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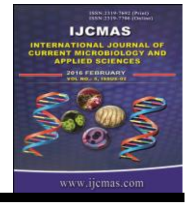

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Ethnobotany, Phytochemical Screening and Toxicity Risk of *Cleome gynandra* and *Cleome viscosa*, two traditional leafy vegetables consumed in Benin

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A B S T R A C T

Cleome gynandra (L.) Briq and *Cleome viscosa* (L.) Briq.,of the Capparaceae family are two wild or semi-domesticated herbaceous highly consumed as traditional leafy vegetables in Southern Benin. They are little studied and belong to the neglected and underutilized species. This study aims to assess the ethnobotanical knowledge and the qualitative phytochemical composition of these species as well as and their toxicity risk in southern Benin. Ethnobotanical data were collected using participatory appraisal tools and techniques such as group and individual surveys and field visits using questionnaires. The phytochemical screening was carried out using leaves powders of both species and standard methods based on colourand precipitation reactions. Collected data were analyzed with both descriptive statistics and multivariate (Principal Component Analysis- PCA)analysis using SAS V.9.2 software.PCA helped to examine the relationship between ethnic groups and usages of *C. gynandra* and *C. viscosa*. Results obtained, indicated that C. *gynandra* is more used for food (70% of responses) than *C. viscosa* which is more used for traditional medicine (45% of responses). Preferred habitat of both species are agglomerations, near households, home gardens and road sides. *C. gynandra* and *C. viscosa* are nutraceuticals and used can treat, through consumptions, malaria, icterus, anemia, fever, chronic malaria, eyes' disorders, chronic constipation, hypotension and hypertension, ears aches and neuralgia. The study revealed that these two plant species would have medico-magic protection power. Mina, Adja and Holly ethnic groups use more *C. gynandra* for food than Fon, Sahoue and Cotafon ethnic groups. In the other hand, Goun, Adja and Nago ethnic groups use more *C. viscosa* in traditional medicine than Cotafon, Holly and Fon ethnic groups. The qualitative phytochemical screening carried out on *C. gynandra* and *C. viscosa* using leaves extracts, revealed the presence of tannins, flavonoids, anthocyanin, leuco-anthocyanin, steroids, mucilage, reducing compounds and quinone derivatives which varies according to the plant species. The LC50 of *C. viscosa* and *C. gynandra* noted were respectively 0.78 mg/ml and3.125mg/ml indicating non toxicity. The nutritional value of the two species should be determined and there domestication accelerated and promoted.

Introduction

Many plants are used by local communities to satisfy their food needs, nutrition and health and constitute a source of additional income and employment (Sandhya *et al.*, 2006; FAO, 2012; Visweswari *et al.*, 2013).Generally, plants produce metabolites that confers to them, many medicinal virtues that are at the basis of their utilization by people (Zwenger and Basu, 2008; Visweswari *et al.*, 2013; Adjatin *et al.*, 2013). Among them, certain are consumed as traditional leafy vegetables (LFTs) which play an important role in food, nutrition and health of populations because of their availability and richness in nutrients. In Benin, Dansi *et al.*, (2012) reported 187 plant species consumed as leafy vegetables among which *Cleome gynandra* L. (Briq.) and *Cleome viscosa* L. (Briq.) classified in the group of theso-called neglected and underutilized species of high priority on which further study must be performed. In Asia and Africa, leaves and seeds are used to treat different infections including fever, anemia, rheumatism and headaches (Grubben *et al.*, 2004; Jane *et al.*, 2012).

In Benin, *Cleome gynandra*L. (Briq.) and *Cleome viscosa* L. are wild or semi-domesticated species (Vodouhe *et al.*, 2012). Contrary to other LFTssuch as *Crassocephalum rubens* and *Crassocephalum crepidioides* that are well studied (Adjatin *et al.*, 2012), few study were carried out on these two species. In order to fill this gap and facilitate the valorization of their genetic resources, it is necessary to conduct a thorough study ontheir ethnobotany. Moreover, it is important to do a comparative phytochemical evaluation of *C. gynandra* and *C. viscosa* to rightly appreciate their increased use in the prevention or treatment of certain illness. Accurate knowledge of the habitat, ecological requirements and usefulness of a plant species is the cornerstone of any program focusing on the conservation and domestication of this species. These studies are necessary for a better knowledge of the species in order to valorize, promote and conserve them forthe benefit of rural populations (Dansi *et al.*, 2008).

The general objective of this study is to contribute to food security and health of the population in Benin through a better knowledge of *Cleome gynandra* and *Cleome viscosa* for their valorization and sustainable use. Specifically, the study aims to document the ethnobotanical knowledge of *Cleome gynandra* and *Cleome viscosa*in southern Benin; and to determine the qualitative phytochemical compositions of both species in relation with their medicinal use assess the toxicity of the leaves of the two species

Materials and Methods

Study Area

Benin is Located in West Africa between Nigeria and Ghana., Southern Benin (Figure 1) grouping the administrative departments of Mono, Couffo, Atlantique, Littoral, Oueme and Plateau constitutes the study area. Southern Benin is inhabited by about 9.9 millions of habitants (INSAE, 2013) belonging to 14 (Adja, Aizo, Cotafon, Fon, Goun, Holly, Mina, Nago, Peda Sahouè, Tori, Watchi, Xwla, Yoruba) ethnic groups (Akoègninou *et al.*, 2006). The climate is sub-equatorial and the average annual rainfall varies from 900-1500 mm per year (Akoègninou *et al.*, 2006). The average annual temperature is 26.5°C and the relative humidity is, on average, 75% per year (Akoègninou *et al.*, 2006). The soil is ferruginous, lateritic, vertisols or hydromorphic soils (Akoègninou *et al.*, 2006). The vegetation is constituted of mosaic of forest and savannah (Akoègninou *et al.*, 2006).

