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Preventive effects of the sesquiterpenic compounds of essential oil from *Greenwayodendron suaveolens* (Engl. & Diels) Verdc. Subsp. *suaveolens* stem barks on free radicals and proteins inflammation

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Abstract
The present study was undertaken to determine the chemical composition and evaluate the *in vitro* antioxidant, anti-inflammatory activities of essential oil (EO) of *Greenwayodendron suaveolens* (Engl. & Diels) Verdc. Subsp. *suaveolens*. The determination of chemical composition of EO had been carried out by Gas Chromatography and Gas Chromatography coupled to Mass Spectrometry. The total antioxidant content was determined by the Folin-Ciocalteu method. The antioxidant activities of EO were evaluated using DPPH, ABTS and FRAP methods. The anti-inflammatory effects of EO were evaluated according to *in vitro* Bovine Serum Albumin (BSA) denaturation and Proteinase Inhibitory Action assays. The results obtained showed the presence of α-muurolene, 14-hydroxy-9-*epi*-(*E*)-caryophyllene, α-humulene, 14-hydroxy-(*Z*)-caryophyllene, davanone and cubeban-11-ol with respectively proportions of 15.5%, 13.8%, 7.9%, 7.3%, 7.2% and 6.8% as major compounds in *G. suaveolens* EO. The EO of *G. suaveolens* exhibited a total antioxidant of 239.89 ± 2.13 µg EGA/mg of dry weight. Meanwhile, the free radical $DPPH^{\cdot}$and $ABTS^{+\cdot}$ scavenging activities (SC_{50}) of 15.60 ± 1.65 µg/mL and 4.69 ± 0.96 µg/mL respectively. We found a high reducing activity of *G. suaveolens* EO at 94.75 ± 1.66 µg EGA/mg dry weight. The *G. suaveolens* EO has shown a high anti-inflammatory activity through bovine serum albumin denaturation and proteinase inhibitory action with the inhibitory concentration 50 (IC_{50}) of 183.30 ± 3.15 µg/mL and 33.62 ± 0.95 µg/mL respectively. These results suggest that *G. suaveolens* essential oil may offer a new source of potential therapeutic agents to prevent inflammation induced by oxidative stress, starting point of several diseases.

Keywords: *Chemical composition, essential oil, Greenwayodendron suaveolens, antioxidant activity, anti-inflammatory effects*

1. Introduction
Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation [1]. While, oxidative stress, is defined as a disturbance in the balance between the production of reactive oxygen species ($O_2^{\cdot-}$, $OH^{\cdot}$, H_2O_2, $NO^{\cdot}$) and antioxidant defenses. These two mechanisms are a great contribution in the maintenance of body homeostasis [2]. Therefore, the dysfunction of these are linked with a number of diseases including diabetes, hypertension, cardiovascular diseases, cancer and mainly respiratory infections like acute or chronic pneumonia [1–4].
The inflammatory response may be physiologically appropriate in the presence of an infection and cellular damage and stress [5]. It's also induced during the increase in vascular permeability, the increase in protein denaturation and, membrane alteration. Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by interaction of reactive oxygen species produced by pro-inflammatory cytokines or various compounds, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat [4-7].
Available treatment is divided into different classes of drugs such as non-steroidal and steroidal anti-inflammatory drugs (NSAIDs/SAIDs) [5]. However, the use of NSAIDs has gastrointestinal side effects, which includes irritation of the gastric mucosa, belching, gastric ulceration, and bleeding.

Long-term use of NSAIDs may impair renal and hepatic functions, predisposing the patient to cardiovascular diseases [5]. As a result of this, much attention has been focused on the use of novel sources of antioxidant and anti-inflammatory drugs, especially natural antioxidants which protect from damage due to free radicals and to inhibit lipid peroxidation, protein denaturation and membrane alteration. A great number of aromatic and other medicinal plants contain chemical compounds that exhibit antioxidant and anti-inflammatory properties, contrary to the standard anti-inflammatory drugs [8]. Sources of volatile compounds are primarily, plant phenolic and terpenes compounds that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks known for their antimicrobial, antioxidant and anti-inflammatory potentials [8, 9].

Greenwayodendron suaveolens (Engl. & Diels) Verdc. Subsp. *suaveolens* is a tropical long tree that measures up to 35−45 meters and belongs to the *Annonaceae* family. It is used by the population of Cameroon to treat malaria, gonorrhoea and infertility, stomach-ache and other pains; it's also considered as diuretic, purgative and aphrodisiac, and as facilitating childbirth. Nyegue *et al*. study revealed that the essential oil of *P. suaveolens*, also known as *G. suaveolens* is a rich source of complex compounds such as phenolic compounds, hydrocarbon monoterpenes and, hydrocarbon and oxygen sesquiterpenes, which are known to present antioxidant and antimicrobial potentials [9]. This study has highlighted the ability of essential oil of *P. suaveolens* to reduce the radical DPPH with SC_{50} of 710 ± 40 µg/mL and showed antimicrobial effects against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* (with a maximum activity >90% at 2000 µg/mL) [9].

To date, to the best of our knowledge, no data are available on the antioxidant and anti-inflammatory efficiency of *G. suaveolens* species. Therefore, the aim of the present study was to investigate the chemical composition and evaluate the *in vitro* antioxidant, anti-inflammatory properties of essential oil of *Greenwayodendron suaveolens* (Engl. & Diels) Verdc.

