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Phytochemical screening, antioxidant and cytotoxic activity of different morphotypes of *Corchorus olitorius* L. leaves in the central region of Benin Republic (West Africa)

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Corchorus olitorius L. which belongs to Tiliaceae family is a cultivated herbaceous plant highly consumed as traditional leafy vegetables in Benin. Extracts from samples of different morphotypes leaves of this species were analysed for their phytochemical compositions, antioxidant and cytotoxicity activities. On each sample extract, qualitative phytochemical tests were used to detect the presence of bioactive molecules and evaluate their cytotoxicity using brine shrimp lethality bioassay. Antioxidant activity was assessed using the 2,2-diphenyl-1-picryl-hydrazyl assay. Phytochemical screening revealed the presence of several phytochemical compounds in all samples of morphotypes studied but these have not a similar profile. Morphotypes with shiny rounded leaves have more phytochemicals (58% of phytochemicals compounds) than the other samples. Analysis of the potential antioxidant reveals a first category of morphotypes with high potential antioxidant capacity ranged from 27,859 to 32,985 mM EAA/g followed by second class of morphotype which ranged from 17,527 to 23,098 mM EAA/g. Regular consumption of *C. olitorius* such as varieties with rounded and non-shiny leaves, varieties with elongated and shiny leaves, varieties with red stems, varieties with elongated and non-shiny leaves with lateral lobes, would be useful for the treatment of various diseases induced by free radicals. The LC₅₀ of morphotypes *C. olitorius* varies between 0.78 and 3.125 mg/ml indicating non toxicity. From this study, results justify the use of some morphotypes of *C. olitorius* in traditional medicine. Hence, these morphotypes are nutraceuticals leafy vegetables that must be promoted through development of food based products.

Key words: Phytochemical analysis, antioxidant activity, cytotoxic activity, *Corchorus olitorius*, morphotypes, Benin.

INTRODUCTION

Human diets are becoming more varied and allow to cover the energy needs of human organism and the

basal metabolism but without taking into account the nutritional and health security aspects (Mulungulungu et al., 2015). Nowadays, the light is focused on foods rich in nutraceutical and functional properties. Nutraceutical combining the words “nutrition and pharmaceutical” is a food or food product that provides health and medicinal benefits including the prevention and disease’s treatment (Pepsi et al., 2012). The nutraceutical is used to accomplish desirable therapeutic outcomes with reduced side effects as compared with other therapeutic agents with great monetary success (Pepsi et al., 2012).

Edible plants have been used as nutraceutical in Benin and contributed to reduce health risks (Djengue et al., 2017a; Koukoui et al., 2015; Adjatin et al., 2013). The consumers’ interest has been the use of foods with more natural antioxidants, dietary fibers, natural colorants, minerals, vitamins and synthetic additives free, etc. Thus, the consumption of fruits, vegetables and other nutraceutical foods is increasing. High consumption of fruits and vegetables is associated to the reduction of cardiovascular disease and some cancers risks (Lazou and Kronida, 2010). Generally, leafy vegetables are prominently used in the diets of people in the world particularly in Africa, Asia and Oceania, where they provide an essential part of nutritional and medicinal purposes (Dansi et al., 2008). *Corchorus olitorius* L. is traditional leafy vegetable belongs to the family of Malvaceae (Whitlock et al., 2003). Recently, Heywood et al. (2007) split the large Malvaceae and classified *Corchorus* within the family Sparrmanniaceae. Genus *Corchorus* consists of annual or short-lived perennial herbs and shrubs with many agriculturally important species. It consists of some forty species of which about thirty are found in Africa (Mbaye et al., 2001). In Benin, it is represented by five species *Corchorus aestuans* L., *Corchorus fascicularis* Lam., *C. olitorius* L., *Corchorus tridens* L. and *Corchorus trilocularis* L. (Akoegninou et al., 2006). *C. olitorius* L. is the most popular in Benin because it is cultivated countrywide as vegetable and its local name varies according to the regions (Dansi et al., 2008; Adjatin et al., 2017). It is recognized for its high intra-specific diversity and a wide distribution (Benor et al., 2011). In fact, the leaves are alternate, oval, lance-shade and toothed, allowed to identify different morphotypes (Adebo et al., 2015; Adjatin et al., 2017). The leaves of *C. olitorius* can be eaten fresh like spinach, cooked or stored as a dried powder for at least six months without any major loss of its nutritional value (Choudhary et al., 2013). In Benin, its leaves are used to make a sticky cooked sauce that accompanies the main courses (Adjatin et al., 2017). It is of high social economic importance especially for the local farmers for which, the livelihoods depend essentially on *C. olitorius* production

