IN VITRO ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF ALCHORNEA CORDIFOLIA (SCHUMACH AND THONN.) MULL. ARG. AND ANTROCARYON KLAINEANUM PIERRE EXTRACTS

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ABSTRACT

Objective: This study aims to evaluate the in vitro antioxidant, anti-inflammatory activities of the aqueous and hydroethanolic extracts recipe of Alchornea cordifolia and Antrocaryon klaeaneum. A preliminary phytochemical screening was carried out.

Methods: The total phenols content was determined by the Folin Ciocalteu reagent method, while the antioxidant activity of both extracts was characterized by the 2,2-diphenyl-1-picrylhydrazil (DPPH) and β-carotene assays. The anti-inflammatory activity of the extracts was evaluated as the inhibition of Bone Serum Albumin (BSA) denaturation and antiprotease activity.

Results: The aqueous extracts of Alchornea cordifolia and Antrocaryon klaeaneum contained more polyphenols [270 mg Ascorbic acid equivalent (AAE)/g dry weight (d.w)] than the hydroethanolic recipe extract (262.41 mg AAE/g d.w) at the same concentration level. On the other hand, the aqueous and hydroethanolic recipe extract had the same radical scavenging activity with the antiradical power of 0.851 and 0.830, respectively. Similarly, the recipe extract had the same reducing activity with reducing the power of 94.2±2.03 mg EAA/g d.w and 97.4±4.16 mg EAA/g d.w for the aqueous and hydroethanolic recipe extract of Alchornea cordifolia and Antrocaryon klaeaneum respectively. For the anti-inflammatory activity it was observed that both extracts possess the same activity as Diclofenac® with an IC50 of 50.21 μg/ml. The phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, carbohydrates, phenols and tannins, which may account for their activities.

Conclusion: The plant recipe extract studied possess antioxidant and anti-inflammatory potentials, which may be beneficial to its consumers.

Keywords: Antioxidant, Anti-inflammatory, Alchornea cordifolia, Antrocaryon klaeaneum, Phytochemical study, Recipe

INTRODUCTION

Free radicals are known as Reactive Oxygen Species (ROS) are produced by the human body in a normal metabolic process and its environment. There is a permanent system of antioxidant defense of the body against ROS, but an imbalance between antioxidants and pro-oxidants is in favor of pro-oxidants leads to oxidative stress [1], oxidative stress and inflammation are strongly linked [2, 3]. Inflammation, when it reacts disproportionately, it may be inappropriately, pathological and altering homeostasis thus, contributing to the onset of disease [4]. The synthetic anti-inflammatory and antioxidants are currently used are associated with serious side effects like weight gain, gastric irritation [5]. Therefore, the development of anti-inflammatory drugs with little or no side effects is needed from medicinal plants. Alchornea cordifolia (Schumach. and Thonn.) Müll. Arg. (Euphorbiaceae), also called “aboe” in ewondo, “dibobon” in Doula, is one of such plants which is widely distributed in tropical and subtropical regions of Africa. In the central and coastal regions of Cameroon, Dibong et al. [6] reported that the leaves of this plant are used for the treatment of anemia. This plant has many order traditional applications, including treatment of pain and inflammatory disorders, hormonal-related gynecological disorders, infertility, urinary, respiratory and intestinal problems as well as malaria-like fevers and anti-HIV1 integrase activity [7-16]. Earlier studies on the chemical constituents of A. cordifolia identified compounds such as tannins, phenolic acids, flavonoids and alkaloids [11]. The variety of popular uses of different parts of A. cordifolia have led to many modern pharmacological investigations like antibacterial [16], antifungal [17], spasmolytic [18] and reproductive activities [13].

