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Chemical Constituents of *Clerodendrum splendens* (Lamiaceae) and Their Antioxidant Activities

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Abstract: The purpose of this study was to evaluate the antioxidant activity of compounds isolated from *Clerodendrum splendens* leaves. The leaves of *Clerodendrum splendens* are used in traditional medicine by indegenous people to treat shingles, spleen in children, asthma, rheumatism, ulcers and malaria. In vivo and in vitro studies carried out by many researchers have shown that *Clerodendrum splendens* has antioxidant properties. The chemical study of the methanol extract of *Clerodendrum splendens* leaves (Lamiaceae) led to the isolation of three compounds: Triancontanol (1), (22E, 24S) - Stigmasta - 5, 22, 25 - trien – 3β-ol(2); 3-O-D-glucopyranoside of (22E, 24S) - Stigmasta - 5,22,25 - trien - 3β-ol (3). Their structures were elucidated on the basis of a spectroscopic analysis and a comparison of their data spectral with those reported in the literature. The results of the antioxidant activity have shown that the compounds 1 and 2 inhibit the peroxidation of the hepatic lipids, they also show that the compounds 1, 2 and 3 have a reducing effect on Fe^{2+}. However, the compounds 1, 2 and 3 have an OH reduction power which is directly proportional to the concentration of these compounds compared to that of vitamin C, which made it possible to determine the IC_{50} of the different compounds. Furthermore, the compounds 1 and 2 have higher IC_{50} than that of vitamin C (5.613 ± 0.117). 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: *Clerodendrum splendens*, Lamiaceae, Antioxidant Activities, Secondary Metabolites

1. Introduction

Plants play a very important role in the daily life of men because for a long time they are used as firewood, raw materials in real estate, decoration and in the care of diseases. The use of extracts of different parts of plants in the preparation of therapeutic potions is a mark more cultural than social [1]. Today, these are a true hive for drugs because they are fully integrated into the African way of life. And are involved in traditional pharmacopoeia in the fight against many diseases such as cancer, malaria, dysentery, yellow fever, ulcers, gonorrhea [2]. If medicinal plants are widely used in African regions, and in particular 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 dose active and their toxicity often unknown. It is in this context, that in the framework of this work, the study focused on Clerodendrum splendens, a plant

of the Lamiaceae family. *Clerodendrum splendens* is a shrub about 3.7 m high. It has simple and opposite dark green leaves [3]. *Clerodendrum splendens* leaves are used in traditional medicine by local people to treat shingles, spleen in children, asthma, rheumatism, ulcers and malaria. [4]. In vivo and in vitro studies conducted by many researchers have shown that *Clerodendrum splendens* has anti-inflammatory [3], antioxidant [5, 6], antimicrobial [7, 8] properties. Previous chemical studies performed on *Clerodendrum splendens* have leads to the isolation and characterization of some secondary metabolites among which, Carbohydrates, Steroids, Terpenoids and Flavonoids [9]. Thus, the general objective of this study is to evaluate the antioxidant activity of secondary metabolites isolated from *Clerodendrum splendens* (Lamiaceae) leaves.

2. Material and Methods

2.1. Apparatus and Equipment

(1) After drying, grinding of the splendid sheets of paper is done using a crushing machine. The maceration of powder in methanol is made in a hermetically sealed 20L can.

(2) An electronic balance of the type MARQLUTAN GM-300P made it possible to bring out the raw extract and the different masses of the fractions.

Flash chromatography was performed with the VELP Scientifica vacuum cleaner, a Buchner and a vacuum flask;

(3) A Büchi brand Heidolph WB 200 rotary evaporator made it possible to separate the extract from the solvent;

Column chromatographies were carried out in a column 3 cm in diameter and 60 cm long and a small column with a diameter of 2 cm and 50 cm in length. We used the type of KIESELGEL 60 (0.04 - 0.063 mm) as a phase;

(4) The ^{1}H NMR spectrum was obtained using a BRUKER DRX600 spectrometer at a frequency of 600 MHz for the proton and 150 MHz for the carbon. This was done in the laboratory of the Institute for Environmental Research, Faculty of Chemistry, University of Technology Dortmund, Germany.

