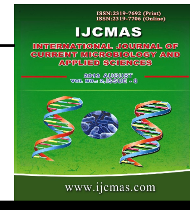

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Original Research Article

Phytochemical screening and toxicity studies of *Crassocephalum rubens* (Juss. ex Jacq.) S. Moore and *Crassocephalum crepidioides* (Benth.) S. Moore consumed as vegetable in Benin

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A B S T R A C T

Keywords

Crassocephalum crepidioides; *Crassocephalum rubens;* leafy vegetable; nutraceutical; phytochemical screening; toxicity; Benin.

Gbolo (*C. crepidioides* and *C. rubens*) is a wild or semi-domesticated aromatic traditional leafy vegetable highly consumed in Benin. The vegetable was said to have medicinal values and locally used as nutraceutical. Considering the scientifically established potential toxicity of some vegetable species to humans and animals, leaves' extracts of *C. crepidioides* and *C. rubens* were subjected to qualitative phytochemical screening and testing for cytotoxicity using the brine shrimp lethality bioassay. The phytochemical screening recorded the presence of pharmacologically important substances such as tannins, coumarins, combined anthracene derivatives C-heterosides, flavonoids, mucilage, reducing compounds and Steroids. Alkaloids, anthocyanins, quinone derivatives, saponins, triterpenoids, cyanogenic derivatives, cardiac glycosides and anthracene derivatives (except anthracene derivatives C-heterosides) were not detected. The LC50 values of the leaves' extracts were found to be 0.901 mg/ml for *C. crepidioides* and 0.374 mg/ml for *C. rubens* hence indicating the non-toxicity of both species. The outcome of this study lends support to the trado-medicinal uses of vegetable Gbolo in the treatment of various ailments in Benin and indicates the potential usefulness of this vegetable as nutraceutical to prevent or treat various diseases. However more studies are needed for the validation of the antimicrobial, anti-diabetic, anti-inflammatory and blood pressure regulation properties attributed by the local communities to this vegetable Gbolo

Introduction

Traditional vegetables from the wild or home gardens are mutually important for humans both in rural and urban set ups (Dansi *et al.*, 2008). Traditional leafy vegetables (TLVs) are those plants whose leaves or aerial parts have been integrated in a community's culture for use as food over a large span of time (Adeoti *et al.*, 2012). TLVs are highly recommended because they have a relatively high nutritional value compared to the introduced varieties and their consumption gives diversity to daily food intake, adding flavour and zest to the diet (Ahohuendo *et al.,* 2012). According to Dansi *et al.*, (2008) many traditional leafy vegetables has some curative, regulative and stimulative properties besides food qualities and are used as nutraceutical. Though the bulk of the weight of traditional leafy vegetables is water, they represent sometimes a veritable natural pharmacy of minerals, vitamin and phytochemical compounds such as alkaloids, flavonoids, glycosides and tannins. However several studies have established that some vegetable species are potentially toxic to humans and animals (Agbaire *et al.,* 2013). Plant chemical compounds, toxic to humans and livestock, are produced as part of the plant's defence against being eaten by pests and herbivores or to gain an advantage over competing plants (Bharathi 2008; Asaolu *et al.,* 2009; Andzouana and Mombouli 2012; Agbaire *et al.,*2013). Plant poisons are highly active substances that may cause acute effects when ingested in high concentrations and chronic effects when accumulated (Agbaire *et al*., 2013). Under stress conditions, brought on by food shortage, consumption of large amounts of vegetable toxins can have negative consequences (Orech *et al.*, 2005).

In Benin, a biodiversity inventory and documentation survey recently conducted on TLVs throughout the country revealed a total of 187 plant species among which the vegetable locally known as Gbolo was found to be of paramount interest (Dansi *et al.*,2008). Gbolo comprising two species namely *Crassocephalum rubens* (Juss. ex Jacq.) S. Moore and *C. crepidioides* (Benth.) S. Moore (Figure 1a, b), is highly consumed throughout Benin. According to Adjatin *et al.*, (2013), the nutritional values of these two species although slightly different are of high importance. The contents of the leaves in raw proteins and in crude lipids expressed in % of dry matter are, respectively, 27.13%, 3.45 for *C. crepidioides*; 26.43% and 2.75% for *C. rubens*. The content of vitamin C for 100 g of fresh leaf is of 9.17 mg for *C. crepidioides* and 3.60 mg for *C. rubens*. The content of ash is of 19.76% and 19.02% for *C. rubens* and *C. crepidioides*, respectively. The contents for sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), Manganese (Mn), and copper (Cu) are 2129.04 mg, 4469.91 mg, 434.13 mg, 3845.88 mg, 1.6 mg, 8.22 mg, and 2.6mg, respectively, for *C. rubens* and these are higher than those in *C. crepidioides* (Adjatin *et al.,*2013). Traditionally, Gbolo is used as a nutraceutical and believed to have antibiotic, anti-helminthic, anti-inflammatory, anti-diabetic, anti-malaria and blood regulation properties and also treats indigestion, liver complaints, colds, intestinal worms, and hepatic insufficiency in addition to its nutritional value (Adjatin *et al.,*2012). Since Gbolo is highly consumed (leaves eaten raw or cooked in sauce) by Benin local populations, its phytochemical screening need to be carried out and its toxicity examined. The phytochemical screening and the toxicity results in addition to the already established nutritional value of the two species of Gbolo will be used to create awareness of their values and, when possible (i.e. if not toxic and trado-medicinal uses supported), popularize their usage in diets as nutraceutical.

