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In vitro antioxidant activity of extracts of the root *Cochlospermum planchonii* Hook. f. ex. Planch (Cochlospermaceae)

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Abstract
In the present study, we have evaluated the antioxidant activity of four root extracts of *Cochlospermum planchonii* successively obtained by the solvent extraction method of increasing polarity. These are Dichloromethane extracts (EDCm), Acetatic (EACe), Ethanolic (EEth 96 %) and Aqueous (EAq). The determination of total phenols and total flavonoids by the colorimetric method showed that respectively the EEth 96% contains 476 ± 1.15 mg GAE/g of extracts and EACe have 24.63 ± 0.28 mg QE/g of extracts. In addition, evaluation of antiradical power extracts towards the DPPH confirms that EEth 96% and EACe are also the most active; with IC_{50} respectively, of the order of 01.83 ± 0.74 and 03.7 ± 0.98 mg / mL compared to vitamin C (01.25 ± 0.02 mg / mL) standard used as reference molecule. Furthermore, testing the ability of chelating iron ions to extract by EEth 96% better with an IC_{50} in the range of 03.42 ± 0.14 mg / mL as vitamin C to IC_{50} = 04, 08 ± 0.01 mg / mL. Similarly, the EACe, EDCm and EAq have shown good chelators, but less active than vitamin C. In addition, the EACe, EEth 96 % and EAq themselves have so good reducing the ferric ion to ferrous ion respectively Concentrations Effective (EC_{50}) of approximately 16.80 ± 0.06; 06.50 ± 0.03 and 22.50 ± 0.06 mg / mL.

Keywords: *Cochlospermum planchonii*, Antioxidant activity, Chelating and Reducing power.

1. Introduction
Antioxidant molecules are currently the subject of numerous studies. They have an interest in the conservation of food, edible and food protection against the oxidative damage (Caillet and Lacroix, 2007) [10]. So they are useful in the prevention and treatment of cancer, diabetes, cardiovascular diseases, inflammatory diseases, neurodegenerative and other diseases in which oxidative stress is implicated (Pincemail *et al*., 2002; Koechlin-Ramonatxo, 2006) [20, 34]. To escape the consequences of oxidative stress, it is necessary to restore the balance oxidant / antioxidant to preserve the physiological performance of the organization.
However, the use of synthetic antioxidant molecules is currently challenging because of potential toxicological hazards and carcinogenic effects (Bougadoura and Bendimerad, 2012) [8]. Hence the need to substitute natural antioxidants. Now, new plant sources of antioxidants are sought. (Suhaj M., 2006; Tadhani and *al.*, 2007) [36, 38]. Medicinal plants are an inexhaustible source of substances with varied biological and pharmacological activities. Scientific research has been developed for the extraction, identification and quantification of these compounds from medicinal plants (Huang et *al*., 2005; Bidié et *al.,* 2011) [6, 15].
Endowed with these natural substances have antioxidant activity with socioeconomic interest in the field of bio- pharmacological research (N'guessan *et al*., 2009) [31]. It is in this context that the present work focused on *Cochlospermum planchonii* Hook f. ex. Planch (Cochlospermaceae), a plant species of the savannas of West Africa up to 0.5 to 1.5 m in height. It grows from Guinea to eastern Tchad. This plant has several medicinal and traditional food applications in these African countries for the treatment of malaria, hemorrhoids, gall fever, jaundice, back pain, intestinal pain and stomach worms, bilharzias, diarrhea, infertility and hepatitis (Adjanohoun and Aké-Assi, 1979; Burkill 1985; Koné and *al.,* 2002; Koné and *al.,* 2004; Igoli and *al.,* 2005; Blench, 2007) [2, 9, 21, 22, 16, 7]. In addition, scientific studies have demonstrated its effectiveness against certain associated with oxidative stress, such as hepatitis (Aliyu *et al.,* 1995) [3].Diseases depression of the central nervous system, analgesic and anti-inflammatory (Anaga and Oparah, 2009) [5], diabetes (Yakubu and Nafiu, 2010) [40],

hyperglycemia (Nafiu, 2011a) [29] and infertility (Abu *et al.*, 2012) [1]. This present work is essential to evaluate the antioxidant activity of four root extract *Cochlospermum planchonii* goal.