2.2. Plant Material

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

2.3. Extraction and Isolation

The leaves of *Clerodendrum splendens* were dried, crushed and a powder of 2836.76 g was obtained. This powder was triple extracted 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 were obtained. 100.08 g of this crude extract was cold-fixed on 90 g of silica gel (SiO_2) (0.063-0.200 mm) and the Buchner was charged 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. After flash chromatography, 120 fractions of about 400 mL each were collected. The 120 fractions were pooled into eight major fractions (A, B, C, D, E, F, G and H) based on the analytical TLC.

2.4. Purification of the Different Fractions

2.4.1. Chromatography of Fraction B

Fraction B (9.18 g) was fixed on 7.04 g of silica gel and then column chromatographed with 60 g of silica (0.063-0.200 mm) as stationary phase. Elution of this was done with hexane and hexane / ethyl acetate mixture by gradient of increasing polarity; 134 fractions were collected and grouped into 6 major fractions (B1, B2, B3, B4, B5 and B6) under the base of the analytical TLC.

B2 fraction led to the isolation of Triancontanol (1) soluble in hexane / ethyl acetate 4%.

Fraction B5 led to the isolation of a steroid (22E, 24S) - Stigmasta - 5,22,25 - trien - 3β – ol (2) soluble in ethyl acetate and crystallized in MeOH.

Fraction G led to the isolation of 3–O-D - glucopyranoside from (22E, 24S) - Stigmasta - 5,22,25 - trien - 3β – ol (3) in the hexane / ethyl acetate 70% system soluble in the hot ethyl acetate mixture.

2.4.2. Physical and Spectral Data of the Compounds (1, 2 and 3)

Triancontanol (1) Calculated mass of $C_{30}H_{62}O$; m/z = 434 g/mol. ^{1}H NMR (600 MHz, $CDCl_3$)

δ(ppm) 3,64; 1,56; 1,20; 1,38; 0,88.

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 63,13; 32,82; 31,94; 29,38; 29,68; 25,75; 22,71; 14,14.

(22E, 24S)- Stigmasta − 5,22,25 - trien - 3β– ol (2): Calculated mass of $C_{29}H_{46}O$; m/z =410 g/mol. white needle; ^{1}H NMR (600 MHz, $CDCl_3$): d 0.68 (3H, s, H-18), 0.83 (3H, t, J = 7.6 Hz, H-23), 1.00 (3H, s, H-19), 1.01 (3H, d, J = 6.4 Hz, H-21), 1.68 (1H, s, H-27), 3.53 (1H, m, H-3), 4.70 (2H, bs, H-26), 5.17 (1H, dd, J = 15.6, 7.2 Hz, H-23), 5.24 (1H, dd, J = 15.6, 7.6Hz, H-22), 5.34 (1H, d, J = 5.6 Hz, H-6).

^{13}C NMR ($CDCl_3$, 150 MHz) δ(ppm) 37.24 (C-l), 148.60 (C-25) 140.74 (C-5), 137.18 (C-22), 130.02 (C-23), 121.67 (C-6), 109.5O(C-26), 71.79(C-3). 56.83 (C-14). 55.87(C-17). 51.98 (C-24), 50.14 (C-9), 42.49 (C-13), 42.24 (C-4), 40.17 (C-20), 39.66 (C-12), 36.49 (C-10), 31.86 (C-7, 8). 31.65 (C-2). 28.68 (C-16)

3-O-D-glucopyranoside of (22E, 24S)-Stigmasta − 5, 22, 25 - trien - 3β – ol (3): Masse Calculated mass of $C_{35}H_{56}O_6$; m/z =572g/mol. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) d 3.97 (1H, s, H-3), 5.35 (1H, d, H-6), 5.33 (1H, m, H-22), 5.38

(1H, m, H-23), 4.88 (2H, m, H-26), 1.73 (3H, s, H-27), 5.05 (1H, d, H-1'), 4.05 (1H, t, H-2'), 4.27 (1H, t, H-3'), 4.30(1H, t, H-4'), 3.97 (1H, s, H-5'), 4.56 (1H, dd, H-6'$_A$), 4.41 (1H, dd, H-6'B).