2. Materiel and Methods

2.1. Plant Material

Stem bark of *Greenwayodendron suaveolens* was collected at Kala Mount (Yaoundé, Cameroon) on 11th January 2018 and identified at Cameroon National Herbarium under the identification number 45578−HNC.

2.2. Essential oil extraction procedure

The essential oil of *Greenwayodendron suaveolens* were extracted by hydrodistillation using a Clevenger-type apparatus during 5 hours then, dried over anhydrous sodium sulfate and further stored at 4 °C until used. The extraction yields were calculated as the ratio of the mass of essential oil to the mass of the starting plant material expressed as a percentage.

2.3. Chemical analysis of essential oils

2.3.1. Essential oils analysis

Essential oils were analyzed by Gas Chromatography (GC) and Gas Chromatography coupled with Mass Spectrometry (GC-MS) as described by Moni *et al*. [10, 11].

2.3.2. Gas Chromatography (GC/FID)

GC Analysis was performed on a Varian gas chromatograph, model CP-3380, with flame ionization detector containing two silica capillary columns: HP5 J&W Agilent (5%-Phenylmethyl polysiloxane) capillary column (30 m × 0.25 mm i.d. × 0.25 µm film) and Supelcowax 10 (polyethylene glycol) fused capillary column (30 m × 0.25 mm i.d. × 0.25 µm film); N_2 was the carrier gas at 0.8 mL/min; injection type 0.1 µL of pure sample, split ratio 1:100; injector temperature 220 °C, detector temperature 250 °C; temperature program 50−200 °C at 5 °C/min, then it was kept at 200 °C for 10 min. The linear retention indices of the components were determined relative to the retention times of a series of *n*-alkanes. The entire set up was coordinated by Chromeleon (version 7.4) software system that ensured its functioning and the follow-up of the chromatographic analysis.

2.3.3. Gas Chromatography-Mass Spectrometry (GC/MS)

GC-MS Analyses were performed using a Hewlett Packard 5890 II gas chromatograph, interfaced with a quadrupole detector (Model 5972) and equipped with a HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25µm). Helium was the carrier gas, at a flow rate of 0.6 mL/min. Injector and MS transfer line temperatures were 220 °C and 250 °C, respectively. The oven program temperature was the same as that used in the GC-FID analyses. Diluted samples (10:100 in CH_2Cl_2, v/v) of 1 µL were injected manually and in a split mode (1:100). The MS was operated in the EI mode at 70 eV, in the m/z range 35-300; electron multiplier 1460 eV; scan rate, 2.96 scan/s.

2.3.4. Qualitative analysis

The identification of the constituents was assigned on the basis of a comparison of their relative retention indices, calculated with reference to a series of *n*-alkanes (C9–C22), and their mass spectra with those of the standards (for main components) and those found in the literature and supplemented by the NBS75K database and Wiley 7th NIST 2014 EPA/NIH Mass Spectral Library Upgrade (provided by Hewlett Packard with the GC/MS control and data processing software) [12, 13].

2.3.5. Quantitative analysis

The percentage composition of the essential oils was computed by the normalization method from the GC/FID peak areas, assuming an identical mass response factor for all compounds [12, 13].

2.4. *In vitro* Antioxidant activity

2.4.1. Determination of total antioxidant content

The total antioxidant was evaluated according to the spectrometric method using the Folin-Ciocalteu reagent as described by Chew *et al*. [14]. About 200 µL (1.5 mg/mL) essential oil solution was mixed with 1 mL 10-fold diluted Folin-Ciocalteu reagent. Following 4 min of incubation, 0.8 mL of 7.5% Na_2CO_3 solution was added and the mixture was allowed to stand for 120 min at room temperature and the absorbance measured at 765 nm. Calibration was performed using a freshly prepared aqueous solution of gallic acid.

2.4.2. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging test

To prepare a standard solution of 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$), 3 mg of DPPH was dissolved in 75 mL of methanol [15]. This solution was taken and 2-fold diluted with methanol to obtain 225 mL of final solution. After preparing the different solutions, 2000 µL of the DPPH solution was pipetted into test tubes and 500 µL of essential oil and gallic acid at different concentrations was then added

to each test tube to a final volume of 2.5 mL per tube. All tests were carried out in triplicate in a dark room. The optical density was measured at a wavelength of 515 nm using a spectrophotometer of the brand Thermo-Fisher-Scientific: Evolution 300 UV−VIS, after 30 min of incubation.

2.4.3. ABTS radical scavenging activity

The most widely used methods for determining the antioxidant activity of plant extracts, it consists in following the kinetics of discoloration of the $ABTS^{+\cdot}$ ion as described by Re *et al.* [16]. ABTS (2,2'-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid)) was prepared by mixing 0.0384 g of ABTS and 0.00662 g of potassium persulfate ($K_2S_2O_8$) with 10 mL of distilled water. The mixture was incubated for 16 hours at room temperature, protected from light before use. For the actual analysis, the ABTS solution was diluted with ethanol and the absorbance adjusted to 0.700 (± 0.02) at 734 nm and stable at 30 °C (initial optical density). In a test tube, 3.0 mL of this diluted ABTS solution were added in the 30 µL of the essential oil of varying concentration (1.5 mg/mL, 0.75 mg/mL, 0.375 mg/mL, 0.1875 mg/mL and 0.0937 mg/mL). The tubes were agitated to homogenate the mixture. Absorbance reading was taken at 734 nm immediately after agitation. Gallic acid was used as an antioxidant reference at the same concentrations as the essential oil. The percent inhibition was calculated according to the formula:

$$I\ (\%) = \frac{\text{Abs control} - \text{Abs essential oil/gallic acid}}{\text{Abs control}} \text{ x } 100$$

Where, $Abs_{control}$ is the absorbance of control tube and $Abs_{essential\ oil/gallic\ acid}$ is the absorbance of sample tube.