Site Selection and Data Collection

Based on results of research carried out by Dansi *et al.*, (2008), Codjia *et al.*, (2009) and Akoègninou *et al.*, (2006), 9 villages (Table 1, Figure 1) were selected for the study (Figure 1). Preliminary surveys were carried out in order to determine the size (**n)** of the sample (interviewees) to be considered for the survey following Dagnelie (1998) formula which is:

$$n = \frac{U^2_{1-\alpha/2} \times p(1-p)}{d^2}$$

n = sample size; U1-α / 2 is the value of the normal variable reduced for a probability value; α = 0.05; U1-α / 2 = 1.96; p is the proportion of individuals who know the species (p varies from a locality to another); d is the marginal error set at 0.08. In total 260 people were surveyed in nine villages. Data collected from individual surveys were related to the knowledge or not of *C. gynandra* and *C. viscosa*, the vernacular names and their meanings, the habitats of the species and the farmers' perception of their the abundance in these habitats, the organs harvested for use, the use patterns (food, medicinal and medico-magical) of the species, the mode of utilisation and the domestication level according to the model proposed by Vodouhe *et al.*, (2012) that defines six levels of domestication: Level 0: wildlife species; Level 1: Species just spared in the fields during field works; Level 2: Species spared in the fields but benefits from some care for its growth; Level 3: Species transplanted from the nature to the cultivated fields or home gardens; Level 4: Species well cultivated and reproduced; Level 5: Species cultivated with some selection activities; Level 6: Pests and diseases are knownas well as their means of control.

Phytochemical Analysis

Samples (stems and leaves) of the plant (*Cleome gynandra* and *Cleome viscosa*) were obtained from the germplasm maintained at the Faculty of Science and Technology of Dassa (FAST Dassa) in Benin. They 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.

Qualitative phytochemical screening 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 and ammonia), Combined anthracene derivatives (test with chloroform and ammonia).

Brine Shrimp Lethality Assay

The cytotoxic activity of the extracts of the two species was evaluated using Brine shrimp lethality bioassay. Brine shrimp (*Artemiasalina* Leach) also known as sea monkey are marine invertebrates of about 1mm in size. The test is basedon 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 Cleome on the body. A solution was prepared by moderate heating for 20minutes, the mixture of 1gof powdered leaves of *Cleome gynandra* or *Cleome viscose* in 20ml of distilled water following Dougnon *et al.,* (2013). The concentration of 50mg/ml was obtained and arrange often successive dilutions (49µg/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 seawater from decoction. Eggs of *A.salina* were grown in an Erlenmeyer containing seawater 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 *Cleome gynandra* and *Cleome viscosa*. These solutions and the controls containing no extract of the Vegetable Cleome were left stirring and read after 24 hoursof incubation. The total death and percentage mortality (death) at each dose level and control were determined. To assess the degree of toxicity of the different species, the LC50and toxicity corresponding table (Table 2) was used following Agbaire *et al.,* (2013) and Dougnon *et al.,* (2013).

Data Analysis

Data collected were analyzed by both descriptive statistic and multivariate analysis and the results are presented in the form of table and figure. The index of Cultural importance (CIs) of each species was calculated by the formula of Tardio and Pardo-de-Santayana (2008) indicating the most important uses of the species. SAS V 9.2 software was used to perform PCA (Principal component Analysis) in order to assess the relationship between the different uses and the ethnic groups surveyed.

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

Ethnobotanical Investigation

For each species considered, the index of Cultural Importance (CI) was calculated (Table 3) to appreciate the relative importance of each species for the local populations. The results obtained (Table.3) indicate that the cultural importance of *C. gynandra*is greater than the one of *C. viscosa*. *C. gynandra* has more food importance (CI_{UA} = 0.7) while *C. viscose* shows greater medicinal importance (CI_{UM} = 0.45). Indeed, 70% of the surveyed population use *C. gynandra* for food against

38% who use it for traditional medicine and only 6% for medico-magic matter. In contrast, 45% of this same population use *C. viscosa* for traditional medicine, 2% use it for food and 4% use it for medico-magic.

The vernacular names of each species vary according to the ethnic groups surveyed (Table 5). Across all ethnic groups *C. gynandra* is considered as "female" *Cleome* or domesticated *Cleome* while *C. viscosa* is called "male" or wild Cleome. However, the female and male terminologies are not linked to the sexuality of the species. The study revealed that *C. gynandra* and *C. viscosa* are used to prevent or cure 20 illnesses (Table 5). The leaves are the most organ used for the different treatments.

The medico-magical use of *C. gynandra* and *C. viscosa* is low. Sauce of *C. gynandra* or *C. viscose* eaten regularly would give longevity while their leaves harvested, dried and burned as incense move away evil spirits. *C. viscosa* is also a luck plant. The leaves crushed with perfume and passed on the body would attract happiness.

In terms of habitat, *C. gynandra* and *C. viscose* were found in agglomeration, homes and roadsides as well as in fields and in fallows. In their preferred habitats, their domestication levels vary from one village to another. *C. viscosa* is at level 0 of domestication in all villages except Okebodé and Koutongbé where it was found respectively at domestication levels 3 and 1 (Figure 2). In the villages Issaba, Okebodé, Niaouli and Zohoudji, *C. gynandra* was found at domestication levels 0 and 3 with level 3 predominant at Issaba and Zohoudji. In Ahouango and Koutogbé villages, the species is still in the wild (level 0 and 1). In the market garden of Houéyiho and in the Adromè-Kpovidji and Ahouamè, domestication of *C. gynandra* is advanced (level 4).

