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Phytochemistry and Antioxidant Activities of the Methanolic Leaf Extract of *Clerodendrum splendens* (Lamiaceae)

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Abstract

This paper aimed at studying the antioxidant efficacy of the methanolic leaf extract of *Clerodendrum splendens*, a plant of the Lamiaceae family. Phytochemical tests carried out on extracts of *Clerodendrum splendens* leaves have been able to detect the presence of secondary metabolites such as Flavonoids, Tannins, Saponins and Terpenoids. The results of the antioxidant activity have shown that CSF2, CSF3 fractions and CSB, CSG fractions similarly inhibited hepatic lipids but significantly less than vitamin C. Compared to all fractions, the CSB fraction shows the best inhibitor on the peroxidation of hepatic lipids because at 150 µg/mL, there is a maximum activity (2.5 µg/mL of protein). However, it is found that CSF3, CSF2 and CSG have higher IC50 values than vitamin C (5.613±0.117) while CSEB, CSB and CSC fractions showed lower IC50 values than vitamin C, which is used as the reference reducing compound. The lower the IC50 value compared to vitamin C, the greater the antioxidant capacity of the plant extract. The results of this study suggest that *Clerodendrum splendens* represents an untapped source of compounds with potential antioxidant activity that could be explored in the development of new therapeutic natural products.

Keywords: Antioxidant activities, *Clerodendrum splendens*, Lamiaceae, Phytochemistry.

Introduction

Since ancient times, plants play a very important role in the daily life of men because they are used as firewood, raw materials in real estate, decoration and in the treatment of diseases. The use of extracts from different parts of plants in the preparation of therapeutic potions is a more cultural than social attribute (Falkenberg et al., 2002). Today, these are a hive of drugs because they are fully integrated into the morals of Africa and intervene in traditional pharmacopoeia in the fight against many diseases such as cancer, malaria, dysentery, yellow fever, ulcers, gonorrhea (Berhaut, 1975). If medicinal plants are widely used in African regions and particularly in Cameroon by traditional healers to solve public health problems, their use requires the expertise of researchers to study the properties of these plants, to assess the active dosage and their toxicity which is often unknown.

In recent years, the natural antioxidants found in medicinal plants are the subject of several scientific researches as a result of their ability to prevent the oxidative stress which is the root cause of several cardiovascular, neurodegenerative and certain types of cancer. Among the medicinal plants known for their antioxidant activity, the genus *Clerodendrum* is one of them. Interest in the role of antioxidants in human health has prompted research in the fields of food science and medicinal herbs to evaluate the role of herbs as antioxidants.

Clerodendrun splendens, family Lamiaceae is a shrub about 3.7m tall. It has dark green leaves that are simple and opposite (Oppong, 2014). The leaves of *Clerodendrum splendens* are used in traditional medicine by indigenous people to treat shingles, spleen in children, asthma, rheumatism, ulcers and malaria (Nnanga et al., 2016). In vivo and in vitro studies conducted by many researchers have shown that *Clerodendrum splendens* has antioxidant properties (Okwu and Iroabuchi 2009; Amal, 2014). The concentration dependent activity of the methanolic leaf extract of *Clerodendrum splendens* annulled the oxidative stress resulting from aggressions. These results can be used as a prerequisite for screening of plants for bioactive compounds for medicinal purposes. Antioxidants are compounds that stop molecular oxidation and play a vital role in protecting the body's defense mechanism against free radicals and reactive oxygen species, which are generated continuously in the body, because of both normal metabolism and some diseases. (Gate, 1999; Gutteridge and Halliwell

2000) Vitamin C (ascorbic acid), its antioxidant properties are attributed to its ability to be reduced to ascorbyl radical after the loss of an electron or a proton. This radical can easily oxidize by capturing the superoxide anion and some radical species (perhydroxyls and peroxyls) (Valko et al., 2006; Antwerpen, 2006). These exogenous antioxidants are also known as natural antioxidants that exist in food and medicinal plants. Empirical use of natural antioxidants is a very old practice for food preservation. The search for new natural antioxidants has been gaining in popularity in recent years, as the synthetic antioxidants currently used, such as Butylhydroxytoluene (BHT) and Butylhydroxyanisole (BHA), are not devoid of toxicity (Barlow, 1990). Phytochemical studies carried out on *Clerodendrum splendens* have shown the presence of secondary metabolites including Carbohydrates, Steroids, Terpenes and Flavonoids (Rohitash et al., 2012). Thus, the aim of this study was to evaluate the antioxidant activity of the methanolic leaf extracts of *Clerodendrum splendens* (Lamiaceae).

Material and methods

Apparatus and equipment

After drying, the leaves of *Clerodendrum splendens* were crushed using a grinding machine. Maceration of the powder was done in methanol in a tightly closed 20 L container. A MARQLUTAN GM-300P electronic scale was used to weigh the raw extract and the different masses of the fractions while separation of the extract from the solvent was carried out using a Büchi brand Heidolph WB 200 rotary evaporator.

Plant material

The leaves of *Clerodendrum splendens* were harvested by Mrs. NDZANA Marie and LEKINI Gisele at Mount Mbankolo in Yaounde in the Centre Region of Cameroon in September 2016. This plant was identified in comparison with the sample of *Clerodendrum splendens* (G Don) A. Koufani 2009 collector of *Clerodendrum splendens* (G. Don) of the specimen with a sample from the National Herbarium of Cameroon under the number 41512/HNC.

Maceration of the Plant Material

The leaves of *Clerodendrum splendens* were dried, crushed and a powder of 2836.76 g was obtained. This powder underwent triple extraction by maceration with pure methanol for 72 hours. The filtrate obtained was evaporated to dryness using a rotary evaporator under reduced pressure and 188.92 g of crude extract was obtained. 100.08 g of this crude extract was cold fixed on 90 g of silica gel (SiO2) (0.063-0.200 mm) and the Buchner was filled with 101.02 g of silica as a stationary phase to undergo flash chromatography. Elution of this extract was done with solvents and gradient solvent systems of increasing polarity such as: hexane, hexane/ethyl acetate, ethyl acetate, ethyl acetate/methanol. We collected 120 fractions of about 400 mL each. The 120 fractions were regrouped into 8 major fractions (A, B, C, D, E, F, G and H) based on TLC analyses. From these fractions, we had the following different fractions CSB, CSEB, CSG, CSEB, CSC, CSF2, CSF3. This study took place at the laboratory of the University of Maroua in Cameroon.

