

**STUDY OF ANTIOXIDANT ACTIVITY LEAVES OF *CORCHORUS OLITORIUS* AND
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ABSTRACT

The promotion and consumption of leafy vegetables could help not only to reduce food insecurity but also to prevent metabolic diseases associated with oxidative stress. This study was therefore conducted to value some leafy vegetables such as the *Corchorus olerius* (cp) and the *Solanum macrocarpon* (Fa) leaves consumed by Ivorians. The analysis of the biochemical composition allowed on the one hand to determine the levels of total phenols and flavonoids and on the other hand to evaluate the antioxidant power. The evaluation of the antioxidant activity was carried out by measuring the antiradical power, the inhibition of lipid peroxidation and the reducing power. The results showed that leaves extracts contain high levels of total phenols and total flavonoids. However, there is a significant difference between the total phenol and flavonoid contents of these leaves extracts. Thus, *Corchorus olerius* has the highest total phenol content (866.6 ± 15.3 mgEAG / g leaves) and total flavonoids (72.0 ± 10.5 mgEQ / g leaves) and leaves of *Solanum macrocarpon* (Fa) the lowest total phenol content (423.3 ± 10.0 mgEAG / g leaves) and total flavonoids (28.0 ± 2.0 mgEQ / g leaves). Also, the evaluation of antioxidant activity revealed that these leafy vegetables have a good inhibitory activity of lipid peroxidation, a good anti-radical and reducing power. It appears that all these vegetables have antioxidant activities that increase with their polyphenol content. Thus, these leafy vegetables could therefore represent a source of natural antioxidant substances to contribute to the well-being of the body.

KEYWORDS: antioxidant activities, oxidative stress, polyphenols, total flavonoids, *Solanum macrocarpon* and *Corchorus olerius*.

INTRODUCTION

Diet is a basic human need since it provides to the body with the essential nutrients and energy it needs to function (Rigaux, 2013). It is also an essential factor to take into account in the appearance and development of most pathological conditions. Indeed, the body is exposed to free radicals whose great reactivity and deleterious action on biological systems promote the aging and genesis of several diseases such as cardiovascular events and certain cancers (Pastre, 2005). The phenomenon of oxidation, generated by these free radicals, creates an imbalance in the balance oxidants / antioxidants when there is an exaggerated production of free radicals or a decrease of the antioxidant defenses and causes the oxidative stress (Baudin, 2006). To escape the serious consequences of oxidative stress, there is a need to maintain the balance between oxidants and antioxidants, in order to preserve the physiological performance of the body and health (Derbel and Ghedira, 2005). Consumption of fruits and vegetables, particularly

leafy vegetables, would therefore be an alternative to strengthen the body's antioxidant defenses (Massot, 2011). Indeed, leafy vegetables contribute to the well-being of the body because according to Blanc (1996) and Soro *et al.* (2012), they contain significant levels of vitamin, beta-carotene and various polyphenolic type molecules that have antioxidant activity to fight against free radicals. Leafy vegetables play an important role in the dietary habits of Ivorian populations (Agbo *et al.*, 2009). Indeed, in addition to their nutritional interest, they are of great importance because of their taste, their relatively low cost, the ease and speed of their preparation, their great diversity, and their availability during most of the year. The problem lies in the fact that in Côte d'Ivoire, leafy vegetables are consumed secondarily despite their richness in micronutrients and polyphenol antioxidants (anthocyanins, flavonoids ...) likely to bring health benefits to the body. To appreciate these properties, an evaluation of their antioxidant activity is necessary. It is with this in mind that the

present study was conducted and set as an objective to assess the antioxidant power of two leafy vegetables (*Solanum macrocarpon* and *Corchorus olitorius*).

I. MATERIAL AND METHODS

1. Biological material

The biological material used consists of two leafy vegetables namely *Solanum macrocarpon* and *Corchorus olitorius*.



Solanum macrocarpon



Corchorus olitorius

2-METHODS

2-1. Sampling

The fresh leaves were purchased at the market in Adjamé (Abidjan). Then they were cleaned and 400 g of each leaves were dried at room temperature away from the sun at the Laboratory of Food Science and Technology at Nangui Abrogoua University. After drying, they were made into powder separately using a mechanical grinder type IKAMAG to obtain four powders that were used to aliquot. After extraction, the determination of polyphenols and the measurement of antioxidant activity were carried out at the Department of Medical and Basic Biochemistry at the Institut Pasteur in Côte d'Ivoire.