and utilization (Komlan et al., 2013). *C. olitorius* is rich in protein, iron, calcium, vitamin (B, C), Beta-caroten and folic acid and constitute good nutritional supplements (Choudhary et al., 2013). Its leaves, roots and fruits are used in traditional medicine to treat various diseases such as gonorrhoea, chronic cystitis, pain, fever and tumors (Kumawat et al., 2012). A regularly consumption of *C. olitorius* leaves helps to control blood pressure, cholesterol and lowers the risks of asthma, cancer, diabetes and heart disease (Handoussa et al., 2013). Also, decoctions of these leaves would treat many diseases such as typhoid fever, anemia, malaria and ulcer (Adjatin et al., 2017). Consequently, this vegetable is considered as an important medicinal taxon because of its several medicinal properties. Based on the aforementioned uses of this plant to cure many diseases, a qualitative phytochemical screening of leaves’ extracts from different morphotypes of *C. olitorius* was carried out in order to identify phytochemical elements which confer to species, its several medicinal properties and also to investigate their antioxidant and cytotoxic activities.

MATERIALS AND METHODS

Collection and extraction of plant material

A total of twelve accessions from seven morphotypes *C. olitorius* were collected from six districts of Central Benin (Table 1). Morphotypes included in the study were identified from study conducted by Adjatin et al. (2017) on phenotypic diversity of *C. olitorius* in the same region.

Fresh leaves samples of each accession were washed thoroughly under running tap water followed by sterile distilled water. Each sample was then cut into smaller pieces and dried under shade for two weeks. The dried leaves were ground with electric blending machine and the obtained powdery samples were sieved using a sieve of 0.2 mm (mesh size) and stored in air tight sterile containers for the analysis.

Phytochemical analysis

Qualitative phytochemical screening of *C. olitorius* was carried out on the powdery samples, after extraction with aqueous solvent (distilled water), using the standardly employed precipitation and coloration reactions as described by Houghton and Raman (1998) and used by Djengue et al. (2017b). Major secondary metabolites essayed and the used methods were as follows: 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 anhydride 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

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Table 1. List of morphotypes, samples and collection areas.

Accession	Morphotypes/Distinctive traits	Local name	Collecting sites	Districts
Cor1	Rounded leaves, non-shiny	Ayoyo okpolo	Ouissi	Dassa
Cor2	Elongated leaves, elliptical, shiny	Ayoyo edjo	Gamba	Dassa
Cor3	Elongated leaves, non-shiny, red stem	Yoyo olèssèkpikpa	Gamba	Dassa
Cor4	Serrated leaves, rounded, shiny	Aladjèlè	Aklamkpa	Glazoué
Cor5	Rounded leaves, non-shiny	Ninnouwi	Doissa	Savalou
Cor6	Elongated leaves, lobed lateral, non-shiny	Afimblipotomé	Doissa	Savalou
Cor7	Elongated leaves, non-shiny	Ninnouwi kpahè	Bobè	Bantè
Cor8	Elongated leaves, non-shiny	Ninnouwi	Bobè	Bantè
Cor9	Rounded leaves, shiny	Yoyo doundoun	Oké Owo	Save
Cor10	Elongated leaves, shiny	Yoyo okpolo	Oké owo	Save
Cor11	Serrated leaves, elongated, shiny	Yoyo oyimbo	Atchakpa	Save
Cor12	Rounded leaves, shiny	Krimlin	Zogbagaou	Ouesse

compounds (test with fehling solution), Coumarins (test with ether and ammonia), Free anthracene derivatives (test with chloroform and ammonia), and Combined anthracene derivatives (test with chloroform and ammonia).