Harvest method influences the availability and conservation of leafy vegetable species (Dansi *et al.*, 2008; Adjatin *et al.*, 2012; Vodouhe *et al.*, 2012). *C. Gynandra* and *C. viscosa* were harvested by cutting leaves and rarely by uprooting. They are available in abundance during the rainy season. In the dry season, *C. viscose* becomes scarce but *C. gynandra* remains available in home gardens.

According to the results obtained, four (04) types of habitat are known for *C. gynandra* and *C. viscosa*. Similar results were reported by Akoègninou *et al.*, (2006), Codjia et al (2009), Soro *et al.*, (2012) and Bâ (2013). The low distribution of *C. gynandra* in the fields and in the fallows in southern Benin can be explained by the demographic growth but also by urbanization. Population growth causes devastating cultural practices (Adegbola *et al.*, 2002; Goussanou and al. 2011) while increasing urbanization leads to loss of habitats and plant resources. This is confirmed by the reports of Kateb (2004) and Djègo *et al.*, (2011) according to which urbanization is the main reason of the plant genetic resources erosion raised by the traditional healers and farmers. Our results are also consistent with those obtained by Sinsin *et al.*, (2009) indicating that plant resources are subject to high human pressures through agriculture, transhumance, pruning, bush fires, collection of NTFPs and construction of houses.

In southern Benin, *C. gynandra* has a food and cultural importance. This confirms the work of Dansi *et al.*, (2008) which showed that *C. gynandra* is consumed as a leafy vegetable in Benin and help understanding its advanced domestication level. *C. gynandra* is already transplanted from the wild status to fields and home gardens. In the study area farmers reported that the consumption of *C. gynandra* in sauce is a guarantee of a good health and longevity and the plant is therefore seen as a nutraceuticals leafy vegetable. The longevity aspect rhymes with the proverb "*C. gynandra* never dies without having white hair." This adage confirms the nickname "the white-haired man" given to this plant by Senegalese as reported by Bâ (2013).

Unlike *C gynandra*, *C. viscosa* has mostly medicinal importance. This can be explained by its potential to cure diseases such as malaria, jaundice whose consequences are feared by the populations. Akoegninou *et al.*, (2006) and Bâ (2013) reported that *C. viscosa* is indicated against eye disorders, chronic constipation, hypo / hypertension and neuralgia.

Relationship between ethnic groups and uses of *C. gynandra* and *C. viscose*

To examine the relationship between ethnic groups and uses of the species, a Principal Component Analysis (PCA) was performed by considering the ethnic groups as individuals and the three types of use (food, medicinal use, medico-magic use) as variables. In *C. gynandra* and *C. viscose* species, analyses revealed that the first two axes are highly significant and account for 78.02% and 72.22% of the available information respectively.

With *C. gynandra* the correlations between the main components and these variables (Table 6) show that UA, UM and UMM are better represented on axis 1 while UA is the best shown in the axis 2. On axis 1 of the PCA, the Fon, Sahouè, Cotafon, Holly and Aizoethnic groups located at the positive side of the axis are clearly opposed to Goun, Nago, Mina and Adja ethnic groups located at the negative side of the axis (Figure 4). The first ethnic group uses more *C. gynandra* for medicine and medico-magic matters than Goun, Nago, Mina and Adja ethnic groups which use *C. gynandra* only for food. Considering the axis 2, the ethnic groups Mina, Adja, Holly, Cotafon and Sahouè, located at the positive side of the axis and which use more *C. gynandra* for food are clearly opposed to Goun, Nago, and Fon that use *C. gynandra* for medicine and medico-magic problems (Figure. 4). In summary, Fon ethnic group uses *C. gynandra* for its medicinal and medico-magical importance whereas Adjaand Mina ethnic groups use it for its food importance. The Goun, Nago, Sahouè, Cotafon, Holli and Aizo ethnic groups use *C. gynandra* for its food, medicinal and medico-magical importance.

With *C. viscosa* the first two axes are highly significant and account for 72.22% of the information related to 3 the variables (UA, UM, UMM) considered. Correlations between these variables and principal components revealed that the AU and UMM variables are better represented on axis 1. UA is positively correlated (r = 0.66) to that axis while UMM is negatively correlated (r = -0.71). UM is best shown on the axis 2 and is positively correlated (r = 0.83) to this axis (Table 7). On the axis 1, the Cotafon, Sahouè, Goun and Adja ethnic groups located at the positive side of the axis are clearly opposed to Nago, Holly, Fon and Mina at the negative side of the axis (Figure 5). This therefore results that Cotafon, Sahouè, Goun and Adjause more *C. viscose* for food and medicine while Nago, Holly, Fon and Minause *C. viscose* for medico-magic problems. On the axis 2, the ethnic Sahouè, Goun, Adja and Nago at the positive side of the axis are clearly opposed to Cotafon, Fon and Holly located on the negative side of the axis (Figure 5).

Therefore, the Sahouè, Goun, Adja and Nago ethnic groups use more *C. viscosa* in traditional therapy (UM and UMM)while Cotafon, Holly and Fon use *C. viscosa* for food. Aizo and Minaare on the two axes. They moderately use *C.viscosa* for food, traditional medicine and medico-magic problems.