2.4.4. Test of FRAP (ferric reducing antioxidant power) assay

The ferric reducing antioxidant power assay (FRAP) is based on the reduction of the tripyridyltriazine ferric complex (Fe^{3+}-TPTZ) to the tripyridyltriazine ferrous complex (Fe^{2+}-TPTZ) in the presence of an antioxidant, 2000 µL of FRAP solution was pipetted into different test tubes, followed by 75 µL of extracts or gallic acid at different concentrations [17]. The tests were done in triplicate, and the mixture was incubated for 30 min. The optical density was measured at 593 nm using a spectrophotometer of the brand Thermo-Fisher-Scientific: Evolution 300 UV−VIS. The FRAP solution was prepared as follows: 14.1 mg of TPTZ was diluted in 9 mL HCl at 40 mM then ferric chloride ($FeCl_2$ at 20 mM) and acetate buffer 300 mM at pH = 3.6 mixed in the ratio of 1:1:10 respectively to form the FRAP solution.

2.5. *In vitro* Anti-inflammatory activity

2.5.1. Inhibition of bovine serum albumin denaturation

Method of Sakat *et al.* [18] followed with slight modifications. The reaction mixture was consisting of test essential oil or diclofenac sodium and 5% aqueous solution of bovine serum albumin. The mixture was incubated at 37 °C for 20 min and then heated to 70 °C for 15 min, after cooling the samples the turbidity was measured at 660 nm using a spectrophotometer of the brand Thermo-Fisher-Scientific: Evolution 300 UV−VIS. The experiment was performed in triplicate. The percent inhibition of bovine serum albumin denaturation was calculated as follows, where $Abs_{control}$ is the absorbance without sample, Abs_{sample} is the absorbance of sample (essential oil/diclofenac sodium).

$$I\ (\%) = \frac{\text{Abs control} - \text{Abs essential oil/diclofenac sodium}}{\text{Abs control}} \text{ x } 100$$

2.5.2. Proteinase inhibitory action

The test was performed according to the modified method of Oyedepo *et al.* [19]. The reaction mixture (2 mL) was containing 0.06 mg trypsin, 1 mL of 20 mM Tris-HCl buffer (pH = 7.4) and 1 mL test essential oil or diclofenac sodium of different concentrations. The reaction mixture was incubated at 37 °C for 5 minutes and then 1 mL of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min, 2 mL of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated.

$$I\ (\%) = \frac{\text{Abs control} - \text{Abs essential oil/diclofenac sodium}}{\text{Abs control}} \text{ x } 100$$

Where, $Abs_{control}$ is the absorbance of control tube and $Abs_{essential\ oil/diclofenac\ sodium}$ is the absorbance of sample tube.

2.6. Statistical analysis

Each data was expressed as mean ± SD (n = 3). The analysis was done by paired t-test. The difference between standard and essential oil concentrations was considered significant at $p<0.05$. The graphical representation of the data was performed using the Graph Pad Prism 8.0.1 software (Microsoft, USA). The IC_{50} and SC_{50} were determined using the SPSS Statistic Software version 23.0.

3. Results and Discussion

3.1. Extraction yield and chemical composition of essential oil

The extraction yield of the essential oil of *Greenwayodendron suaveolens* is shown in Table 1. The EO extraction from *G. suaveolens* stem barks produced the lowest yield (0.02%) compared to EOs extracted from *G. suaveolens* stem barks harvested from two others localities of Cameroon (Mfou and Afanoyo IV) with yields of 0.05% and 0.23% respectively [9]. The difference between the extraction yields could be due to the impact of site and period of harvest [11]. Nyegue *et al.* demonstrated that the extraction yield of essential oils of plants, obtained by hydrodistillation can be influenced by the duration of hydrodistillation and conditions of the plant materials, the hydrodistillation time and hydrolysis mechanisms involving chemical skeleton of the compounds and pH in the environmental conditions [21]. The soil type, vegetative cycle of plants and climate are the factors that can also influenced the extraction yields of essential oils [11].

The results from the chemical composition of essential oil of *G. suaveolens* stem barks are presented in Table 1. This table showed aromatic components correlated with their relative percentage. The results of our study showed that EO of *G. suaveolens* contained forty-five biomolecules, which represent 99.9% of total compounds of EO. These results showed high content of cyclic sesquiterpene hydrocarbons, dominated by α-muurolene (15.5%), α-humulene (7.9%); of the oxygenated sesquiterpenes, 14-hydroxy-9-*epi*-(*E*)-caryophyllene (13.8%), 14-hydroxy-(*Z*)-caryophyllene (7.3%), davanone (7.2%) and cubeban-11-ol (6.8%).

Table 1: Relative percentages of constituents of essential oils from *G. suaveolens* stem barks obtained by GC and GC/MS.