Preliminary phytochemical analysis

The phytochemical screening of methanolic extracts of *Clerodendrum splendens* was performed according to the standard procedure by Harbone (Harbone, 1998). All extracts of *Clerodendrum splendens* prepared were subjected to a preliminary phytochemical screening for the presence of phenolic compounds, glycosides, Anthraquinones, Terpenoids, Flavonoids, Tannins, Carbohydrate, Lignans and Saponins.

Antioxidant activities
Catalase activities

Catalase activity procedure

In the spectrophotometric vats (white and tests), 200 µL of H2O2 (9 %) were introduced 1150 microliters and 1135 µL of phosphate buffer (0.1 M pH 7.2) were respectively introduced into the white test tanks. 15.5 microliters of DMSO 0.025 % (white tank), Vitamin C or CSB, CSEB, CSG, CSEB, CSC, CSF2 (for final concentrations of 0, 50, 100, 150, 200 and 250 µg/mL) were added in the vats. Two hundred microliters of hepatic PMS were then added to the test tanks and after homogenization, the ODs were read at 240 nm at 30, 60 and 90 s. The enzymatic activity of catalase, expressed in IU/mg of testicular PMS protein, was calculated from the formula:

$$\text{Activity} = \frac{M.V.10^3}{t.\varepsilon.l.Cp.v}$$

M: OD difference at 240 nm; **V:** total volume of the reaction medium **t:** reaction time in min; ε: molar extinction coefficient (40.M-1cm-1) **l:** optical path of the vessel; **Cp:** protein concentration of the PMS; **v:** volume of the PMS used.

Trapping activity of the radical DPPH° (1, 1-diphenyl-2-picrylhydrazyl)

Principle

The DPPH° radical is trapped directly by an antioxidant (AH) which gives it a hydrogen atom and reduces it to DPPH-H. This results in a color change in the DPPH methanolic solution that gradually changes from purple to yellow. This color change is measured at λ=517 nm (Gokhan et al., 2010).

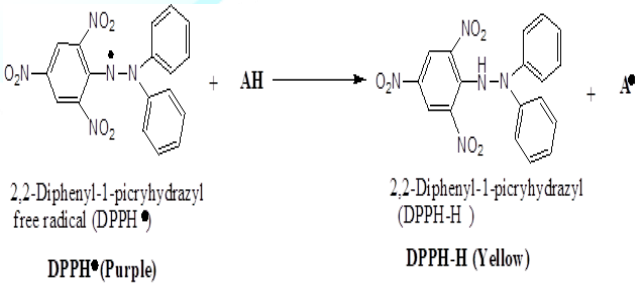

Figure: 1 DPPH Free radical conversion to DPPH-H by antioxidant compounds.

Procedure

50 µL of extract was added to each test tube containing 3.1 mL of the methanolic solution of DPPH 40 µg/mL. In negative control tubes, the extract was replaced with 50 µL of DMSO. The mixtures were well homogenized and incubated in the dark for 30 minutes at room temperature. The absorbances read at λ=517 nm was used to calculate

the trapping percentages, the trapping concentrations fifty (CP50), the effective concentrations fifty (EC50) and finally the anti-free radical powers (PA) according to the following formulas:

$$\%\ piegeage = \frac{DOtémoin - DOessai}{DOtémoin} \times 100 \quad (1)\ CE_{50} = \frac{CP_{50}}{C}\ ;\ PA = \frac{1}{CE_{50}}$$

C: Concentration of DPPH mol/mL

Trapping activity of the OH° radical

Principle

In the presence of FeSO4 and H2O2, the hydroxyl radicals (OH°) are formed. The latter, coupled with sodium salicylate, form a violet complex which absorbs at λ=562 nm. The intensity of the coloration is inversely proportional to the amount of free radical in the medium (Zhenwei et al., 2009).

Procedure

In each test tube, 50 µL of polyphenol extract, 0.7 mL of FeSO4 (3 mM), 1 mL of H2O2 (1 mM), 1 mL of distilled water and 0.4 mL of sodium salicylate were added (10 mM). In the negative control tubes, the extract was replaced with 50 µL of DMSO while the white contained distilled water instead of sodium salicylate. The mixtures were incubated at 37°C for 1 hour and the absorbances were read at λ=562 nm against the white. The different percentages of entrapments were calculated using formula (1) (Zhenwei et al., 2009).

Ferric ion reduction capacity (FRAP)

Procedure of ferric ion reduction capacity (FRAP)

In test tubes (blank, assays), 200 µL of freshly prepared FRAP reagent (potassium ferricyanide 1 %) and 500 µL of phosphate buffer (white test tube), vitamin C or CSB, CSEB, CSG, CSC CSF2 (for final concentrations of 0, 50, 100, 150, 200 and 250 µg/mL) are introduced. In parallel, 200 µL of FRAP reagent and 10 µL of a ferrous sulphate solution (to obtain the final concentrations of 0, 50, 100, 150, 200 and 250 µg/mL) are introduced into the standard test tubes. All the tubes are incubated for 20 min at 50°C, 500 µL of 10 % TCA added and the ODs read at λ=593 nm. The Fe2+ TPZ concentrations characterizing the reducing activity of the products are determined from the OD calibration curve as a function of the ferrous sulphate concentration.

Reducing activities Potassium ferricyanide reduction test

Principle

This test is based on the reduction of potassium ferricyanide K3[Fe(CN)6] to potassium ferrocyanide K4[Fe(CN)6] by an antioxidant. This reduction results in the change of the yellow color of the solution to green in the presence of ferric chloride (FeCl3) and the absorbance of the solution is read at λ=700 nm (John et al., 2010).