^{13}C NMR (101 MHz, CDCl$_3$) δ (ppm) 71.79(C-3), 42.24 (C-4), 140.74 (C-5), 121.67 (C-6), 50.14 (C-9), 39.66 (C-12), 42.49 (C-13), 56.83 (C-14), 20.54 (C-15), 29,09 (C-16), 55.87(C-17), 12.17 (C-18), 40.17 (C-20), 40.50 (C21), 137.18 (C-22), 130.02 (C-23), 51.98 (C-24), 148.60 (C-25) 110.19(C-26),.20,34 (C-27), 26,06 (C-28), 12,42 (C-29).

2.5. Antioxidant Activities

2.5.1. Activity of Trapping of the Radical DPPH ° (1, 1-Diphenyl-2-Picrylhydrazyl)

i) 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 [10].
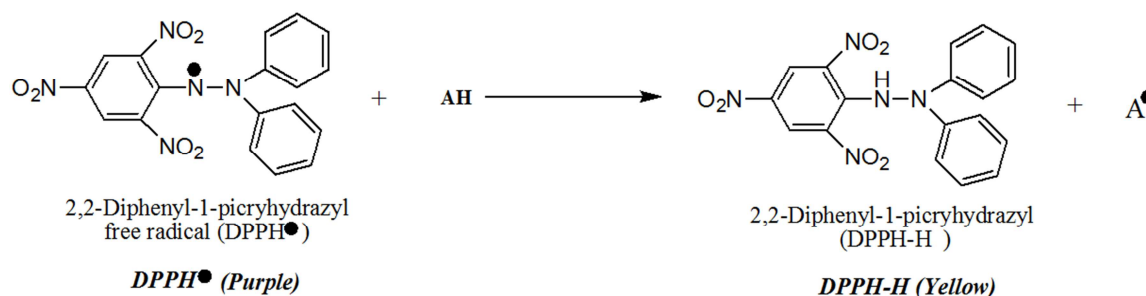

DPPH● (Purple)   DPPH-H (Yellow)

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

ii) Operating mode

Test tube containing 3.1 mL of methanolic solution of DPPH 40 μg / mL, 50 μL of extract added. 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 at λ = 517 nm are authorized to calculate the trapping percentages, the trapping concentrations fifty (CP$_{50}$), the effective concentrations fifty (EC$_{50}$) and finally the anti-free radical powers (PA) according to the following formulas [10, 11]:

$$\% \text{ trapping} = \frac{DOwitness - DOtest}{DOwitness} \times 100 (1) \quad CE_{50} = \frac{CP_{50}}{C}; \quad PA = \frac{1}{CE_{50}}$$

C = Concentration of DPPH in mol/mL

2.5.2. Trapping Activity of the OH° Radical

i) Principle

In the presence of FeSO$_4$ and H$_2$O$_2$, the hydroxyl radicals (OH$^·$) are formed. The latter, coupled with sodium salicylate, form a purple complex which absorbs at λ = 562 nm. The intensity of the coloration is inversely proportional to the amount of free radical in the medium [12].

ii) Operating mode

In each test tube, 50 μL of polyphenol extract, 0.7 mL of FeSO$_4$ (3 mM), 1 mL of H$_2$O$_2$ (1 mM), 1 mL of distilled water and 0.4 mL of sodium salicylate were added. (10 mM). In 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 read at λ = 562 nm against the blank. The different percentages of entrapments were calculated using formula (1) [12].

2.6. Reducing Activities

2.6.1. Potassium Ferricyanide Reduction Test

i) Principle

This test is based on the reduction of potassium ferricyanide K$_3$ [Fe (CN) $_6$] to potassium ferrocyanide K$_4$ [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 (FeCl$_3$) and the absorbance of the solution is read at λ = 700 nm [13].

ii) Operating mode

In each test tube were successively introduced 50 μL of extract, 1mL 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 [13].

