 100 g of pulp. Indeed, soursop is considered a low glycemic index food, with a GI of 35% [18]. Thus, it is better tolerated by diabetics and would cause less glycemic peak when consumption is moderate.

According to official USFDA data, 100 g of soursop pulp provides 66 Kcal of energy to the body. Our results indicate that 100 g of soursop pulp provides 49.3 Kcal of energy, in parallel with Neela B. *et al.* found a value of 53.1 Kcal per 100 g of soursop pulp. This makes soursop a less caloric fruit, and is recommended for a weight loss diet, because it contains a lot of water and dietary fiber.

The results of the phytochemical screening carried out on the seeds of *Annona muricata* L. show the presence of alkaloids, flavonoids, tannins, saponins, steroids and terpenoids, glycosides and the absence of anthocyanins. These results confirm those reported by Peace Doe [19] in a similar study on the seeds of *Annona muricata* L.

This study highlighted the presence of chemical compounds that have interesting biological activities, in particular polyphenolic substances (Flavonoids, Tannins and Saponins).

After analysis of the sample, we found a particularly high content of potassium and sodium, 275 mg/100g and 49.2 mg/100g respectively, compared to other minerals present in the fruit. According to USFDA data, 100 g of soursop contains 278 mg of potassium and 14 mg of sodium, a markedly different value for sodium.

The variability of the content can have several sources, in particular the sources of biological variations of physiological order, the sources of variations of agronomic and environmental order [17].

After the oral administration of a single dose of 5000 mg/kg of the ethanolic extract of seeds of *Annona muricata* L. during the limit test which resulted in the death of the mouse, we moved on to the main test. During this trial, the lethal dose 50 that we obtained was 3320 mg/kg. Our results are in agreement with those found by Peace Doe [19] which indicate that following the limit test at 2000 mg/kg, there were no mouse deaths, which would literally translate that the LD50 is greater than 2000 mg/kg.

In another study conducted on other parts of the plant, we note that the aqueous extracts showed an LD50 > 5000 mg/kg, while the methanolic and ethanolic extracts of leaves, flowers and pulps had an LD50 > 2000 mg/kg (Ana V. *et al.*, 2018). The study conducted by Bertin A. *et al.* [8] with the leaves of *Annona muricata* L. found an LD50 of 3750 mg/kg. This reflects lower toxicity of leaves than of seeds.

In view of the results of this study, the toxicity index of the seeds is 4 according to the Hodger and Sterner toxicity scale depending on the LD50 and the route of administration [20], which reflects mild toxicity. However, certain signs of toxicity such as a decrease in sensitivity to pain or noise, a decrease in motility, tremors were observed, particularly at high doses in mice.

The biochemical analyzes carried out on the serum did not show a significant difference between the control batch and the batches having received different doses when applying a statistical test (Student test). This means that the extract does not cause a change in kidney and liver function.

5. Conclusions

This study consisted of the determination of the nutritional value of *Annona muricata* L. (Soursop) consumed in Kinshasa, as well as the acute toxicity of the seeds of this fruit. The results of this research allow us to conclude the following:

The white pulp of the soursop offering a distinct sweet and sour flavor, compared to certain tropical fruits, the soursop is low in carbohydrates and contains dietary fiber and mineral salts, which are essential elements for the proper functioning of the human organism. They, therefore, make soursop an excellent nutritional element that we can use for a weight loss diet (49.3 Kcal) and without disturbing the glycemic balance.

The phytochemical screening carried out on the seeds of *Annona muricata* L. by classic reactions in solution revealed the presence of polyphenolic substances, the majority of which are powerful antioxidants with preventive effects against certain forms of cancer, inflammatory, cardiovascular, neurodegenerative diseases and diarrhea.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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