2. Materials and Methods

2.1 Plant material

Fresh roots of *Cochlospermum planchonii* Hook f. ex. Planch (Cochlospermaceae) were collected in September 2012 in the north of Côte d' Ivoire specifically near the village of Kapissorivogo, located at about 3 km north of the city of Ferkessédougou. After harvesting the roots, along with samples of leaves and fruits, the plant was identified by Professor Aké-Assi Laurent National Centre Floristic (CNF) of the Félix Houphouët- Boigny University of Cocody, where a specimen is under number 14643 of Cochlospermaceae family

2.2 Technical equipment

The technical equipment includes a mechanical grinder type IKAMAG, a magnetic stirrer RCT type IKAMAG a rotating evaporator Heidolph Type; a UV-Vis spectrophotometer (biomérieux), a P-type oven SELECTA and a precision balance (Denver Instrument)

2.3 Reagents

The reagents used are mainly the Folin-Ciocalteu reagent, sodium carbonate, methanol, aluminum trichloride, potassium acetate, 2, 2-diphenylpicrylhydrazyl (DPPH), phosphate buffer, ethanol, hydrochloric acid, ferrous chloride, trichloroacetic acid, the ferrosine, potassium ferricyanide, quercetin and gallic acid provided by RYCA-PHARMA and CLE (Chemical Laboratory Equipment).

2.4 Preparation of plant extracts

Roots of *Cochlospermum planchonii* are harvested, cleaned, cut, dried in sunlight for two weeks and made into powder using a grinder IKAMAG kind. The preparation of crude extracts of the plant was carried out by the extraction method of increasing polarity solvents described by Diallo et *al.* (2004) **[12].** We used successively in the order four solvents: dichloromethane, ethyl acetate, 96 % ethanol and water. Two hundred and fifty grams of plant powder is soaked in 2 L of dichloromethane for 24 h under a magnetic stirrer type IKAMAG-RCT. The homogenate obtained is filtered successively with a clean cloth, twice cotton wool and once on Whatman paper N°2. The filtrate gave after drying 28.4 g (11.40%) of dichloromethane extract (EDCm). The same operation as above is repeated on the dried pomace to room temperature with 2 L each of ethyl acetate, 2 L of 96 % ethanol and 2 L of distilled water. We obtained 12.7 g (05.08 %) acetate extract (EACe) and 11.20 g (04.48 %) ethanolic extract (EEth 96%). The solutions of the previous extracts are distilled in vacuo in a rotating evaporator Heidolph-type respectively at a temperatures of 40 °C ; 43 °C and 45 °C while the aqueous solution is dehydrated by the means of a P-type SELECTA oven at 50 °C to obtain the aqueous extract (EAq) giving 17.40 g (06.96 %).

2.5 Doses of polyphenols

2.5.1 Determination of total phenols

The total phenolic contents of four extracts of *Cochlospermum planchonii* were determined by the Folin-Ciocalteu method (Mc Donald et *al*., 2001) **[25].** To 0.5 mL of each plant extract of concentration 0.1 mg / mL, respectively were added 5 mL of Folin-ciocalteu diluted 1/10 in distilled water and 4 mL of sodium carbonate (1M). The whole is incubated at room temperature for 15 minutes. The optical densities (OD) are then read in a spectrophotometer at 765 nm against a blank. Gallic acid was used as standard and prepared under the same conditions as above with a solvent mixture of methanol / water (50:50, V / V) at concentrations ranging from 0 to 0.5 mg / mL. The total phenolic contents of the extracts are expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). (Graph 1)

