British Biotechnology Journal
9(4): 1-13, 2015, Article no.BBJ.19123
ISSN: 2231–2927, NLM ID: 101616695

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Phytochemical Screening and Cytotoxic Analysis of Three Local Vegetables Used in the Treatment of Bacterial Diarrhoea in Southern Benin (West Africa): A Comparative Study

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Authors' contributions

This work was carried out in collaboration between all authors. This work for a part of PhD study of author AJA. Author SHB coordinated and supervised the whole study. Authors FA and JG performed the analyses. Authors TVD, SHB, BY and LBM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2015/19123
Editor(s):
(1) Giuliana Napolitano, Department of Biology (DB), University of Naples Federico II, Naples, Italy.
Reviewers:
(1) Sukesh Voruganti, Texas Tech University Health Sciences Center, USA.
(2) Triveni Krishnan, National Institute of Cholera and Enteric Diseases, India.
(3) Anonymous, The Foundation for Medical Research, Mumbai, Maharashtra, India.
Complete Peer review History: http://sciencedomain.org/review-history/11549

Short Communication

Received 26th May 2015
Accepted 22nd July 2015
Published 27th September 2015

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ABSTRACT

The use of plants in traditional medicine become very common nowadays throughout the world and in developing countries in particular. The current study was carried out aiming to compare the chemical features of three vegetables (*Vernonia amygdalina*, *Crateva adansonii* and *Sesamum radiatum*) mostly used for human consumption and traditional medicine to treat bacterial diarrhoea in Benin. These vegetables were selected among the 27 species obtained after ethno-botanical investigations in southern Benin. Therefore, the major chemical groups contained in these vegetables were detected by solubility assays together with coloration and precipitation reactions. Furthermore, cytotoxicity of the plant extracts was assessed on shrimp larvae (*Artemia salina*). The results showed that gallic tannins, flavonoids, alkaloids, mucilages, coumarins and reducing compounds were detected in all samples (100%) followed by cathechic tannins (66.66%) and saponins (33.33%). The study also revealed that none of the studied vegetables is cytotoxic (LC_{50} > 0.01 mg/ml). This study was conducted in the perspective of establishing innovations capable of assisting to; manufacture Improved Traditional Medicines for the treatment of bacterial diarrhoea in the future. Besides, it pointed out the presence of secondary metabolites and the cytotoxicity of these vegetables interesting for further antibacterial, antidiarrheal and pharmacological studies.

Keywords: Vernonia amygdalina; Crateva adansonii; Sesamum radiatum; phytochemical screening; cytotoxicity.

1. INTRODUCTION

Local vegetables are either wild or semi-cultivated species and important in both rural and urban areas of the world [1]. They are plants which leaves or aerial parts are integrated in the food habits of communities [2] and are strongly recommended because of their high nutritive values against the exotic varieties [3]. According to [4], most of local vegetables have medicinal properties and are used as medicinal foods. Although they are constituted mostly of water, they are valuable reserves of minerals, vitamins and phytochemical compounds such as alkaloids, flavonoids, glycosides and tannins that are very beneficial for health maintenance and the prevention of some illnesses [5-10]. Furthermore, some vegetables serve in phytomedicine for the treatment of various affections including stomach aches [11,12]. However, several studies established that some plant species are potentially toxic for human and animals [13]. In fact, phytochemical compounds are produced within the plants as poisons to fight pests and herbivores. Such poisons are very active substances that can cause acute effects in human and animals when they are ingested at high concentrations and chronic effects when they are accumulated [13]. Under stressful conditions caused by food scarcity, the consumption of high amounts of plant toxins can have serious consequences [14].

In Benin, 14.44% of the 27 local vegetable species recorded by [4] , are traditionally used as nutritious food by populations and would serve in phytomedicine by traditional healers to treat bacterial diarrhoea [15]. Among these vegetable species, the most cited and used by traditional healers in the treatment of bacterial diarrhoea are *Ocimum gratissimum* (29.05%), *Vernonia amygdalina* (16.80%), *Crateva adansonii* (13.49%) and *Sesamum radiatum* (11.41%). These vegetables leaves are not only used to treat bacterial diarrhoea, but they are also used in treatment of high blood pressure, constipation, malaria, dysentery, candidoses and the delivery at pregnant woman [15-17]. Nevertheless, in these plants, the chemical compounds responsible for biological activities as well as their toxicity remain unknown. This could cause enormous prejudices to prescribers, leading to issues like overdose, under-dose and poisonings and even death. The used doses remain inaccurate [18]. This imprecision constitutes a serious problem to traditional medicine improvement. Therefore, a surveillance of the toxicity and posology of plant extracts that are empirically administered is needed in order to avoid adverse therapeutic risks that can sometimes be tragic [19,20].