C. viscosa is more used by Goun, Sahouè and Adja ethnic groups to cure several diseases like fever, chronic malaria and especially to facilitate the first step to the newborn. The low use of *C. viscosa* by Aizo and Mina is explained by their low knowledge of the species. Similar results were obtained by Koura *et al.*, (2011) who showed that Lokpa, Waama and Bariba ethnic groups have a great knowledge of *Parkiabiglobosa* and use it more for food and medicinal unlike other ethnic groups in northern Benin who have a restricted knowledge for this plant.

The study revealed that the ethnic groups Mina, Adja and Holly in one hand and Goun and Nago in the other hand, are particularly involved in the valorization process of *C. gynandra* and *C. viscosa* respectively through domestication practices.

Phytochemical Screening of *C. gynandra* and *C. viscosa*

Phytochemical analysis revealed that *C. gynandra* and *C. viscosa* have a similar composition (Table 7). Indeed, both species contain tannins, flavonoids, anthocyanins, leuco anthocyanin, steroids, mucilage, reducing compounds, anthracene combined with the C-heteroside derivatives and the quinone. In the two species, saponins, triterpenoids, cardenolides, cyanogenic, coumarins, free anthracene and anthracene combined with O-heteroside are absent. *C. gynandra* contains alkaloids while this is absent with*C. viscosa*.

The phytochemical analysis showed that *C. gynandra* and *C. viscose* do not contain saponoside, triterpenoid, cadenolides, cyanogenic, coumarins, free anthracene and anthracene combined with O-heterooside. Cyanogenic derivatives and anthracene are real poisoning metabolites (Okwu, 2004) and their absence in the leaves of both species studied, reassures consumers of the risks associated with their use. However, saponinshas expectorant effects in the treatment of respiratory infections(Okwu, 2004). The absence anthracene and cadenolides in *C. gynandra* is in agreement with the report of Ajaiyeoba (2000). The absence of saponins and triterpenoid in *C. viscosa* is contrary to the results of Jane *et al.,* (2012), Siddiqui *et al.,* (2009) and Koche *et al.,* (2010). This variation may be associated to agro-ecological conditions, varieties, extraction methods and solvents used. Similar results were obtained by Adjatin *et al.,* (2013) on the leaves of *C. crepidioides* and *C. rubens* indicating the absence of saponins unlike those of Arawande *et al.,* (2013) and Gbadamosi *et al.,* (2012) who found saponins in the leaves of *C. crepidioides* and *C. rubens*. The author explained the differences by the fact that the study was conducted in Beninwith aqueous solvent, while the other two were conducted in different parts of Nigeria with other solvents.

The determination of chemical compounds in the leaves powder of the two species showed the presence of tannins, flavonoids, anthocyanin, leucoanthocyanin, steroids, mucilage of reducing compounds and quinone derivatives. Hydrolysable tannins present in many plant foods were identified as being responsible in large doses, of the reduction of growth rate and protein digestibility in laboratory animals. The incidence of certain cancers including esophageal cancer is related to the consumption of foods rich in tannins (Shils *et al.,* 2006). However the consumption of *C. gynandra* with the presence of this anti-nutritional compound could be explained by the fact that it is triturated before preparation and the dose is reduced.

Some authors have indicated that the low concentration of a vegetable tannin is associated with positive effects on digestion (Guimarães-Beelen *et al.,* 2006; Emebu and Anyika, 2011). Quinone derivatives have irritant laxatives or drastic effects on the intestines causing contractions of the intestinal walls (Cowan, 1999). Mucilage have laxative properties, anti-inflammatory, anti-diarrhea and decreases the sensation of pain (Lin *et al.,* 2005).The flavonoids (flavones) are particularly active in maintaining good blood flow (Park *et al.,* 2008) and have a high antioxidant or anti- radical, anti-proliferative and anti-carcinogenic potential and can inhibit the growth of prostate tumors (Tomofuji *et al.,* 2009;. Jane *et al.,* 2012). As for anthocyanins, they slow down the aging of cells, including skin cells by improving the elasticity and skin density (Afaq *et al.,* 2005). This corroborates with consideration given to *C. gynandra* by the population as "plant giving life." The strong presence of these compounds in both the leaves of *C. gynandra* and *C. viscosa* bring benefits to the body because they have antioxidant properties. The alkaloids are phytochemicals most effective in therapeutic uses (Ayoola and Adeyeye, 2010; Okwu, 2005).Our results revealed that only the leaves of *C. gynandra* contain alkaloids. These results partly confirmed those of Jane *et al.*, (2012) and Koche *et al.*, (2010) who reported the presence of flavonoids and tannins in the leaves of *C. viscosa* but contrasted those specifying the presence of alkaloids in *C. viscosa*.

Similarly Siddiqui *et al.*, (2009) revealed the presence of alkaloids and tannins but the lack of flavonoids in the leaves of *C. viscosa*. This absence could be explained by high pollution in the area where sample were collected. Similarly, Mamadou (2011) in Malinoted on *Nauclealati folia* a high presence of alkaloids in the leaves and fruits of Kanadjiguila, Lafiabougou and Lassa sites and a very low presence of these molecules on the organs of the same species at Koulouba site. According to the author this low presence of alkaloids in Koulouba site could be explained by the exposure of the area to a high degree of pollution. Indeed, the alkaloids may be very sensitive to the presence of certain exhaust gas as source of pollution (Mamadou, 2011). Alkaloids also have anti-malarial, anti-microbial and analgesic activity, which could justify the prescription of crushed leaves or decoction of *C. gynandra* to cure jaundice and other infection. The presence of reducing compounds was confirmed by the work of Sievanesan *et al.*, (2007) showing that the aqueous extract of *C. gynandra* has a net modulatory effect on glucose metabolism in liver cancer cells.