2-2. Analyzes

The study of the antioxidant activity of the two leafy vegetables is based on the analysis of the two parameters such as the determination of the total polyphenols (total phenols and total flavonoids) and the evaluation of the antioxidant activity by measurement of the anti-oxidant power. Radical, the inhibition of lipid peroxidation and the reducing power of the leaves.

2-2-1. Extraction

A quantity of 10 grams of each leaves powder was diluted; in 100 mL of a methanol / water hydroalcoholic mixture (90:10, V / V). The mixture was stirred with a rotary shaker for 24 h. The mixture was then filtered through Whatman N° 1 paper. The collected filtrate was oven dried at 50 ° C for 24 h to evaporate the organic solvent. And the obtained paste constituted the total extract (Bala *et al.*, 2014).

2-2-2. Determination of total polyphenols

The total phenol content was determined in the total extract by the Folin-Ciocalteu method (Mc Donald *et al.*, 2001). The aluminum chloride colorimetric method was used to determine total flavonoid content in total leaves extracts (Chang *et al.* 2002).

2-2-3. Evaluation of antioxidant activity

The measurement of the antiradical activity of the leaves extracts was carried out by the 2,2-diphenyl-1-picrylhydrazyl inhibition test (DPPH) according to the method described by Parejo *et al.* (2000), which assesses the ability of extracts to fix free radicals by measuring the decrease in purple color due to the reduction of radicals produced by DPPH. The percentages of inhibition of the DPPH radicals are calculated by the following formula:

$$\text{Inhibition (\%)} = [(\text{white ABS} - \text{ABS sample} / \text{white ABS})] \times 100$$

NB: **Inhibition (%)**: the percentage of inhibition of DPPH radical.

White ABS: the absorbance of the blank. (No excerpt).

ABS sample: the absorbance of the root extracts of the plant and vitamin C.

The concentration of extract which causes 50 % inhibition of the DPPH radicals (IC₅₀ in µg extract / mL solution) was determined on a curve representing the inhibition of the DPPH radicals as a function of the concentration of extract and then compared with that of vitamin C.

Measurement of inhibition of lipid peroxidation by leaves extracts was performed by the ammonium thiocyanate test (Lee *et al.*, 2009) with slight modifications. The reducing power of the leaves extracts was determined according to the method described by Oyaizu (1986) using potassium ferricyanide.

2-3. Statistical analysis

Statistical analysis of the results was performed using the SPSS.20 software. The one-way analysis of variance (ANOVA I) was performed using the Duncan test at the 5% threshold. Graphical representations of the data were performed using Graph Pad Prism 5.0 software (Microsoft U.S.A). The results were averaged with the standard error on the mean (mean ± ESM).

II-RESULTS

1. Polyphenol content of leaves

The total phenol contents of the leaves extracts are shown in Table I. The extracts of *Solanum macrocarpon* (Fa) and the *Corchorus olitorius* (Cp) of concentration 0.1 g / mL give respectively 423.3 ± 10.0 and 866.6 ± 15.3 mg of gallic acid per gram of Fa and Cp. There is a significant difference between the total phenol contents of these leaves extracts at the threshold of p < 0.0 5. The results obtained show that the *Corchorus olitorius* has the highest total phenol content (866.6 ± 15.3 mgEAG / g of leaves) and the *Solanum macrocarpon* leaves has the lowest content (423.3 ± 10.0 mgEAG / g of leaves). The total flavonoid contents of the leaves extracts are shown in Table II. Extracts of *Corchorus olitorius* (Cp) and *Solanum macrocarpon* leaves (Fa) of concentration 0.1 g / mL give respectively 72.0 ± 10.5 and 28.0 ± 2.0 mg of quercetin per gram of Cp and Fa. There is a significant

difference between the total flavonoid contents of these extracts. The results obtained show that the Cp has the highest content of total flavonoids (72.0 ± 10.5 mg EQ/g

of leaves) and the lowest Fa leaves (28.0 ± 2.0 mg). EQ/ g of leaves).

