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Phytochemical and acute toxicity of ethanolic extract from leaves of *Annona muricata* (L.) from Benin in experimental albino rats

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Abstract

The aims of this study was to evaluate the phytochemical composition and acute toxicity of ethanolic extract from leaves of *Annona muricata* (L.) from Benin. The results of phytochemical analysis indicated that tanins gallics, tanins catechics, flavonoids, leuco-anthocyan and mucilages are presents in leaves of *Annona muricata*. However, quinonics and cyanogenics derivatives as well as saponosides are not detected. The results of acute oral toxicity of ethanolic extract from leaves of *Annona muricata* indicated that the LD₅₀ value of the ethanolic extract from leaves of *Annona muricata* was 3750 mg.kg⁻¹, corresponding to the low class toxicity substance.

Keywords: *Annona muricata*, phytochemical, ethanolic extract, acute toxicity, Benin

1. Introduction

For a long time, medicinal plants are considered as the basis for health preservation and care worldwide. The use of complementary traditional medicine which include herbal medicines in the treatment of various diseases has expanded rapidly in both developed and developing countries, attributable to affordability, accessibility and efficacy [1]. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value.

Annona muricata L. is a species of the Annonaceae family that has been widely studied in the last decades due to its therapeutic potential. The medicinal uses of the Annonaceae family were reported long time ago [2], and since then, this species has attracted the attention due to its bioactivity. Ethnobotanical studies have indicated that *A. muricata* has been used as insecticide [3] and parasiticide [4]. Fruit juice and infusions of leaves or branches have been used to treat fever [5], sedative [6], respiratory illness [7], malaria [8], hypoglycemic and hypotensive [9] and cancer treatments [10]. Previous studies have upgraded a special interest on acetogenins as principal bioactive compounds [11]. Indeed, considerable information, both formal and informal, is available on the relation of the consumption of *A. muricata* with the appearance of an atypical Parkinson's disease [12, 13]. The association of the consumption of fruit and homemade preparations of *A. muricata* with the appearance of atypical Parkinsonism in the Caribbean Island of Guadeloupe is based on a case study published in 1999 [12]. This association has also been reported in New Caledonia and Caribbean patients living in London [14]. From these studies, assessment of the neurotoxic effect of the main bioactive compounds of *A. muricata* alkaloids and acetogenins was initiated. Several studies have reported that some phytochemicals, such as acetogenins present in fruit of *A. muricata*, have shown neurotoxicity in vitro and in vivo studies. Then, due to the wide use of this plant in folk medicine, more researches are needed to investigate the toxicity of leaves extracts and determine the level of human exposure. Despite the probable presence of toxic compounds, metabolic studies are also necessary to determine whether digestive processes decrease or increase bioactivity and/or neurotoxicity of the active compounds. Then the aim of the study was to evaluate the acute toxicity of ethanolic extracts from leaves of *A. muricata*, in animals and also to carry out the preliminary phytochemical screening.

2. Material and methods

2.1 Collection of plant leaves

Plant materials used for ethanolic extraction were leaves of *Annona muricata* L. (Figure 1).

Plants were collected at Abomey-calavi (Southern Benin) and identified at the Benin national herbarium, where voucher specimens are deposited.



Fig 1: Plant of *Annona muricata* L.

2.2 Identification of the secondary metabolites

The procedure used for the research of every compound enumerate as follows:

- The gallic tannins has been characterized by an aqueous solution of ferric chloride (FeCl_3) to 2% driving to the development of a coloration bruise-black or green black characterizing the presence of tannoïdes;
- The catechic tannins has been put in evidence by the reagent of Stiasny (formalin 30% in HCl extract: 2/1 v/v)^[16];
- The flavonoïdes as for them are revealed by the reaction to the cyanidine^[16];
- The anthocyanes have been revealed by hydrochloric acid addition to 5% and some drops of ammonia water to infuse it. A red coloration that turns to the purplish or greenish bruise indicates the presence of anthocyanes;
- The leuco-anthocyanes is put in evidence by the hydrochloric alcohol (ethanol to distilled 95° alcool/water; HCl concentrated in proportion equivolumic) and heating to the bath gets married to 90°C. A coloration red cherry or purplish indicates the presence of leuco-anthocyanes;
- The alkaloids have been identified by the test of Meyer and have been confirmed by the test of Bouchardât^[17, 18];
- The derivatives quinoniques have been identified by the test of Bornträger^[19]
- Saponins were determined by the test of moss; degree of dilution of an aqueous décoction giving persistent moss after shaking^[16, 19];
- The steroids have been put in evidence by the test of Liebermann-Burchard^[20];
- The Coumarineses were identified by fluorescence in UV at 365nm^[15];
- Cyanogenic derivatives were detected by the picric acidic test 1% resulting in a brown coloration characteristic of the presence of cyanogenic derivatives;
- Mucilages were revealed by the obtaining a flaky precipitate of a décoction in ethylic ether indicating the presence of mucilages^[15].