2.5.2 Determination of total flavonoids

The technique used for the determination of the levels of total flavonoids extracted from *C. planchonii* is the colorimetric method of aluminum trichloride described by Yi et *al.*, (2007) **[41]**. Thus , 0.1 mL of 5 mg / mL of each extract plant are collected , to which are successively added 1.5 mL of methanol , 0.1 mL of 10 % aluminum trichloride , 0.1 mL of potassium acetate (1M) and 2.5 mL of distilled water. After incubation at room temperature for 30 minutes, the optical densities were measured in a spectrophotometer at 415 nm. A methanolic solution of quercetin with concentrations ranging from 0 to 100 mg / mL is used as a standard. The contents of flavonoids extracts are expressed in milligrams of quercetin equivalent per gram of extract (mg QE / g extract). (Graph 2)

2.6 *In vitro* evaluation of the antioxidant activity

2.6.1 Measurement of anti-radical power

The measurement of the antiradical activity of plant extracts was performed by testing the 2, 2- diphenyl-1-picrylhydrazyl (DPPH) according to the method of Parejo et *al.,* (2000) **[32]**. From a stock solution of each plant extract to 0.1 mg / mL, a concentration range is prepared by successive doubling dilution of 1.56 mg / mL to 100 mg / mL. Then, each extract concentration, the same volume of a methanolic solution of DPPH is added. After 30 minutes of incubation at room temperature (37 °C) and protected from light, the absorbance is read in a spectrophotometer at 517 nm against a blank sample (0 mg / mL of extract). Vitamin C (100 mg / mL) which is the reference material is prepared in the same conditions. (Graph 3)

The percentage inhibition of DPPH radicals is calculated by the following formula:

Inhibition (%) = [(white ABS - ABS sample / white ABS)] x 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.

2.6.2 Chelating power measurement

The colorimetric method of Le et *al*., (2007) **[24]** based on the determination of the complex formed by the ferrous ion (Fe^{2+}) and which was used to measure ferrosine the chelating power plant extracts. Thus, 3.7 mL of methanol , 0.1 mL of iron II chloride (2 mM) and 0.2 mL of ferrosine (5 mM) were added successively to 1 mL of each sample at various concentrations achieved by dilution of the order of from 2 100 to 0.78 mg / ml to initiate the reaction . After vigorous stirring and then incubated at room temperature for 10 minutes, absorbance are

read at the spectrophotometer at 562 nm against a blank. Vitamin C is used at different concentrations as compared to the reference solution for the chelating activity of the extracts (Graph 4). Chelation samples can be determined using the following formula:

Power chelator (%) = [(white ABS - ABS sample) / ABS white] x 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.

2.6.3 Measurement of reducing power

The method described by Yildirin et *al.,* (2001) [42] was used for determining the reducing power of the plant extracts. It measures the ability of the extracts to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). Thus, 1 mL of each extract and vitamin C at different concentrations are mixed separately with one mL of phosphate buffer (0.2 mM, pH 6.6) and 1 mL of 1 % potassium ferricyanide. The mixture was incubated in a water bath at 50 ° C for 30 minutes. After adding 1 mL of 10 % trichloroacetic, the reaction mixture was centrifuged at 3000 revs / min for 10 minutes acid. Supernatant are added to 0.1 mL of ferric chloride and 2 % to 0.1 mL of distilled water. After 10 minutes incubation at room temperature, the absorbance is measured with a spectrophotometer at 700 nm against a blank, prepared in the same conditions. The increase in absorbance of the sample indicates an increase in reducing power, according to the concentrations of the extracts and vitamin C. The concentrations inducing an absorbance of 0.5 nm (EC_{50}) of the extracts were determined and compared to that of vitamin C. (Graph 5)

