International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Evaluation of the Antioxidant Activity of Aqueous and Methanolic Extracts of Various Organs of *Alchornea cordifolia* by Scavenging the Radical Ion ABTS$^{\bullet+}$

N'Negue ép Mezui-Mbeng M.-A.[1], Ella Ndong J. G.[2], Lendoye E.[3], Nguema Edzang R. W.[4], Mackosso A. H. K[5]

Laboratoire de Chimie-Biochimie de la Faculté de Médecine. Université des Sciences de la Santé B. P.4009. Libreville-Gabon.

[2]Corresponding Authors Email: *ella_ndong[at]yahoo.fr*

Abstract: ***Alchornea cordifolia is a shrub very widespread in sub-Saharan Africa where it is used in traditional medicine in the treatment of several aliments. Indeed, in vitro and in vivo studies on animal models have demonstrated therapeutic properties such as the antioxidant properties of extracts from A. cordifolia leaves, the antioxidant activity being variable with the extraction solvent. However, the antioxidant activity has not been evaluated on other parts of the plant. The aim of this work is to evaluate the antioxidant activity of different concentrations of aqueous and methanolic extracts of different A. cordifolia organs, namely: leaves, trunk, fruit pulp and stem. The main goal is to determine the most antioxidant organ of the plant and the extraction solvent to observe a better yield in terms of activity. The method is based on the measurement of the free radicals of the radical cation of 2, 2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid] (ABTS$^{\bullet+}$) by comparison with that of gallic acid, a reference antioxidant. The results showed on the one hand that the extraction in methanol of the bioactive compounds was superior to the aqueous extraction. In general, the methanolic extracts exhibited better antioxidant activity than the aqueous extracts. On the other hand, we have shown that the leaf is the most active organ followed by the bark of the trunk and the pulp of fruit whose activities are similar. The stem appears to be the least active organ. The IC50 values are as follows; methanolic extract of leaves "IC50 of 1 µg. mL^{-1}"; methanolic extracts of trunk bark (IC50 = 3 µg. mL^{-1}); methanolic extract of fruit pulp (IC50 = 3.75 µg. mL^{-1}); 9 µg. mL^{-1} IC50 aqueous leaf extract; the aqueous extract of the stem (IC50 of 100 µg. mL^{-1}). Gallic acid, the standard antioxidant, showed an IC50 of 0.35 µg. mL^{-1}.***

Keywords: *Alchornea cordifolia*; aqueous extracts; methanolic extracts; gallic acid; antioxidant activity

1. Introduction

The ecosystem includes a large number of plants that play an important role in the life of humans and animals by constituting food, cosmetic and even therapeutic source (Cseke et al., 2016). Indeed, these plants contain bioactive secondary metabolites responsible for their biological activities such as antioxidant (Sinan et al., 2021), antidiabetic (Agbor et al., 2007), anticancer (Aholia Adepo et al., 2019) activities, etc. Also, traditional African medicine uses many of these plants called medicinal plants in the treatment of several diseases. On the other hand, the food, cosmetic and now pharmaceutical industry is interested in these plants with the aim of extracting a very wide variety of natural antioxidants (Ma et al., 2016) inexpensive and potentially less toxic than synthetic antioxidants, to fight against the formation of reactive oxygen species and the oxidative disorders caused by them in the human body and in food (Huang et al., 2005).

Alchornea cordifolia is a medicinal plant, found in rain forests or gallery forests of tropical Africa, but also along rivers. It is an Euphorbiaceae widely used in traditional medicine as an anti-dysenteric, antidiarrheal, antispasmodic, anti-inflammatory (Mavar-Manga et al., 2008; Pompermaier et al., 2018), analgesic, veinotonic, astringent (Dibong et al., 2011), antimalarial (Mustofa et al., 2000), antisalmonella (Djague et al., 2020), and anti-arthritis (Duke et al., 2002; Adeneye et al., 2014), anxiolytic (Kamenan A, 2013) again in herbal medicine for hemorrhoidal disease (Nga Nnanga et al., 2017). In Gabon, the leaves are ingested for their abortive properties and a cold infusion of these dried and crushed leaves is believed to have diuretic properties (Akendengue et al., 1994). Decoctions of *A. cordifolia* leaves are also used for the treatment of ulcers, rheumatism, pain and convulsive fevers (Adeneye et al., 2014).

Several studies have shown that *A. cordifolia* is a strong antioxidant plant. An antioxidant activity of ethanolic extracts and that of aqueous leaf extracts has been demonstrated by scavenging of the DDPH radical (Agbor et al., 2007; Aholia Adepo et al., 2019). Methanolic extracts of *A. cordifolia* leaves, rich in antioxidant phenolic compounds, have an antioxidant activity equivalent to that of ascorbic acid (Osadebe et al., 2012). Antioxidant activity comparable to that of vitamin C from the hydroethanolic extract of the trunk bark was demonstrated by scavenging free radicals from DDPH and ABTS.