The objectives of this study were two folds: Carry out a qualitative phytochemical screening of the leaves' extract of *C. crepidioides* and *C. rubens* and investigate the cytotoxic activity of *C. crepidioides and C. rubens* leaves using brine shrimp assay

Materials and Methods

Source and Preparation of vegetable Gbolo samples

Samples (stems and leaves) of Gbolo (*Crassocephalum rubens* and *Crassocephalum crepidioides*) were obtained from the germplasm maintained at the Faculty of Science and Technology of Dassa (FAST Dassa) in Benin. The stems and leaves of Gbolo were washed thoroughly under running tap water followed by sterile distilled water, cut into smaller pieces and dried under shade during for 9 days. The dried plant parts were ground using electric blending machine and the powdery samples obtained were sieved using two sieves of 0.2 mm (mesh size) and stored in air tight sterile containers until needed.

Phytochemical analysis

Qualitative phytochemical screening of Gbolo species was carried out on the powdery samples, after extraction with aqueous solvent, using the standardly employed precipitation and coloration reactions as described by Houghton and Raman (1998) and Dougnon *et al.,*(2013). Major secondary metabolites essayed and the methods used were as follow: Alkaloids (Mayer's test), Quinone derivatives (born-trager reaction), Cathetic tannins (stiasny test), Gallic tannins (ferric chloride test after saturation with sodium acetate), Flavonoids (shinoda test and magnesium powder), Cyanogenic derivatives (picric acid test), Triterpenoids (acetic acid test + mixture of acetic an hydride and sulfuric acid), Steroids (kedde reaction), Saponins (test index foam), Cardiac glycosides (Raymond Marthoud reaction), Anthocyanins (test with hydrochloric acid and ammonia diluted to half), Leucoanthocyanes (shinoda test), Mucilage (test of absolute alcohol), Reducing compounds (test with fehling's solution), Coumarins (test with ether andammonia), Free anthracene derivatives (test with chloroform andammonia), Combined anthracene derivatives (test with chloroform andammonia).

Brine Shrimp Lethality Assay

The cytotoxic activity of the extracts of the two species of Gbolo was evaluated using Brine shrimp lethality bioassay. Brine shrimp (*Artemia salina* Leach) also known as sea monkey are marine invertebrates of about 1mm in size. The 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 leaves of vegetable Gbolo on the body. A solution was prepared by moderate heating for 20 minutes, the mixture of 1 g of powdered leaves of *C. rubens* or *C. crepidioides* in 20 ml of distilled water following Dougnon *et al.,*(2013). The concentration of 50 mg/ml was obtained and a range of ten successive dilutions (49 µg/ml, 98 µg/ml, 195 µg/ml, 391 µg/ml, 781 µg/ml, 1582 µg/ml, 3125 µg/ml, 6250 µg/ml, 12500 µg/ml, 25000 µg/ml) were made with sea water from decoction. Eggs of *A. salina* were grown in an erlenmeyer containing sea water taken from the Atlantic Ocean and filtered before use. The mixture (eggs and sea water) was left under stirring for 48 hours. Meanwhile, the eggs were hatched to give birth to young larvae (nauplii). Using a pipette, a colony of 16 live larvae was placed in contact with the series of solutions of graded concentrations of decoction of *C. rubens* and *C. crepidioides*. These solutions and the controls containing no extract of Vegetable Gbolo were left stirring and read after 24 hours of incubation. The total death and percentage mortality (death) at each dose level and control were determined (Tables 1 and 2). To assess the degree of toxicity of the different species of vegetable Gbolo, the LC50 and toxicity corresponding table (Table 4) was used following Agbaire *et al.*, (2013) and Dougnon *et al.*, (2013).

Statistical analysis

For each extract or sample the lethal concentration that causes 50% death (LC50) was calculated at 95% confidence interval by linear regression analysis and also by using the probit analysis method following Ullah *et al.*,(2013). A regression line equation was derived for each extract with the mortality data obtained and, it was then used to calculate the LC50 value. The detailed mathematical steps used to derive the regression line equation are reported in the literature (Hubert, 1980; Vincent, 2012).