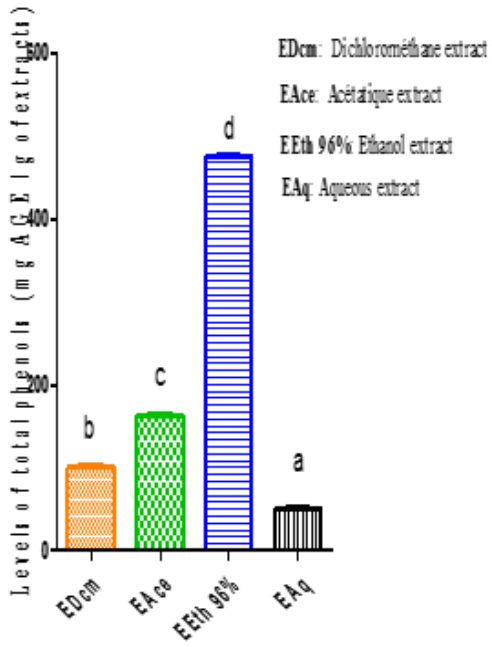
2.7 Statistical Analysis

Statistical analysis was performed by Graph Pad Prism 5 statistical software. Results are expressed as mean ± SD and analyzed by ANOVA and Tukey tests with the univariate rate determination of significance with $P \leq 0.05$ considered statistically significant.

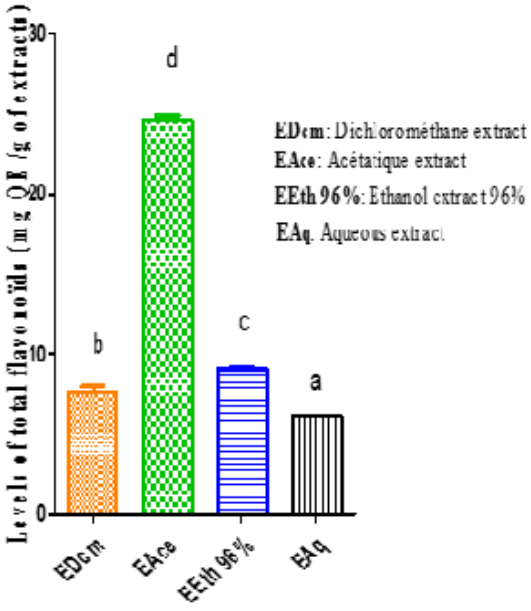
3. Results and Discussion

3.1 Contents of total phenols and flavonoids root extracts of *C. planchonii*

The levels of total phenols and total flavonoids of root extracts of *C. planchonii* are determined from the calibration line y = 0.004 x + 00; $R^2 = 0.998$ and y = 0.037 x + 00; $R^2 = 0.997$ plotted using standard as gallic acid and quercetin, respectively.



Graph 1: Levels of total phenols and total flavonoids of root extracts of *C. planchonii*. (Mean ± SD of three trials).



Graph 2: Levels of total phenols and total flavonoids of root extracts of *C. planchonii*. (Mean ± SD of three trials).

mg GAE / g of extracts: milligrams gallic acid equivalent per gram of extract.
mg QE / g of extracts: milligrams of quercetin equivalent per gram of extract.

Table 1: Levels of total phenols and total flavonoids of root extracts of *Cochlospermum planchonii*. (Mean ± SD of three trials).

Extracts	total phenols mg GAE / g of extract	total flavonoids mg QE / g of extract
EDcm	100.66 ± 3.48^{b}	07.62 ± 0.35^{b}
EAce	162.66 ± 2.60^{c}	24.63 ± 0.28^{d}
EEth 96%	476 ± 1.15^{d}	09.12 ± 0.05^{c}
EAq	50.33 ± 2.60^{a}	06.15 ± 0.11^{a}

Medium extracts the same column with different letters (a, b, c, d) with superscript are significantly different from the smallest to the largest average at $P \leq 0.05$.

The results of determination of total phenols by the Folin-Ciocalteu reveal that EEth 96% is the richest in phenolic compounds (476 ± 1.15 mg GAE / g extract), followed by the EACe (162 66 ± 2.60 mg GAE / g of extract), and of which contains EDCm (100.66 ± 3.48 mg GAE / g of extract), while only contains EAq (50. 33 ± 2.60 mg EAG / g extract). (Table1)

The quantitative estimation of total flavonoids by aluminum trichloride method shows that the EACe (24.63 ± 0.28 mg QE / g extract) and EEth 96% (9.12 ± 0.05 mg QE / g of extract) are the richest in flavonoids , thereafter comes the EDCm (07.62 ± 0.35 mg QE / g extract) followed by EAq (06.15 ± 0.01 mg QE / g of extract). (Table 1)