Regarding the fact that these local vegetables are widely consumed as food and used as medicine by local populations in Benin, their phytochemical characteristics and cytotoxicity need to be investigated. Results of their phytochemical screening and cytotoxicity in addition to the nutritional value of these three vegetables (*V. amygdalina* is very rich in magnesium (9000±1 mg/kg), sodium (2870±2 mg/kg), potassium (48200±1 mg/kg) and phosphorus (8738±2 mg/kg); *C. adansonii* has high calcium (24000±1 mg/kg), proteins (25248±1 mg/kg) and zinc (22±1 mg/kg) contents; *S. radiatum*, it is rich in iron (1360±1 mg/kg) and copper (38±1 mg/kg) [21]) will be used to impart knowledge on their nutritional and sanitary importance and promote their use. The objectives of the present study were to conduct a qualitative phytochemical screening of the leaves of *V. amygdalina*, *C. adansonii* and *S. radiatum* and to assess the cytotoxic activity of the leave extracts of *V. amygdalina*, *C. adansonii* and *S. radiatum* on shrimp larvae.

2. MATERIALS AND METHODS

2.1 Materials

The material was basically plant material constituted of powders of the leaves of *Vernonia amygdalina*, *Crateva adansonii* and *Sesamum radiatum*. During two weeks, these leaves were collected from Ouèdo, Abomey Calavi Municipality, Pahou, Ouidah Municipality and Savalou Municipality respectively and identified at the National herbarium of Benin.

2.2 Methods

2.2.1 Preparation of the leave powders

Leaves of the three vegetables were thoroughly washed with distilled water containing Bleach (1/100) then dried at laboratory temperature for 20 days. The dried leaves were ground using mechanical grinder. The obtained powders were sieved using a 0.2 mm stitch sifter. They were then stored in sterile containers at laboratory temperature until use.

2.2.2 Phytochemical analysis

The chemical analyses were carried out at the laboratory of Pharmacognosy and essential oils of the institute of Applied Biomedical Sciences based on a phytochemical screening. It is a qualitative analysis based on coloration and precipitation reactions. This was performed on the crude powders of the three vegetables following the methodology described by [22] and repeated by [23]. This method permits to determine some constituents such as Alkaloids, Polyphenol, Triterpenoids and Reducing compounds.

3. LARVAL CYTOTOXICITY

3.1 Extracts Preparation

3.1.1 Aqueous extract

100 g of plant powder were boiled for 30 minutes in 1000 ml distilled water. The decoction, after cooling was filtered once on absorbent cotton and once on filter paper Whatman N°1. The obtained filtrate was then dried in an oven at 50℃, to produce the aqueous extract (AE).

3.1.2 Hydro-ethanolic extract

100 g of plant powder were agitated for 72 hours in 1000 ml 70% ethanol then filtered once on absorbent cotton and once on filter paper Whatman N°1. The hydro ethanolic phase was dried in an oven at 50℃, to give the hydro ethanolic extract (HE).

The output (R) every extract of each vegetables leaves studied was calculated and was expressed in relation to plant material extracts (powder). Its calculation makes itself of following manner:

$$R = \frac{\text{Mass of extract obtained}}{\text{Mass of plant powder used}} \times 100$$

3.2 Principle of the Test

This test is based on the survival of the shrimps' larvae (*Artemia salina*) in sea water along with the tested solution. It was carried out at the Laboratory of Pharmacognosy and Essential Oils of the Institute of Applied Biomedical Sciences on the various extracts of three studied vegetables. This is a primary test of non-clinical toxicity which was proposed by [24], and applied by [25] on one hand and [26] on the other hand. The test was proposed as a simple bioassay method of preliminary toxicity assessment in active natural products investigations [27].

Artemia salina eggs were incubated at laboratory temperature (28℃-30℃) in a conical flask of 1000 ml containing sea water fetched from Atlantic Ocean and filtered before use. The solution was continuously agitated for 48 hours. During that period, the eggs hatched and gave birth to larvae. Stock solutions of the diverse vegetable extracts were then prepared by mixing 200 mg of extracts in 4 ml of sea water giving a concentration of 50 mg/ml. Ten serial dilutions of 1:2 were then made from the decoctions using sea water. The concentrations (mg/ml) of diluted solutions contained in test tubes numbered from 1 to 10 were 25 – 12.5 – 6.25 – 3.12 – 1.56 – 0.78 – 0.39 - 0.19 – 0.09 and 0.05 respectively.