Many phytochemical studies have shown that leafy vegetables with medicinal properties used for various therapeutic purposes contain important phytochemical compounds (Okwu *et al.*, 2006; Adinortey *et al.*, 2012;Gbadamosi al. 2012; Dougnon *et al.*, 2012; Agbaire *et al.*, 2013; Arawande et al 2013;. Ondo *et al.*, 2013; Adjatin *et al.*, 2013).Because of the pharmacological properties of these various chemical compounds found in these plants, their regular consumption would help prevent or cure various diseases (Adjatin *et al.*, 2013). This is consistent with the perception of the population on the nutraceuticals utilities of these two species (Adjatin *et al.*, 2012).

Table.1 List, Administrative Localization and Ethnic Groups of the Villages Surveyed

N°	Villages	Districts	Department	Ethnic group
1	Adromè-kpovidji	Houéyogbé	Mono	Sahouè
2	Ahouamè	Lokossa	Mono	Cotafon
3	Ahouango	Kpomassè	Atlantique	Aïzo
4	Houéyiho	Cotonou	Littoral	Fon
5	Issaba	Pobè	Plateau	Holly
6	Koutongbé	Porto Novo	Ouémé	Goun
7	Niaouli2	Allada	Atlantique	Aïzo
8	Okébodé	Ketou	Plateau	Nagot
9	Zohoudji	Aplahoué	Couffo	Adja

Figure.1 Map of Southern Benin Showing Surveyed Localities

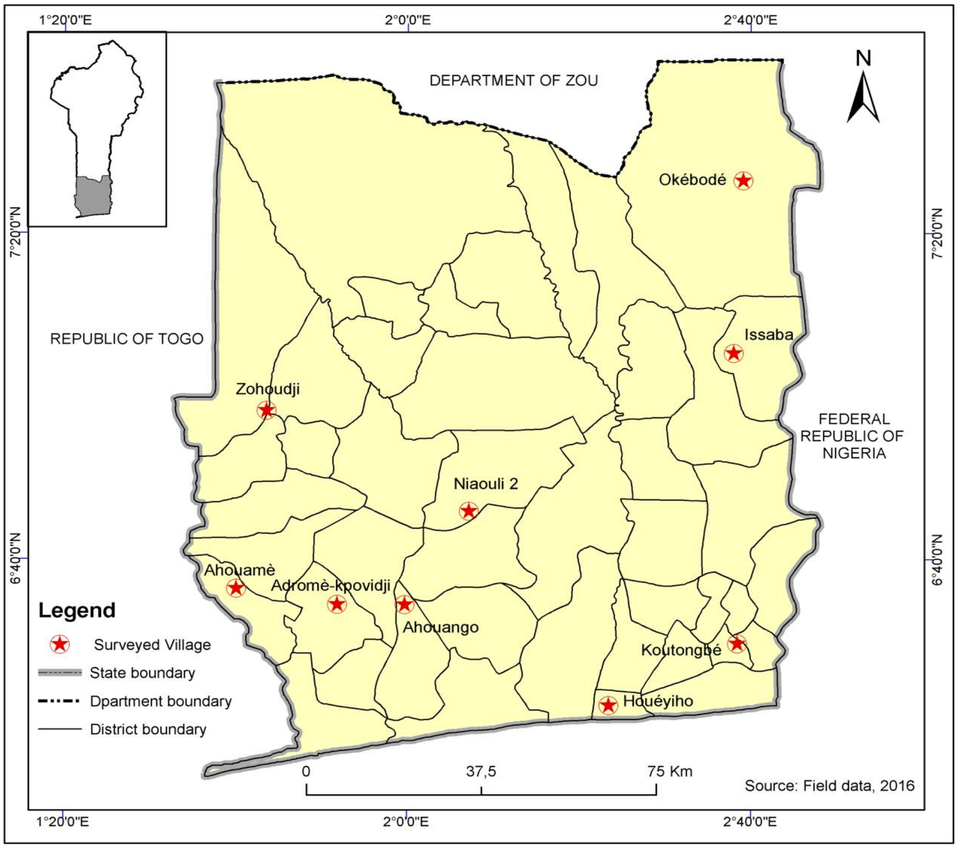


Table.2 Correspondence between Lc50 and Toxicity

LC_{50}	Toxicity
$LC_{50} \geq$ 0.1mg/ml	- (Non-toxic)
0.1mg/ml>$LC_{50} \geq$ 0.050 mg/ml	+ (Lowtoxicity)
0.050mg/ml>$LC_{50} \geq$0.01mg/ml	++ (Moderate toxicity)
LC_{50}<0.01mg/ml	+ + + (Hightoxicity)

Table.3 Cultural Importance Index (Ci) of the Two Cleome Species in Southern Benin

Species	Number	ΣUR_{UA}	CI_{UA}	ΣUR_{UM}	CI_{UM}	ΣUR_{UMM}	CI_{UMM}	CI
C.gynandra	260	181	0,70	98	0,38	16	0,06	1,14
C.viscosa	260	4	0,02	118	0,45	10	0,04	0,51

Legend: UA = food use, UMM = medicinal use, UMM = medico-magic use; CI= cultural index

Table.4 Vernacular Names of *C. gynandra* and *C.viscosa* According to the Ethnic Groups