By comparing the assay results to those of Nafiu et *al*., (2011b) [30] who estimated 3.16% of polyphenols after the saponins to 07.5 % (major component) in root extracts *C. planchonii* and 0.07 % content of flavonoids; we can say that the content of our extracts, especially that of EEth 96% (476 ± 1.15 mg GAE / g) and the EACe (162.66 ± 2.60 mg AGE / g extract) have much higher values. It is the same for flavonoid levels. However, it is difficult to compare these results with those of the bibliography for the use of the different extraction methods reduces the reliability of comparisons between studies. Several factors can affect the content of phenolic compounds (Pedneault et *al.,* 2001) [33]**.** Recent studies have shown that extrinsic factors (such as geographic and climatic factors), genetic factors, but also the degree of maturation of the plant and the storage time has a strong influence on the content of polyphenols and flavonoids (Fiorucci, 2006) [14]**.**

In addition, work conducted by Katalinic et *al.,* (2010) [18]**,** confirms our results indicate that ethanol enables better extraction of total polyphenols. This largely explains the richness of the ethanol extract of the roots of *C.planchonii* compared to other plant extracts.

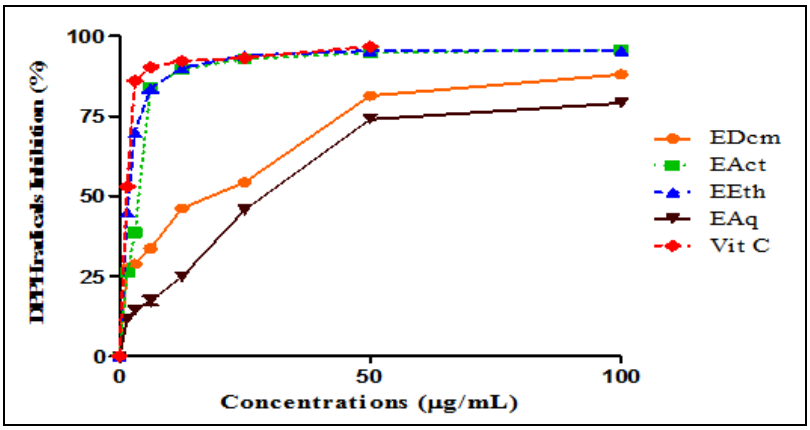
3.2 Anti-radical activity, chelating and reducing powers root extracts of *C. planchonii*

The anti-radical activity obtained reveal that EDCm, EACe, EEth 96% and EAq and have a dose-dependent activity. All extracts have good anti-radical. For the comparison between these extracts, reveals that EEth 96%represents the most active extract with an IC_{50} of 01.83 ± 0.74 µg / mL , followed by the EACe with an IC_{50} of about 03 70 ± 0.98 µg / mL and an IC_{50} EDCm with approximately 18.9 ± 0.50 µg / mL. EAq represents the lowest (IC_{50} = 28.70 ± 0.50 µg / mL) anti-radical activity. (Table 2)