The antioxidant activity by superoxide anion scavenging has also been observed with aqueous extracts and ethyl acetate from *A. cordifolia* leaves (Aholia Adepo et al., 2019; Kouakou Siransy et al., 2010). A reduction in lipid peroxidation of *A. cordifolia* has also been demonstrated in rats (Olalye MT and Rocha JB, 2007). According to some authors, the antioxidant activity of the methanolic extract of the leaves is greater than that of the aqueous extract, which is greater than that of the extract of ethyl acetate (Sinan et

al., 2021). This could be explained by a higher level of phenolic compounds such as flavonoids in the methanolic extract. Several studies have demonstrated the antioxidant character of extracts from *A. cordifolia* leaves, however, very few studies have evaluated the antioxidant activity of other plant organs.

The aim of this work was to evaluate the antioxidant activity of aqueous and methanolic extracts of different organs of *A. cordifolia* native to Gabonese soil, namely: leaves, trunk, fruit pulp and stem. We will thus assess the antioxidant capacity of parts of the plant other than the leaves; to determine the most active part of the plant and the solvent for extracting these bioactive compounds that provides the best yield. The anti-free radical activity is determined by scavenging the radical cation of 2, 2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid] ($ABTS^{\bullet+}$) according to the method developed by Re *et al.* (Re et al., 1999) and optimized by N'negue *et al.* (N'negue et al., 2020). Gallic acid is used as a benchmark antioxidant.

Image 1: *A. cordifolia (leaves)*

2. Materials and Methods

2.1 Materials

The leaves, trunk bark, stem and fruit pulp of *A. cordifolia* come from an *A. cordifolia* shrub gathered in Okala in Northern Libreville, Gabon. ABTS (2, 2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]), gallic acid, potassium persulfate ($K_2S_2O_8$) and hydrated sodium dihydrogen phosphate were purchased from Sigma-Adrilch (Saint-Quentin Fallavier, France). The water used was distilled by the equipment of the "Milli-Q Labo" laboratory (Millipore Japan, Tokyo, Japan). All these products are suitable quality for analysis. The antioxidant activity was determined by UV spectrophotometry: V-200 spectrophotometer (BOECO, Germany). The optical density was read at 734 nm, which is the maximum absorption wavelength of the radical cation $ABTS^{\bullet+}$.

2.2 Preparation of methanolic and aqueous extracts of *A. cordifolia*

Direct extraction of the drug into water or methanol, after drying and grinding the plant into powder in a grinder. The methanolic extracts of fruit leaves and pulp in the form of tar and the extracts of trunk bark in the form of powder of *A. cordifolia* are diluted in DMSO.

2.3 Preparation of "reference antioxidant" gallic acid solutions

Gallic acid (3, 4, 5-trihydroxybenzoic acid) is an aromatic organic compound, used as a reference anti-free radical compound (Yehye et al., 2015; Pisoschi AM and Pop A, 2015; Siti et al., 2015; Oroian M and Escriche I, 2015). Ten working solutions, of decreasing concentrations, ranging from 0.94 to 0.094 µM, were prepared by diluting gallic acid in distilled water.

2.4 Measurement of anti-free radical activity

The principle of the test for measuring the anti-radical activity by the ABTS method is based on the decrease in the absorbance at 734 nm of the radical cation $ABTS^{\bullet+}$ (blue-green coloration) in the presence of a potentially anti-compound. radical which reduces the cation radical (Re et al., 1999). The reduction in the radical form of $ABTS^{\bullet+}$ leads to a discoloration of the solution.

The $ABTS^{\bullet+}$ radical ion is obtained by reacting the ABTS molecule (7 mM) with potassium persulfate (2.45 mM), in distilled water for 16 hours at room temperature and under cover light. The $ABTS^{\bullet+}$ solution obtained is diluted with sodium phosphate buffer (5 mM, pH = 7.4), in order to obtain a stock solution having an initial absorbance value at 734 nm between 0.65 and 0.70. The radical cation ($ABTS^{\bullet+}$) is stable for more than 2 days when stored at room temperature and protected from light. All the assays were carried out three times and the anti-free radical activity is calculated according to the formula below (Re et al., 1999; Long et al., 2000). Anti-radical activity (%) = [1-(Ar-Ab) / (Ai-Ab)] x 100. (Ar, absorbance of the remaining $ABTS^{\bullet+}$ radical / Ai, absorbance of the initial $ABTS^{\bullet+}$ radical / Ab, absorbance of the phosphate buffer).

In fact, the reduction of the $ABTS^{\bullet+}$ radical cation therefore amounts to determining the anti-free radical activity and in total, the antioxidant properties of *A. cordifolia* extracts compared to the antioxidant properties of gallic acid (standard). The antioxidant activity was determined by UV spectrophotometry in cuvettes with an optical path of 1 cm (reaction volume of 2 mL). The incubation time is 6 minutes (N'negue et al., 2020).

3. Results

3.1 Relationship between concentration and antioxidant activity of gallic acid

The percentage of antioxidant activity increases linearly as a function of the gallic acid concentration (Figure 1); or a disappearance of the $ABTS^{\bullet+}$ radical in the presence of the reference antioxidant "gallic acid".