Antrocaryon klaeaneum Pierre (Anacardiaceae) called “ongongu” in ewondo, “nongoa” in bassa is an evergreen tree, deciduous, medium-sized up to 35 to 45 m tall [19]. The barks of Antrocaryon klaeaneum are used as a decoction for the treatment of painful periods, sales ailments and even sexually transmitted infections in Cameroon [6]. The crude extract and the isolated compounds of this plant were evaluated for their ability to inhibit the 3D7 strain of Plasmodium falciparum and were evaluated to treat liver complaints and to facilitate the production of breast milk [20, 21]. Phytochemical studies of A. klaeaneum extracts have shown the presence of secondary metabolites with antimalarial properties [22]. As of now, very few authors reported the evaluation of the antioxidant and anti-inflammatory activities of the recipe from these plant. Therefore, the aim of the present study was to evaluate the antioxidant and anti-inflammatory activities of the aqueous and hydroethanolic extracts recipe of Alchornea cordifolia (Schumach and Thonn.) Müll. Arg. and Antrocaryon klaeaneum Pierre.

MATERIALS AND METHODS

Plant extracts

The leaves of Alchornea cordifolia were collected in the locality of Mfou Center Region of Cameroon in July 2017. The plant identification was done at the Cameroon National Herbarium by comparison with specimen number 9657/SRF/Cam of Alchornea cordifolia (Schumach and Thonn.) Müll. Arg. (Euphorbiaceae).

The barks of Antrocaryon klaeaneum were collected in July 2017, in Ebolowa (Southern Region of Cameroon). The plant identification...
was done at the Cameroon National Herbarium by comparison with specimen number 1742/SRF/Cam of Antrocaryon klaineanum Pierre (Anacardiaceae).

The fresh vegetal material collected was dried in the shade at room temperature (25 °C) for two weeks to constant weight. The aqueous extracts were prepared by boiling 100 mg of plant material in 1000 ml of water. The hydroethanolic extract was obtained from a maceration of 100 g of plant material in 1000 ml of water-ethanol [70:30].

The boiled extracts obtained was allowed to cool and then filtered using a 0.23 mm diameter filter. The filtrate is then left on the bench for 24 h and the supernatant dried in a lyophilizer.

The macerate obtained is filtered on hydrophilic cotton and then on Whatman No. 1 paper, then the filtrate is dried in an oven at 70 °C for 48 h. The dried extracts obtained were weighed and stored in the refrigerator at 4 °C.

As indicated in an ethnobotanical study, Alchornea cordifolia and Antrocaryon klaineanum are the most plants used in the treatment of infectious diseases in four cities of Cameroon as reported by Ndjob et al. [23]. For the preparation of the recipe solutions from the obtained plant extracts, the same amount of mass of each extract is weighed and dispersed in 3 ml of distilled water to obtain a final concentration of 100 mg/ml [24].

Determination of the total phenols content by the Folin-Ciocalteu method

The total polyphenols were evaluated according to the spectrophotometric method using the Folin-Ciocalteu reagent as described by Cheew et al. [25]. In this method, 1817 µl of distilled water was introduced into the test tube, 115 µl of Folin Ciocalteu diluted 1:10 and 345 µl of 15% sodium carbonate (Na2CO3) were added. Tubes were vortexed and incubated for 2 h, and the absorbance measured at 765 nm. Calibration was performed using a freshly prepared aqueous solution of Ascorbic acid.

DPPH radical scavenging test

The molecule of 1,1-diphenyl-2-picrylhydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecular does not dimerize, as would be the case with most other free radicals. To prepare a standard solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH), 10 mg of DPPH was dissolved in 25 ml of methanol [26]. From this solution, 5 ml was taken and mixed with 45 ml of methanol. After preparing the different solutions, 1950 µl of the DPPH solution was pipetted into test tubes and 50 µl of each extract at different concentrations was then added to each test tube to a final volume of 2 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 Jenway 6305, Germany, after 120 min of incubation.

Kinetik study, development of study conditions

At the beginning of this evaluation, a kinetik study of the reactivity of DPPH with the different extracts recipe was carried out. It consisted in monitoring the reactivity of the DPPH over time by measuring the absorbance every 15 min for 120 min [27].

β-carotene bleaching coupled the auto-oxidation of linoleic acid assay

The reductive capacity of extracts was estimated by the method [27] as described previously by Moure et al. [28] and Djova et al. [29].

Anti-inflammatory assays

Bovine serum albumin (BSA) denaturation assay

Protein denaturation was performed as described by Djova et al. [30].

Proteinase inhibitory action

The anti-proteinase activity of ASE was determined using the Oyedepo and Famurewa method [31] with slight modifications. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 mmol Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations. The reaction mixture was incubated at 37 °C for 5 min and then 1 ml of 5% (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.