Total antioxidant capacity (TAC) assay

Antioxidant activity was related to the capacity of plant extract to trap the free radical molecules. The technique applied for this determination used the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Velazquez et al. (2003). A volume of 1.5 mL of DPPH solution (4mg of DPPH dissolved in 10 mL of methanol) was mixed with 0.75 mL of leaves extract of each accession at different concentration (0.5 - 3.5 mg/mL). After 15 min of incubation in the dark, at the room temperature, absorbance of the mixture was read at the wavelength of 517 nm with a spectrophotometer using ascorbic acid as blank and standard. The total antioxidant activity was expressed as Equivalent of Ascorbic acid per gram of aqueous extract (mmol EqAA/g).

Brine shrimp lethality assay

The cytotoxic activity of the extracts from the twelve accessions was assessed using Brine shrimp lethality bioassay. Brine shrimp (*Artemia salina* Leach) also known as sea monkey are marine invertebrates of about 1 mm in size. The test is based on the survival of shrimp larvae in sea water in the presence of the test solution. Its interest is linked in the understanding of the possible side effects that would result in consumption of *C. olitorius* leaves on the organism. A solution was prepared by moderate heating of the mixture of 1 g of powdered leaves in 20 ml of distilled water according to Agbankpé et al. (2015). The concentration of 50 mg/ml was obtained and from this decoction, a range of ten successive dilutions (49, 98, 195, 391, 781, 1582, 3125, 6250, 12500, and 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 incubated during 48 h. Meanwhile, the eggs were hatched to give birth to young larvae (nauplii). When using a pipette, a colony of 16 living larvae was placed in contact with the series of solutions of graded concentrations of *C. olitorius* decoction. These solutions and the control containing no extract of *C. olitorius* were incubated and read after 24 h. The total death and percentage of mortality at each dose level and control were

determined.

Statistical analysis

Descriptive statistic of data from screening phytochemical was presented in the form of figures and tables. For the toxicity assessment, counting the number of survival larvae in each solution was allowed to evaluate the toxicity of the solution. The percentage of death was then calculated according to the following formula:

$$\% \text{ Death} = [(test - control) / control] \times 100.$$

For each sample, the lethal concentration that causes 50% death (LC_{50}) was calculated at 95% confidence interval by linear regression analysis and also by using the probit analysis method following Djengue et al. (2017b). A regression line equation was derived for each extract with the mortality data obtained and, it was then used to calculate the LC_{50} . The detailed mathematical steps used to derive the regression line equation are reported in the literature (Vincent, 2012). The correlation table established by Mousseux (1995) was used to assess the degree of toxicity of the different samples from LC_{50} values (Table 2).

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of the leaves extracts of the twelve samples of *C. olitorius* revealed the presence of several phytochemical compounds (Table 3). Flavonoids, leuco-anthocyanes, cathetic and gallic tannins, mucilage, quinone derivatives and reducing compound were present in all the studied extracts whereas saponins, triterpenoids, cardenolid derivatives, cyanogenic derivatives, combined anthracene O-heterosides and glycosides cardiotoxic were not detected in any of the analysed extracts (Table 3). In addition, the phytochemical compounds such as alkaloids, steroids, coumarins, free anthracene derivative and anthracene C-heterosides were present in some morphotypes and

Table 2. Correspondence between CL50 and toxicity.

CL50	Toxicity
CL ₅₀ ≥ 0.1 mg/ml	-
0.1 mg/ml > CL ₅₀ ≥ 0.050 mg/ml	+
0.050 mg/ml > CL ₅₀ ≥ 0.01 mg/ml	++
CL ₅₀ < 0.01 mg/ml	+++

- = No toxic; += low toxicity; ++ = moderate toxicity; +++ = high toxicity.

Table 3. Qualitative assessment of phytochemical compounds in the extracts of *C. olitorius*.