An antioxidant activity of 15.98 (± 3.58) was recorded for a concentration of 0.094 µg. mL^{-1} of gallic acid. This antioxidant activity is 74% (± 4.68) for a concentration of 0.56 µg. mL µg. mL^{-1} and 100% for a concentration of 0.84

µg. mL^{-1} (4.5 µM) gallic acid; or an IC50 of 0.35 µg. mL^{-1} (1.8 µM).

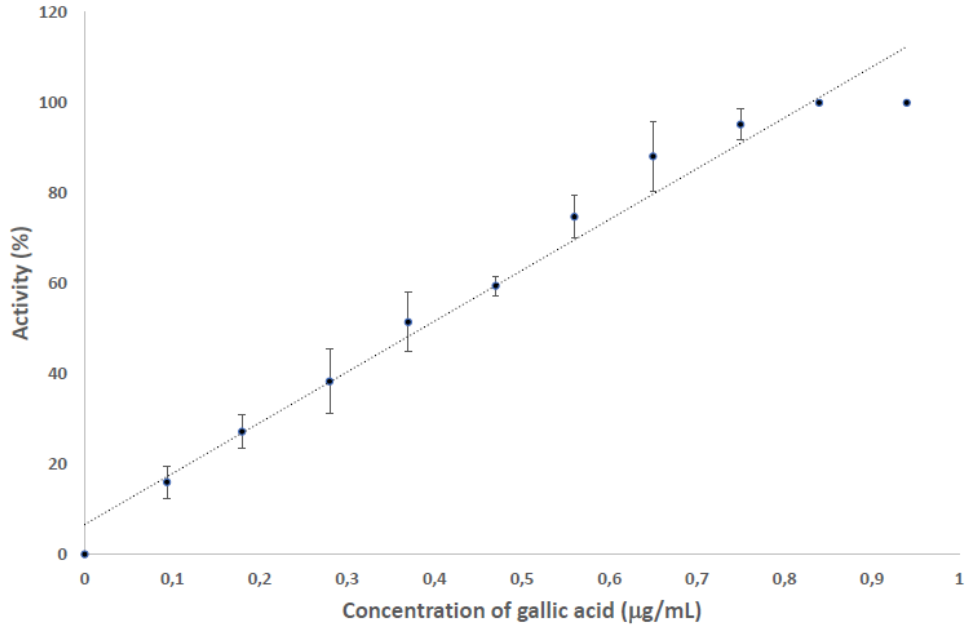

Figure 1: Relationship between antioxidant activity and the concentration of gallic acid after 6 minutes of incubation. The proportion ABTS•$^{+}$ transformed into ABTS^{+} in the presence of gallic acid is calculated from the change in absorbance at 734 nm measured by spectrophotometry. The equation on the right is: y = 112.55x + 6.602 (R2 = 0.98); n = 3.

3.2. Antioxidant activity of aqueous and methanolic extracts of *A. cordifolia* leaves

3.2.1. Evaluation of the anti-free radical activity of the aqueous extract of the leaves

According to the results obtained (Figure 2), the antioxidant activity of the aqueous extract of the leaves increased from 0.2 µg. mL^{-1} to 20 µg. mL^{-1}. Above 20 µg. mL^{-1}, the antioxidant activity stabilizes around a percentage ranging from 99.61% ± 0.12 to 99.78% ± 0.31. According to these same results, the IC50 of the aqueous extract of *A. cordifolia* leaves is 9 µg. mL^{-1}.