Table 1: Levels of total phenols of leaves extracts of *Solanum macrocarpon* (Fa) and *Corchorus olitorius* (Cp) (mgEAG/g).

Echantillon	Moyenne \pm Ecart-type
Cp	$866,6 \pm 15,3^a$
Fa	$423,3 \pm 10,0^b$

Table 2: Levels of total flavonoids of leaves extracts of *Solanum macrocarpon* (Fa) and *Corchorus olitorius* (Cp) (mg EQ/g).

Echantillon	Moyenne \pm Ecart-type
Cp	$72,0 \pm 10,5^a$
Fa	$28,0 \pm 2,0^b$

Numbers affected by the same letter are not significantly different.

2. Antioxidant activity of the leaves

➤ Anti-radical activity of leaves vegetables

The results of the antiradical activity of vitamin C and leaves extracts on the DPPH free radical are shown in Figure 1. This activity increases with increasing concentration of vitamin C and leaves extracts. Concentrations of vitamin C and leaves extracts resulting in 50% inhibition of the DPPH radical (IC50) are $1.74 \pm$

0.33 ; 41.1 ± 0.19 and 115.00 ± 0.59 $\mu\text{g} / \text{mL}$ for vitamin C, Cp and Fa respectively. These results show that these two leafy vegetables have antiradical activities. However, the leaves of *Corchorus olitorius* (Cp) has the best antiradical activity compared to that of the *Solanum macrocarpon* (Fa) leaves which results in its low IC50 while the Fa leaves, has a slightly low anti-radical activity by its high IC50.

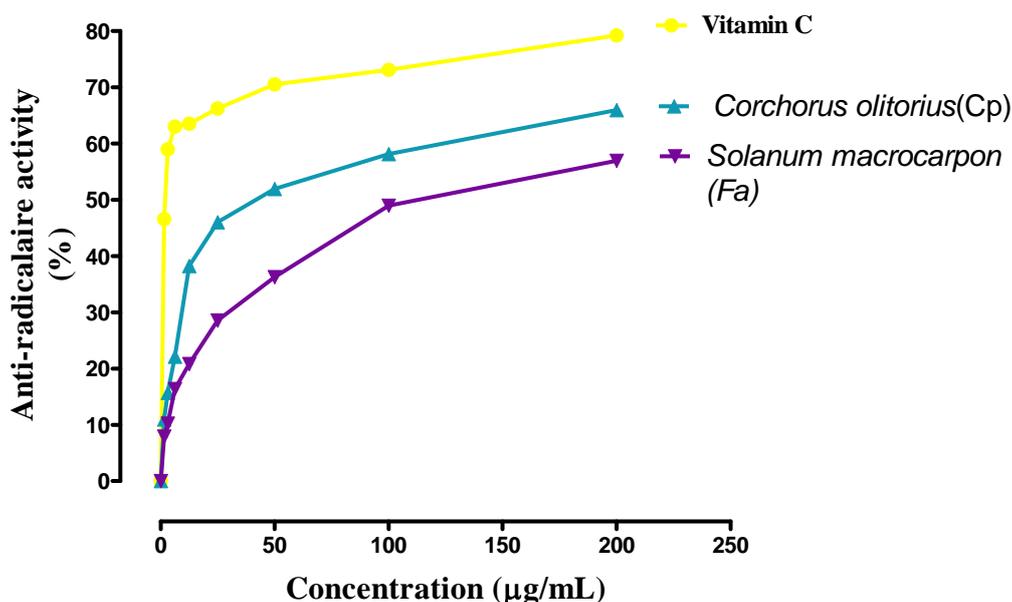


Figure 1: Evolution of the antiradical activities of Vitamin C and leaves extracts.