2.6.2. Inhibition of Lipid Peroxidation

i) Initiation and inhibition of lipid peroxidation in rat liver homogenate.

ii) Principle

Coupled with Fe^{2+}, H$_2$O$_2$ liberates the hydroxyl radical (HO°) which attacks the ethylenic bonds of unsaturated fatty acids (AGI) to oxidize them. Thiobarbituric acid reactive substances (ATB) are formed, including malondialdehyde (MDA), which reacts in acid and heat with two ATB molecules to form a pink complex that absorbs at λ = 532 nm according to the equation. Below [14, 15].
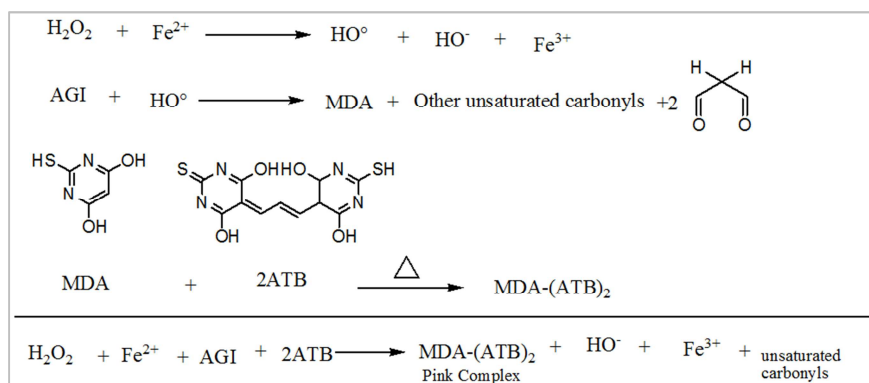
Figure 2. *Scheme representing formation of aldehydic product of lipid peroxidation induced by iron or hydroxyl radicals.*

iii) Operating mode

In each test tube, were successively introduced 50 μL of polyphenol extract, 1 mL of 10% rat liver homogenate, 50 μL of 0.5 mM FeCl. sub.2 and 50 μL of 0.5 mMH₂O₂. In the white tube, $FeCl_2$ and H_2O_2 were replaced by 100 μL KCl 1.15% while in the negative control tube, DMSO was used in place of the extract. The mixtures were incubated (1 hour, 37°C). After incubation, 1mL of trichloroacetic acid (15% ATC) and 1mL of 0.67% ATB were added to all tubes and boiled in a water bath for 15 minutes. After cooling and centrifugation (3000 rpm, 5 min, 4°C), the supernatants were recovered and the absorbance of the pink color read at λ = 532 nm against the white. Inhibition percentages were calculated using formula (1) [14, 15].

3. Results and Discussion

3.1. Structure Elucidation

The leaves of *Clerodendrum splendens* harve sted at Mount Mbankolo in Yaounde capital of Cameroon in September 2016, were cut, dried, crushed and extracted with methanol at room temperature. Several chromatographic techniques carried out on this extract made it possible to isolate three compounds Triancontanol(1),(22E, 24S) - Stigmasta - 5,22,25 - trien - 3β - ol(2) and 3– O-D - glucopyranoside from (22E, 24S) - Stigmasta - 5,22,25 - trien - 3β - ol (3). The structures of the isolated compounds were established using spectroscopic analysis, in particular, ¹H NMR, ¹³C NMR and two-dimensional NMR, COSY, HSQC, HMBC and a direct comparison with the literature reference data available.

Compound (1) was obtained as a white powder in the Hexane / ethyl acetate system (4%). It is soluble in ethyl acetate, crystallizes in methanol and responds positively to the Jones test characteristic of primary and secondary alcohols. Its ¹H NMR spectrum shows three families of protons in the weak fields: a broad triplet of 2H at 3.64 ppm, attributable to 2 protons at the base of a hydroxyl group (-CH₂-CH₂OH); CH₂ between 1.20 - 1.38 ppm A 3H-wide triplet at 0.88 ppm attributable to the fragment; (CH₃-CH₂ -). This information suggests a long chain of linear fatty alcohol.

Analysis of the ¹³C NMR spectrum of the compound (1) reveals the presence of seven carbon signals, there fore three attributable respectively to: the sp^3 hybridized methyl terminus at δC = 14.14 ppm, a carbon (C1) at δC = 63,13 ppm which allows to confirm the presence of a hydroxylated carbon (CH₂-OH), with carbon signals of C22.7 at 32.8ppm corresponding to a symmetrical (CH₂)ₙ sequence around 29 ppm. On the ¹H NMR spectrum, the intense peak at [1.20-1.38] ppm informs that for a carbon, there is two protons, therefore an integral of 48 protons. Hence the equivalence of 24 carbons. However, on the ¹³C NMR spectrum of (1), we observe 6 carbon signals in addition to the intense peak so the chemical shift is in the range of [29.38-29.68] ppm which allows us to deduce that the corresponding 30-membered alkyl chain of this fatty alcohol is triancontanyl. On the basis of this data, (1) comprises about 62 percent of the total number of ethylene groups, which contains substantially 24 carbons from which the compound (1) is triancontanol.