Using a micropipette, a colony of 16 living larvae was collected and added to each of the dilutions. The obtained Mixture together with the controls were incubated for 24 hours under agitation. In case larval death occurs in the control solutions, data were corrected using the formula of [28]:

Dose-response data were transformed by logarithm and the Lethal Concentration 50 (LC_{50}) was determined by a polynomial or logarithmic regression. In order to appreciate the degree of toxicity from the values of LC_{50}, the correspondence table (Table 1) established by [29] was used. Tests were carried out in triplicate.

3.3 Statistical Analyses

To assess the toxicity of the extracts on *A. salina* larvae, a probit analysis was performed. The LC_{50} values of the tested plant extracts were then calculated, using Microsoft Excel 2010 software. A confidence interval of 95% was defined to conclude statistical differences.

Table 1. Correspondence between LC_{50} and toxicity

LC_{50}	Toxicity
LC50 ≥ 0.1 mg/ml	−
0.1mg/ml> LC50 ≥ 0.050 mg/ml	+
0.050mg/ml > LC50 ≥ 0.01 mg/ml	++
LC50 < 0.01 mg/ml	+++

− = No toxic; + = Low toxicity; ++ = Moderate toxicity; +++ = High toxicity [29]

4. RESULTS

4.1 Phytochemical Screening

The major chemical groups present in the leaves of the three tested plant species are displayed in Table 2. The leaves of *Vernonia amygdalina* contain alkaloids, gallic and cathechic tannins, flavonoids, anthocyanins, mucilages, coumarins, quinone derivatives, reducing compounds, saponins, traces of steroids and cyanogenic derivatives. Those of *Crateva adansonii* contain alkaloids, gallic and cathechic tannins, flavonoids, anthocyanins, mucilages, coumarins, quinone derivatives and reducing compounds. While the leaves of *Sesamum radiatum* contain alkaloids, gallic tannins, flavonoids, leucoanthocyanes, mucilages, coumarins, quinone derivatives and reducing compounds.

Table 2. Chemical groups present in of the three studied plants

Chemical groups	*V. amygdalina*	*C. adansonii*	*S. radiatum*
Alkaloids	++	++	++
Gallic tannins	+++	+++	+++
Cathechic tannins	+++	+++	-
Flavonoids	+++	+++	+++
Quinone derivatives	++	++	+
Cyanogenic derivatives	+	-	-
Triterpenoids	-	-	-
Steroids	+	-	-
Cardenolides	-	-	-
Saponins	++	-	-
Anthocyanins	++	++	-
Leucoanthocyanes	++	++	++
Mucilage	+++	+++	+++
Reducing compound	++	++	+++
Coumarins	+++	+++	+++
Free anthracene derivatives	-	-	-
Combined anthracene derivatives	-	-	-

+ = Weak presence; − = Absence; ++ = Moderate presence; +++ = High presence

4.2 Larval Toxicity of the Tested Vegetable Extracts

For the aqueous extracts (AE) we obtain 10.5 g of *V. amygdalina*, 12.7 g of *C. adansonii* and 12.4 g of *S. radiatum*, either respectively an output (R) extracting of 10.5%, 12.7% and 12.4%. On the other hand we obtain 10.7%, 14.3% and 13.2% as output of hydro-ethanolic extract (HE) of each vegetable leaves, either respectively 10.7 g of *V. amygdalina*, 14.3 g of *C. adansonii* and 13.2 g of *S. radiatum*.

The larvae of *A. salina* (shrimps) were sensitive to the leave extracts of *V. amygdalina*, *C. adansonii* and *S. radiatum* with a mortality threshold of 0.49 mg/ml. A variability of death rate of *A. salina* was observed according to different concentrations used. Compared to the witnesses, the sensitivity curves showed that larval mortality increases with the concentration of extracts. Such sensitivity follows therefore a dose-response relationship (Figs. 1a, 1b, 2a, 2b, 3a and 3b). Furthermore, the half Lethal Concentration (LC_{50}) values were 1.796 mg/ml for the AE of *V. amygdalina* and 9.235 mg/ml for its HE; 2.261 mg/ml for the AE of *C. adansonii* and 2.280 mg/ml for its HE and 2.629 mg/ml for the AE of *S. radiatum* and 3.648 mg/ml for its HE) (Table 3). Besides, these values were greater than 0.1 mg/ml, which is the limit of toxicity [29]. It comes out of such result that the leaves of the three studied vegetables (*V. amygdalina*, *C. adansonii* and *S. radiatum*) are harmless to the shrimp's larvae according to the range of concentrations used.

