Research Journal of Recent Sciences _____ ISSN 2277-2502
Vol. **2(5),** 6-9, May **(2013)** *Res. J. Recent Sci.*

Cytotoxicity of Leaves and Fruits of *Solanum macrocarpon* Linn (Solanaceae) against shrimp larvae (*Artemia salina* Leach)

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Available online at: www.isca.in
Received 3th October 2012, revised 5th November 2012, accepted 7th February 2013

Abstract

Traditional leafy vegetables are these plants whose leaves or aerial parts have been integrated in a community's culture for use as food over a long span of time. These vegetables are highly recommended due to their relatively high nutritional value compared to the introduced varieties, and are also important in food security. They are also used as medicines. This study aims to assess the potential cytotoxicity of S. macrocarpon, a vegetable highly used in Africa. After hatching shrimp larvae for 48 hours, they were brought into contact with aqueous dilutions of the leaves as well as fruit for 24 hours. The variation in larval mortality as a function of concentration has been translated by a curve and semi-lethal concentrations were determined. In addition, the values of the half-lethal concentration (LC50 = 1.33 mg / ml for leaves and 1.51 mg / ml for fruit) were all greater than 0.1 mg / ml, the upper limit of toxicity. It follows then that the leaves and fruits were not toxic on shrimp larvae for the range of explored concentrations. These parts of vegetable can therefore be used both in traditional medicine and nutrition without immediate or medium-term major risks.

Keywords: *S. macrocarpon*, cytotoxicity, LC_{50}, vegetable.

Introduction

Vegetables are excellent contributions enrichment and diversification of food. They are the primary source of mineral nutrients, vitamins and other compounds involved in the health and human nutrition[1]. In fact, vegetables are important in food security during periods of drought or crop failure and are also important sources of income. In addition to their nutritional value, vegetables are also sources of drugs[2-4]. In Benin, *S. macrocarpon* called "Gboma" in the local language Fon is grown by nearly of 95% of market gardeners[5]. This high production is justified by the high consumption of this vegetable by people[6]. Thus, the leaves and young fruits are prepared and eaten as a vegetable in several African countries[7]. In addition, this plant appears to have an interest in traditional medicine in the treatment of various disorders[7].

Despite the importance of the nutritional and medicinal vegetable, no scientific work to date, has provided scientific evidence that consumption of *S. macrocarpon* would have no effect on human cells. This is why the present study evaluated the cytotoxicity of leaves and fruits of S. *macrocarpon* against larvae of shrimp. It is justified by the fact that previous studies have revealed that the fruits of *S. macrocarpon* especially should be consumed with caution because of some chemical groups that phytochemical screening revealed[8-9]. This study is a contribution to a better understanding of this vegetable and to control possible risks associated with its use.

Material and Methods

Material: The leaves and mature fruit of *S. macrocarpon* (figure 1) were the plant material used in this study. They were purchased in July 2012 on the gardener site of Houéyiho at 6° 21' 20" North latitude and 2° 21' 35" East longitude in Benin. The plant was identified and authenticated by the National Herbarium of Benin under N° AA 6423/ HNB of 31 July 2012. Toxicity was evaluated at Laboratory of Pharmacognosy and Essential Oils with shrimp larvae whose eggs are marketed by the German Company JBL Gmbh & Co.KG.

Plant preparation: The leaves were dried at room temperature of 16°C in Research Laboratory in Applied Biology (University of Abomey-Calavi) for 17 days while fruits were finely cut into small pieces and dried for 09 days. Dried leaves and fruits were ground using a SAYONA Moulinex commercially purchased. The powders obtained were sieved using sieves of 0.2 mm and stored in sterile containers until needed.

Cytotoxicity test on shrimp larvae: This test is based on the survival of shrimp larvae in sea water in the presence of the test solution. Its interest lies in understanding the possible side effects that would result from consumption of fruits and leaves of *S. macrocarpon* on the body. It is a test of primary non-clinical toxicity proposed by Michael et al.[10] and later developed by Vanhaecke et al.[11], Sleet and Brendel[12]. This test is proposed

as a simple method of bioassay for preliminary assessment of toxicity in search of natural active products[13]. Eggs of *A. salina* were grown in an erlenmeyer containing sea water taken from the Atlantic Ocean and filtered before use. The mixture was left under stirring for 48 hours. Meanwhile, the eggs hatched to give birth to young larvae. Using a pipette, a colony of 16 live larvae was placed in contact with a series of solutions of graded concentrations of decoction of *S. macrocarpon*. These solutions and controls containing no solutions of *S. macrocarpon* were left stirring and read twenty-four hours later. In case of death in the control medium, the data are corrected by Abbott's formula[14]:

% Death = [(test-control) / control] × 100

The dose-response data were log-transformed and the Lethal Concentration 50 (LC_{50}) determined by a linear regression study[15].

To assess the degree of toxicity from LC_{50} values, the correlation table (table 1) established by Mousseux[16] was used.

Preparation of test solutions: A solution was prepared by moderate heating for 20 minutes the mixture of 1 g of powdered leaves and fruits of *S. macrocarpon* in 20 ml of distilled water. The concentration of 50 mg/ml was obtained. A range of ten successive dilutions were made with sea water from decoction. Concentration values expressed in mg/ml of the dilutions contained in test tubes were 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.09, and 0.04.