Procedure

In each test tube were successively introduced 50 µL of extract, 1 mL of phosphate buffer (0.2 mM, pH 6.6) and 1 mL of potassium ferricyanide (1 % w/v). The mixtures were incubated (50 °C, 20 minutes). After incubation, 1 mL of trichloroacetic acid (TCA) 10 % w/v was added and the mixtures centrifuged (3000 rpm, 10 minutes). To 1 mL of aliquot of each mixture, 1 mL of distilled water and 0.2 mL of ferric chloride (0.1 % w/v) were added and the absorbances were read at λ=700 nm (John et al., 2010).

Inhibition of lipid peroxidation

Initiation and inhibition of lipid peroxidation in rat liver homogenate

Principle

Coupled with Fe2+, H2O2 releases the hydroxyl radical (HO°) which attacks the ethylenic bonds of unsaturated fatty acids (AGI) to oxidize them. Reactive acid substances are formed with Thiobarbituric acid (ATB), including malondialdehyde (MDA), which reacts in acid medium and when heated with two molecules of ATB to form a pink complex that absorbs at λ=532 nm according to the equation below (Xiao-Yu et al., 1989).

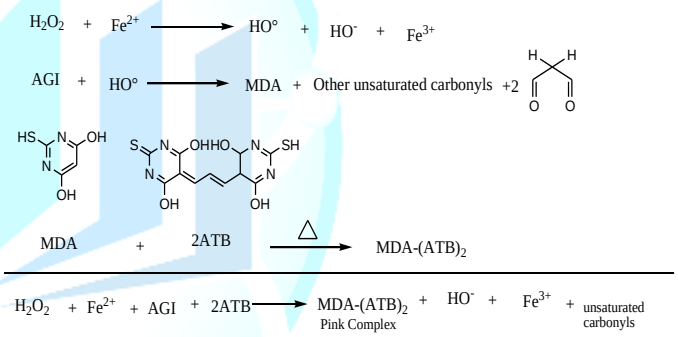

Figure 2: Scheme representing formation of aldehydic product of lipid peroxidation induced by iron or hydroxyl radicals.

Procedure for the inhibition of lipid peroxidation

800 µL, 1000 µL and 900 µL of Phosphate buffer (50 mM, pH 7.4) were introduced into test tubes: blank, control and test, respectively. One hundred microliters of hepatic homogenate (volume for a final protein content of 2.3 mg/mL in the tube) are added to the test tubes followed by 100 µL of Vitamin C, or CSB, CSEB, CSG, CSC, CSF2 (for final concentrations of 0, 50, 100, 150, 200 and 250 µg/mL). Lipid peroxidation is initiated by adding 100 µL 1 M Fe(SO4)2 to all tubes. After a 15 min incubation period at 37°C, the lipid peroxidation is stopped by adding 1 mL of 20 % TCA and then revealing the malonic aldehydes by adding 1 mL of 0.67 % TBA to the tubes. One hundred microliters of hepatic homogenate is then added to the control tube. The tubes are heated at 90 °C for 10 min, cooled and centrifuged at 3000 rpm for 15 min at 4 °C. The supernatants are removed and the ODs are read at 530 nm. The percent inhibition of lipid peroxidation (% I) is calculated using the following formula (Xiao-Yu et al., 1989).

$$\% I = \frac{DO_{witness} - DO_{essai}}{DO_{witness} - DO_{white}}$$

Results and discussion

Results

The leaves of *Clerodendrum splendens* harvested at Mount Mbankolo in Yaounde capital of Cameroon in September 2016, were cut, dried, crushed and extracted with methanol at room temperature. These different extractions made it possible to split into several extracts that the following codes were assigned: CSB, CSEB, CSG, CSEB, CSC, CSF2, CSF3. CSB: Fraction B ; CSC : Fraction C ; CSG : Fraction G ; CSF2 et CSF3 : Fraction F.

Phytochemical content of methanolic extracts of *Clerodendrum splendens*

Qualitative phytochemical screening of *Clerodendrum splendens* crude extracts showed the results presented in **Table 1**. From these results, a number of classes of metabolites were identified among all extracts of *Clerodendrum splendens*, among which Sterols, Alkaloids, Flavonoids, Sugar glycosides, Saponins, Quinones, Polyphenols, Triterpenes and Saponins were tested on extracts of *Clerodendrum splendens*.

Chemical compounds	Methanolic extract of *Clerodendrum splendens*					
	CSEB	CSF_3	CSB	CSG	CSC	CSF_2
Phenolic compounds	-	+	-	-	-	+
Flavonoids	+	++	+	+	+	++
Terpenoids	++	++	++	++	++	++
Tanins	+	++	+	+	+	++
Saponines	++	++	++	++	++	++
Alkaloids	-	-	-	-	-	-
Glucosides	+	++	+	+	+	++
Carbohydrates	+	+	+	+	+	++

Table 1: Phytochemical Screening of Methanolic extracts of *Clerodendrum splendens*.
++: abundant, +: trace, - : absent.

From the table above, the results show that *Clerodendrum splendens* leaves contain in abundance secondary metabolites such as Terpenoids, Saponins, carbohydrates and glucosides. They contain traces of Flavonoids and Phenolic compounds and alkaloids are completely absent. These results agree with the conclusions of many authors who identified the same classes of chemicals in extracts of roots, bark stems and leaves of *Clerodendrum splendens* (Burkill, 1988).

Antioxidant activities
Catalase activity

Principle

Catalase is an enzyme in the antioxidant cell system that catalyzes the decomposition of hydrogen peroxide (H2O2) to give water and oxygen. The higher the catalase activity as compared to vitamin C (the reference antioxidant compound), the more beneficial the plant is to the human system. At 240 nm, the absorbance of the non-degraded hydrogen peroxide is directly proportional to the catalase concentration of the sample (Misra and Fridovich 1972).