Table 3. LC_{50} values of the different tested vegetable leave extracts

	Species	Regression	Values of LC_{50} (mg/ml)
AE	*V. amygdalina*	$Y = -0.4598x^2 + 9.4869x + 34.44$ ($R^2 = 0.9725$)	1.796
	C. adansonii	$Y = 13.528\ln(x) + 38.962$ ($R^2 = 0.9868$)	2.261
	S. radiatum	$Y = 0.1685x^2 + 6.7137x + 31.987$ ($R^2 = 0.9435$)	2.629
HE	*V. amygdalina*	$Y = 0.0939x^2 + 1.6266x + 26.962$ ($R^2 = 0.9448$)	9.235
	C. adansonii	$Y = -2.5525x^2 + 23.286x + 10.169$ ($R^2 = 0.985$)	2.280
	S. radiatum	$Y = 0.8929x^2 + 8.3929x + 7.5$ ($R^2 = 0.9584$)	3.648

AE = Aqueous extract; HE = Hydro-ethanolic extract

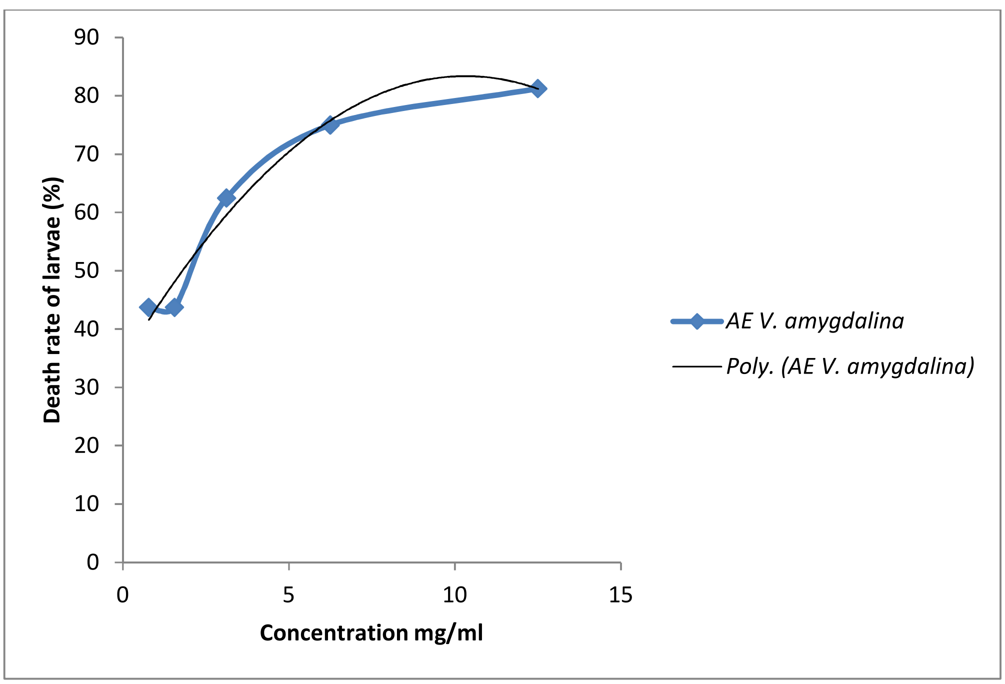

Fig. 1a. Curve of larval toxicity of aqueous extract (AE) of leaves of *Vernonia amygdalina*