Results and Discussion

Phytochemical compounds identified and their importance

Vegetables are important sources of protective foods which are highly beneficial for the maintenance of good health and prevention of some diseases (Dansi *et al*., 2013). The qualitative phytochemical screening of the powdery samples of the two species (*C. crepidioides* and *C. rubens*) of vegetable Gbolo revealed the presence of cathetic tannins, gallic tannins, coumarins, combined anthracene derivatives C-heterosides, flavonoids, mucilage, reducing compounds and Steroids (Table 2) that are known to exhibit medicinal properties (Anandhi and Revathi, 2013). Both species showed similar phytochemical profile and the active compounds they contain were somewhat similar to those reported on *Crassocephalum crepidioides* (Arawande *et al.,* 2013) and on other traditional leafy vegetables such as *Launeae taraxacifolia* (Adinortey *et al.*, 2012; Olalekan *et al.*, 2013) and *Moringa oleifera* (Shahriar *et al.*, 2012).

The medicinal properties of the different compounds identified are reported in the literature. Tannins are well known for their antioxidant, antimicrobial, anti-inflammatory, antiviral, antifungal, anthelmintic and anti-tumor properties as well as for soothing relief, skin regeneration and diuresis (Domart 1981; Agbaire *et al.,* 2013). Tannins are generally abundant in leafy vegetables and have been traditionally used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, hemorrhoids and diarrhea (Konig *et al.,* 1994; Vijayameena *et al*., 2013). Their presences in the leaves of both species of Gbolo well justify the use of this vegetable by the Benin local communities (most often as nutraceutical) in healing wounds and stomach ulcers and to combat intestinal worms and fungus diseases (Adjatin *et al.*, 2012). In Central Africa, particularly in

Table.1 Correspondence between **LC_{50}** and toxicity

LC_{50}	**Toxicity**
$LC_{50} \geq 0.1$ 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)

Table.2 Secondary metabolites examined and detected in the leaves of vegetable Gbolo

Chemical compounds	***C. crepidioides***	***C. rubens***
Alkaloids	-	-
Polyphenol compounds		
Gallic tannins	+	+
Cathetic tannins	+	+
Flavonoids	+	+
Anthocyanins	-	-
Leucoanthocyanes	-	-
Quinone derivatives	-	-
Saponins	-	-
Triterpenoids	-	-
Steroids	+	+
Cyanogenic derivatives	-	-
Mucilage	+	+
Coumarins	+	+
Reducing compounds	+	+
Anthracene derivatives		
Free anthracene derivatives	-	-
Combined anthracene derivatives O-heterosides	-	-
Combined anthracene derivatives C-heterosides	+	+

Congo and Uganda, *C. crepidioides* and *C. rubens* are used to treat stomach disorders and fresh wounds as well as lip swellings (Bosch, 2004).

Flavonoids have antioxidant and detoxification activities and many health promoting effects (Akroum, 2011). Some of the other activities attributed to flavonoids include: anti-allergic, anti-cancer, anti-inflammatory, anti-fungal, anti-viral, anti-diabetic and anti-malarial (Morel, 2011). Flavonoids protect against free radicals, platelet aggregation, microbes, ulcers, hepatoxins, liver injury, viruses and tumours and have ability to relieve hay fever, eczema, sinusitis and asthma (Adedapo *et al*., 2013). Flavonoids protect against heart disease and epidemiological studies have even illustrated that heart diseases are inversely related to flavonoid intake (Chakraborty *et al.,* 2004; Morel, 2011). These known properties justify the traditional use (as nutraceutical) of vegetable Gbolo for the regulation of blood pressure (hypertension and hypotension) and the treatment of diabetes, malaria and various infections as reported by Adjatin *et al.,* (2012). Gbadamosi *et al.,* (2012) did not detect flavonoids in the leaves of *C. rubens* in Nigeria. This difference in our results may be due to the geographical origin of the samples or the extraction methods used.