Compound (2) was obtained as a white powder in the system hexane ethyl acetate 30%; It is soluble in ethyl acetate and crystallizes in methanol. ¹H NMR spectrum, Low Value spectrum: A large multiplet at δH = 3.52 ppm attributable to the proton carried by a hydroxyl carbon, A large doublet of an ethylene proton at δH = 5.35 ppm and two doublets resolved at 5H = 5.24; 5.17 ppm. 3.5H 3.52 signals; 5.35; 5.24 and 5.17 attributable to H3, H6, H22, and H23 protons. Three singlet signals indicate angular methyls at δH = 0.69 ppm (H18); 1.01 ppm (H19); 1.65 ppm (H27). Finally, a triplet at δH = 0.83 ppm attributable to H29. These data clearly could (2) a skeleton of stigmasterol type [16-18]. The ¹³C NMR spectrum, completely decoupled from the presence of 29 carbon signals, including: Six ethylenic carbons at δC = 140.90 ppm; 121.84 ppm; 137.35 ppm; 130.17 ppm; 148.78 ppm and 109.66 ppm attributable respectively to C5, C6, C22, C23, C25 and C26. Hydroxyl hydroxide at δC = 71.96 ppm attributable to C3. To finish a set of carbon sp^3 at δC between 56.99 to 12.21ppm. The COSY ¹H-¹H spectrum is found between the correlations between the strongly depressed methyl protein H-3 (3.52ppm) and the protons H-2 (1.15ppm), H-4 (2.25ppm). A correlation is also observed between H-2 (1.50 ppm) and H-4 (2.25 ppm). Analysis of the HSQC spectrum shows that we observe a correlation with the H-22 nucleus resonant at 5.24ppm the carbone 22 (C-22) appearing 137.35ppm. Then there is a correlation between the proton (H-6) at 5.35ppm and the carbon 6 (C-6),

appearing at 6.84ppm. Similarly, the 3.52 ppm H-3 proton correlates with carbon 3 (C-3) resonating at 71.96 ppm. In addition, we observe the correlations between the proton (H-23) resonant at 5.17 ppm it is carbone 23 at 130, 17 ppm. On the other hand, the proton (H-26) resonating at 4.69 ppm and carbon monoxide having a chemical shift at 109.66 ppm. Finally, we see that the C-25 at 148.70 ppm is not bound to any proton so it is a quaternary carbon. The information supported corroborated by the spectra HSQC et HMBC. On its spectrum of HMBC, on the view H-3 and carbon C-1. Also the correlations between the proton H-6 and the carbons C-4, C-8 and C-10. Also, the correlations of the protons H-22/23 and the carbons C-17, C-20, C-21, C-22, C-23, C-24, C-25 and C-28. Moreover, we also observe the correlations between the proton H-24 and the carbons C-22, C-23, C-25 and C-26 which justifies the position of the double bonds. This spectrum also shows the correlations between the H-26 protons and the C-25, C-24, C-23 and C-27 carbons. C-6 and C-7 and H-5 carbons and C-8 carbon. On the basis of these data and in comparison with the data of the literature the compound (2) has the following name (22E, 24S) - Stigmasta - 5, 22, 25 - trien - 3β – ol (2).