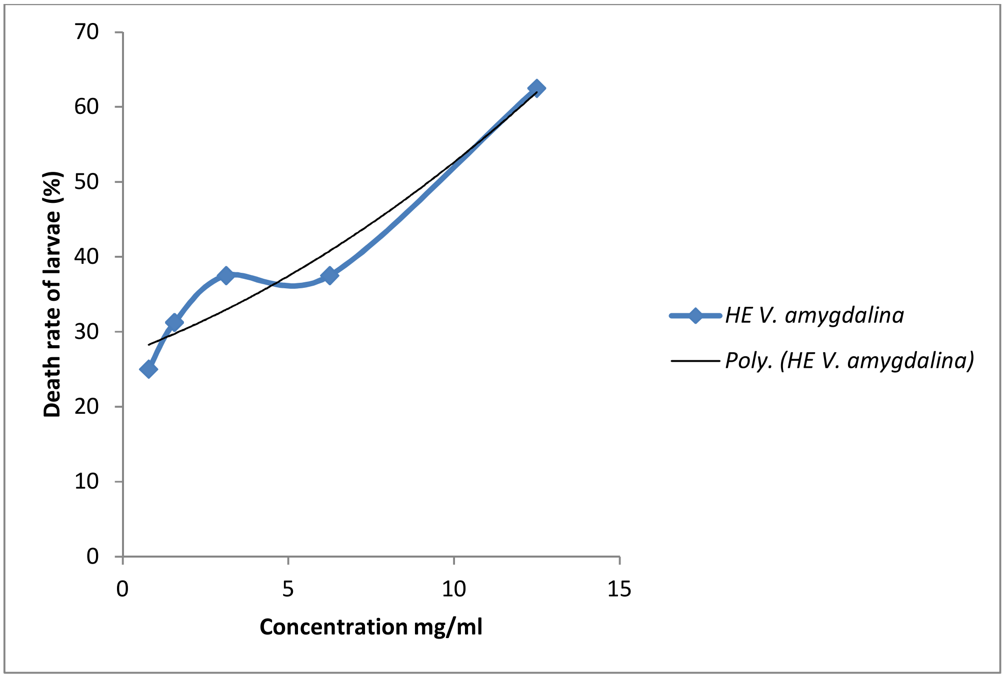

Fig. 1b. Curve of larval toxicity of hydro-ethanolic extract (HE) of leaves of *Vernonia amygdalina*

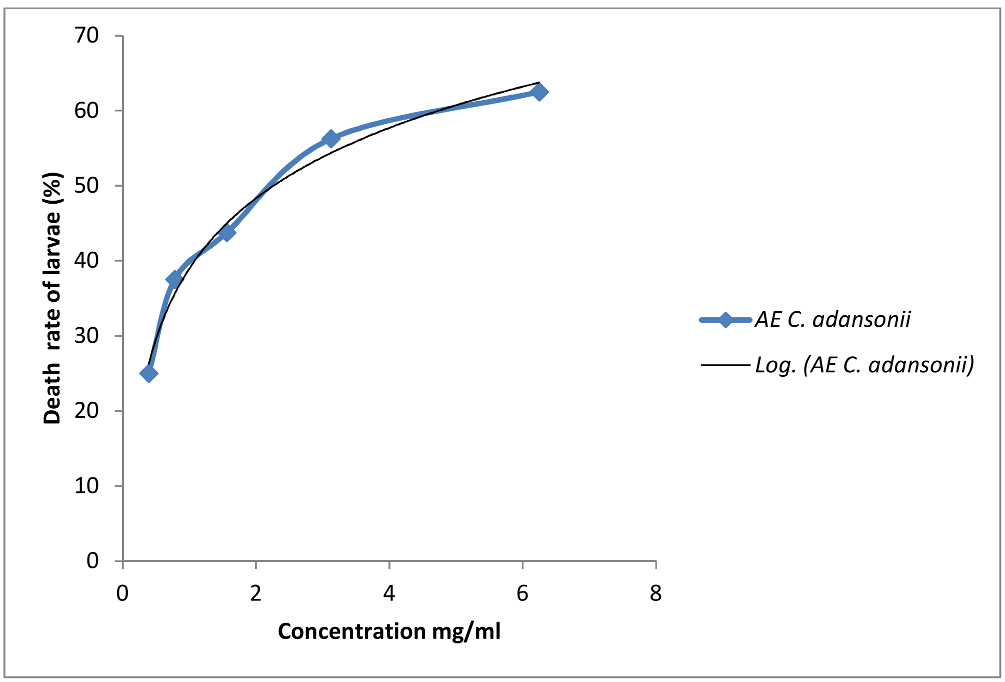

Fig. 2a. Curve of larval toxicity of aqueous extract (AE) of leaves of *Crateva adansonii*