Natural coumarins (Aromatic phytochemical compounds with a vanilla like flavour) have recently drawn much attention due to its broad pharmacological activities (Bruneton, 2009). Many coumarins and their derivatives exert anti-fungal, anti-coagulant, anti-tumor, anti-malaria, anti-viral, anti-inflammatory, diuretic, analgesic, anti-oedema, anti-oxidant and anti-microbial effects and have enzyme inhibition properties. Coumarins increase the blood flow in the veins and decreases capillary permeability. Adjatin *et al.,* (2012) and Dansi *et al.,* (2013) reported that Gbolo is aromatic and locally used against malaria and oedema. The smell of this vegetable and its medicinal properties may be also linked to the presence of coumarins. The antioxidant property of tannins, flavonoids and coumarins make them capable of protection against free radicals which are responsible of more than 200 human diseases including cardiovascular disease, cancer, arthritis, sight disorder and ageing (Datta *et al.*,2000; Adeoye *et al.*,2005; Oszmianski *et al.*,2007; Ouali *et al.*,2007). Steroids are known for their analgesic, anti-microbial, anti-inflammatory and cardiotonic properties (Hossain *et al*., 2013). They regulate carbohydrate and protein metabolism, increase muscles and bone synthesis and are also associated with hormonal control in women (Hossain *et al.,* 2013). As reported by the local communities (Adjatin *et al.,* 2012), vegetable Gbolo, when regularly taken, enhances lactation in newly born mothers. Therefore, one understands the reason why the leaves of Gbolo are locally used as vegetable for expectant and breast feeding mothers.

Mucilage (soluble fibres) also has several medicinal properties (Lin *et al*., 2005; Dougnon *et al*., 2012). It is anti-cholesterol (aid to lower cholesterol in the blood), anti-constipation (helps to prevent the appearance of intestinal decomposition), anti-cancer, anti-diabetic agents and stomachic (has ability to protect internal mucous membranes). Their presence in *C. crepidioides* and *C. rubens* leaves explains their use in the treatment of diarrhoea and indigestion (Bosch, 2004; Adjatin *et al.,* 2012). Reducing compounds and combined anthracene derivatives C-heterosides also have interesting medicinal value (Dougnon *et al.,* 2012)

Unrevealed Phytochemical compounds and significance of their absence

Our study revealed that *C. crepidioides* and *C. rubens* do not contain alkaloids, anthocyanins, quinone derivatives, saponins, triterpenoids, cyanogenic derivatives, cardiac glycosides and anthracene derivatives except anthracene derivatives C-heterosides (table 1). Anthocyanins are natural pigments responsible of the colour of vegetative organs, especially petals. Quinone derivatives have irritating or drastic laxative effects on the intestines, causing contractions of the intestinal walls. Their absence in vegetable Gbolo was confirmed by Gbadamosi *et al.* ,(2012). Cyanogenic derivatives and anthraquinones are real metabolites poisons (Agbaire *et al*., 2013) and their absence in the leaves of both species of vegetable Gbolo somehow ensure consumers on the inexistence of risk associated with their consumption. Alkaloids are the most effective phytochemicals compounds in therapeutic uses (Okwu, 2005; Ayoola and Adeyeye, 2010). Their absence in Gbolo was also confirmed by the results of Arawande *et al.,*(2013). Cardiac glycosides are natural substances that act on the heart by regulating its contractions without increasing the amount of oxygen in the heart muscle (Ayoola and Adeyeye, 2010). However, these compounds could become real poisons by blocking relaxation during diastole because of the very narrow margin between the therapeutic and the toxic doses. Consequently, a high dose can cause a cardiac arrest (Ayoola and Adeyeye, 2010). Given the very high frequency of the consumption of both species of vegetable Gbolo mainly during their abundance period as reported by Adjatin *et al.,* (2012), the absence of Cardiac glycosides is a relief for the consumers. Saponin is being used as mild detergent and in intracellular histochemistry staining to allow antibody access to intracellular proteins (Okwu and Okwu, 2004). The most important properties of the saponins were reported to include expectorant (useful in the treatment of respiratory infections), diuretic, analgesic and promotion of wound healing (Arawande *et al.,*2013). It is of great importance in medicine because it is used in hypercholesterolaemia, hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory and body loss. Steroidal saponins are used as contraceptive and precursors for sex hormones while glycosidal saponins are cardiotonic (Gbadamosi *et al.,* 2012). Saponins are responsible for the bitter taste of the leaves that contain them and are linked to sex hormones like oxytocin involved in the control of the birth inducement and subsequent release of milk (Okwu and Okwu, 2004). The current study did not reveal their presence in Gbolo leaves. These results are contrary to the reports of Arawande *et al.,* (2013) and of Gbadamosi *et al.,* (2012) according to which leaves of *C. crepidioides* and *C. rubens* contain saponins. The differences in the results of the diverse studies with regards to saponins content of Gbolo could be explained by the genetic differences of the plant materials (plants from Benin and from different regions of Nigeria) used. Moreover, this study was carried out with the aqueous solvent which is not necessarily the case in the other studies. Denton (2004) and Adjatin *et al.,* (2012) signalled that *C. crepidioides* and *C. rubens* are used to treat cough and to stop nasal haemorrhage. This medicinal value of vegetable Gbolo would probably be due to the presence of saponins in its leaves. In that case, further research on the presence of saponins should be carried out with other solvents such as methanol, ethanol and acetone to confirm or deny our results.