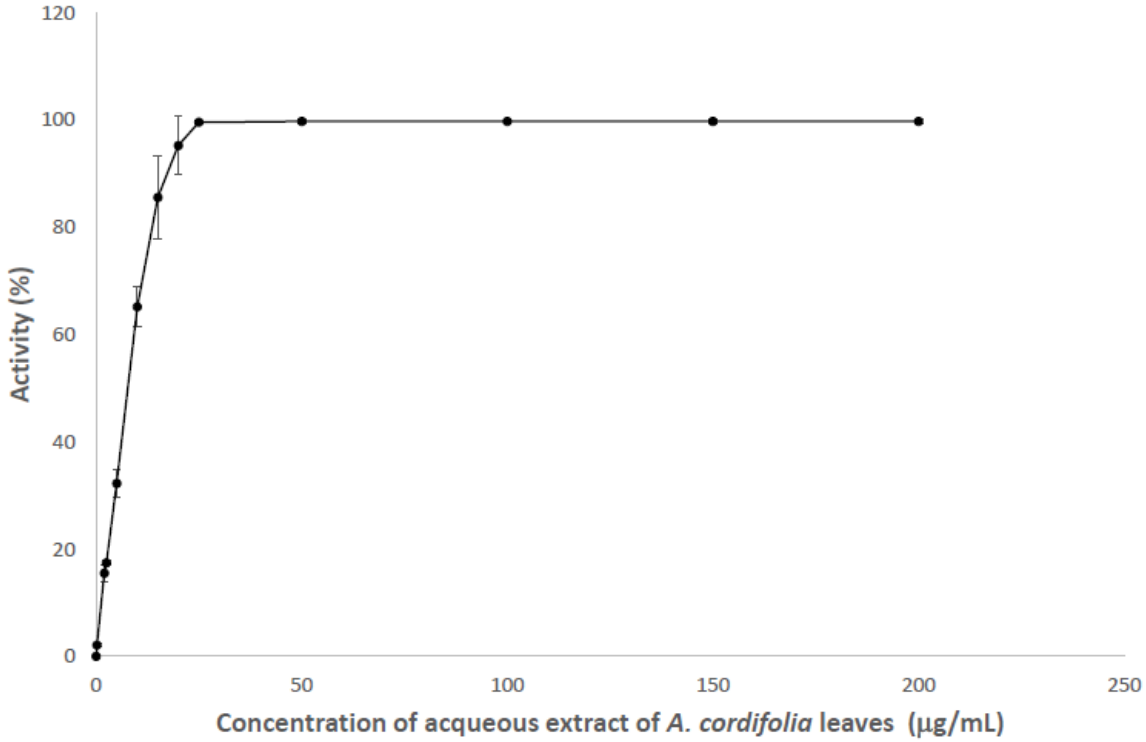

Figure 2: Relationship between Antioxidant activity and the concentration of aqueous extract of *A. cordifolia* leaves after 6 minutes of incubation. The proportion ABTS•$^{+}$ transformed into ABTS^{+} is calculated from the change in absorbance at 734 nm measured by spectrophotometry; n = 3.

3.2.2. Evaluation of the antioxidant activity of the leaves methanolic extract

The results (Figure 3) show an increasing antioxidant activity with the concentration of methanolic extract of *A. cordifolia* leaves. The antioxidant activity is 90.92 ± 7.34% for a concentration of 2.5 µg. mL^{-1} and 100% for a concentration of 5 µg. mL^{-1} of methanolic extract of leaves of *A. cordifolia*. These results give an IC50 of 1 µg. mL^{-1} of the methanolic extract of *A. cordifolia* leaves.

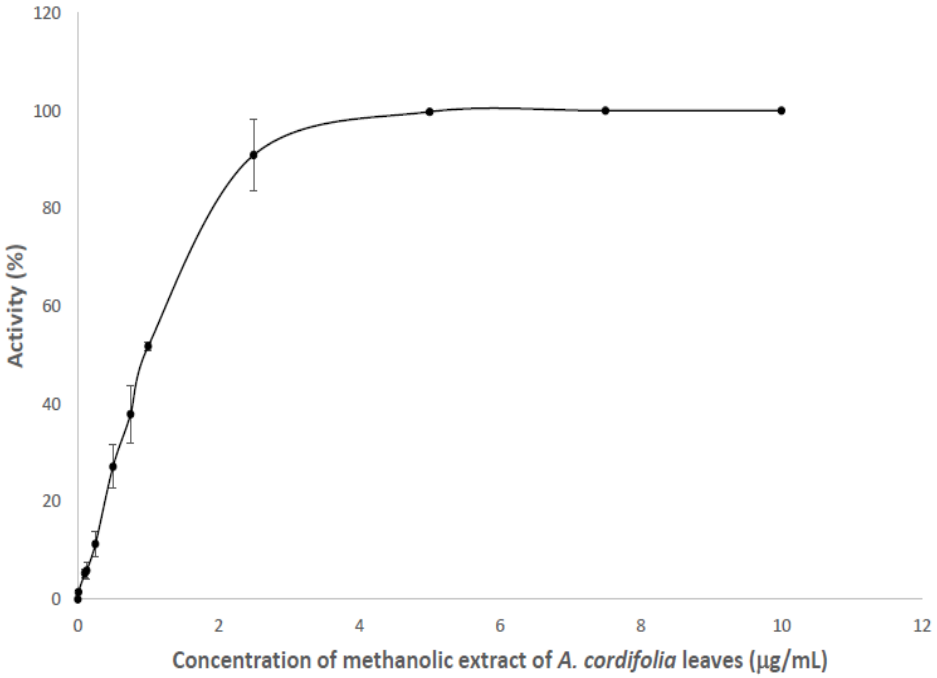

Figure 3: Relationship between antioxidant activity and the concentration of methanolic extract of *A. cordifolia* leaves after 6 minutes of incubation. The proportion $ABTS^{\bullet+}$ transformed into ABTS + is calculated from the change in absorbance at 734 nm measured by spectrophotometry; n = 3.

3.3 Antioxidant activity of aqueous extract of *A. cordifolia* stem

According to the results observed (Figure 4), the antioxidant activity increases with the concentration of aqueous extract of *A. cordifolia* stem. This activity reaches 100% for an aqueous stem extract concentration of 200 µg. mL^{-1}; the IC50 being 100 µg. mL^{-1}.