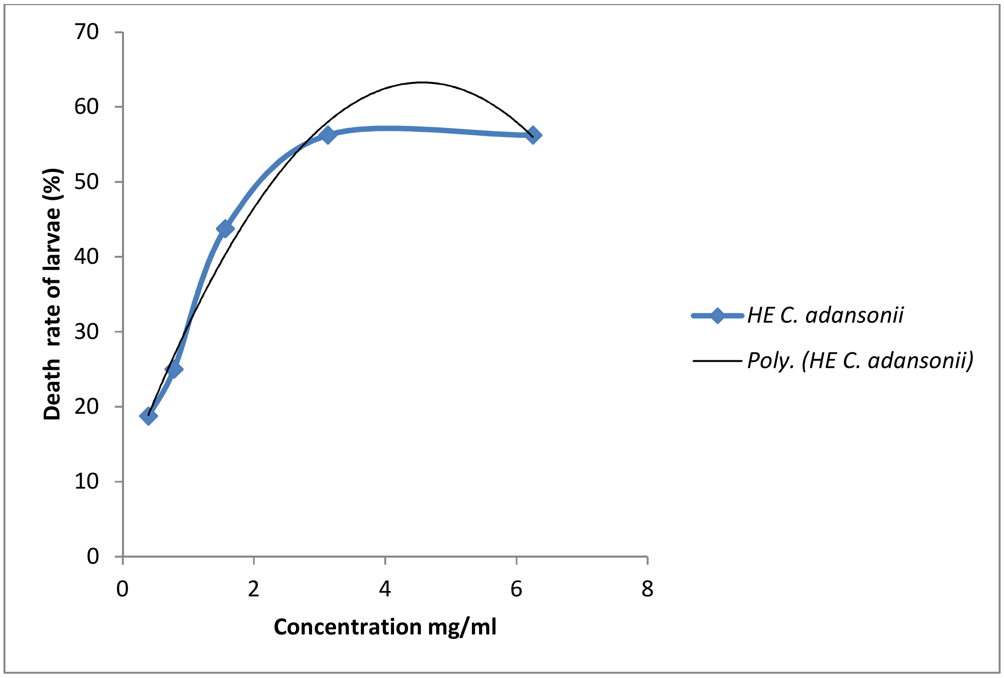

Fig. 2b. Curve of larval toxicity of hydro-ethanolic extract (HE) of leaves of *Crateva adansonii*

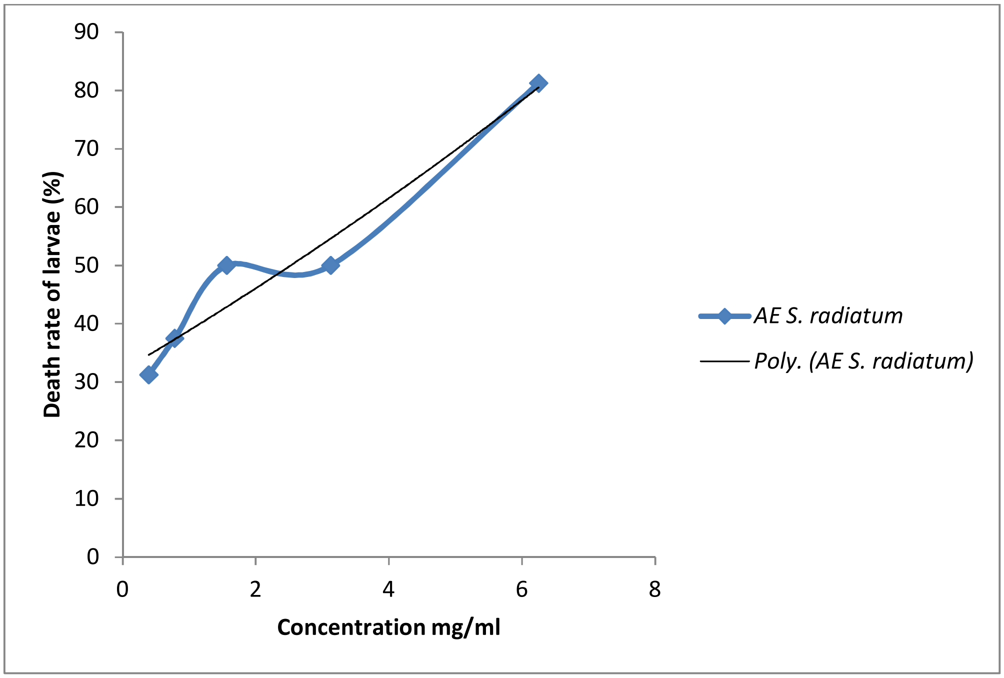

Fig. 3a. Curve of larval toxicity of aqueous extract (AE) of leaves of *Sesamum radiatum*