Degree of toxicity of *C. crepidioides* and *C. Rubens*

The extracts of the two species (*C.crepidioides* and *C. rubens*) showed positive results (lethality) on the Brine Shrimp nauplii indicating that the test samples are biologically active (table 3, Table 4). Varying degree of lethality of *Artemia salina* was observed with exposure to different dose levels. The lethal concentration (LC50) graphically determined (figure 2a, b) was 0.391 mg/ml for *C. rubens* extract and between 0.781 and 1.562 mg/ml for *C. crepidioides.* The determination of LC50 obtained using the regression line is 0.901 mg/ml for *C. crepidioides* and 0.374 mg/ml for *C. rubens*. These values which are very close those determined graphically confirm the reliability of the determination methods. The study revealed, based on the LC50 and toxicity table of correspondence (Table 1) set by Mousseux (1995), that none of the species of vegetable Gbolo investigated was toxic to shrimp larvae as their LC50 are greater than 0.1 mg/ml. However extract of *C.crepidioides* appeared more toxic for Brine shrimp (*Artemia salina*) larvae than the one of *C. rubens*. This observation supports Ahmed *et al.*,(2012) who reported a variability of toxicity between species of the genus Amaranthus. Taking into account the established correlation between the toxicity of shrimp larvae and that of human cells, the two species of Gbolo can be considered as leafy vegetable with no risk of toxicity. One understands why species of vegetable Gbolo were eaten as raw green salads in some areas in Nigeria (Denton, 2004) and Benin (Adjatin *et al.*, 2012). Studies conducted by Mouekeu *et al.*, (2011) on *Crassocephalum bauchiense* (Hutch.), Mukazayire *et al.*,(2010) on *Crassocephalum vitellinum* and Musa *et al.*,(2011) on *C. crepidioides* confirm the non-toxicity of species of the genus *Crassocephalum*. Similar results were obtained on other leafy vegetables such as *Boerhavia diffusa* Linn (Apu *et al.*,2012), *Moringa oleifera* (Shahriar *et al.*,2012), *Launaea taraxacifolia* (Adinortey *et al.*,2012) and *Solanum macrocarpum* (Dougnon *et al.*,2013).

Adjatin *et al.*,(2012) reported that to prepare Gbolo in Benin, thoroughly washed fresh leaves are generally simply cut into small slices (or grinded) and directly introduced into boiling palm nut or Egusi (seeds of *Citrullus lanatus, Cucumeropsis mannii* and *Lagenaria siceraria*) sauces. With regard to the non-toxicity highlighted above and to the high nutritional value of Gbolo (Adjatin *et al.*,2013), this cooking method which also preserves the aroma of the vegetable sauce could be recommended as blanching reduces the nutrient content, especially vitamin C (Gil *et al.*, 1999).

The phytochemical screening of the two species of vegetable Gbolo revealed the presence of cathetic tannins, gallic tannins, coumarins, combined anthracene derivatives C-heterosides, flavonoids, mucilage, reducing compounds and Steroids indicating that they have high medicinal properties and confirming their use in the management of various ailments in Benin. In addition to these results, the no-toxicity of the species revealed by the study and their already established nutritional value will be used to advise

Table.3 Cytotoxicity of the extract of *C. crepidioides*

Concentration (mg/ ml)	Log concentration	% mortality	Probit
0.049	1.690	18.75	51.39
0.098	1.991	31.25	52.30
0.195	2.290	31.25	53.18
0.391	2.592	37.5	54.08
0.781	2.893	37.5	54.97
1.582	3.199	75	55.86
3.125	3.495	81.25	56.76
6.25	3.796	100	57.65
12.5	4.097	100	58.55
25	4.398	100	59.44