N°	Compounds	ILR_X	ILR_A	Relative percentage (%)	Identification methods
1	α-Pinene	940	939	0.1	GC, RI
2	Sabinene	978	975	0.2	GC, RI, MS
3	δ-3-Carene	1011	1011	0.9	GC, RI
4	δ-Limonene	1027	1029	0.1	GC, RI
5	δ-Elemene	1330	1338	0.7	GC, RI
6	α-Cubebene	1342	1351	0.1	GC, RI, MS
7	Isoledene	1363	1376	0.3	GC, RI
8	α-Copaene	1373	1376	1.1	GC, RI
9	β-Elemene	1387	1390	1.0	GC, RI
10	Italicene	1394	1405	0.2	GC, RI
11	Sesquithujene	1405	1405	0.1	GC, RI
12	β-Caryophyllene	1418	1419	3.9	GC, RI
13	(*E*)-Caryophyllene	1424	1419	0.5	GC, RI
14	β-Copaene	1435	1432	0.3	GC, RI
15	α-Humulene	1456	1454	7.9	GC, RI
16	*cis*-Muurola-4(14),5-diene	1473	1466	0.3	GC, RI
17	Germacrene D	1482	1481	3.7	GC, RI, MS
18	γ-Curcumene	1487	1482	0.3	GC, RI
19	α-Muurolene	1498	1500	15.5	GC, RI, MS
20	β-Bisabolene	1507	1505	0.7	GC, RI
21	β-Curcumene	1515	1515	2.4	GC, RI
22	(*E*)-γ-Bisabolene	1529	1529	0.1	GC, RI
23	α-Cadinene	1536	1538	0.1	GC, RI
24	α-Calacorene	1545	1545	2.1	GC, RI
25	*trans*-Cadineneether	1556	1558	1.5	GC, RI
26	Davanone D	1568	1566	1.3	GC, RI, MS
27	Davanone	1585	1587	7.2	GC, RI, MS
28	Glenool	1592	1587	4.6	GC, RI
29	Cubeban-11-ol	1594	1595	6.8	GC, RI, MS
30	1,3,5-Bisabolatrien-7-ol	1601	1601	1.7	GC, RI
31	Humuleneepoxide II	1609	1608	0.9	GC, RI
32	1-*epi*-Cubenol	1623	1628	1.5	GC, RI
33	*epi*-α-Cadinol	1639	1640	3.8	GC, RI
34	α-Muurolol	1646	1646	4.8	GC, RI
35	α-Cadinol	1652	1654	1.0	GC, RI
36	14-Hydroxy-(*Z*)-Caryophyllene	1667	1667	7.3	GC, RI
37	14-Hydroxy-9-*epi*-(*E*)-Caryophyllene	1669	1669	13.8	GC, RI, MS
38	*epi*-α-Bisabolol	1682	1684	0.1	GC, RI
39	*cis*-14-nor-Muurol-5-en-4-one	1687	1689	0.1	GC, RI
40	14-Hydroxy-4,5-dihydro-caryophyllene	1704	1706	0.1	GC, RI
41	(*2Z,6E*)-Farnesol	1725	1723	0.2	GC, RI
42	(*2E,6E*)-Farnesol	1743	1743	0.2	GC, RI
43	α-Bisabololoxide A	1749	1749	0.1	GC, RI
44	14-Hydroxy-δ-cadinene	1808	1803	0.2	GC, RI
45	(*2E,6E*)-Farnesoicacid	1814	1817	0.1	GC, RI
Total of compounds (%)				99.9	
Extraction yield (%)				0.02	

Legend: N°: elution order given on a polar column (HP-5); **LRI_X**: Linear Retention Index of compounds on a polar (HP-5) column; **LRI_A**: Linear Retention Index of compounds in Adams database on polar (DB-5) column; Identification methods: **GC-FID** (identification based on co-injection with authentic sample); **RI** (Retention Indices); **MS** (identification based on comparison of mass spectrum with literature data); **%**: relative percentage.

The results obtained by Nyegue *et al*. showed a different chemical composition with the predominance of sesquiterpene hydrocarbons, dominated by β-caryophyllene (16.0%), germacrene D (8.5%) and cadinene (7.0%); of the oxygenated sesquiterpenes, caryophyllene oxide (7.3%), salvial-4(14)-ene-1-one (7.3%) and *epi*-α-cadinol (8.3%) [21]. However, the comparative study has shown the presence of hydrocarbons and oxygenated sesquiterpenes, at 23.4% and 35.1% for this study; and 31.5% and 23.1% for Nyegue *et al.* study [21], but at different percentage.

The results obtained by Fekam differ significantly from those previously obtained for the same species, also collected in Cameroon, which showed an essential oil with only one major compound such as β-caryophyllene at 36.4% [22]. The variation of major compounds found in essential oil of plants could depend to the geographical origin, type of climate and soil [11]. Moreover, Nyegue *et al*. put in evidence that the chemical profile of *G. suaveolens* essential oil could have slight variations depending to the process by which it is extracted [9].