The compound (3) was obtained in the form of a white powder in 70% hexane ethyl acetate system after evaporation. It is soluble in the mixture AcOEt-MeOH (1:1) to lime. It gives a blue color to the Liebermann-Burchard test and a purple crown to the Molish test characteristic of steroidal glycosides. Its ^{1}H NMR spectrum shows in the weak fields: a signal in the form of a multiplet centered at 5.16 ppm, indicating the presence of a trisubstituted double bond. Two doublets resolved at δH = 4.81; 4.6 ppm. The signals at δH 3.52; 5.16; 4.81; 4.6 ppm attributable to H3, H5, H22 and H23 protons. Three angular methyl signals at δH = 0.63 ppm (H18); 0.92 ppm (H19); 0.92 ppm (H27), and a triplet at δH = 0.83 ppm attributable to H29. The existence of several signals

between 3 and 4 ppm testifies to the presence of heteroatoms of osidic type in the structure of the compound. Moreover, the signal of a methine proton not assigned to glucose in the zone of chemical shifts between 3 and 4 ppm appears as a multiplet centered at δH = 3.52 ppm, belongs to the proton H-3 of the aglycone. δH sugar protons = 2.07 at 3.60 ppm, among which the anomeric proton at δH = 2.07ppm. The protons H-1 '(δ 2.07) and H-2' (δ 2.36), indicates that we have more precisely β-D-glucose. These data make it possible to deduce the carbon skeleton of this part of the molecule, and its chemical nature which is glucose. The ^{13}C NMR spectrum, completely decoupled reveals the presence of 35 signals. Indicating the presence of 35 carbon atoms.

Six methyls that appear at δ$_C$ 40,65 (Me-18); 12,30 (Me-29); 21,22 (Me-21); 110,47 (Me-27); 50,0 (Me-19); 109,66 (Me-26). Twelve methylenes to δ$_C$ 21,22 (C-11); 25,86 (C-28); 24,47 (C-15); 130,10 (C-23); 28,86 (C-16); 31,82 (C-2); 31,82 (C-7); 137,33 (C-22); 37,40 (C-1); 42,40 (C-4); 39,82 (C-12); 61,62 (C-6'). Fourteen methines so the one to δ$_C$ 148,18 (C-25); 31,82 (C-8); 40,35 (C-20); 50,29 (C-24); 50,29 (C-9); 56,02 (C-17); 56,99 (C-14); 73,99 (C-4'); 77,47 (C-2'); 70,62 (C-5'); 77,29 (C-3'); 77,51 (C-3); 101,34 (C-1') et 121,70,82 (C-6) Three quaternary carbons to δ$_C$ 36,66 (C-10); 42,40 (C-13); 140,99 (C-5), an aglycone carbon at δC = 71.96 ppm, six ethylenic carbons at δC = 140.99 ppm; 121.70 ppm; 137.337 ppm; 130.101 ppm; 148.197 ppm and 110.479 ppm attributable respectively to C5, C6, C22, C23, C25 and C26. Six carbons of sugar at δC = 101.34; 77.47; 77.29; 73.99; 70.62, 61.62 ppm attributable to C'1 to C'6 including anomeric carbon (δC = 101.34 ppm). These physical and spectroscopic data compared to those described in the literature [19] made it possible to identify the compound (3) with 3–O-D - glucopyranoside from (22E, 24S) - Stigmasta - 5,22,25 - trien - 3β - ol (3)de formula (C$_{35}$H$_{62}$O$_6$) having eight unsaturations.
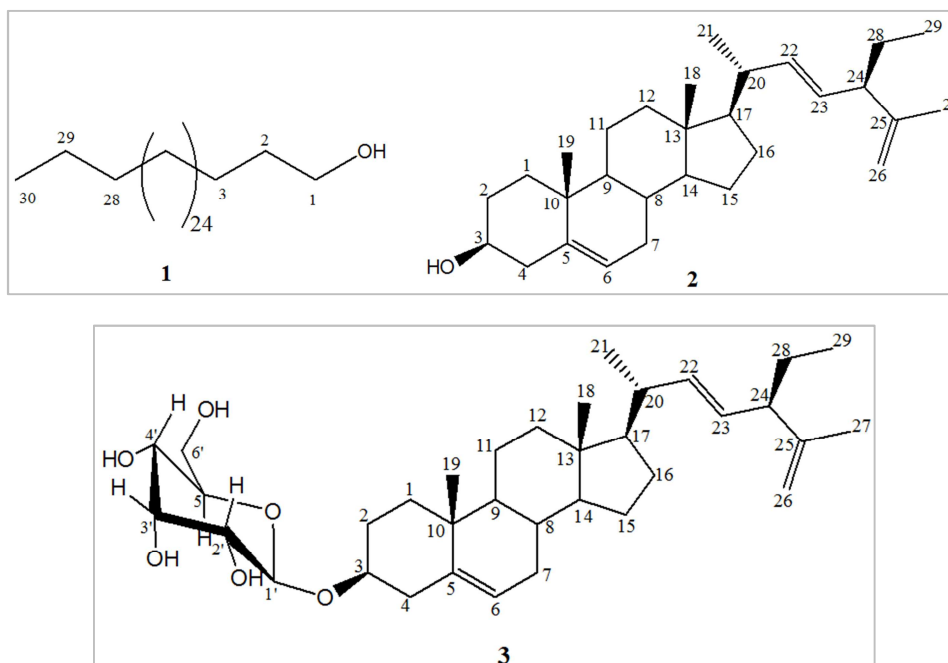