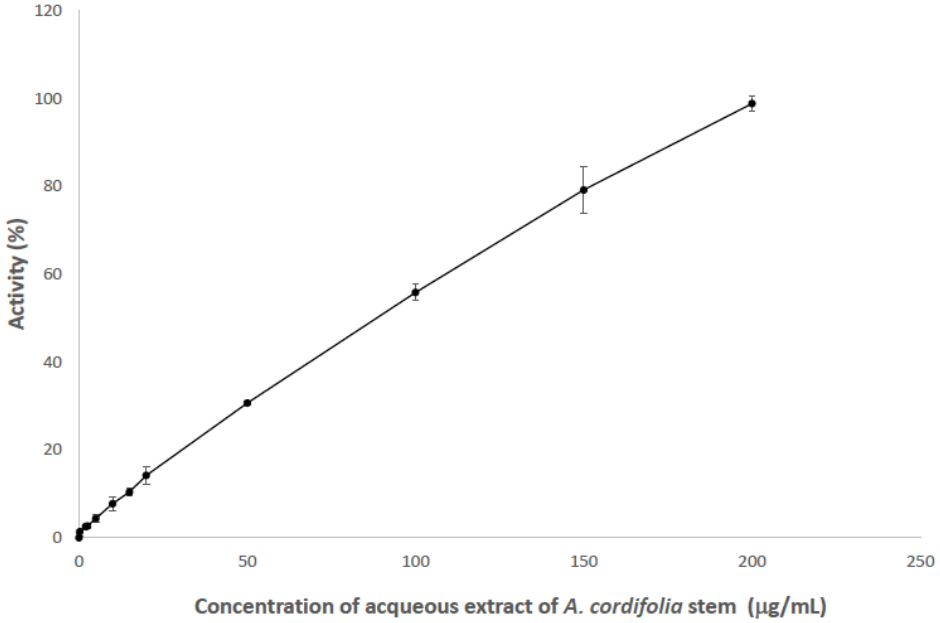

Figure 4: Relationship between anti-oxidant activity and the concentration of aqueous extract of *A. cordifolia* stem after 6 minutes of incubation. The proportion $ABTS^{\bullet+}$ transformed into $ABTS^{+}$ is calculated from the change in absorbance at 734 nm measured by spectrophotometry; n = 3.

3.4 Anti-free radical activity of methanolic extract of *A. cordifolia* trunk bark

According to the results obtained (Figure 5), the antioxidant activity increases with the concentration of methanolic extract of *A. cordifolia* trunk bark. It reaches 100% for a concentration of 12.5 µg. mL^{-1}. The IC50 is 3 µg. mL^{-1}.

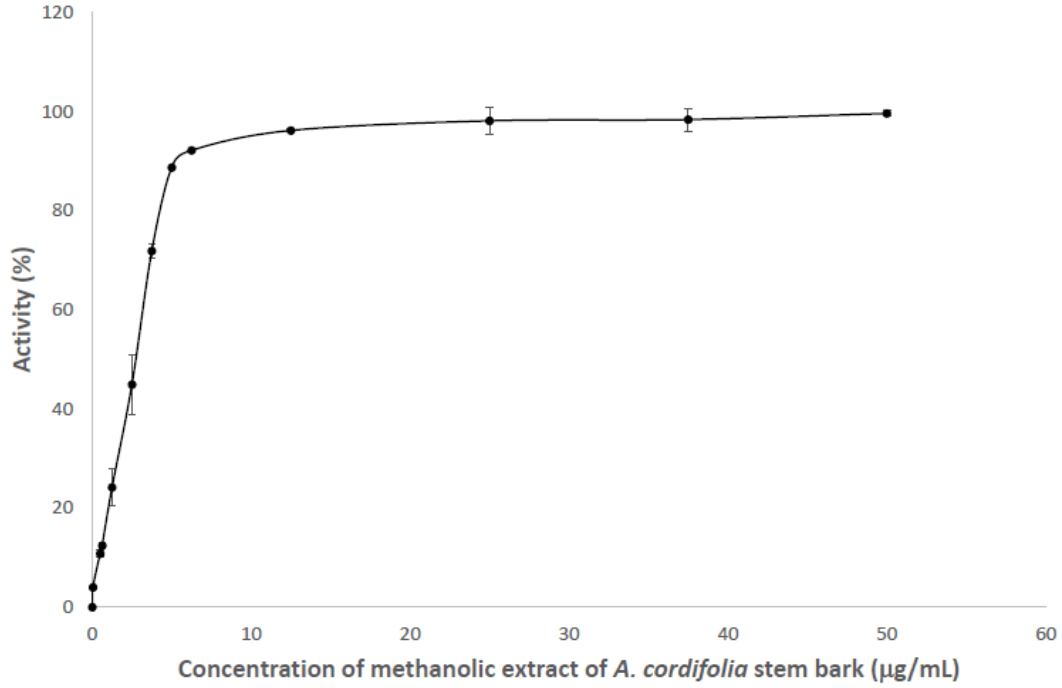

Figure 5: Relationship between concentration antioxidant activity and the concentration of methanolic extract of *A. cordifolia* trunk bark after 6 minutes of incubation. The proportion $ABTS^{\bullet+}$ transformed into $ABTS^{+}$ is calculated from the change in absorbance at 734 nm measured by spectrophotometry; n = 3.

3.5. Antioxidant activity of the methanolic extract of the fruit pulp of *A. cordifolia*

According to the results obtained (Figure 6), the antioxidant activity increases with the concentration of methanolic extract of the fruit pulp of *A. cordifolia*. This activity is approximately 100% for a concentration of 7.5 µg. mL^{-1}, with an IC50 of 3.75 µg. mL^{-1}.