Table.4 Cytotoxicity of the extract of C. *rubens*

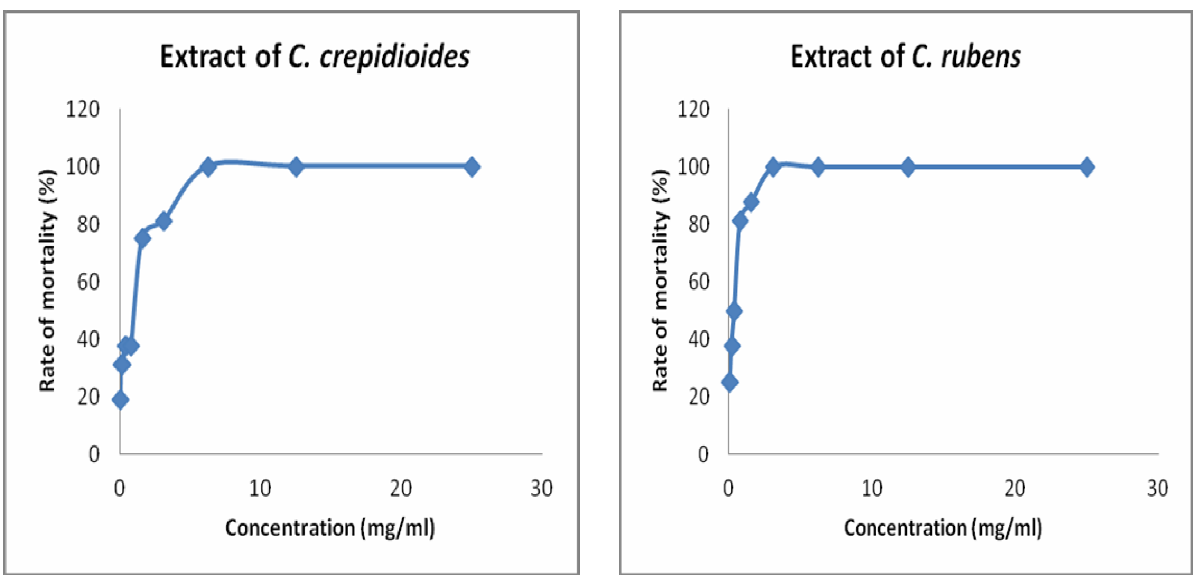
Concentration (mg/ ml)	Log concentration	% Mortality	Probit
0.049	1,690	25	62.74
0.098	1.991	25	63.46
0.195	2.290	37,5	64.17
0.391	2.592	50	64.88
0.781	2.893	81,25	65.60
1.582	3.199	87,5	66.34
3.125	3.495	100	67.05
6.25	3.796	100	67.75
12.5	4.097	100	68.47
25	4.398	100	69.18

Tableau.5 LC50 values of the extracts of *C. crepidioides* and *C. rubens*

Species	Regression line	LC_{50} values (mg/ml)
C. crepidioides	y= 2.98 x +46.36	0.901
C. rubens	y = 2.38 x + 58.72	0.374

Figure.1 Flowering plants of vegetable Gbolo

Figure.2 Sensivity curve of the extracts of Gbolo (*C. crepidioides* and *C. rubens*) leaves against shrimp larvae



a) ***C. crepidioides*** **b)** ***C. rubens***

people on their values and popularize their usage in diets as nutraceutical to prevent or treat various diseases. However more studies are needed for the validation of antimicrobial, anti-diabetic, anti-inflammatory and blood pressure regulation properties attributed by the local communities to this vegetable Gbolo.

References

Adedapo, A., T. Adewuyi and Sofidiya, M. 2013. Phytochemistry, anti-inflammatory and analgesic activities of the aqueous leaf extract of *Lagenaria breviflora* (Cucurbitaceae) in laboratory animals. Int. J. Trop. Biol. 61 (1): 281-290.

Adéoti, K., A.VDansi L. Ahoton , R. Vodouhè , B.C. Ahohuendo, A. Rival and Sanni, A. 2012. Agromorphological characterization of *Sesamum radiatum* (Schum. and Thonn.), a neglected and underutilized species of traditional leafy vegetable of great importance in Benin. African. J. Agricult. Res. 7(24): 3569-3578

Adinortey, M.B., J.K. Sarfo , E.T. Quayson, A. Weremfo, C.A. Adinortey, W. Ekloh and Ocran, J. 2012. Phytochemical screening, proximate and mineral composition of Launeae taraxacifolia leaves. Res.J.Med. Plant. DOI: 10.3923 p:9.

Adjatin A., A. Dansi, C.S. Eze,P. Assogba, I. Dossou-Aminon, K. Akpagana, A. Akoègninou and Sanni, A. 2012. Ethnobotanical investigation and diversity of Gbolo [*Crassocephalum rubens* (Juss. ex Jacq.) S. Moore and *C. crepidioides* (Benth.) S. Moore], a traditional leafy vegetable under domestication in Benin. Genet. Resour. Crop. Evol. 59 (8):1867-1881.

Adjatin A., A. Dansi, A.F. Sanoussi, E. Badoussi, F. Gbaguidi, P.Azokpota, R. Vodouhe , A. Akoègninou, K. Akpagana and Sanni A, 2013. Proximate, mineral and vitamin C composition of vegetable Gbolo [*Crassocephalum rubens* (Juss. Ex Jacq.) S. Moore and *C. crepidioides* (Benth.) S. Moore] in Benin. J. Biol. Life Sci.7 (1):319-331

Agbaire, P.O., J.O.T. Emudainohwo and Peretiemo-Clarke, B. O. 2013. Phytochemical screening and toxicity studies on the leaves of *Manniophyton fulvum*. Inter. J. Plant. Ani.Environ. Sci. 3 (1): 1-6.