Figure 3 below shows that the different fractions stimulate the catalase activity, but to a lesser extent compared to the reference compound, vitamin C. The fractions CSC, CSG and CSB have a stimulating effect in a dose-dependent manner on the activity of Catalase, an effect clearly comparable to that of Vitamin C.

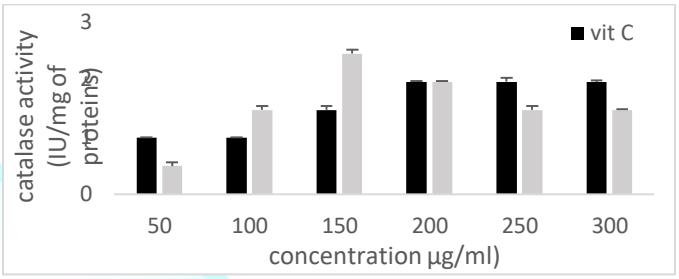

Figure 3: Effect of CSB crude extract.

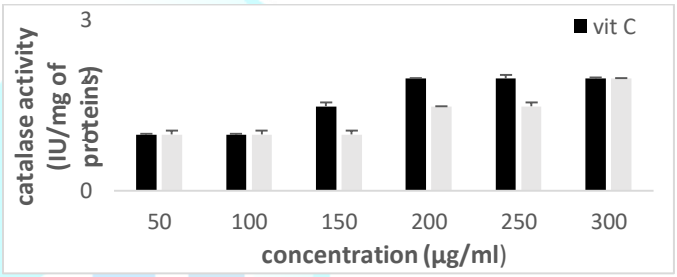

Figure 4: Effect of CSE3 crude extract.

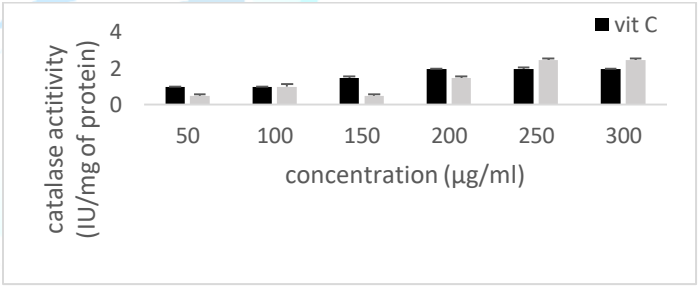

Figure 5: Effect of CSG crude extract.

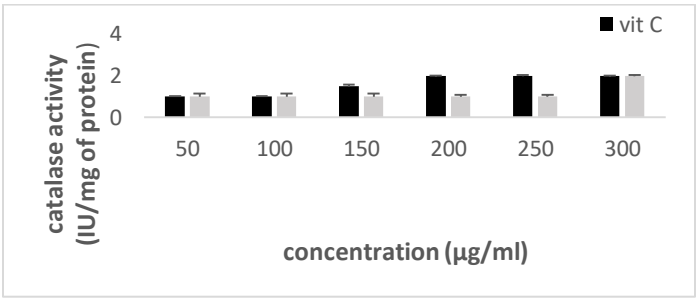

Figure 6: Effect of CSEB crude extract.

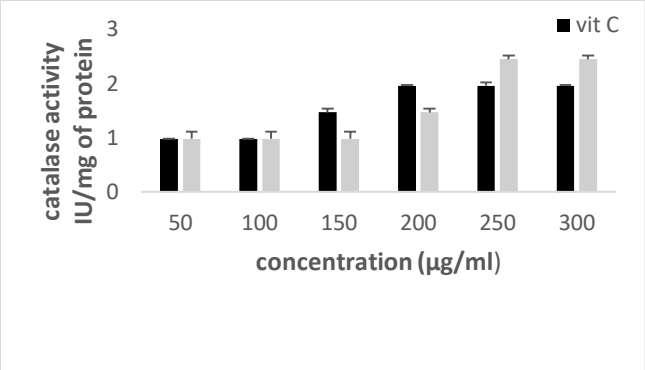

Figure 7: Effect of CSC crude extract.

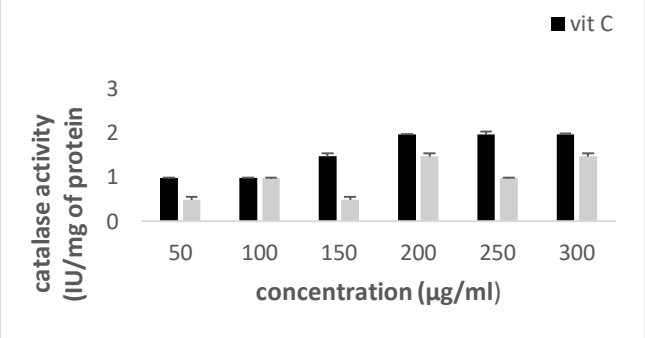

Figure 8: Effect of CSF2 crude extract.

Inhibition of the peroxidation of hepatic lipids

The different fractions inhibit the peroxidation of hepatic lipids (**Table 2**). Compared with each other, CSF2, CSF3 and CSB, CSG fractions similarly inhibit hepatic lipids but significantly less than Vitamin C and CSB. Compared to all the fractions, the CSB fraction shows the best inhibitor on the peroxidation of hepatic lipids because at 150 µg/mL, there is a maximum activity (2.5 µg/mL of protein).

From this table, it appears that extracts of *Clerodendrum splendens* significantly inhibited lipid peroxidation in a concentration dependent manner. This allowed us to determine the IC50 of the different extracts.

The CSB, CSG, CSC, CSF2 and CSF3 extracts had higher IC50 values than vitamin C (2.047±0.003) while the CSEB fraction had a lower IC50 value than vitamin C (1.45±0.07). Inhibition of lipid peroxidation was done according to method by Pilipenko (Pilipenko, 1990).