➤ Inhibitory activity of lipid peroxidation of leaves

The results of the inhibition of the peroxidation of linoleic acid by gallic acid and extracts of the leaves are shown in fig. 2. This activity increases with the increase in the concentration of the extracts. For a concentration of $125 \mu\text{g} / \text{mL}$ of gallic acid and extracts of the leaves, the percentages of inhibition are $13.70 \pm 1.37\%$; $30.20 \pm 2.70\%$ and $17.46 \pm 3.45\%$ respectively for gallic acid, Cp and Fa. For a concentration of $250 \mu\text{g} / \text{mL}$ of gallic acid and leaves extracts, the inhibition percentages are $23.58 \pm 2.58\%$; $56.11 \pm 2.72\%$ and $26.32 \pm 3.24\%$ respectively

for gallic acid, Cp and Fa. For a concentration of $500 \mu\text{g} / \text{mL}$ of gallic acid and extracts of the leaves, the percentages of inhibition are $34.20 \pm 3.42\%$; $90.79 \pm 2.69\%$ and $58.03 \pm 3.77\%$ respectively for gallic acid, Cp and Fa. These results show that leaves extracts all have inhibitory activities for lipid peroxidation. However, the *Corchorus olitorius* (Cp) has a very high inhibitory power which far exceeds that of gallic acid while that of the *Solanum macrocarpon* (Fa) leaves although higher than that of gallic acid is average compared to that of the *Corchorus olitorius* (Cp).

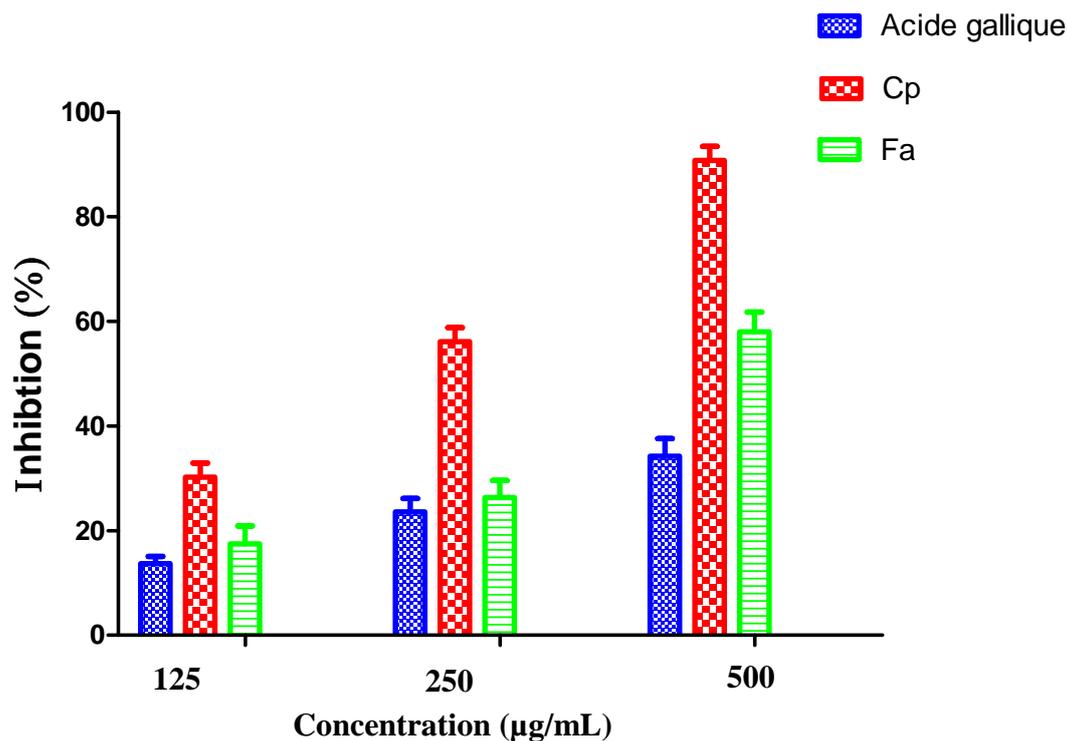


Figure 2: Inhibition of lipid peroxidation by gallic acid and leaves extracts.

1-2-3. Reducing activities of vitamin C and leaves extracts

The results of the reduction of Iron III to Iron II by vitamin C and leaves extracts are shown in Figure 3. The reducing capacity of vitamin C increases with

concentration as well as that of leaves extracts. These results show that vitamin C has a good reducing activity than leaves extracts. However, the reducing power of *Corchorus olitorius* (Cp) is greater than that of *Solanum macrocarpon* (Fa) leaves.