Table-1
Correspondence between LC_{50} and toxicity

LC_{50}	Toxicité
$LC_{50} \geq 0.1$ mg/ ml	- (Non-toxic)
0.1 mg/ ml $> LC_{50} \geq$ 0.050 mg/ ml	+ (Low toxicity)
0.050 mg/ ml $> LC_{50} \geq$ 0.01 mg/ ml	++ (Moderate toxicity)
$LC_{50} < 0.01$ mg/ ml	+ + + (High toxicity)

Statistical analysis: For the toxicity study, *A. salina* larvae were exposed to different concentrations of powdered decoction of leaves and fruits to determine the concentration that will kill 50% of larvae in 24 hours. Probit analysis was used to analyze data from toxicity tests. LC_{50} values of tested powders were calculated with Microsoft Excel 2010. A confidence interval of 95% has been set.

Figure-1
Leaves and fruits of *S. macrocarpon*

Results and Discussion

Several studies have shown that larval toxicity test is an excellent method for preliminary investigations of toxicity[17]. Para et al.[18] have also demonstrated a positive correlation between larval toxicity test and the oral lethal dose of medicinal plants in mice. Shrimp larvae remained sensitive to leaves and fruits of *S. macrocarpon* with a threshold dose of mortality equal to 0.39 mg / ml (figures 2 and 3). Sensitivity curves showed that the death of the larval population increases with the concentration. This sensitivity thus followed a dose-response relationship.

In addition, the values of the half-lethal concentration (LC_{50} = 1.33 mg / ml for leaves and 1.51 mg / ml for fruit) were all greater than 0.1 mg / ml, the upper limit of toxicity[16]. We then deduce that the leaves and fruit are not safety on shrimp larvae for the range of concentrations explored. In addition, taking into account the correlation between larval toxicity and toxicity to cells, leaves and fruits of *S. macrocarpon* does not have harmful effects on human cells namely PS 9 and 9 KB (human carcinoma) on one hand[19]; A-549 cells of lung carcinoma and HT-29 cells of colon carcinoma on the other part[20]. In sum, the organs studied for this vegetable can be used for daily basic diet without exposure to adverse effects in short and medium term. This work reinforces the phytochemical study recently conducted by Dougnon et al[9] who showed the richness of fruits in secondary metabolites. The non-cytotoxicity of *S. macrocarpon* justifies that the leaves and fruits are widely consumed as a vegetable in Africa.

In addition, the medicinal properties of this plant recognized in Africa can be seriously explored, since there is no cellular toxicity associated with its use. *S. macrocarpon* has better harmlessness compared to *Tamarindus indica*, a plant of the family Leguminoseae, which is also used as food and in traditional medicine[21-23]. Indeed, *T. indica* used in high doses, seems to have a certain cell toxicity[24]. It is the same with plants as *Lannea barteri* (K & Gillet), *Sclerocaria birrea* A. Rich, *Annona senegalensis* Pers., *Kigelia africana* (Lam). Benth, *Ananas comosus* L. Merr., etc. used in the treatment of malaria and various cancers in Nigeria[25], *Muntingia calabura* used for its antibacterial properties in Brazil[26], *Abroma augusta*, *Acanthus ilicifolius* used in traditional medicine in Bangladesh[27]. This study classified *S. macrocarpon* among the non-cytotoxic vegetables such as *Amaranthus hybridus*, a highly consumed vegetable in Kenya[28].

Conclusion

The present study scientifically demonstrated that both leaves and fruits of *S. macrocarpon* grown in Benin can be eaten and used in traditional medicine in short and medium term without major risk. However, long-term use should be done with caution, since no scientific study has explored this aspect.

Conflict of interest: Authors declare no conflict of interests.

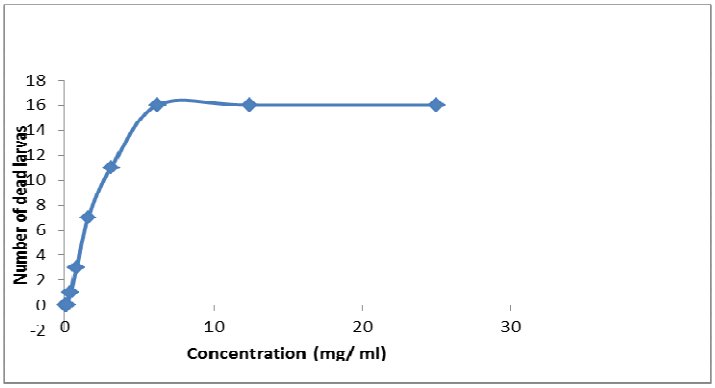

Figure-2
Sensivity curve of the decoction of leaves against shrimp larvae

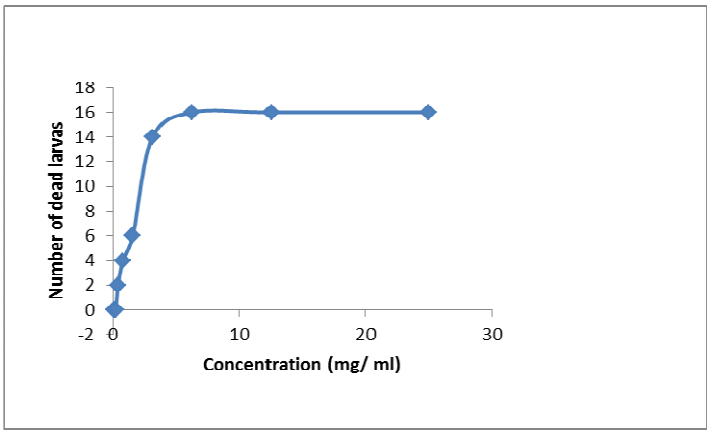

Figure-3
Sensivity curve of the decoction of fruits against shrimp larvae

Acknowledgment

We sincerely thank all the technicians of the Laboratory of Pharmacognosy and Essential Oils at Benin Center of Scientific and Technical Research for their various contributions. Authors also thank the National Program of Traditional Medicine and Pharmacopoeia of Benin for its support.

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