Ahmed F., F. Islam, N. Nahar, M. Ahmed, A. H. Rana, K.M., HasanurRahman, Md. MumitHossain, M. Rahmatullah, 2012. Assessment of Bioactivity of Bangladeshi Medicinal Plants Using Brine Shrimp Lethality Assay. Advances in Natural and Applied Sciences, 6(8): 1325-1329.

Ahohuendo, B., C.O. Sinébou, L.E. Ahoton, A.C. Etèka, A. Dansi, A. Ahanchédé, J.D. Hounhouigan, A. Sanni and Foucault, B. 2012. Study of the phenology and floral biology of *Sesamum radiatum* Schumach. & Thon., a traditional leafy vegetable in domestication in Benin. Acta. Botanica. Gallica. 159(3): 335-344.

Anandhi D., and Revathi, K. 2013.Phytochemical analysis of *Caesalpinia coriaria* (Jacq.)Wild. Int. J. Bioscience Res. 2(1): 1-7

Andzouana, M., and Mombouli, J.B. 2012. Assessment of the chemical and phytochemical constituents of the leaves of wild vegetable Ochthocharis dicellandroides (Gilg).Pak. J. Nutrit. 11 (1):94-99.

Apu, A.S., M.S. Liza, A.T.M. Jamaluddin, A. Howlader Md, R.K. Saha, F. Rizwan and Nasrin, N. 2012. Phytochemical screening and in vitro bioactivities of the extracts of aerial part of Alvarez A., Real R., Perez M., Mendoza G., Prieto J.G. and Merino G, 2010. Modulation of the activity of ABC Transporters (P-Glycoprotein, MRP2, BCRP) by flavonoids and drug response. J.

Pharma. Sci. 99: 598-617.

Arawande, J. O., E.A. Komolafe and Imokhuede, B. 2013. Nutritional and phytochemical compositions of fireweed (*Crassocephalum crepidioides*). Inter. J. Agricul. Technol. 9 (2): 371-381.

Asaolu, M.F., O.A. Oyeyemi and Olanlokun, J.O. 2009. Chemicals compositions, phytochemical constituents and in vitro biological activity of various extracts of Cymbopognon citrates. Pak. J. Nutr. 8:1920-22

Ayoola, P.B., and Adeyeye, 2010. Phytochemical and nutrient evaluation of *Carica papaya* (pawpaw) leaves. IJRRAS 5(3):325-328

Bharathi, V.T., 2008. Evaluation of antioxidant properties of *Canthiumparvifloram* Lam. Leaves. Nat. Prod. Rad. 7(2): 122-126

Bosch C.H., 2004. *Crassocephalum rubens* (Juss .ex Jacq.) S. Moore. In Grubben G. J. H & Dentor, O. A (Editors) PROTA 2: Vegetables / Legumes [CD-ROM] PROTA, Wageningen Nertherlands.

Bruneton J., 2009. Pharmacognosie. Phytochimie. Plantes médicinales, 4e édition. TEC & DOC, Paris, 1269 p.

Dansi A., A. Adjatin, H. Adoukonou-Sagbadja, V. Faladé, H. Yedomonhan, D. Odou and Dossou, B. 2008. Traditional leafy vegetables and their use in the Benin Republic. Genet. Resour. Crop Evol. 55**:** 1239-1256

Dansi A., R. Vodouhè, P. Azokpota, H. Yedomonhan, P. Assogba, A. Adjatin Y.L. Loko, I. Dossou-Aminon and Akpagana K. 2012. Diversity of the Neglected and Underutilized Crop species of importance in Benin. TheScienti. World. J. 2012, ID 932947, DOI 10.1100/2012/932947, 19.

Dougnon, T.V., H.S. Bankolé, A.P. Edorh, T.J. Dougnon, J.R. Klotoé, F. Loko and Boko M. 2013. Cytotoxicity of Leaves and Fruits of *Solanum macrocarpon* Linn (Solanaceae) against shrimp larvae (*Artemia salina* Leach). Res. J. Recent Sci. 2(5):6-9

Dougnon,T.V., H.S. Bankolé, R.S. Johnson, J.R. Klotoé, G.D. Fernand and Assogba, G.F. 2012. Phytochemical Screening, Nutritional and Toxicological Analyses of Leaves and Fruits of *Solanum macrocarpon* Linn (Solanaceae) in Cotonou (Benin).Food Nutr. Sci. 3:1595-1603.

Egharevba, H.O., and Kunle, F.O. 2010. Preliminary Phytochemical and Proximate Analysis of the leaves of *Piliostigma thonningii* (Schumach) Milne-Redhead. Ethnobotanical Leaflets. 14: 559-569.