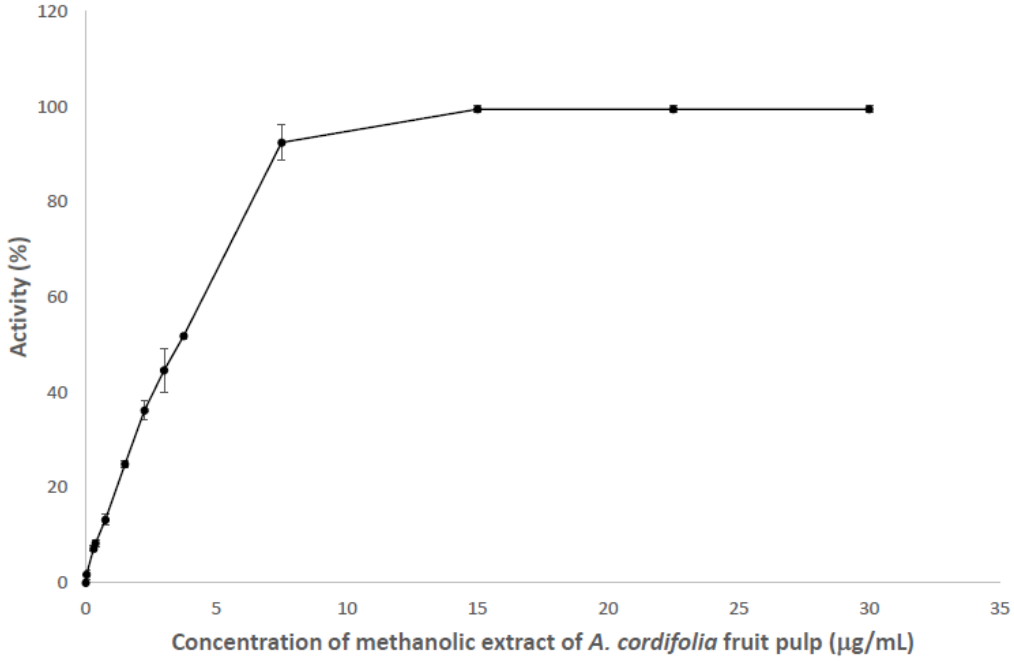

Figure 6: Relationship between Antioxidant activity and the concentration of methanolic extract of the fruit pulp of *A. cordifolia* after 6 minutes of incubation. The proportion $ABTS^{\bullet+}$ transformed into $ABTS^{+}$ is calculated from the change in absorbance at 734 nm measured by spectrophotometry; n = 3.

4. Discussion

In our study, we evaluated the antioxidant activity of aqueous and methanolic extracts of different organs of *A. cordifolia* by scavenging the radical ion ABTS•$^{+}$ according to the method of Re *et al.* (Re et al., 1999) optimized by N'negue *et al.* (N'negue et al., 2020) with gallic acid as the benchmark antioxidant.

The results of the evaluation of the antioxidant activity of *A. cordifolia* extracts showed that all the organs tested here namely; leaves, stem, trunk bark, and fruit pulp have an antioxidant activity, which varies in percentile depending on the organ but also depending on the type of extraction.

Indeed, if we first compare the antioxidant activity and the method of extraction, we observe that the methanolic extract of *A. cordifolia* leaves has an antioxidant activity greater than the aqueous extract. It only takes 5 µg. mL^{-1} of methanolic leaf extract against 25 µg. mL^{-1} of aqueous extract of *A. cordifolia* leaves to achieve 100% anti-free radical activity; the aqueous extract should be 5 times more concentrated than the methanolic extract. Considering the IC50s, and according to our results, the methanolic leaf extract exhibited antioxidant activity with an IC50 of 1 µg. mL^{-1}; while the aqueous extract of the leaves showed antioxidant activity with an IC50 of 9 µg. mL^{-1}. The methanolic extract of *A. cordifolia* leaves is therefore approximately 9 times more active than the aqueous extract of the leaves. These results are in agreement with the literature. Indeed, several studies have validated the significant antioxidant activity of *A. cordifolia* leaves (Sinan et al., 2020; Agbor et al., 2007; Aholia Adepo et al., 2019; Kouakou Siransy et al., 2010). The antioxidant activity of leaf extracts is linked to their richness in flavonoids and tannins (Nga Nnanga et al., 2020; Osadebe et al., 2012), polyphenolic compounds capable of scavenging free radicals. These phenolic compounds would therefore be found at a higher rate in the methanolic extract than in the aqueous extract due to better solubility. These results agree with those of other authors who have also shown that methanolic leaf extract was more antioxidant than aqueous extract and, than leaf extract and ethyl acetate extract (Sinan et al., 2020).

The second step in comparing the antioxidant activity of the different organs of *A. cordifolia* shows that the leaf is the most active organ, with an IC50 of 1 µg. mL^{-1} of its methanolic extract; followed by trunk bark (IC50 of methanolic extract = 3 µg. mL^{-1}) and fruit pulp (IC50 of methanolic extract = 3.75 µg. mL^{-1}).