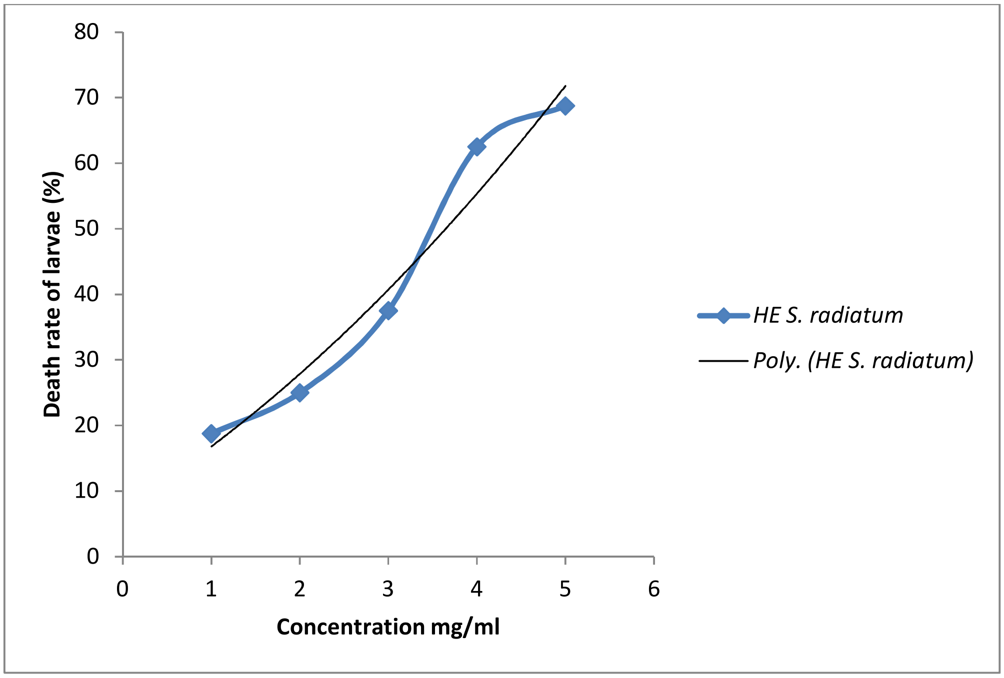

Fig. 3b. Curve of larval toxicity of hydro-ethanolic extract (HE) of leaves of *Sesamum radiatum*

5. DISCUSSION

5.1 Phytochemical Screening

Rural populations use various plant species for medicinal purposes of which most are available in their immediate environment, thus limits human pressure on the environment [30-*32*]. Vegetables are important source of protective elements that contribute to health maintenance and prevention of some illnesses [33].

The phytochemical analysis of the three studied vegetables (*Vernonia amygdalina*; *Crateva adansonii* and *Sesamum radiatum*) revealed the presence of alkaloids, tannins, flavonoids, mucilages, coumarins, reducing compounds, quinone derivatives, anthocyanins and leucoanthocyanes. These results are similar to those obtained by [34] and [35]. However, saponins, traces of steroids and cyanogenic derivatives were detected in the powder of the leaves of *Vernonia amygdalina*. [36] also obtain the same results and so did [37]. Nevertheless, analyses of [37] revealed in addition to other compounds the presence of anthraquinonic compounds. This difference could be due to geographical origin and genetic differences of the samples especially because [37] conducted their study in Nigeria.

The presence of tannins in the studied vegetables is a particular advantage since tannins are well known for their relatively high antioxidant, antibacterial, anti-inflammatory, deworming, antiviral, blood pressure regulation and antidiarrheal properties [13,18,38,39]. Tannins are generally abundant in vegetables and are used for lips protection against inflammations, cataract treatments, wounds healing, haemorrhoid and diarrhoea treatments [40,41]. Their presence in the leaves of the studied vegetables justifies the use of these vegetables by local communities of Benin in diarrhoea, stomach ulcers, abscesses, blood pressure and intestinal worms control [15].

Alkaloids are the most effective phytochemical compounds in therapeutic uses [5,42]. The presence of alkaloids in the studied vegetables is an advantage to consumers who can benefit from the analgesic, anti-inflammatory, antibacterial and resistance to illnesses and stress properties of these compounds [18,43,44]. Moreover, flavonoids have antioxidants activities and detoxification as well as many favourable effects on health [45]. Flavonoids are anti-allergic; anti-cancer, antibacterial, antifungal, anti-inflammatory, antiviral, ant-diabetic, anti-malaria and anti-diarrheal [18,46,47]. The studied vegetables contain high quantities of coumarins. Many coumarins and their derivatives have various activities: antifungal, anti-tumorous, antiviral, antimalarial, anti-inflammatory, antioxidant, antimicrobial, diuretic, analgesic and enzymes inhibitory [23,48,49]. Mucilages that are soluble fibres also possess several medicinal properties [23,50]. They are anti-cholesterols, anti-constipation, anti-cancer, anti-diabetics and stomachic. The leaves of *V. amygdalina*, *C. adansonii* and *S. radiatum* are rich in reducing compounds. These reducing sugars are monosaccharides and disaccharides [18,51]. Most of the vegetables contain a lot of reducing compounds. This is why it is recommended to have a diet rich in vegetables.