Gbadamosi, I.T., A.E. Alia and Okolosi O. 2012. In-vitro Antimicrobial Activities and Nutritional Assessment of Roots of Ten Nigerian Vegetables. New York Sci. J. 5(12): 234-240.

GilM, I., F. Ferreres, and Tomas-Barberan, F.A. 1999.Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. J. Agric. Food Chem. 47: 2213–2217.

Grubben, G. J. H., and O. A. Denton, 2004.Plant Resources of Tropical Africa vegetables. PonennadLooijenhv, Wageningen, pp. 667 .

Hossain, H., I.A. Jahan, S.I. Howlader, S.K. Dey, A. Hira and Ahmed A. 2013. Phytochemical Screening and Anti-nociceptive Properties of the Ethanolic Leaf Extract of Trema Cannabina Lour. Advan. Pharma. Bull. 3(1):103-108

Houghton P. J., and A. Raman, "Laboratory Handbook for the Fractionation of Natural Extracts," Chapman and Hall, New York, 1998, pp. 130-207. doi:10.1007/978-1-4615-5809-5

Hubert J. J., 1980. Bioassay. Kendall Hunt Publishing Company.

Morel S., 2011. Etude phytochimique et évaluation biologique de *Derrisferruginea* Benth. (Fabaceae).Université d'Angers, thèse de doctorat. pp. 245.

Mouokeu, R. S., R. AN Ngono, P.K. Lunga, M. MKoanga, A. T. Tiabou, Guy, S.S. Njateng, J. D.D. Tamokou and Kuiate, J.R. 2011. Antibacterial and dermal toxicological profiles of ethyl acetate extract from *Crassocephalum bauchiense* (Hutch.) Milne-Redh (Asteraceae). Complemen. Alter. Medi. 11:43.

Mukazayire, M.J., V. Allaeys, P. Bue Calderon, C. Stevugny, M.J. Bigendako and Duez, P. 2010. Evaluation of the hepatotoxic and hepatoprotective effect of Rwandese herbal drugs on in vivo (guinea pigs barbiturate-induced sleeping time) and in vitro (rat precision-cut liver slices, PCLS) models. Exp.Toxicol. Pathol., 62(3):289-99

Musa A.A., 2012. Cytotoxicity activity and phytochemical screening of *Cochlospermum tinctorium* Perr ex A. rich rhizome. J. App. Pharma. Sci. 02 (07): 155-159.

Okwu, D.E., and Okwu, M.E. 2004. Chemical composition of *Spondiasmombia* L., plant parts. J. Sustain. Agric. Environ. 6: 140-147.

Okwu, D.E., 2005. Phytochemicals, vitamins and mineral contents of two Nigeria medicinal plants. International J. Mole. Medi. Advan. Sci. 1(4):375-381.

Olalekan, A.J., A.I. Adekunle and Labunmi L. 2013. Chemical and phytochemical composition of wild lettuce (*Launaea taraxacifolia*). J. App. Phytotechnol. Environ. Sani. 2 (1): 25-30.

Orech, F.O., T. Kenga, J. Ochora, H.Friis, and Aagaard-Hansen, J. 2005. Potential toxicity of some traditional leafy vegetables consumed in Nyang'Oma division, Western Kenya. 11th NAPRECA Symposium. Book of Proceedings, Antananarivo, Madagascar.pp. 78-87.

Oszmianski, J., A. Wojdylo, E. Lamer-Zarawska and Swiader, K. 2007. Antioxidant tannins from Rosaceae plant roots. Food Chem. 100 (2): 579-83.

Ouali, K., F. Trea, L. Toumi, A. Bairi, D. Maurel and Guellati, M.A. 2007. L'hespéridine, un antioxydant flavonoïde qui diminue le stress oxydatif et prévient les malformations fœtales au cours du diabète gestationnel expérimental. Phytothér. 5 (4): 204-9.

Shahriar, M., I. Hossain Md, A.N. Bahar Md, S. Akhter, A. Haque Md. and Bhuiyan M. A. 2012. Preliminary phytochemical screening, *in-vitro* antioxidant and cytotoxic activity of five different extracts of *Moringa oleifera* leaf. J. App. Pharma. Sci. 02 (05): 65-68.

Ullah, M.O., M. Haque, K.F. Urmi, A.H. Zulfiker, E.S. Anita, M. Begum and Hamid, K. 2013. Anti-bacterial activity and brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables from Bangladesh. Asian Pacific. J. Trop. Biomedi. 3(1): 1-7.

Vijayameena, C., G. Subhashini, M. Loganayagi and Ramesh, B.2013. Phytochemical screening and assessment of antibacterial activity for the bioactive compounds in *Annona muricata*. Int. Curr. Microbiol. App. Sci, 2(1): 1-8

Vincent, K., 2012. Probit Analysis.http://userwww.sfsu.edu