2.3 Plant preparation and extraction

Collected Leaves of *Annona muricata* L., were washed, shade-dried, milled and macerated in 6 L of 95% ethanol at room temperature for 4 days. Extraction was repeated until the last extract turned colourless. Then, the extract was then filtered through cotton wool.

By the use of the rotary evaporator at 45°C and reduced pressure, most of the ethanol solvent was removed and the concentrated *A. muricata* extract was obtained. The crude extract was dried in the oven at 60 °C until a constant weight was obtained. The extract was stored in a desiccator from where it was used when required.

2.4 Acute oral toxicity study

The toxicity study was carried out using Swiss albino mice (20 – 25 g). The animals were divided into five groups of five animals per group; control group and 4 treated groups. They were maintained on standard feed and water; and allowed to acclimatize for seven days to the laboratory environment before the experiment. After an overnight fast, the control group received 0.3 ml sterile distilled water while each treated group received 100, 1000, 2500 and 5000 mg/kg b.wt. administered orally with the aid of a feeding needle connected to syringe at stated doses in appropriate volume of sterile distilled water. The animals were observed for signs of toxicity and mortality for the first critical 4 hours and thereafter daily for 7 days. Signs of toxicity included paw-licking, stretching, respiratory distress, diarrhea and death were observed. The oral median lethal dose (LD) was calculated as the geometric mean of dose that caused 0 % and 100 % mortality respectively.

2.5 Statistical analysis

Experiments were performed in triplicate, and data analyzed are means \pm SE subjected to one-way Anova. Means are separated by the Tukey's multiple range test when Anova was significant ($P < 0.05$) (SPSS 10.0; Chicago, IL, USA).

3. Results and discussion

The results of phytochemical analysis of leaves of *Annona muricata* collected in southern Benin are presented in Table 1. These results indicated that tanins gallics, tanins catechics, flavonoids, leuco-anthocyanes and mucilages are presents in leaves of *Annona muricata*. However, quinonics and cyanogenics derivatives as well as saponines are not detected in leaves of *Annona muricata*. These results were similar to those reported by Arthur *et al.*^[22], in *Annona muricata* leaves collected at Kumassi (Ghana). Patel and Patel^[23] also reported the presence of various phytoconstituents and compounds, including alkaloids, megastigmanes, phenolics, flavonol, triglycosides, cyclopeptides in leaves of *Annona muricata*. The presence of these phytochemical compounds could justified the biological activities of this plant, such as, insecticide^[3], parasiticide^[4], sedative^[6], hypoglycemic, hypotensive and cancer treatments^[10].

The results of acute oral toxicity tests of ethanolic extract from leaves of *Annona muricata* are presented in Table 2. In oral acute toxicity studies, no untoward clinical signs were observed in the rats at doses of 100, 1000, 2500 mg/kg b.wt. There were no changes in the nature of stool, urine and eye color. No mortality was also observed at these doses levels from the critical 24 hours post administration to the end of the seventh day. Orally, these dose were well tolerated in mice even after 7 days. However, at dose of 5000 mg/kg b.wt, clinical signs were observed in the rats and the mortality rate recorded were 100%. Based on these results, the LD_{100} value of the plant extract, was 5000 mg.kg^{-1} and the Maximal Tolerate Dose (MTD) was 2500 mg.kg^{-1} . The integration of these results in the formula of Dragestedt and Lang^[24], lead to determinate that the LD_{50} value of the ethanolic extract from leaves of *Annona muricata* was 3750 mg.kg^{-1} .

According to the toxicity scale of Diezi ^[25], the LD₅₀ value of this plant extract was between 500 and 5000 mg.kg⁻¹ which correspond to the low class toxicity substance.

Table 1: Phytochemical composition of *Annona muricata* leaves

Species compounds	Results
Tanins gallics	+
Tanins catechics	+
Flavonoids	+
Leuco-anthocyanins	+
Quinonics derivatives	-
Saponines	-
Cyanogenics derivatives	-
Mucilages	+

+: presence; -: absence

Table 2: Results of acute oral toxicity tests

Lots	Doses of extracts (mg.kg ⁻¹)	Mortality rate (%)
1	0	0
2	100	0
3	1000	0
4	2500	0
5	5000	100

Conclusion

This work underlined the phytochemical composition of leaves of *Annona muricata* from Benin, and presence of compounds with high biological activities. The high LD₅₀ value obtained was an indication of safety of *A. muricata* for internal and external use. However, more attention should be paid to the use of higher doses of this extract, which could induce negative effect on organs.

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