Figure 3. Structure of compounds 1 to 3 isolated from Clerodendrum splendens.

3.2. Antioxidant Results

3.2.1. Inhibition of the Peroxidation of Hepatic Lipids

The various compounds 1 and 2 inhibit the peroxidation of hepatic lipids (Table 1). Compared with one another, compounds 1 and 2 inhibit hepatic lipids in a comparable way but significantly less than vitamin C. With respect to all the compounds, the compound (2) shows the best inhibitor on the peroxidation of hepatic lipids because at 150 µg / mL, on maximal activity (2.5 IU/ mg protein).

Table 1. Inhibition of lipid peroxidation of compounds 1, 2 and 3 and vitamin C.

Concentration (µg/mL)	Inhibition of lipid peroxydation (%)			
	(1)	(2)	(3)	Vit C
300	45.24± 5.05	66.07 ± 2.53	3,415±1,0127	42.30± 0.90
250	32.74± 0.84	52.38 ± 1.68	2,845±0,8439	38.66 ± 0.79
200	22.85± 1.36	40.76 ± 0	2,276±0,6751	23.81 ± 0.00
150	14.30 ± 0.70	38.69 ± 0.84	1,7075±0,6751	16.75 ± 0.12
100	10.12± 0.84	22.85 ± 1.36	1,1383±0,3373	8.69 ± 0.5
50	5.952 ± 0.34	14.40 ± 2.36	0,924±0,199	-7.74 ± 0.253
0	0	0	0	0
IC_{50}	2.49± 0.02[b]	2.59 ± 1.73[b]	1.69± 2.15[b]	2.047± 0.003[c]

The different letters show significant differences at P <0.05.

From this table, it appears that compounds 1 and 2 significantly inhibited lipid peroxidation and thus in a concentration-dependent manner. Against compound 3 does not inhibit. Inhibition of compounds 1 and 2 of the lipid peroxidation allowed to determine the IC_{50} of the various compounds. The compounds 1 and 2 showed higher IC_{50} than vitamin C (2.047 ± 0.003). Inhibition of lipid peroxidation was done according to the ULF method [20].

3.2.2. Evaluation of Ferric Ion Reduction Capacity (FRAP)

This method evaluates the ability of an antioxidant to transfer electrons to Fe^{3+} ions. These ions are in solution in the form of complex Fe^{3+}/ 2, 4, 6-tripyridyl-S-triazyn (TPZ) and their reduction gives the complex Fe^{2+}/ 2, 4, 6-tripyridyl -S-triazyne blue color Intense absorbing at 593 nm, the intensity of the blue color depends on the reducing power of the molecule tested [21].

The set of compounds 1, 2, 3 of the reduction effect (Table 2). Compound 1 has a reducing effect compared to Fe^{2+} ions, compared to Vitamin C.

Table 2. Reduction of ferric ions by extracts of compounds 1, 2 and 3 and vitamin C.

Concentration (µg/mL)	Vit C	(1)	(2)	(3)
300	0.477±0.017	0.135±0.001	0.28±0.013	0,137±0,004
250	0.463±0.018	0.114±0.001	0.243±0.073	0.114±0.003
200	0.478±0.017	0.106±0.001	0.315±0.014	0.091±0.002
150	0.356±0.0367	0.087±0.001	0.225±0.009	0.068±0.002
100	0.277±0.010	0.093±0.001	0.145±0.124	0.045±0.001
50	0.226±0.0127	0.059±0.001	0.152±0.047	0,130±0,003
IC50 (µg/mL)	5.613±0.117b	6.887±0.001a	5.847±0.045b	4.806±0.001c

The different letters show significant differences at P <0.05.