3.2. *In vitro* antioxidant studies

3.2.1. Total antioxidant content

Total antioxidant content of *G. suaveolens* essential oil was

determined by a method using Folin-Ciocalteu and was expressed in micrograms of equivalent gallic acid per milligram of dry weight. The results were obtained from the calibration curve of gallic acid presented in figure 1. The differences in total antioxidant content among essential oil used were statistically significant (p<0.05). A rich literature study is available showing that the antioxidant potential is mainly due to presence of phenolic compounds and others compounds which have a hydroxyl group on aromatic ring [23, 24]. The essential oil has a content of total antioxidant of 240.87 ± 1.70 µg EGA/mg of dry weight. This essential oil has 35.07% of oxygenated sesquiterpenes that can contribute to the antioxidant properties [21]. The antioxidant activity of these compounds is due to the reactivity of phenol moiety (hydroxyl group on aromatic ring). Phenol moiety has the ability to scavenge free radicals via hydrogen donation or electron donation [25]. Antioxidants play a vital role in the control of reactive oxygen species (ROS), produced during metabolic diseases and infectious infections.

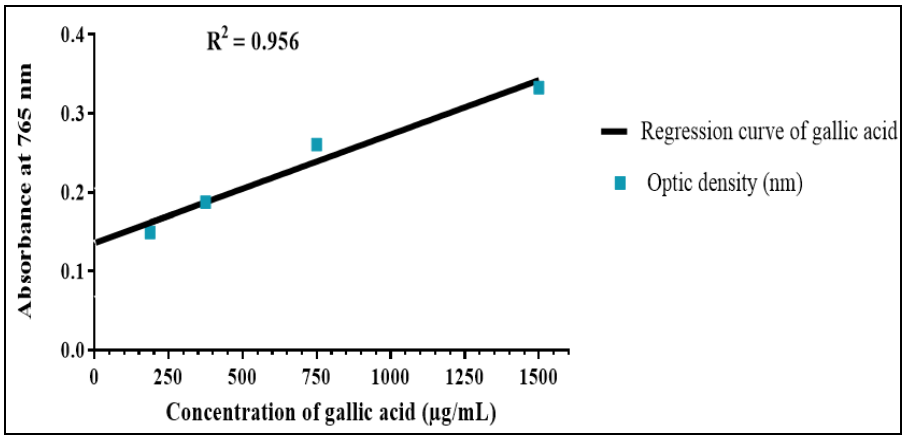

Fig 1: Standard calibration curve of gallic acid for total antioxidant content in essential oil of stem barks of *G. suaveolens*.

3.2.2. DPPH radical scavenging activity

The figure 2 shows the dose-response curve of DPPH radical scavenging activity of essential oil of *G. suaveolens*, compared with gallic acid. It was observed that the standard molecule had higher activity than essential oil. At the concentration of 300 µg/mL, the inhibition percentage was 76.51% and 94.10% respectively for *G. suaveolens* EO and standard. The scavenging capacity 50 (SC_{50}), effective capacity 50 (EC_{50}) and antiradical power (AP) is presented in Table 2. These results showed that, the higher antioxidant activity is found in gallic acid compare to *G. suaveolens*. Nyegue *et al.* in 2008 has reported that *P. suaveolens* essential oil had shown a SC_{50} value of 710 ± 40 µg/mL compared to *G. suaveolens* at 15.60 ± 1.65 µg/mL in this study. The difference between the scavenging abilities could be probably influenced by chemical composition of essential oil [21].

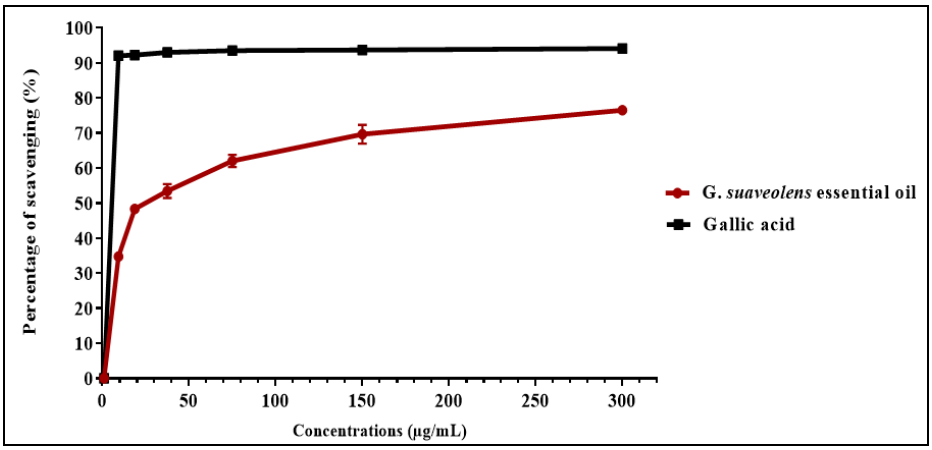

Fig 2: Curve of variation of the percentage of scavenging of the DPPH˙ radical as a function of the concentration of essential oil of stem barks of *G. suaveolens*.

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability [25]. Though the DPPH radical scavenging abilities of the essential oil (0.85 ± 0.00) were less than that of gallic acid (3.38 ± 0.00), the study showed that the essential oil have the proton-donating ability and could serve as free radical inhibitors or scavengers [26].

Table 2: Summary of *in vitro* antiradical activity by the DPPH assay.