The stem would be the least antioxidant organ with an IC50 of its aqueous extract of 100 µg. mL^{-1} (by extrapolation would be divided by 9 if methanolic extract, or an IC50 of 11 µg. mL^{-1}). These results can be explained by a higher concentration of anti-free radical compounds in the leaves compared to other organs. Indeed, the leaves are rich in flavonoids which are polyphenols with antioxidant properties (Sinan et al., 2020; Nga Nnanga et al., 2020; N'guessan et al., 2011). The presence of gallic acid in the leaves of *A. cordifolia* (Sinan et al., 2020) would help to increase the antioxidant capacities of the leaves of *A. cordifolia*. In fact, gallic acid, the benchmark antioxidant, showed an IC50 of 0.35 µg. mL^{-1}, an antioxidant activity barely 3 times that of *A. cordifolia* leaves. Therefore, for an antioxidant activity identical to that of gallic acid, it would only take 3 times more methanolic extract from the leaves of *A. cordifolia*. Given the pure character of gallic acid and the presence of non-active compounds in the methanolic extract of *A. cordifolia* reducing the concentration of the active principle of the leaves of *A. cordifolia*, we can say that the methanolic extract of the leaves of *A. cordifolia* has an antioxidant activity equivalent to that of gallic acid. This could explain the strong interest shown in this plant organ in traditional medicine (Nga Nnanga et al., 2020; N'guessan et al., 2011).

However, the leaves, the bark of the trunk as well as the stems require processing for the recovery of the active principle. These transformations into digestible elements for humans are likely to lead to losses or dilutions of the active ingredient with the consequences of a significant decrease in the antioxidant activity once the mixtures or potions have been ingested. Since the fruit pulp does not require chemical treatment, it would probably be the most suitable organ for antioxidant supply in humans.

5. Conclusion

A. cordifolia from Gabonese soil has an antioxidant activity which varies from one part of the plant to another. The way in which the antioxidant compounds are extracted appears to alter the antioxidant properties of the plant. Indeed, aqueous extracts do not have as many antioxidant molecules as methanolic extracts. Although all the tested organs of the plant namely; stem, trunk bark, fruit pulp and leaves have antioxidant power, the maximum anti-free radical activity is observed in the methanolic extract of the leaves, which has the highest number of antioxidant compounds. A number of studies have revealed the important role that antioxidants play in our body. *A. cordifolia*, through its antioxidant properties, therefore has therapeutic potential against pathologies associated with oxidative stress (cardiovascular diseases, aging, cancer, and inflammation, neuronal or genetic diseases). In addition, the methanolic extract of the leaves could be used in the food and cosmetic industry as a natural antioxidant.

6. Acknowledgements

We thank Professor AKendengugué Blandine for providing the plants.

References

[1] Adeneye, A. A., Oreagba, A. I., Ishola, I. O., Kalejaiye, H. A., 2014. Evaluation of the anti-arthritic activity of the hydroethanolic leaf extract of Alchornea cordifolia in rats. Afr J Tradit Complement Altern Med.11 (2): 402-10.

[2] Agbor, G., Vinson, J., Oben, J., Ngogang, J., 2007. In Vitro Antioxidant Activity of Three Piper Species. Journal of herbal pharmacotherapy.7: 49-64. Doi: 10.1300/J157v07n02_04.

[3] Aholia Adepo, J.-B., Manda, P., Ngbé, J. V., Diakité, A., Tigori Sangaré B, Djédjé Dano, S., 2019. Study of aflatoxicosis reduction: effect of Alchornea cordifolia on biomarkers in an aflatoxin B1 exposed rats. Drug Chem Toxicol.42 (3): 243-251.

[4] Akendengue, B., Louis, A. M., 1994. Medicinal plants used by Masango people in Gabon. Journal of Ethnopharmacology.37: 165-173.

[5] Cseke, L. J., Kirakosyan, A., Kaufman, P. B., Warber, S., Duke, J. A., Brielmann, H. L., 2016. Natural products from plants. CRC Press.

[6] Dibong, S. D., Mpondo, M. E., Ngoye, A., Kwin, N. F., 2011. Plantes médicinales utilisées par les populations bassas de la région de Douala au Cameroun. Int J Biol Chem Sci.5 (3): 1105-1117.

[7] Djague, F., Lunga, P. K., Toghueo, K. R. F., Melogmo, D. Y. K., Fekam, B. V., 2020. Garcinia kola (Heckel) and Alchornea cordifolia (Schumach. & Thonn.) Müll. Arg. from Cameroon possess potential antisalmonellal and antioxidant properties. PLoS One.15 (8): e0237076.

[8] Duke, J. A., Mary, J. B., and Judi, D., 2002. Handbook of medicinal herbs. Boca Raton: CRC Press.

[9] Huang, D., Ou, B., Prior, R. L., 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem.53: 841-1853.

[10] Kamenan, A., 2013. Activité anxiolytique d'un extrait aqueux des feuilles d'Alchornea cordifolia. Journal Africain de Pharmacie et Pharmacologie.7 (16): 816-821.

[11] Kouakou Siransy, G., Sahpaz, S., Nguessan, GI., Yao Datté, J., Kablan Brou, J., Gressier, B., Bailleul, F., 2010. Effects of Alchornea cordifolia on elastase and superoxide anion produced by human neutrophils. Pharm Biol.48 (2): 128-33.