Phytochemical compound	Cor1	Cor2	Cor3	Cor4	Cor5	Cor6	Cor7	Cor8	Cor9	Cor10	Cor11	Cor12
Cathetic tanins	+	+	+	+	+	+	+	+	+	+	+	+
Poly-phenolic compounds												
Gallic tannins	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Anthocyanins	-	-	-	-	-	-	-	-	-	-	-	-
Leuco-anthocyanes	+	+	+	+	+	+	+	+	+	+	+	+
Mucilage	+	+	+	+	+	+	+	+	+	+	+	+
Reducing compound	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	-	+	-	-	-	+	+	+	+	+	-	+
Saponins	-	-	-	-	-	-	-	-	-	-	-	-
Triterpenoids	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	+	+	-	+	+	-	-	-	-	+	-	+
Quinone derivatives	+	+	+	+	+	+	+	+	+	+	+	+
Cardenolid derivatives	-	-	-	-	-	-	-	-	-	-	-	-
Cyanogenic derivatives	-	-	-	-	-	-	-	-	-	-	-	-
Coumarins	+	+	-	-	+	+	+	+	-	-	+	-
Glycosides cardiotoxic	-	-	-	-	-	-	-	-	-	-	-	-
Anthracene derivatives												
Free anthracene derivatives	+	+	+	+	+	-	-	+	-	+	-	-
Combined anthracene O-heterosides	-	-	-	-	-	-	-	-	-	-	-	-
Combined anthracene C-heterosides	+	-	+	+	+	+	+	-	+	+	-	+

absents in others (Figure 1). The most of phytochemical elements found in all morphotypes are phenolic compounds with high antioxidant

properties (Kumar et al., 2018).

Moreover, it could be observed that the morphotypes Cor1, Cor2, Cor5 and Cor10 have

more phytochemicals (58%) than the others (Figure 2). Cor1 and Cor5 varieties have non-shiny rounded leaves while Cor2 and Cor10

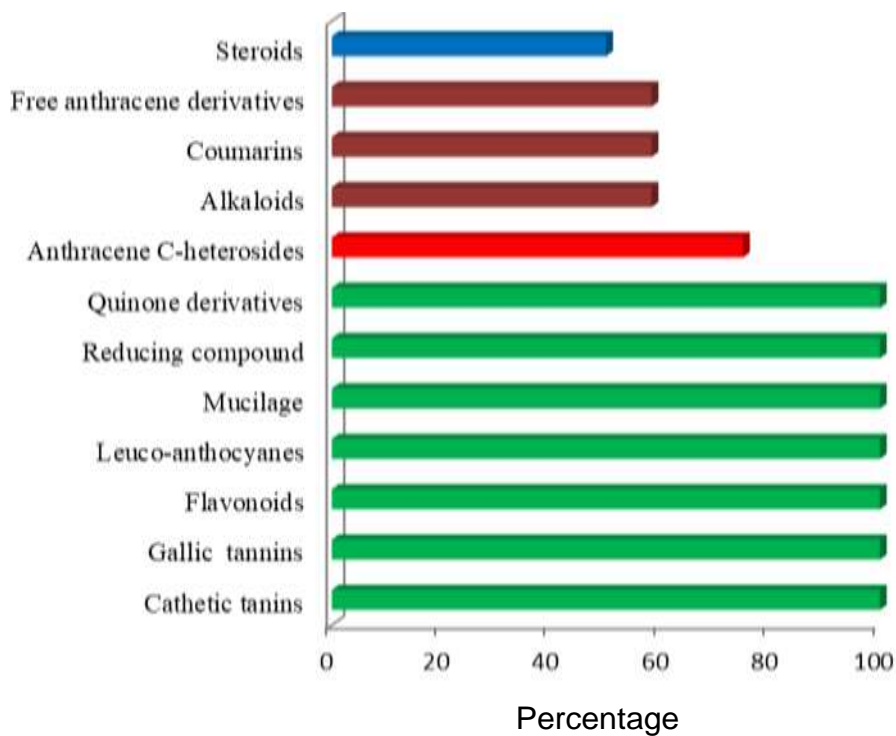


Figure 1. Importance of phytochemicals through the studied *C. olitorius* morphotypes.