From this table, it is composed 2 have shown a reducing power that is directly proportional to what was added to vitamin C. 1 and 2 on the IC_{50}, insights of vitamin C (5,613 ± 0,117), while the compound IC_{50}, is one of vitamin C (5,613 ± 0,117), is available to buy the Reductor Reference. The lower the IC_{50} value compared to vitamin C, the greater the antioxidant capacity of the compound.

Table 3. DPPH° (1, 1-diphenyl-2-picrylhydrazyl) by compounds 1, 2 and 3 and vitamin C.

Concentration (µg/mL)	Vit C	(1)	(2)	(3)
300	85.120±0.096	5.251±0.009	7.308±0.075	11.334±0.083
250	70.933±0.080	4.375±0.007	6.090±0.062	9.445±0.069
200	56.746±0.064	3.500±0.006	4.872±0.005	7.556±0.055
150	42.560±0.048	2.625±0.004	3.654±0.037	5.667±0.041
100	28.373±0.032	1.750±0.003	2.436±0.025	3.778±0.027
50	14.18±0.016	0.875±0.001	1.218±0.012	1.889±0,013
IC50 (µg/mL)	7.613±0.117b	8.887±0.001a	7.847±0.045b	6.806±0.001c

The different letters show significant differences at P <0.05.

From this table, it appears that all compounds have a DPPH that is directly proportional to the concentration of these

compounds compared to that of vitamin C which allowed to determine the IC_{50} of the various compounds. Compounds 2 and 3 are found to have higher IC_{50} than vitamin C (7.613 ± 0.117), where as compound 3 has a lower IC_{50} than vitamin C (7.613 ± 0.117), which is used as the reducing compound of reference.

Table 4. Reduction of the radical OH of compounds 1, 2 and 3 and vitamin C.

Concentration (µg/mL)	Vit C	(1)	(2)	(3)
300	13,284±1,084	3,111±0,199	3,912±1,013	5,860±1,144
250	11,070±0.900	2,592±0,165	3,260±0.844	4.883±0.953
200	8.856±0.722	2.074±0,132	2.608±0.675	3.906±0.762
150	6,642±0.542	1.555±0,099	1.956±0.506	2.93±0.572
100	4.428±0.361	1.037±0,066	1.304±0.337	1.953±0.381
50	2,214±0.180	0.518±0,033	0.652±0.168	0.976±0.190
IC50 (µg/mL)	8.613±0.117b	9.887±0.001a	8.847±0.045b	7.806±0.001c

The different letters show significant differences at P <0.05.

From this table, it appears that all the compounds 1, 2 and 3 have an OH reduction power which is directly proportional to the concentration of these compounds compared to that of vitamin C, which made it possible to determine the IC_{50} of the different compounds. Compounds 1 and 2 are found to have higher IC_{50} values than vitamin C (8,613 ± 0.117), while compound 3 has an IC_{50} lower than that of vitamin C (8,613 ± 0.117), which is used as the reducing compound of reference.

4. Conclusion

The objective of this work was to determine the antioxidant capacity of *Clerodendrum splendens* compounds 1, 2 and 3. Catalase is an enzyme that is responsible for the degradation of hydrogen peroxide in water and molecular oxygen. This enzyme is involved in the defense mechanism of the body against infectious agents. Indeed, the more a compound increases the activity of this enzyme is high compared to that of the reference compound (Vit C) this compound will be beneficial for the body. Compound 3 exhibited an IC_{50} lower than that of vitamin C which shows that this compound may consist of glucose steroids which is recognized in the literature for its great antioxidant power. Since lipid peroxidation has been induced in the rat liver homogenate by Fe^{2+} and H_2O_2 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 $HO^.$ issue of Fenton's reaction. The reducing activity is generally associated with their inhibitory action of chain reactions and precursor of peroxides. On the other hand, compounds 1 and 2 have had reducing capacities greater than that of vitamin C which is the reference compound. Compounds 1, 2 and 3 have reductive activities greater than that of the reference compound.

Acknowledgements

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