[12] Long, L. H., Thiam Kwee, D. C., Halliwell, B., 2000. The antioxidant Activities of seasonings used in Asian Cooking. Powerful antioxidant activity of dark soy sauce revealed Using the ABTS Assay, Free Rad Res.32: 181-186.

[13] Ma, Y., Ke, C. Y., Zi, X., Zhao, F., Yaun, L., Zhu, Y. L., Fan, X. X., Zhao, N. M., Li, Q. Y., Qin, Y. H., Liu, H. M., 2016. Introduction of the mithochondria mediated apoptosis in human esophageal cancer cells by DS2, a newly synthetic diterpenoid analog is regulated by Bax and caused by generation of reactive oxygen species. Oncortarget.7: 86211-86224.

[14] Mavar-Manga, H., Haddad, M., Pieters, L., Baccelli, C., Penge, A., Quetin-Leclercq, J., 2008. Anti-inflammatory compounds from leaves and root bark of Alchornea cordifolia (Schumach. & Thonn.) Müll. Arg. J. Ethnopharmacol.115: 25–29.

[15] Mustofa, A. V., Francoise, B., Yves, P., Koné-Bamba, D., Mallié, M., 2000. Antiplasmodial activity of plant extract used in West African traditional medicine. J of Ethno Pharm.73: 145-151.

[16] N'guessan, A.-H., Dago, D. C. E., Békro, J. A. M., Békro, Y. A., 2011. CCM d'extraits sélectifs de 10 plantes utilisées dans le traitement traditionnel de l'hypertension artérielle en Côte d'ivoire. European Journal of Scientific Research.66 (4); 575-585.

[17] N'negue ép. Mezui-Mbeng, M.-A., Ella Ndong, J. G., Nguema Edzang, R. W., Lendoye, E., Ngou Milama, E., 2020. Cinétique d'étude de l'activité antioxydante d'un extrait aqueux de calices séchés d'*Hibiscus sabdariffa* par piégeage de l'ion radicalaire $ABTS^{\bullet+}$. IJAR.6 (11): 361-367.

[18] Nga Nnanga, E., Yinyang, J., Baran à Bidias, E., Etame-Loe, G., Dibong, D. S., 2017. Etude phytochimique et pharmacologique d'*Alchornea cordifolia* (Schum. & Thonn.) Mull. Arg. et de *Mangifera indica* dans le traitement traditionnel de la maladie hémorroidaire. J. Appl. Biosci.109: 10649-10661.

[19] Olalye, M. T., Rocha, J. B., 2007. Commonly used tropical medicinal plants exhibit distinct in vitro antioxidant activities against hepatotoxins in rat liver. Exp Toxicol Pathol.58 (6): 433-8. doi: 10.1016/j. etp.2007.01.002.

[20] Osadebe, P. O., Okoye, F. B. C., Uzor, P. F., Nnamani, N. R., Adiele, I. E., Obiano, N. C., 2012. Phytochemical analysis, hepatoprotective and antioxidant activity of Alchornea cordifolia methanol leaf extract on carbon tetrachloride-induced hepatic damage in rats. Asian Pacific Journal of tropical medicine.5 (4): 289-293.

[21] Oroian, M., Escriche, I., 2015. Antioxidants: Characterization, natural sources, extraction and analysis. Food Research International.74: 10-36.

[22] Pisoschi, A. M., Pop, A., 2015. The role of antioxidants in the chemistry of oxidative stress: A review. European Journal of Medicinal Chemistry.5 (97): 55-74.

[23] Pompermaier, L., Marzocco, S., Adesso, S., Monizi, M., Schwaiger, S., Neinhuis, C., Stuppner, H., & Lautenschläger, T., 2018. Medicinal plants of northern Angola and their anti-inflammatory properties. Journal of ethnopharmacology, 216, 26–36. https: //doi. org/10.1016/j. jep.2018.01.019

[24] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio. Med.26: 1231-1237.

[25] Sinan, K. I., Ak G, Etienne, O. K., Jekő, J., Cziáky, Z., Gupcsó, K., João Rodrigues, M., Custodio, L., Mahomoodally, M. F., Sharmeen, J. B., Brunetti, L., Leone, S., Recinella, L., Chiavaroli, A., Orlando, G., Menghini, L., Tacchini, M., Ferrante, C., Zengin, G., 2021. Deeper Insights on Alchornea cordifolia (Schumach. & Thonn.) Müll. Arg Extracts: Chemical Profiles, Biological Abilities, Network Analysis and Molecular Docking. Biomolecules.11 (2): 219. doi: 10.3390/biom11020219.

[26] Siti, H. N., Kamisah, Y., Kamsiah, J., 2015. The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease. Vascular Pharmacology.71: 40-56.

[27] Yehye, W. A., Rahman, N. A., Ariffin, A., Abd Hamid, S. B., Alhadi, A. A., Kadir, F. A., Yaeghoobi, M., 2015. Understanding the chemistry behind the antioxidant activities of butylated hydroxytoluene (BHT). European Journal of Medicinal Chemistry.101 (7953): 295-312.