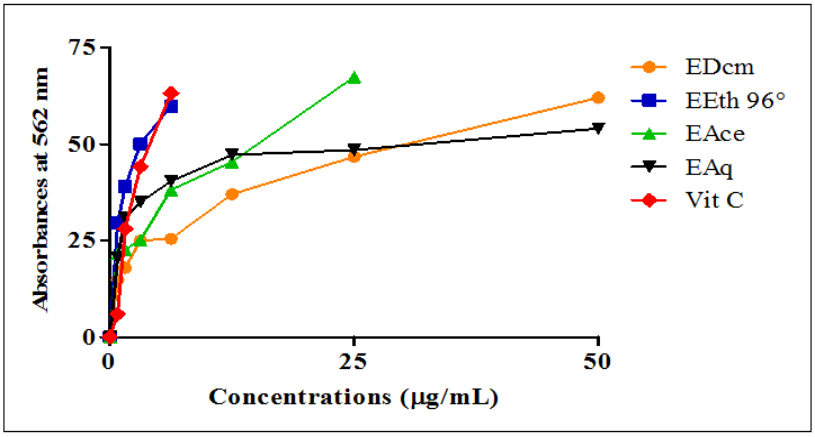
For comparative purposes, we used the standard antioxidant vitamin C as reference molecule. It showed an interesting anti-radical activity with an IC_{50} in the range of 01.25 ± 0.02 µg / mL. When compared with the extract EEth 96% tested with an IC_{50} in the range of 01.83 ± 0.74 µg / mL, the EEth 96% assets are very similar thereto. Especially EACe, EDCm and EAq are respectively less active only 3; 18 and 28 times with respect to vitamin C. The results compared to those of Michel Joyeux (1993) [26] support the activity acetic and ethanolic extracts of *Cochlospermum planchonii* Hook f. ex. Planch, with a difference of superiority of the activity of our extracts. Because according to the author, the aqueous extract of *Cochlospermum planchonii* causes discoloration of DPPH by 78% for a solution of 0.25 g / L and 49% for a solution of 0.125 g / L. It must be emphasized that it is not the same solvent, or even activity protocols and extraction; same shelf life and temperature could explain the discrepancy between our results and those of the bibliography (Klervi, 2005) [19]**.** Also, IC_{50} of EEth 96% and EACe is it superior to methanol extracts of *Chrysophyllum perpulchrum* (IC_{50} = 04.00 ± 0.288 mg / mL) obtained by Bidié et *al.,* (2011) [6] and those of *Combretum sp* (06.0 ± 0.45 g / mL) produced by N'guessan et *al.,* **(2007)** [28]. Phenolic compounds are good candidates for the antioxidant activity due to the presence of numerous hydroxyl groups, which can react with free radicals (Skolt-Letowska et *al.*, 2007) [37]. Good scavenging activity of EEth 96% and EACe is probably related to synergistic action content in polyphenols, flavonoids and carotenoids that abound in the plant. Thus, N'guessan et *al.*, (2007) [28] showed the existence of a correlation between the polyphenol contents and antiradical activity.

Furthermore, as regards the chelating of different extracts power, EEth 96 % seems to have a higher activity with an IC_{50} of 03.42 ± 0.45 µg / mL as vitamin C (IC_{50} = 04.08 ± 0.01 µg / mL). The chelating activity of EEth 96% is surely his explanation in a synergistic effect between the constituents of the extract which would contain polyphenolic compound such as ellagic acid, gallic acid, ellagitannins, Cochlospermines AD and salts zinc (Aliyu et *al.,* 1995) [3]**.** EACe extracts (IC_{50} = 14.90 ± 0.35 µg / mL), EDCm (IC_{50} = 32.40 ± 0.6 µg / mL) and EAq (IC_{50} = 33.10 ± 0.60 µg / mL) prove 3.65; 7.94 and 8.11 respectively times less active than vitamin C. numerous studies have evaluated the effect of chelating ferrous ions by the extracts of various plants. The study by Le et *al*., (2007) [24] shows that the effective concentration of the ethanolic extract of the fruit of *Lycium barbarum* is about 10 mg / mL , this value is significantly lower than the values obtained with different extracts of roots of *C. planchonii* , reflecting a significant chelating activity of the roots of our plant. The chelating ability is very important because it reduces the concentration of transition metal catalysts of the lipid per oxidation. Indeed, the iron can stimulate lipid oxidation by the Fenton reaction, and this also accelerates oxidation by decomposing hydroperoxides into peroxyl and alkoxyl radicals, which in turn can maintain the chain reaction (Elmastas et *al.*, 2006) [13]. According to the literature, the phenolic compounds are found to be good chelating metal ions (Morris, 1995) [27].