Essential oil	SC_{50} (µg/mL)	EC_{50} (µg EO/mg of DPPH)	AP (α x 10^{-3})
***G. suaveolens* essential oil**	15.60 ± 1.65***	1170.75 ± 0.53***	0.85 ± 0.00
Gallic acid	3.94 ± 0.22	295.87 ± 0.27	3.38 ± 0.00***

Legend: µg/mL: micrograms of sample per millilitre of solution; µg EO/mg of DPPH: micrograms of dry sample per milligrams of DPPH; mg/µg: milligrams de DPPH per micrograms of dry weight sample. Data are expressed as mean ± SD; Gallic acid was used as a reference compound. Statistical differences between gallic acid and *G. suaveolens* essential oil are analyzed by the Student t-test (***: p< 0.001).

3.2.3. ABTS radical scavenging activity

The EO of the stem barks of *G. suaveolens* was a fast, effective scavenger of the ABTS radical (Figure 3), and this activity was comparable to that of gallic acid. At 150 µg/mL, the activity of the essential oil was similar to standard molecule with the percentage inhibition of 99.98% and 99.33% respectively for the *G. suaveolens* EO and gallic acid. The SC_{50}, EC_{50} and AP of ABTS assay is presented in Table 3 and showed that ABTS radical scavenging abilities of the essential oil (1.59 ± 0.00) were similar than that of gallic acid (1.72 ± 0.00).

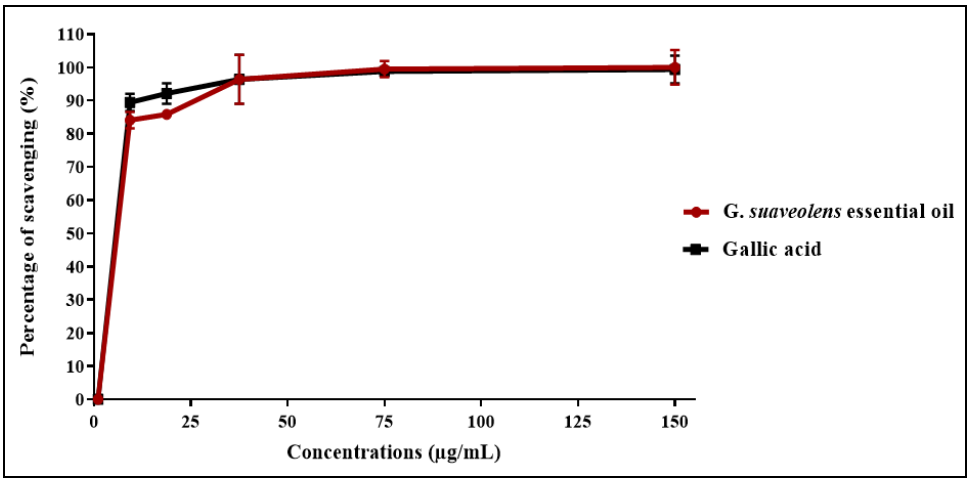

Fig 3: Curve of variation of the percentage of scavenging of the radical ABTS as a function of the concentration of essential oil of stem barks of *G. suaveolens*.

Table 3: Summary of *in vitro* antiradical activity by the ABTS assay

Essential oil	SC_{50} (µg/mL)	EC_{50} (µg EO/mg of ABTS)	AP (α x 10^{-3})
***G. suaveolens* essential oil**	4.69 ± 0.06[a]	627.46 ± 0.27[a]	1.59 ± 0.00[a]
Gallic acid	4.32 ± 0.08[a]	577.92 ± 0.40[a]	1.72 ± 0.00[a]

Legend: µg/mL: micrograms of sample per milliliter of solution; µg EO/mg of ABTS: micrograms of dry sample per milligrams of ABTS; mg/µg: milligrams de ABTS per micrograms of dry weight of sample. Data are expressed as mean ± SD; Gallic acid was used as a reference compound. Statistical differences between gallic acid and *G. suaveolens* essential oil are analyzed by the Student t-test ($p< 0.05$).

The scavenging of the $ABTS^{+\cdot}$ radical by the EO of the stem barks of *G. suaveolens* was found to be much higher than that of DPPH radical. Factors like stereo selectivity of the radicals or the solubility of essential oil in different testing systems have been reported to affect or modulate the capacity of bio-molecules contained in the plant extract to react and quench different radicals [27, 28].

Wang *et al*. [29] reported that some compounds that have $ABTS^{+\cdot}$ scavenging activity did not show DPPH scavenging activity. This is not the case in this study. Nevertheless, this study compared the $ABTS^{+\cdot}$ and DPPH scavenging activity in Table 4. It showed the capability of the essential oil to highly scavenge the free radicals of $ABTS^{+\cdot}$ (1.59 ± 0.00) than DPPH free radical (0.85 ± 0.00). The results obtained are analogous to those of Emad *et al*. in (2012) which showed that DPPH has low solubility in aqueous solutions compared to organic solvents (methanol and ethanol). While, the ABTS showed the advantage of having a better solubility in both [30]. The scavenging capacity of *G. suaveolens* EO in this study indicates that it may be useful therapeutic agents for treating radical-related pathogenic and inflammatory damages.

Table 4: Comparison of scavenging capacity 50 (SC_{50}), effective capacity 50 (EC_{50}) and antiradical power (AP) between ABTS and DPPH assays.