Particularly *Vernonia amygdalina* contains high amounts of saponins traces of steroids and cyanogenic derivatives. Saponins are important nutritional sources [52]. They are glycosidic molecules [53] and have a useful expectorant action in curing infections of the superior respiratory tract. Saponins are implicated in the treatment of hypercholesterolemia and possess antibacterial and anti-diarrheal properties [18,54]. Steroids are known for their analgesic and cardio-tonic properties [55]. They regularize the metabolism of proteins and carbohydrates, increase muscles and bones synthesis and are also associated with hormonal control in women [55]. Cyanogenic derivatives are serious metabolite poisons. However, the very low presence of this anti-nutritional substance in leaves of *V. amygdalina* by the current study can allow its consumption without harm.

5.2 Cytotoxicity of the Studied Local Vegetables

The output (R) of hydro-ethanolic extracts of vegetables leaves is raised more than the one of aqueous extracts of this same vegetables. The ethanol diluted to 70% could be a good solvent of extracting of active principles of *V. amygdalina*, *C. adansonii* and *S. radiatum* leaves.

Several studies proved the relevance of larval toxicity test in exploratory studies of toxicity [56]. A positive correlation was recorded between larval toxicity test and lethal oral dose of medicinal plants in mice [57].

Additionally, with respect to the correlation that exists between larval toxicity and toxicity on human cells, notably the 9 PS and 9 KB cells of human nasopharyngeal carcinoma on one hand [58], the A-549 cells of the pulmonary carcinoma and the HT-29 cells of the colon carcinoma on other hand [59], *V. amygdalina*, *C. adansonii* and *S. radiatum* can be considered as harmless to human cells. In short, these vegetables can be used daily as a basic food, and in traditional medicine as well without any cytotoxic risk. This justifies their excessive consumption by population and by their use by Beninese traditional healers [15].

The Lethal Concentration 50 (LC_{50}) of the aqueous extracts (AE) of each of studied vegetable was lower than the one of their hydro-ethanolic extracts (HE). Such situation shows that the AE present more effect (lethally) on the larvae of (*A. salina)* than their HE. This could be explained by the strong polarity of the AE against the HE. These results are similar to those reported by [60] who demonstrated that the LC_{50} of methanolic extracts of *Fagonia olivieri* was higher than the one of its aqueous extract. Therefore, variations of toxicity can be observed within different extracts of the same plant. The results also revealed that out of the three studied vegetables, *V. amygdalina* showed the strongest larval lethally (LC_{50} = 1.796 mg/ml) followed by *C. adansonii* (LC_{50} = 2.261 mg/ml) and *S. radiatum* with a poor larval lethally (LC_{50} = 2.629 mg/ml).

V. amygdalina, *C. adansonii* and *S. radiatum* are more harmless than *Tamarindus indica*, a plant used as well in nutrition and in traditional medicine [61,62]. In fact, when it is used at high dose, this vegetable presents a cellular toxicity [63]. The harmlessness of each of the three studied vegetables is also better than the one of many other vegetables such as *Boerhavia diffusa* [64], *Moringa oleifera* [8], *Solanum macrocarpon* [65], *Crassocephalum crepidioides* and *Crassocephalum rubens* [66] and *Manihot esculenta* [67]. This study allowed to classify *V. amygdalina*, *C. adansonii* and *S. radiatum* in the group of non-cytotoxic vegetables where are found *Solanum macrocarpon* [65], *Crassocephalum crepidioides* and *Crassocephalum rubens* [66] and *Amaranthus hybridus*, a vegetable mostly consumed in Kenya [14].

6. CONCLUSION

The phytochemical screening of the leaves of *V. amygdalina*, *C. adansonii* and *S. radiatum* revealed the presence of tannins, alkaloids, flavonoids, steroids, mucilages, coumarins, anthocyanins, leucoanthocyanes, quinone derivatives, cyanogenic derivatives and reducing compounds indicating that they have strong medicinal properties. This confirms their use in the treatment of various affections in Benin. Furthermore, the non-toxicity of these vegetables will allow to sensitize populations on their values and to promote their use in food for nutritional purposes and in traditional medicine for the therapeutic properties. However other studies are necessary to assess the antibacterial, antidiarrheal, anti-diabetic, anti-inflammatory and the blood pressure regulation potentials assigned by the local communities to these vegetables. However, the use of sophisticated and more advanced methods for the phytochemical screening and the cytotoxicity tests would be paramount to improve this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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