The supernatants are removed and the ODs are read at 530 nm. The percent inhibition of lipid peroxidation (% I) is calculated using the above formula (Fig. 9): $\% I = \frac{DO_{witness} - DO\ essai}{DO_{witness - DO_{white}}}$

The different letters show the different significance at P< 0.05.
EB: crude extract; **CSB:** Fraction B; **CSC:** Fraction C; **CSE:** Fraction E; **CSF:** Fraction F; **CSG:** Fraction G; **CSG1:** Fraction G; **CSH:** Fraction H; **CSF2** et **CSF3**

Concentration (µg/mL)	lipid peroxydation Inhibition (%)						
	CSC	CSB	CSEB	CSF_2	CSF_3	CSG	Vit C
300	74.41± 5.88	50 ± 3.37	57.44 ±0.42	45.24± 5.05	66.07 ± 2.53	45.24± 5.05	42.30± 0.90
250	54.17± 4.21	36.31±2.53	49.9 ±0.16	32.74± 0.84	52.38 ± 1.68	32.74± 0.84	38.66 ± 0.79
200	17.86± 1.68	21.49 ± 0.84	29.04 ± 0.17	22.85± 1.36	40.76 ± 0	22.85± 1.36	23.81 ± 0.00
150	10.32± 0.98	10.12± 0.84	18.77 ± 1.27	14.30 ± 0	38.69 ± 0.84	14.30 ± 0	16.75 ± 0.12
100	2.98 ± 0.84	4.76± 1.68	13.25 ±0.38	10.12± 0.84	22.85 ± 1.36	6.072 ± 1.01	8.69 ± 0.5
50	-8.69± 0.5	-4.17± 2.5	10.13 ±0.84	5.952 ± 0	14.40 ± 2.36	6.072 ± 2.53	-7.74 ± 0.253
0	0	0	0	0	0	0	0
IC_{50}	3.53± 0.33^a	2.49± 0.06^b	1.45± 0.07^d	2.49± 0.02^b	2.59 ± 1.73^b	2.53 ± 0.19^b	2.047± 0.003^c

Table 2: Inhibition of lipid peroxidation by fractions and vitamin C.

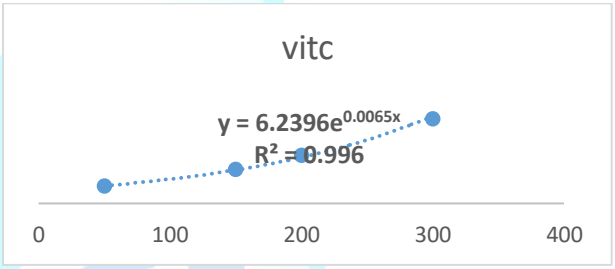

Figure 9a

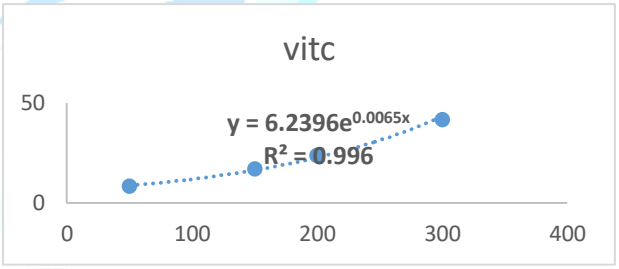

Figure 9b

Figure 9a and 9b: Percentage inhibition of lipid peroxidation (% I) is calculated using the formula above. (Vit C)

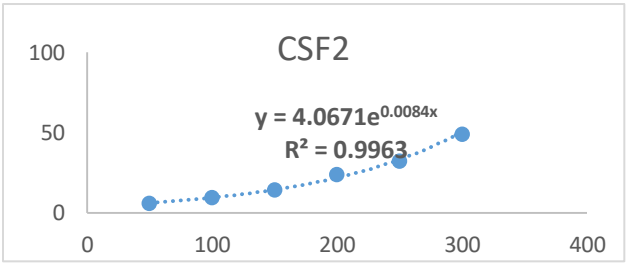

Figure 10a

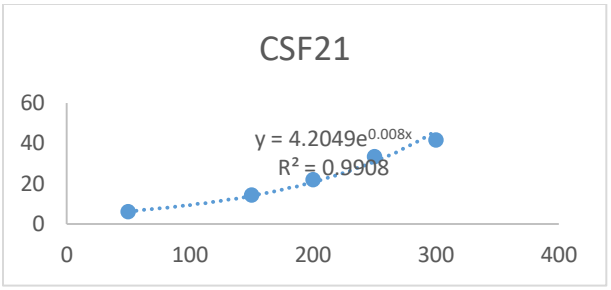

Figure 10b

Figure 10a and 10b: Percentage inhibition of lipid peroxidation (% I) is calculated using the formula above. (CSF2, CSF21).

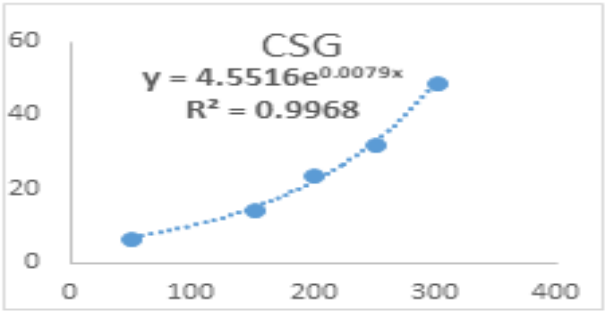

Figure 11a

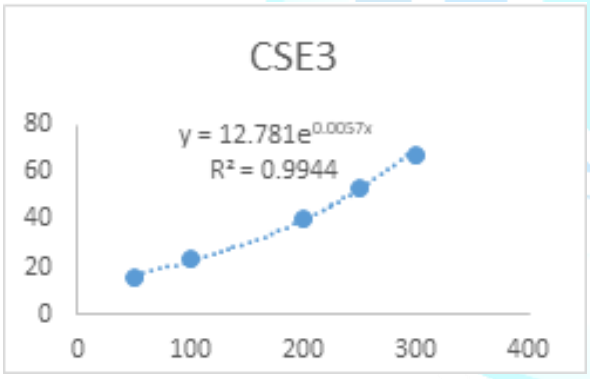

Figure 11b

Figure 11a and 11b: Percentage inhibition of lipid peroxidation (% I) is calculated using the formula above. (CSG, CSE3)