***G. suaveolens* essential oil**	**ABTS**	**DPPH**
SC_{50} (µg/mL)	4.69 ± 0.06	15.60 ± 1.65***
EC_{50}	627.46 ± 0.27	1170.75 ± 0.53***
AP (α x 10^{-3})	1.59 ± 0.00	0.85 ± 0.00***

Legend: Data are expressed as mean ± SD. Statistical differences between ABTS and DPPH are analyzed by the Student t-test (***: $p< 0.001$).

3.2.4. Reducing ability (FRAP)

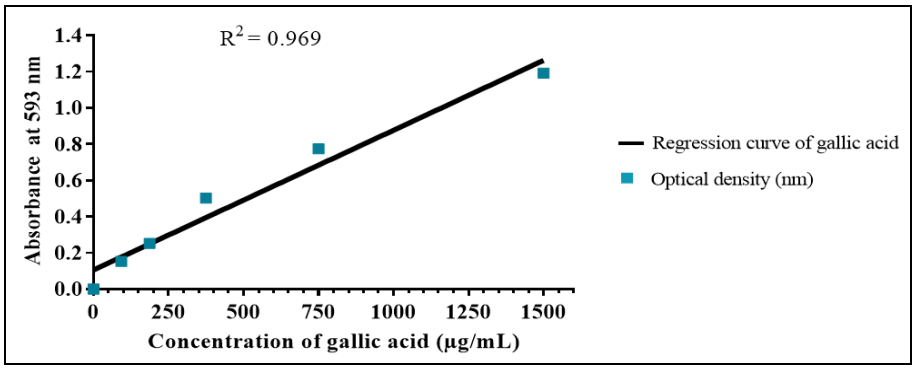

Fig 4: Standard calibration curve of gallic acid for reducing power in essential oil of stem barks of *G. suaveolens*.

The reducing ability of the *G. suaveolens* EO was 94.75 ± 1.66 µg EGA/mg of dry weight. The results were obtained from the calibration curve of gallic acid (Figure 4). The ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II) of essential oil of the stem barks of *G. suaveolens* was proved. The ferric reducing/antioxidant power (FRAP assay) is widely used in the evaluation of the antioxidant component in dietary phenolic compounds and others compounds that having hydroxyl group on aromatic ring. Antioxidant activity increased proportionally to the phenolic compounds content [26, 31].

According to recent reports, a highly positive relationship between total phenols or phenolic compounds and antioxidant activity appears to be the trend in many plant species [32] like *Annonaceae* family.

Djova *et al*. has highlighted the notion of primary and secondary antioxidants. The primary antioxidants or antiradicals also called true antioxidants, which are molecules capable of interrupting the autocatalytic chain by blocking lipid free radicals by transfer of a hydrogen radical. While, the secondary or preventive antioxidants that act on other oxidation factors. They are able to delay the oxidation of lipids by indirect mechanisms such as oxygen reduction or complexation of metal ions [33]. It was noted in Table 5 that the *G. suaveolens* EO had a higher amount of antioxidant chelator (94.75 ± 1.66 µg EGA/mg of dry weight), than the true antioxidants (5.20 ± 0.55 µg EGA/mg of dry weight).The values obtained, were statistically different with a significance level $p<0.05$.

Table 5: Summary of total antioxidants content of essential oil of *Greenyawodendron suaveolens*.

Plant extract	Total antioxidants content	Primary antioxidants	Secondary antioxidants	Pharmacological action
	(µg EGA/mg dw)			
***G. suaveolens* essential oil**	240.87 ± 1.70	5.20 ± 0.55	94.75 ± 1.66***	
Percentage of antioxidant constituents	100%	2.16%	39.35%***	**Preventive**

Legend: µg EGA/mg dw: micrograms equivalent of Gallic acid per milligrams of dry weight. Data are expressed as mean ± SD. Statistical differences between primary antioxidants and secondary antioxidants are analyzed by the Student t-test (***: $p<0.001$).

At the end of antioxidant investigations, the activity of essential oil was deemed preventive or curative depending on the ratio: primary antioxidants/secondary antioxidants. If primary antioxidants/secondary antioxidants is lower than one, the plant extracts is preventive and curative when primary antioxidants/secondary antioxidants higher than one.

3.3. *In vitro* anti-inflammatory analysis

Bovine serum albumin denaturation: The essential oil of *G. suaveolens* displayed significant activity (figure 5). The standard drug and *G. suaveolens* essential oil at 500 µg/mL showed highest inhibition 94.05% and 80.32% of bovine serum albumin denaturation ($p<0.05$) respectively. This essential oil has also showed a higher inhibitory concentration 50 (111.41 µg/mL) compared to the IC_{50} of standard molecule (36.83 µg/mL).

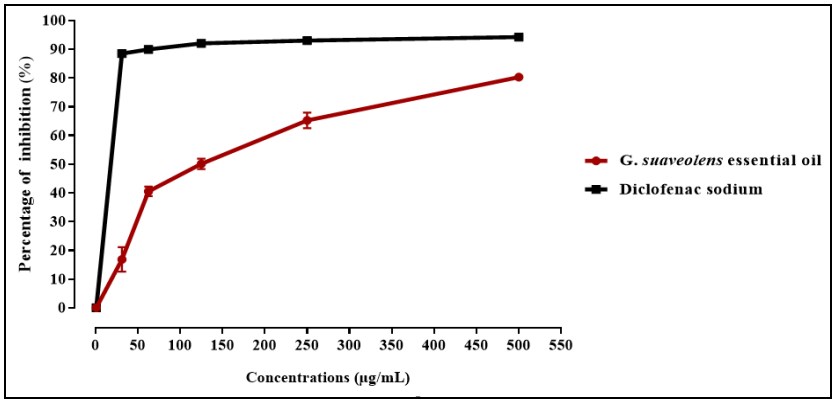

Fig 5: Effect of essential oil of *G. suaveolens* on bovine serum albumin denaturation activity.