Ethnic groups	vernacular names	
	Cleome gynandra	***Cleome viscosa***
Adja	Sabo assi	Sabo assou ; Bomèsabo
Sahouè	Akayaassi	Akaya-assou, klouto kaya
Mina	Samboési	Samboésou
Goun	Akayaassi ; Gbodokaya	Kondo
Fon	Akayaassi	Hêdoulinfiman, Akayaassou
Cotafon	Akayaassi	Akayaassou ou Gbétokaya
Nago	Ewééti ; Efookpoya	Dantcha ; Ewouééyou ; Eyofa
Holly	Efooko	Orokoton
Aïzo	Akayaassi	Akayaassou

Table.5 Medicinal importance of *C.gynandra* and *C.viscosa* in the Study Area

N°	Listed Diseases	Species		Organs used
		C. gynandra	***C. viscosa***	
1	Abscesses and wound	+	+	Leaf
2	Anemia	+	-	Leaves
3	Bleeding after childbirth	-	+	Leaf, stem, fruit
4	Candida	-	+	Leaf
5	Cold	-	+	Leaf
6	Difficult delivery	-	+	Leaf, stem, fruit
7	dizziness	+	-	Leaf
8	Earaches	+	+	Leaf
9	Fever	-	+	Leaf, stem, fruit
10	Fragilityof babies	-	+	Leaf, stem, fruit
11	Haemorrhoid	-	+	Leaf, stem, fruit
12	Headache	+	+	Leaf
13	Hernia	+	-	Leaf
14	Infection	+	+	Root
15	Jaundice	+	-	Leaf
16	Malaria	+	+	Leaf
17	Sexual weakness	+	-	Leaf
18	Tooth sores	-	+	Root
19	Ulcer	-	-	Leaf, stem, fruit
20	Vomiting	-	+	Leaf

Table.6 Correlation between Variables and the Main Factors

Variables	*C. gynandra*		*C. viscosa*	
	Factor 1	**Factor 2**	**Factor 1**	**Factor 2**
Food use (UA)	0.65	0.74	0.66	-0.45
Medicinal use (UM)	0.73	-0.40	0.52	0.83
Medico-magical use (UMM)	0.75	-0.24	-0.71	0.18

Table.7 Organic Compounds Identified in the Two Species Studied

Compounds	***C. gynandra***	***C. viscosa***
Alkaloïd	+ +	-
Catechin tannins	+	+ +
Gallic tannins	+ +	+
Flavonoids	+	+
Anthocyanin	+ +	+ +
Leuco-anthocyanins	+	+ +
Saponins	-	-
Triterpenoids	-	-
Steroids	+	+
Cardenolides	-	-
Cyanogenic derivatives	-	-
Mucilages	+ +	+ +
Coumarins	-	-
Reducing compounds	+ +	+ +
Free anthracene	-	-
Anthracene combined to O-heterosides	-	-
Anthracene combined to C-heterosides	+ +	+
Quinone derivatives	+	+ +