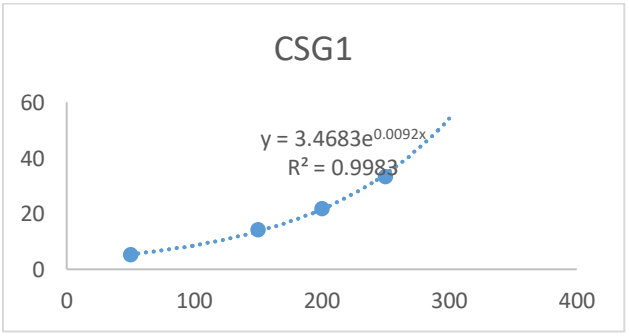

Figure 12a

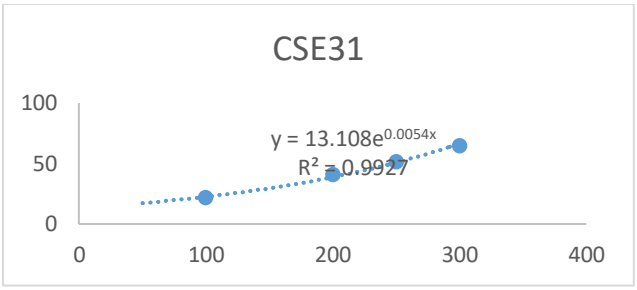

Figure 12b

Figure12a and 12b: Percentage inhibition of lipid peroxidation (% I) is calculated using the formula above. (CSG1, CSE31)

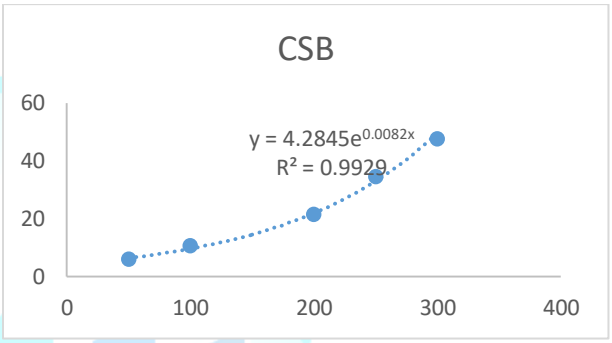

Figure 13a

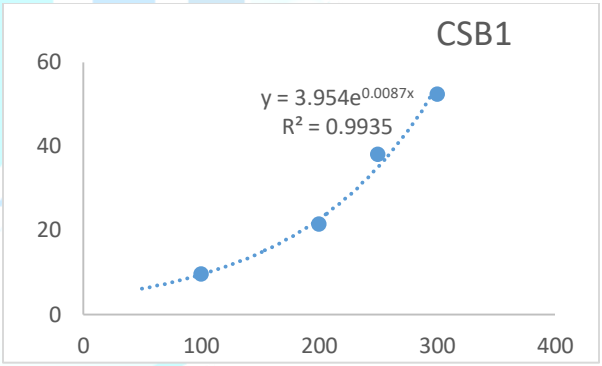

Figure 13b

Figure13a and 13b: Percentage inhibition of lipid peroxidation (% I) is calculated using the formula above. (CSB, CSB1)

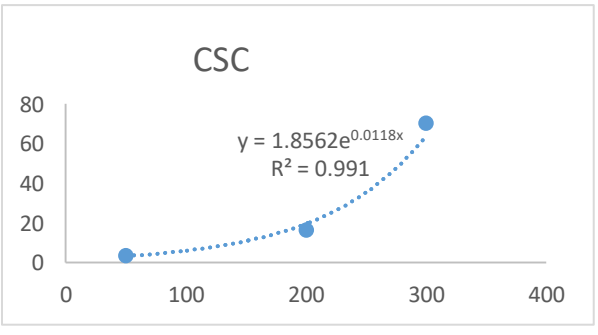

Figure 14a

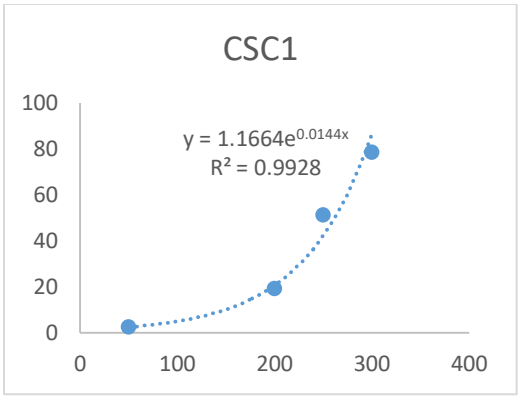

Figure 14b

Figure 14a and 14b: Percentage inhibition of lipid peroxidation (% I) is calculated using the formula above. (CSC, CSC1)

Evaluation of ferric ion reduction capacity (FRAP)

Principle

This method evaluates the ability of an antioxidant to transfer electrons to Fe3+ ions. These ions are in solution in the form of Fe3+/2,4,6-tripyridyl-S-triazyn (TPZ) complex and their reduction gives the blue complex Fe2+/2,4,6-tripyridyl-S-triazyne which absorbs at 593 nm (Benzie and Strain 1972). The intensity of blue color depends on the reducing power of the molecule tested.All the extracts have a reducing effect on Fe2+ (Table 3). The CSG and CSF2 fraction have a comparative reduction effect on Fe2+ ions, a lesser effect compared to Vitamin C and the CSEB fraction. The fractions CSEB, CSB and CSC have better effects on the reduction of ferric ions. Of these fractions, the CSEB fraction is the most reductive of ferric ions.