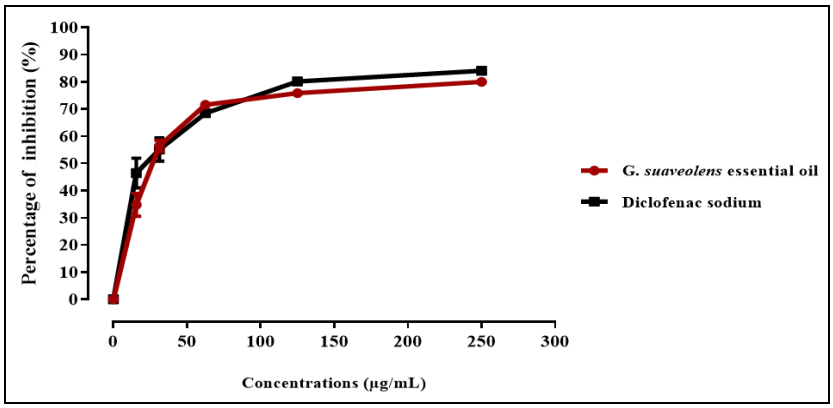

Fig 6: Effect of essential oil of *G. suaveolens* on proteinase inhibitory action activity.

Concerning the proteinase inhibitory action, *G. suaveolens* exhibited significant anti-proteinase activity (figure 6). The inhibitory concentration 50 was observed at 25.85 µg/mL and 19.87 µg/mL for essential oil of *G. suaveolens* and the standard diclofenac sodium drug respectively. Statistically, there is no significance difference between these IC_{50} values. The results are tabulated in Table 6.

Table 6: Summary of the inhibitory concentration of bovine serum albumin denaturation and proteinase inhibitory action assay of *G. suaveolens* essential oil and diclofenac sodium drug.

IC_{50} (µg/mL)	*G. suaveolens* essential oil	Diclofenac sodium
BSA denaturation	$111.41 \pm 0.01^{b\alpha}$	$36.83 \pm 0.74^{a\alpha}$
Proteinase inhibitory action	$25.85 \pm 2.36^{a\beta}$	$19.87 \pm 2.75^{a\beta}$

Legend: IC_{50}: Inhibitory concentration 50. Data are expressed as mean ± SD; Diclofenac sodium was used as a reference compound. Statistical differences (a>b) between diclofenac sodium and *G. suaveolens* essential oil as analyzed by the Student t-test ($p< 0.05$). Statistical differences (β>α) between proteinase inhibitory action assay and BSA denaturation assay as analyzed by the Student t-test ($p< 0.05$).

Denaturation of proteins and proteinase action are well-documented causes of inflammation [18, 34]. Table 6 highlights that the essential oil of *G. suaveolens* has a higher anti-inflammatory potential in the proteinase inhibitory action compared to bovine serum albumin denaturation. While, according to standard drug, it has shown the same activity in these two assays. The essential oil of *G. suaveolens* may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage [35]. Neutrophils are also known to be a source of proteinase that carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors like essential oil compounds [36]. Recent studies have shown that many phenolic compounds, hydrocarbons and oxygenated sesquiterpenes contributed significantly to the antioxidant and anti-inflammatory activities of many plants [21, 37].

Thus, these assays were applied for the detecting essential oil compounds that can stabilize the functional and structural proteins from protein denaturation and proteinase inhibition process. The following activity as cyclooxygenase, lipoxygenase and antimicrobial analysis could make the *G. suaveolens* essential oil a potential candidate for further studies.

4. Conclusion

The present investigation has shown that the *G. suaveolens* essential oil has active chemicals compounds that are able to inhibit oxidative and inflammatory damages. This essential oil showed significantly higher proton-donating ability and effective scavengers of the ABTS radical, which could serve as free radical inhibitors or scavengers. The *G. suaveolens* essential oil was richer in chelating antioxidants than in true antioxidants, which suggests that EO of *G. suaveolens* stem bark can be recommend in the prevention of non-communicable diseases like cancers, cardiovascular diseases, diabetes and infectious diseases common among populations. Strong anti-inflammatory efficiency was confirmed with *G. suaveolens* essential oil. These activities may be due to strong occurrence of phenolic and sesquiterpenic compounds. The anti-inflammatory activity was comparable with standard diclofenac sodium. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antioxidant and anti-inflammatory agent from *G. suaveolens* to stabilize the functional and structural proteins from protein denaturation and proteinase inhibition process.

Abbreviations

GC-FID: Gas Chromatography/Flame Ionization Detector; GC/MS: Gas Chromatography coupled to Mass Spectrometry; NSAID: Non-Steroidal Anti-inflammatory Drug; SAID: Steroidal Anti-inflammatory Drug; HNC: Cameroon National Herbarium; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power Assay; ABTS: 2,2′-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid); EO: Essential oil; LRI_X: Linear Retention Index of compounds on apolar (HP-5) column; LRI: Linear Retention Index; RI: Retention Indices.

Acknowledgments

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