NB : + : present ; ++ : abondant ; - : absent

Figure.2 Level of Domestication of *C.viscosa* in the Villages Surveyed

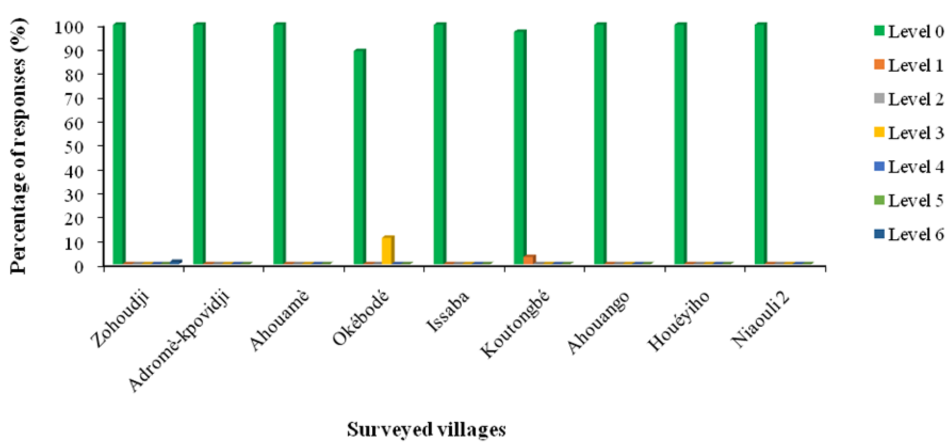


Figure.3 Level of Domestication of *C.gynandra* in the Villages Surveyed

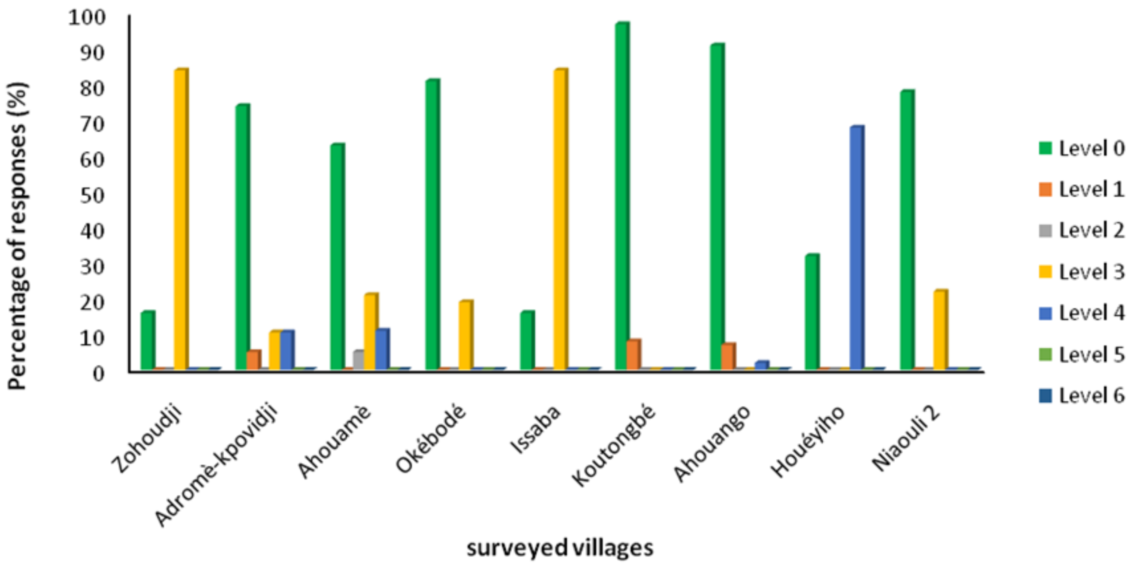


Figure.4 Representation of Ethnic Groups in the Plan Defined by the First Two Major Components with *C. gynandra*

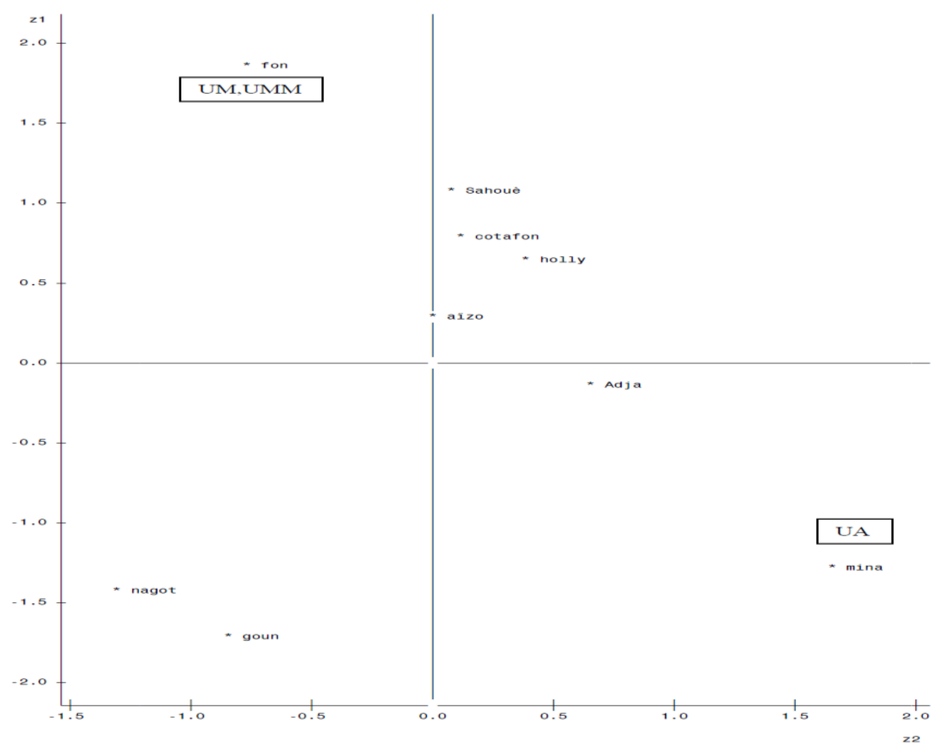


Figure.5 Representation of the Ethnic Groups in the Plan Defined by the First Two Major Components with *C. viscosa*