Concentration (µg/mL)	Vit C	CSEB	CSF_3	CSB	CSG	CSC	CSF_2
300	0.477±0.017	0.998±0.079	0.28±0.013	0.561±0.101	0.618±0.032	0.6275±0.050	0.135±0.001
250	0.463±0.018	0.8495±0.064	0.243±0.073	0.542±0.047	0.514±0.006	0.06045±0.054	0.114±0.001
200	0.478±	0.884±0.102	0.315±0.014	0.429±0.095	0.54±0.073	0.667±0.033	0.106±0.001
150	0.356±0.0367	0.805±0.003	0.225±0.009	0.535±0.035	0.289±0.199	0.56±0.025	0.087±0.001
100	0.277±0.010	0.721±0.041	0.145±0.124	0.438±0.021	0.166±0.004	0.541±0.021	0.093±0.001
50	0.226±0.0127	0.581±0.047	0.152±0.047	0.41±0.014	0.169±0.059	0.455±0.014	0.059±0.001
IC_{50} (µg/mL)	5.613±0.117^b	4.570±0.094^c	5.847±0.045^b	4.982±0.081^c	6.378±0.067^a	4.806±0.001^c	6.887±0.001^a

Table 3: reduction ferric ions by the fractions extracts of Clerodendrum Splendens and vitamin C.

From this table, it appears that all extracts of *Clerodendrum splendens* showed a reducing power which is directly proportional to the concentration of extract compared to that of vitamin C which allowed determines the IC50 of the various extracts.

It is found that CSF3, CSF2 and CSG have higher IC50 values than vitamin C (5.613±0.117) while the CSEB; CSB and CSC fractions had lower IC50 values than vitamin C, the reference reducing compound. The lower the IC50 value compared to vitamin C, the greater the antioxidant capacity of the plant extract.

The different letters show the different significance at P< 0.05
EB: crude extract; **CSB:** Fraction B; **CSC:** Fraction C; **CSE:** Fraction E; **CSF:** Fraction F; **CSG:** Fraction G; **CSG1:** Fraction G (96-98); **CSH:** Fraction H; **CSF2** et **CSF3**.

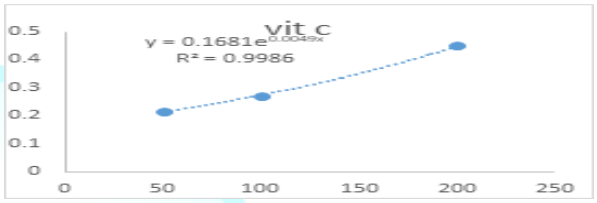

Figure 15a

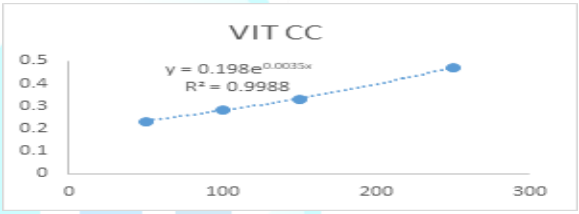

Figure 15b

Figure 15a and 15b: Method of evaluating the ability of an antioxidant to transfer Fe3+ ions. Reduction of ferric ions by the extracts and vitamin C.

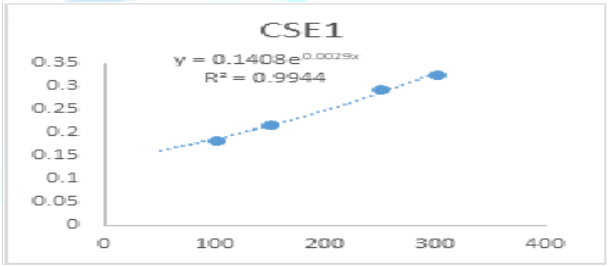

Figure 16a

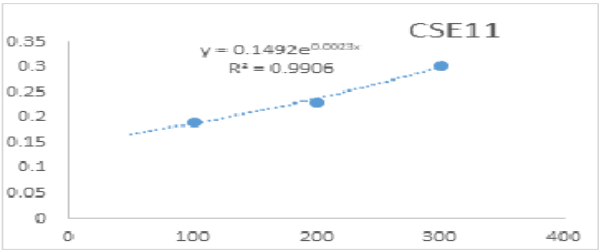

Figure 16b

Figure 16a and 16b: Method of evaluating the ability of an antioxidant to transfer Fe3+ ions. Reduction of ferric ions by (CSE, CSE11) extracts and vitamin C.

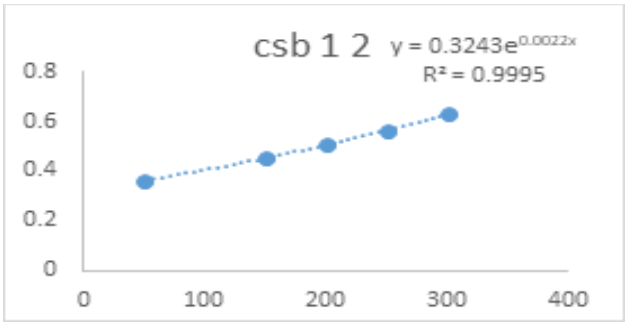

Figure 17a

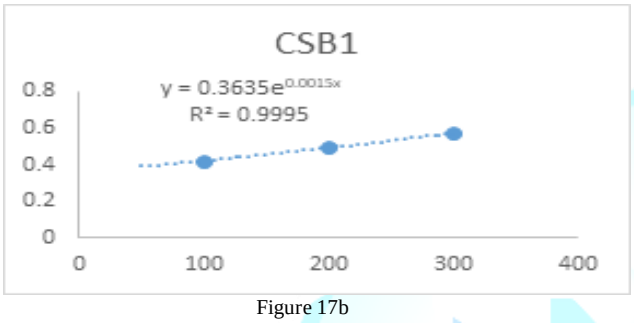

Figure 17b

Figure 17a and 17b: Method of evaluating the ability of an antioxidant to transfer Fe3+ ions. Reduction of ferric ions by (CSB12, CSB1) extracts and vitamin C.