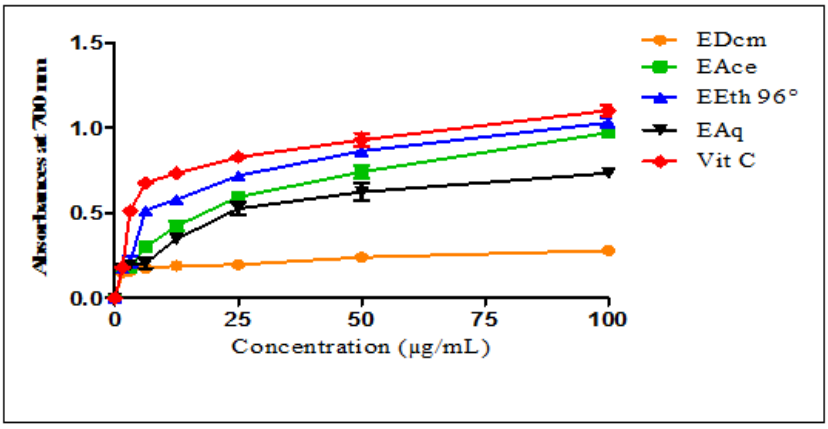
The presence of reducers in plant extracts causes the reduction of ferric iron (Fe^{3+}) with ferricyanide complex to the ferrous form. Consequently, the ferrous ion (Fe^{2+}) can be estimated by measuring and monitoring the increase in the density of the blue color in the reaction medium at 700 nm (Chung and *al*., 2002; Amarowicz and *al.*, 2004) [11, 4]. Reducing the power of plant extracts is dose-dependent. At a concentration of 50 mg / mL, the reducing power of EACe, EEth 96% and EAq is less effective than the vitamin C (EC_{50} = 02.80 ± 0.03 µg / mL). Reductive activity EDCm could be quantified because the highest concentrations tested, it does not reduce more than 30 %. Nevertheless, the EEth 96 % has a reducing activity close to that of Vitamin C. The reducing power of the species *C. planchonii* is probably due to the presence of hydroxyl groups in the phenolic compounds that can be used as the electron donor. Accordingly, antioxidants are considered to reduce oxidizing and inactivators (Siddhuraju et *al.,* 2007) [35]. Some previous studies have also shown that the reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. (Jeong et *al.*, 2004; Kumaran et *al.*, 2007) [17, 23].



Graph 3: Anti-radical power root extract of *C. planchonii*



Graph 4: Chelating power root extract of *C. planchonii*



Graph 5: Reducing power of root extracts of *C. planchonii*

Table 2: Anti-radical, chelating and reducing power root extract of *C. planchonii*

Extracts	Anti-radicalair activity CI_{50} (µg / mL)	Chelating activity CI_{50} (µg / mL)	Reducing power (CE_{50} µg / mL).
EDCm	18.90 ± 0.50[a]	32.40 ± 0.65[b]	Nd[a]
EACe	03.70 ± 0.98[a]	14.90 ± 0.35[b]	16.80± 0.06[b]
EEth 96%	01.83 ± 0.74[a]	03.42 ± 0.14[b]	06.50± 0.03[b]
EAq	28.70 ± 0.16[a]	33.10 ± 0.60[b]	22.50± 0.06[b]
vitamin C	01.25 ± 0.02[a]	04.08 ± 0.01[b]	02.80± 0.03[b]

The average of a column bearing the letters «a, b» superscript is significantly different from the smallest to the largest average P ≤ 0.05 previews.

*¨**ND**¨: Not Detectable; ¨**EC_{50}**¨: Effective concentration 50 mg/mL*

5. Conclusion

At the end of our work, the results can be concluded that the EDCm, EACe, EEth 96% and EAq roots of *Cochlospermum planchonii* containing flavonoids and phenolic compounds at different levels according to their degree of solubility in our solvents. Each extract has a given antioxidant activity which is a function of the content of polyphenol compounds. It is clear from this work that the antioxidant activities of EEth 96% and EACe are the most important and close to that of vitamin C. This is due to the wealth of EEth 96 % total phenols and the EACe flavonoids and carotenoids. This activity supports the use of the roots *Cochlospermum planchonii* in handmade, shea butter and against certain diseases related to oxidative stress.

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