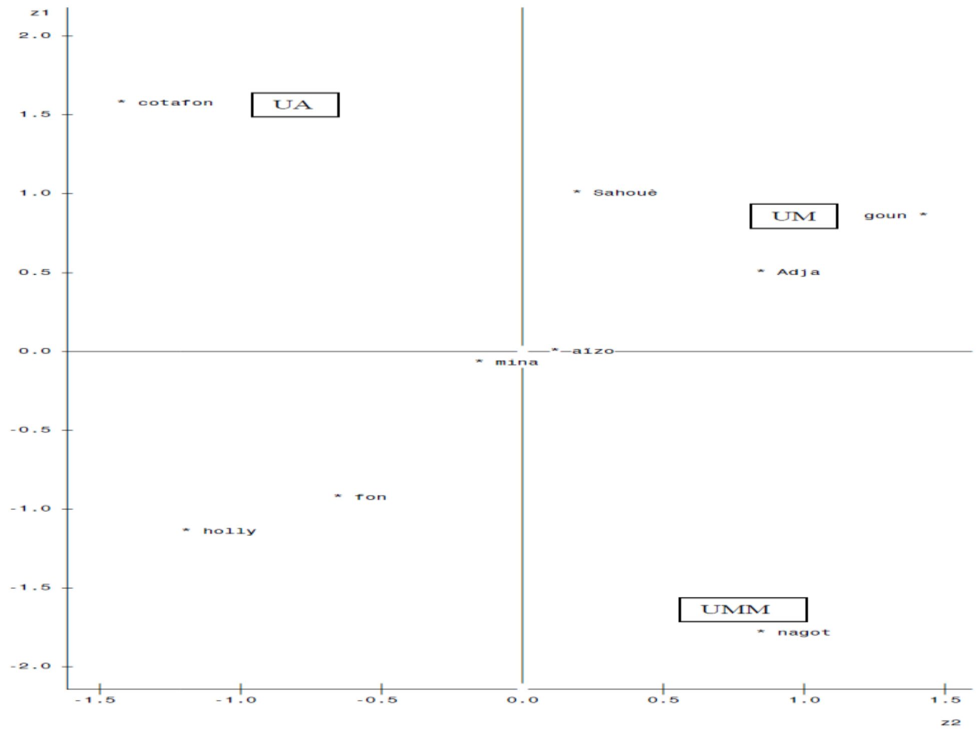


Figure.6 Toxicity Curve of Leaves Extract of *C.gynandra* on Shrimp Larvaes

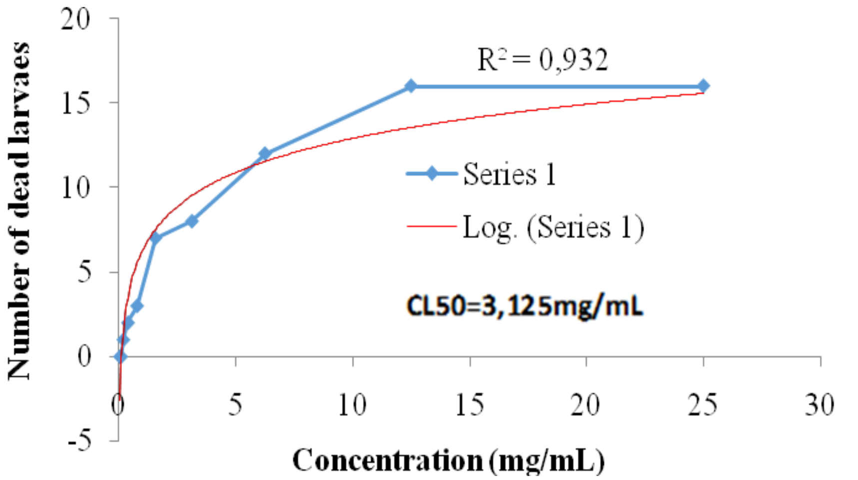
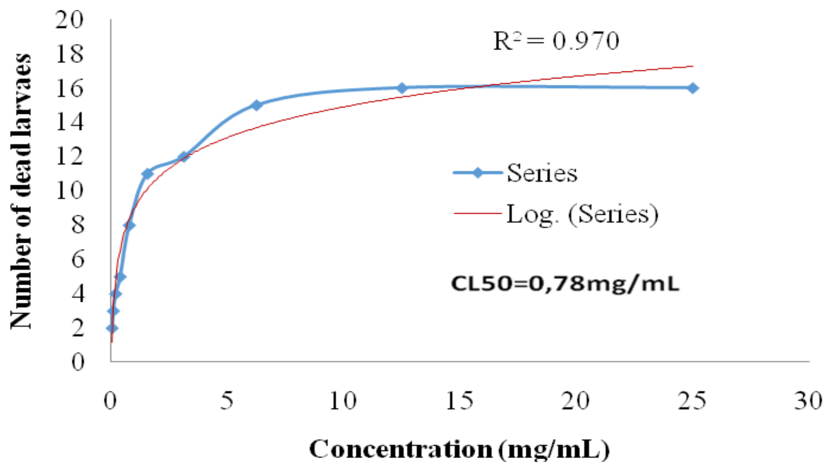


Figure.7 Toxicity Curve of Leaves Extract of C.viscosa on Shrimp Larvaes



Toxicity of *C. gynandra* and *C. viscosa*

The analysis of the sensitivity curves of the figures 6 and 7 shows that the shrimp larvae are susceptible to extracts tested according to the dose-response relationship. Indeed progressively as the aqueous concentration of the leavesof each species increases, the number of dead larvae increases. But this increase is faster with *C.viscosa* aqueous extract than the one of *C. gynandra*. In addition the concentration of 50% (LC50) survival of shrimp larvae is 3.125 mg / ml for *C. gynandra* and 0.78 mg / ml for *C.viscosa*.

Shrimp larvae (*Artemiasalina*) were sensitive to aqueous extracts of the leaves of *C. gynandra* and *C. viscosa* indicating that the samples are biologically active with a limit dose of mortality equal to 0.78 mg / ml for *C. viscosa* and 3,125mg / mL for *C. gynandra*. The sensitivity curves showed that the larval mortality increases with the concentration. This sensitivity thus follows a dose-response relationship. Furthermore, the values of the lethal dose (LC50 = 0.78 mg / ml for the leaves of *C. viscosa* and 3,125mg / ml for leaves of *C. gynandra*) are all greater than 0.1 mg / ml which is the limit dose of toxicity according to Mousseux (1995). These results reveal that none of the two species is toxic. This is similar to the work of Karimulla *et al.*, (2013) which revealed the absence of toxicity sign of *C. gynandra* on rats at a dose of 2000mg / kg. Similarly, Elufioye *et al.*, (2015) showed the non-toxicity of *C. viscosa* on mice because no animal died during 24 hours after oral administration of the extract and the LC50 was greater than 5000mg / kg. These signs are confirmed by Deora *et al.*, (2010) who reported that that the lethal dose is greater than 5000mg / kg. This corroborates with our survey results revealing the food use of *C. viscosa* without a major risk of poisoning.

In conclusion, this ethnobotanical, phytochemical assessment and the risk of toxicity study on *C. gynandra* and *C. viscose* is a starting point of a vast program of domestication of the two species. *C. gynandra* is more used for food while *C. viscosa* is more used for traditional medicine. The qualitative phytochemical screening of *C. gynandra* and *C. viscosa* revealed the presence of many chemical compounds that justified their medicinal values. The toxicity study revealed that none of the species is toxic and can therefore be consumed without risk. *C. gynandra* and *C. viscose* are two nutraceuticals leafy vegetables that must be promoted through domestication and development of food based products.

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