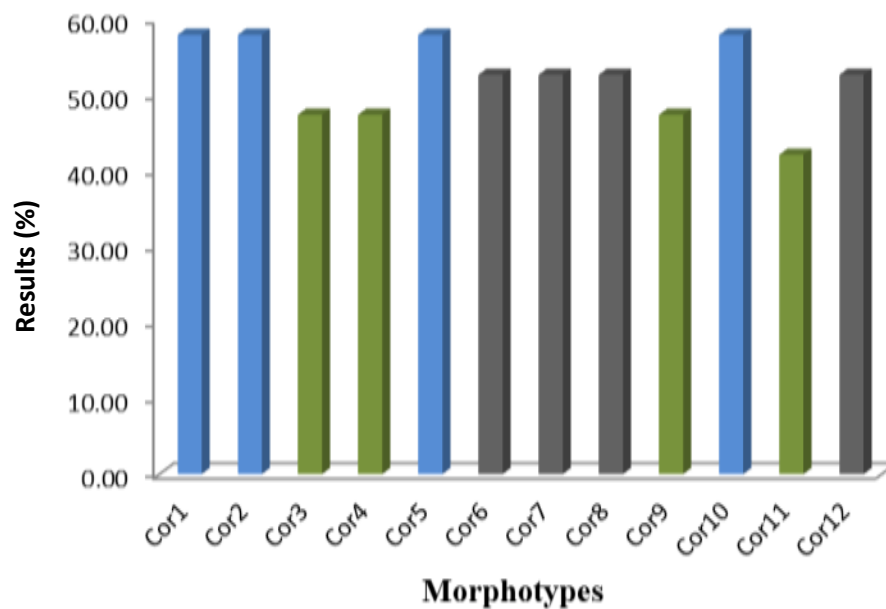


Figure 2. Frequency of phytochemicals compounds identified in morphotypes of *C. olitorius*.

varieties have elongated and shiny leaves. The morphotypes with least phytochemicals were Cor6, Cor7, Cor8 and Cor12 with 53% of phytochemicals elements followed by samples Cor3 and Cor9 and then samples

Cor4 and Cor11 that contain, respectively 47 and 42% of phytochemicals compounds. The Cor6 variety has elongated and non-shiny leaves with lateral lobes while Cor7 and Cor8 varieties have elongated and non-shiny

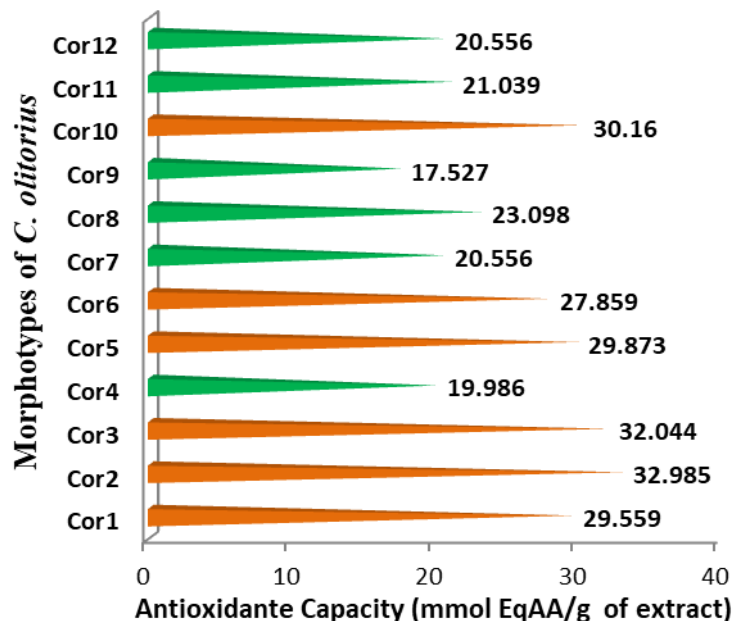


Figure 3. Total antioxidant capacity of extracts of *C. olitorius* morphotypes.

leaves. Cor9 and Cor12 are varieties with rounded and shiny leaves; Cor4 and Cor11 have serrated leaves while Cor3 variety has red stems. Most of the studied samples belong to the same morphotype, same percentages of phytochemical compounds were observed for each morphotype except Cor9 and Cor12 which are varieties with rounded and shiny leaves but with different percentages of phytochemicals elements. Therefore, a variation of qualitative phytochemical composition was observed in different samples in one hand and in the different morphotypes of *C. olitorius* on the other hand. Both the percentage and the qualitative composition of phytochemical vary from one morphotype to another. These results are similar to those obtained by Bouabid et al. (2016) who notified a variability of phytochemical composition between two grapefruit varieties (*Citrus paradisi*). Considering the characteristics of the different morphotypes collected in the study area (Adjatin et al., 2017), the variations observed on the leaves are related neither to the shape and appearance of the leaves nor to the stems colour. These variations could be explained by the physiological stage of the plant during leaf harvesting. In fact, the bioactive constituents or secondary metabolites synthesized by the plants vary according to their different stages of growth (Okunlola et al., 2017).