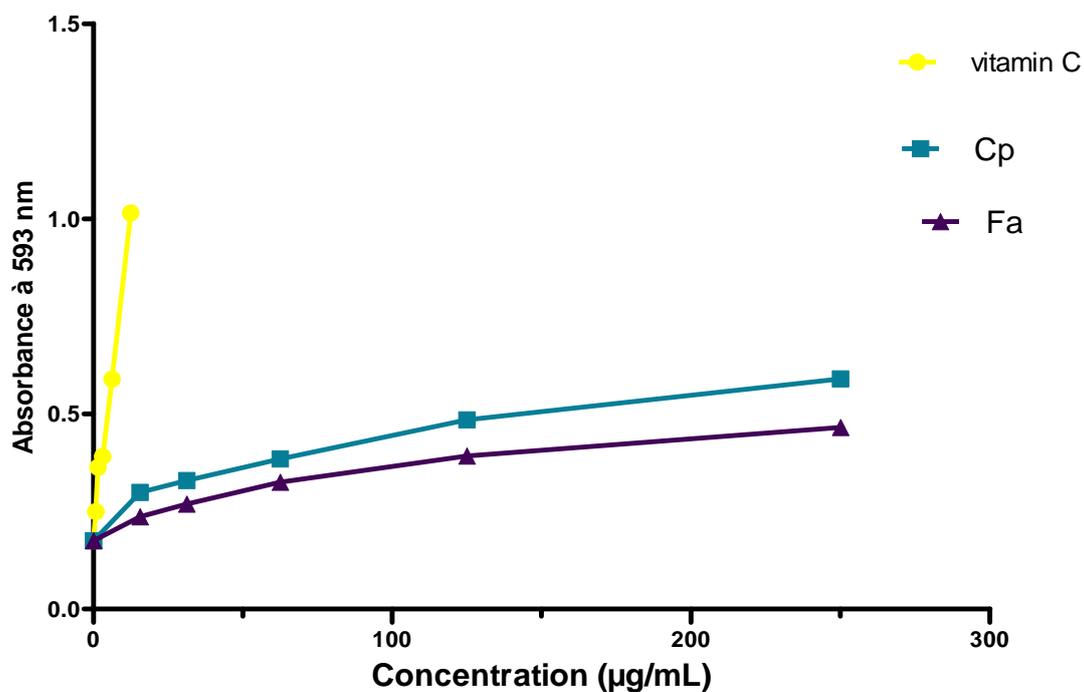


Figure 3: Reducing Activities of Vitamin C and Leaves Extracts.

2. DISCUSSION

This study showed that leafy vegetables contain phenolic compounds (total phenols and total flavonoids). The antioxidant activity of a plant is defined by its polyphenol content. The results showed that the antioxidant activity of leaves extracts increases with increasing polyphenol content. This is explained by Truong *et al.* (2007) in their sweet potato study, which found that the antioxidant activity of sweet potato leaves was very high compared with bark, whole root, and flesh because the phenol content of leaves was about 8, 16, and 18 times larger than that of bark, whole roots, and flesh respectively. Also, Popovici *et al.* (2009), in their work on the evaluation of the antioxidant activity of phenolic compounds by the reactivity with the free radical DPPH, concluded that the antioxidant activity increases with the content of phenolic compounds. Our results are in agreement with those of N'khili (2009) who showed that polyphenols in green tea are able to inhibit oxidative stress by trapping ROS (reactive oxygen species) by electron transfer or hydrogen atoms. The concentrations of vitamin C and extracts of Cp and Fa inhibiting 50% (IC₅₀) the DPPH radical, obtained prove that the reference molecule (Vit C) has an antiradical activity greater than that of leaves extracts. Similar results have been found by Fernando and Soysa (2014), who also indicate that the antiradical activity of vitamin C is superior to that of *Atalantia ceylanica* extracts. However, the Cp extract has the strong reducing activity on the DPPH radicals compared to that of Fa. These reducing activities evolve with their total phenol contents. The more the extract has a high content of phenolic compounds the higher its antiradical activity is high. Indeed, there is a linear correlation between the total phenol content of leaves extracts and their antiradical activity (Meité *et al.*, 2014). These results are the same as those of Popovici *et al.* (2009) confirming a certain correlation between the content of phenolic compounds of grape seed extract (*Vitis Vinifera L.*) and the antiradical activity. Also Khan *et al.* (2012) who showed the same correlation between the antiradical activity and the total phenol content of *Atalantia ceylanica*. According to Bidié *et al.* (2011), the functional groups present in the structures of polyphenols can easily give up an electron or a proton to neutralize free radicals. Several other authors have shown that inhibition of the production of reactive oxygen species (ROS) by polyphenols can proceed directly through enzyme-inhibitor complexes and / or by direct free radical scavenging (Dangles *et al.*, 2006). One of the important activities in this study is the measurement of the inhibition of lipid peroxidation. The results obtained show that leaves extracts inhibit the peroxidation of linoleic acid better than gallic acid in ascending order of their total flavonoid content. This activity increases as gallic acid concentrations and extracts increase. Indeed, there is a close relationship between the total flavonoid content of many plant species and the inhibitory potency of lipid peroxidation (Oktay *et al.*, 2003). Similar results were found by Amieur (2009) who reports that inhibition