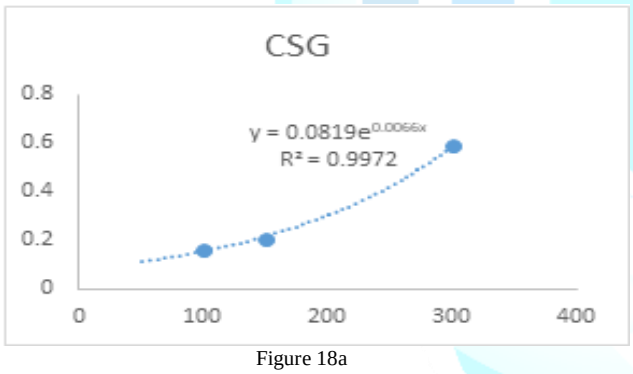

Figure 18a

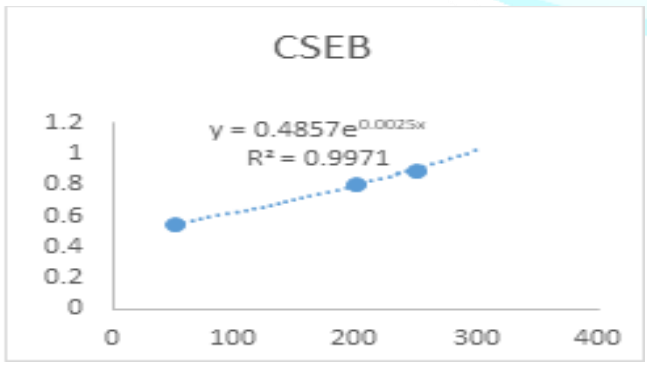

Figure 18b

Figure 18a and 18b: Method of evaluating the ability of an antioxidant to transfer Fe3+ ions. Reduction of ferric ions by (CSG, CSEB) extracts and vitamin C.

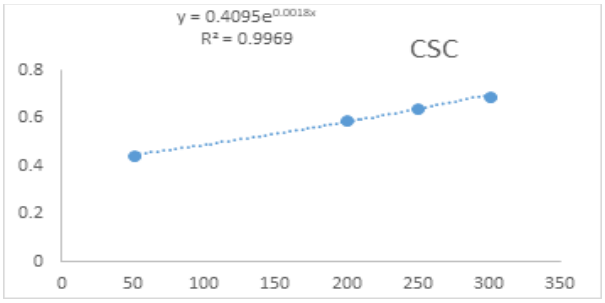

Figure 19a

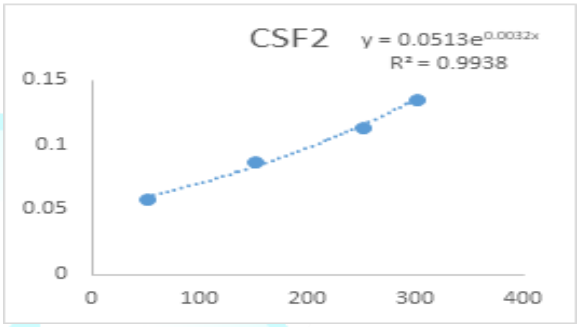

Figure 19b

Figure 19a and 19b: Method of evaluating the ability of an antioxidant to transfer Fe3+ ions. Reduction of ferric ions by (CSC, CSF2) extracts and vitamin C.

Discussion

This work has successfully determined the antioxidant capacity of the methanolic extract of Clerodendron splendens. Catalase is an enzyme that is responsible for the degradation of hydrogen peroxide in water and molecular oxygen. This enzyme is involved in the body's defense mechanism against infectious agents. Hence, the more a plant extract increases the activity of this enzyme is as high as compared to that of the reference compound (Vit C), the more this extract will be beneficial for the body. As part of this work, we were able to determine the IC50 of plant extracts. This result makes it possible to say that the activation of the catalase activity does not depend on the concentration of the extract.

The ability of extracts to protect biomolecules against oxidation was assessed by measuring their ability to inhibit lipid oxidation. Indeed, these lipids are oxidized in a reaction cascade whose increase in concentration is a sign of significant membrane lipid peroxidation. Some extracts showed lower IC50 values than vitamin C, which shows that these extracts may consist of polyphenols and flavonoids which are found in literature review for their great antioxidant power. These results corroborate those of Xiao-Yu (Xiao-Yu et al., 2009) which in fact obtained a strong inhibition of the lipid peroxidation of the rat liver homogenate with the ethanolic extract of *Clerodendrum splendens*. Since lipid peroxidation has been induced in the rat liver homogenate by Fe2+ and H2O2 ions, we can think that its inhibition by plant extracts could be attributed to their ability to either chelate iron or to trap the radical. OH° from the Fenton reaction.

The reducing activity is generally associated with their inhibitory action of chain reactions and precursor of peroxides. Indeed, some extracts have shown reducing capacities greater than that of vitamin C which is the reference compound. These results corroborate those of Rajan (Rajan et al., 2011) who obtained a strong reducing potential of

the aqueous and alcoholic extracts of Hegle marmelos fruits. They showed that extracts of *Clerodendrum splendens* have greater reductive activities than that of the reference compound (vitamin C).

Conclusion

Fractions of the methanolic leaf extract of *Clerodendrum splendens* were found to have pharmacological secondary metabolites; Saponins, Terpenoids and Tannins in abundant quantities, while alkaloids do not exist. This indeed suggests that the methanolic leaf extract of *Clerodendrum splendens* is a relevant and potential antioxidant. Thus, describing the leaf extract of *Clerodendrum splendens* as possessing activities of attenuations of oxidative stress and hepatopathy. Stress related to stress-induced cold stress was confirmed by deleterious effects on the physiological and immune systems against age-related stressors and macular degeneration. However, the concentration-dependent activity of the methanolic leaf extract of *Clerodendrum splendens* annulled the oxidative stress resulting from these aggressions. These results can be used as a prerequisite for *Clerodendrum splendens* screening of plants for bioactive compounds for medicinal purposes.

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