However, some elements are found in the leaves at all stages of species growth and development and could be specific to certain varieties. For example, flavonoids are present in all samples of studies morphotypes. According to Okunlola et al. (2017), the presence of a phytochemical in all the stages of *C. olitorius* growth, showed that this phytochemical is synthesized in the plant at the vegetative, flowering and fruiting stages and

justified then the presence of flavonoids in all samples of these morphotypes. In the current study, saponosides are absent in all studied morphotypes. These results are similar to those of Mohammed (2016). However, the results of Okunlola et al. (2017) showed the presence of saponosides in the leaves of *C. olitorius* during vegetative and flowering stages but indicated their absence at fruiting stage. Therefore, the phytochemical screening of the studies' morphotypes at different development stages of the plant is required to confirm or not the production phytochemicals which are secondary metabolic involved in the plant defence system according to Okunlola et al. (2017).

Total antioxidant capacity (TAC)

Total antioxidant capacity represents both oil soluble and water soluble antioxidants that are capable of scavenging reactive oxygen species (ROS) and protects from chronic diseases such as cancer, diabetics and arthritics. ROS that are produced as a result of cellular metabolism are highly toxic and are involved in the etiology of many chronic diseases due to oxidative damage to lipids, nucleic acids and proteins (Yanishlieva et al., 2006). The results of the antiradical activity of the aqueous extracts of the various *C. olitorius* morphotypes are as shown in Figure 3. Samples exhibited wide range of TAC values, expressed as the number of equivalents of ascorbic acid, from 17,527 mmol EqAA/g for Cor9 which is a variety with cordate and shiny leaves to 32,985 mmol EqAA/g for Cor1 variety with cordate and non-shiny leaves. All studied morphotypes possess an antioxidant potential on

Table 4. LC50 values of the different morphotypes of *C. olitorius*.

Morphotype	Polynomial regressions	R ²	CL ₅₀ (mg/ml)
Cor1	$y = -37.274x^2 + 51.627x - 1.568$	0.999	0.22
Cor2	$y = -0.872x^2 + 7.819x + 0.883$	0.964	1.03
Cor3	$y = -3.542x^2 + 16.099x + 0.048$	0.972	0.56
Cor4	$y = -2.279x^2 + 12.074x + 0.402$	0.982	0.73
Cor5	$y = -3.843x^2 + 17.112x - 0.296$	0.955	0.55
Cor6	$y = -3.151x^2 + 14.968x - 0.256$	0.973	0.64
Cor7	$y = -3.774x^2 + 15.968x - 1.639$	0.983	0.73
Cor8	$y = -7.770x^2 + 23.268x - 1.381$	0.994	0.48
Cor9	$y = -0.734x^2 + 6.9276x + 1.248$	0.979	1.10
Cor10	$y = -1.732x^2 + 11.171x - 2.019$	0.998	1.08
Cor11	$y = -14.238x^2 + 32.699x + 0.541$	0.969	0.30
Cor12	$y = -0.378x^2 + 4.710x + 1.258$	0.968	1.65

DPPH. The good antioxidant property from *C. olitorius* was already reported by several authors (Obob et al., 2009; Handoussa et al., 2013; Youssef et al., 2014). The presence of phytochemicals in extracts of all these morphotypes is in agreement with their antioxidant capacity. Among the phytochemicals, phenolic compounds are reported to be the main contributor of antioxidant activity in plant extracts (Dai and Mumper, 2010). Polyphenolic antioxidants from dietary sources are frequently a topic of interest due to widespread scientific agreement that they may help lower the incidence of certain cancers, cardiovascular and neurodegenerative diseases, and DNA damage and even may have antiaging properties (Obrenovich et al., 2010). Consumption of polyphenols has been linked to lowered risks of many chronic diseases including cancer, cardiovascular diseases, chronic inflammation and many degeneration diseases (Scalbert et al., 2005; Tsao, 2010).