of lipid peroxidation of linoleic acid extracts is positively correlated with their content of phenolic compounds (total flavonoids). Oboh *et al.* (2007) also reported that flavonoids are considered as potential antioxidants exerting their antioxidant activity by radical scavenging and metal ion chelation mechanisms to inhibit lipid peroxidation. Inhibition of lipid peroxidation of linoleic acid of these leafy vegetables varies at 125µg / mL from 17.46 ± 3.45% *Solanum macrocarpon* (Fa)) to 30.20 ± 2.70% (the *Corchorus olitorius* (Cp)) and at 500 µg / mL from 58.03 ± 3.77% to 90.79 ± 2.69% respectively for the eggplant leaves and the vegetable coret, is higher than that of *Gomphrena celosioides* which is 16, 18 ± 1.27% at 125 µg / mL and 48.50 ± 4.74% at 500 µg / mL *Gomphrena celosioides* (Meité *et al.*, 2014). According to N'khili (2009), polyphenols and more precisely flavonoids, are able to reduce peroxy radicals by electron transfer thanks to their low redox potential. The reducing activity of leaves extracts is low compared to the reference molecule, vitamin C, but is proportional to their total phenol content. These results are similar to those of Méité *et al.* (2014) who found a high reducing activity for vitamin C and low activity for *Gomphrena celosioides*, indicating that the reducing power of *Gomphrena celosioides* is lower than that of vitamin C. Among the studied leafy vegetables *Solanum macrocarpon* (Fa) has the lowest reducing activity and the *Corchorus olitorius* (Cp) the highest activity. The reducing power of leaves extracts is probably due to the presence of hydroxyl groups in the phenolic compounds that can serve as electron donors. According to Bidié *et al.* (2011), the functional groups present in the structures of polyphenols can easily give up an electron or a proton to neutralize free radicals. Therefore, antioxidants are considered oxidative reducers and inactivators (Siddhuraju and Becker, 2007). Some previous studies have also shown that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity (Jeong *et al.*, 2004; Kumaran and Karunakaran, 2007).

CONCLUSION

The present study focused on the evaluation of the antioxidant activity of two leafy vegetables, which are quite popular in Côte d'Ivoire, namely *Corchorus olitorius* (Cp) and leaves of *Solanum macrocarpon* (Fa). It revealed that these leafy vegetables have high levels of phenolic compounds including total phenols and total flavonoids. These leaves extracts therefore have important antioxidant activities sometimes approaching that of the reference molecule. Thus, the *Corchorus olitorius* (Cp) has a higher antioxidant activity than the *Solanum macrocarpon* (Fa). These leafy vegetables could therefore represent a source of natural antioxidant substances to prevent metabolic diseases related to oxidative stress. As these leafy vegetables, which may contain a large number of different compounds, it would be interesting, in future research to identify them, to isolate and purify. This will make it possible to obtain the pure molecules or at least the most active fractions

which will subsequently be used to carry out antioxidant tests *in vivo* in order to confirm the antioxidant activities obtained *in vitro*.

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