Moreover, analysis of Figure 3 indicated that all samples have not the same antioxidant capacity and showed two categories of morphotypes. In the first groups, the morphotypes with a better radical scavenging activity whose antioxidant capacities are between 27.859 and 32.985 mM EAA/g of extract. These are Cor1, Cor2, Cor3, Cor5, Cor6 and Cor10 varieties. The second category constituted Cor4, Cor7, Cor8, Cor9 Cor11 and Cor12 varieties, is the one of morphotypes with low antiradical activity whose antioxidant capacities varies from 17.527 to 23.098 mM EAA/g. Among phytochemicals, only free anthracene derivatives are detected in the morphotypes of *C. olitorius* with high potential antioxidant. Then free anthracene derivatives found only in these morphotypes would act with another phytochemicals and might certainly explain their strongest radical scavenging activity of DPPH. In fact, levels of individual antioxidants in food do not necessarily reflect their total antioxidant capacity, which could also depend on synergic and redox interactions between the

different antioxidant molecules (Hodzic et al., 2009). According Yanishlieva et al. (2006), free radicals and reactive oxygen species are involved in a variety of pathological events such as aging, inflammation, cancer, atherosclerosis, and diabetes. Then, regular consumption of *C. olitorius*, especially varieties having rounded and non-shiny leaves, elongated and shiny leaves, red stems or elongated and non-shiny leaves with lobed lateral, would be useful for the treatment of various diseases induced by free radicals as others traditional leafy vegetables such as *Launaea taraxacifolia* (Koukoui et al., 2015), *Hibiscus sabdariffa*, *Solanum scabrum* and *Basella alba* (Konan et al., 2016).

Larval toxicity of the morphotypes *C. olitorius* extracts

All extracts morphotypes of *C. olitorius* tested showed lethality on the shrimp's larvae indicating that the samples are biologically active. The LC values obtained for these extracts are shown in Table 4. These LC50 values ranged between 0.22 mg/ml for Cor1 and 1.65 mg/ml for Cor12. Some extract of samples such as Cor1 (0.22 mg/ml), Cor3 (0.56 mg/ml), Cor4 (0.73 mg/ml), Cor5 (0.55 mg/ml), Cor6 (0.64 mg/ml), Cor7 (0.73 mg/ml), Cor8 (0.48 mg/ml) and Cor11 (0.30 mg/ml) showed the strongest larval lethality than samples Cor2 (1.03 mg/ml), Cor9 (1.10 mg/ml), Cor10 (1.08 mg/ml), and Cor12 (1.65 mg/ml) which have poor larval lethality. Therefore, the morphotypes with rounded and non-shiny leaves, elongated and non-shiny leaves, serrated leaves, elongated leaves with lateral lobes and red stems are more harmless for brine shrimp larvae than morphotypes with rounded and shiny leaves and morphotypes with elongated and shiny leaves.

Besides, these values were greater than 0.1 mg/ml, which is the limit of toxicity (Mousseux, 1995). Then, in this study, that none of the morphotypes of *C. olitorius*

investigated was harmless to shrimp's larvae. Taking into account the established correlation between the toxicity of shrimp larvae and that of human cells, all the morphotypes of *C. olitorius* involved in this study can be used daily both in food and in traditional medicine without risk of toxicity. *C. olitorius* can therefore be considered as a traditional nutraceutical leafy vegetable such as *Lippia multiflora* (Djengue et al., 2017a), *Launea taraxacifolia* (Koukoui et al., 2015), *Crassocephalum crepidioides* and *Crassocephalum rubens* (Adjatin et al., 2013).

Conclusion

The current study offers supporting evidence for medicinal effective use of *C. olitorius*. It revealed the presence of many phytochemical compounds in the studied morphotypes extracts. The number and the nature of these phytochemicals vary from a sample to another. All the morphotypes contained phenolic compounds whose biological activities are well established and possessed each one a total antioxidant capacity on DPPH. The potential antioxidant of the extract of morphotypes of *C. olitorius* is in relation with phenolic compounds that is responsible for their activity. There is a relationship between the antioxidant capacities and phenolic compounds implied that are the major contributors of antioxidant capacities of these plants. Plants naturally possess various therapeutic agents which properties depend on the nature and the variability of the plant. In sum, the morphotype with rounded and non-shiny leaves and the one with elongated and shiny leaves are the richest in phytochemicals and having potential antioxidant; these may be used efficiently in traditional medicine. Furthermore, the non-toxicity of these morphotypes will allow to promote their use in nutraceutical preparations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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