Chemical screening

The secondary metabolites present in the plant extracts studied were investigated through several methods that differ in their reagents and solvents as well as in the coloration observed and their presence was noted (+) and their absence (-) [32].

Data analysis

Graphs were obtained using the Microsoft Excel 2013 Spreadsheet. Statistical analyses were performed in GraphPad/Prism 7 for Windows (San Diego, California, USA) and ANOVA Windows SPSS 23 software. The analysis of the variance (ANOVA) was used to compare the averages between more than two groups. The materiality threshold was set at p<0.05.

RESULTS

Total phenolic content

The evaluation of the total phenolic content in the extracts made it possible to quantify them in two categories of antioxidants as shown in table 1.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total phenols</th>
<th>Primary antioxidants</th>
<th>Secondary antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg EAA/g dw)</td>
<td>(mg EAA/g dw)</td>
<td>(mg EAA/g dw)</td>
</tr>
<tr>
<td>Ac1+Ak1</td>
<td>270.8±0.1**</td>
<td>8.3±0.94</td>
<td>94.22±0.03</td>
</tr>
<tr>
<td>Ac2+Ak2</td>
<td>262.41±0.3***</td>
<td>4.9±0.3*</td>
<td>97.4±4.16</td>
</tr>
</tbody>
</table>

Legend: *(Ac1+Ak1): aqueous extracts Alchornea cordifolia and Antrocaryon klaineanum (v/v); (Ac2+Ak2) hydroethanolic extracts Alchornea cordifolia and Antrocaryon klaineanum (v/v). Values that have the same symbols are statistically identical (p>0.05). Statistically significant differences between total polyphenols content of combined aqueous and hydroethanolic extracts of Alchornea cordifolia and Antrocaryon klaineanum (**p<0.001).

In this table, it can be seen that the aqueous extract (270.8±0.1 mg AAE/g dw) composed of extracts of equal proportions of Alchornea cordifolia and Antrocaryon klaineanum, had a higher total phenolic content than the hydroethanolic extract (262.41±0.3 mg AAE/g dw) composed of extracts of same proportion of the same plants. It was also noted that the extracts had a higher amount of preventative antioxidant with the content of 94.2±2.03 mg AAE/g dw and 97.4±4.16 mg AAE/g dw respectively for the combined aqueous extract of Alchornea cordifolia and Antrocaryon klaineanum and combined hydroethanolic extract of Alchornea cordifolia and Antrocaryon klaineanum than that of the curative antioxidant (8.3±0.94 mg AAE/g dw and 4.9±0.3 mg AAE/g dw) respectively for the combined aqueous extract of Alchornea cordifolia and Antrocaryon klaineanum and combined hydroethanolic extract of Alchornea cordifolia and Antrocaryon klaineanum.

Kinetics of DPPH scavenging

A kinetic study preceded the antiradical analysis by the DPPH method (fig 1):
Nyegue et al.  


Fig. 1: Absorbance curves of the extracts as a function of time

Legend: (Ac1+Ak1): aqueous extracts *Alchornea cordifolia* and *Antrocaryon klaineanum* (v/v); (Ac2+Ak2) hydroethanolic extracts *Alchornea cordifolia* and *Antrocaryon klaineanum* (v/v)

It follows from this curve that the decay of the kinetics curve of the DPPH continues until the 120th min and its stationary state is still not reached.

**DPPH antiradical assay**

The Ascorbic acid calibration curve for the DPPH test obtained is shown in fig. 2 below:

This curve made it possible to obtain the concentration value for trapping 50% of free radicals (SC_{50}) of Ascorbic acid, which is 0.015±0.000 mg/ml.

The following curve represents the percentage of free radical trapping by the extracts as a function of the concentration (fig. 3):

This curve made it possible to obtain the concentrations at which the extracts trap 50% of free radicals (SC_{50}) from which the effective concentrations and the antioxidant power were deduced. The values obtained are recorded in the following table 2.

![Fig. 2: Ascorbic acid calibration curve for DPPH](image)

Table 2: Anti-free radical efficacy of DPPH by extracts compared to ascorbic acid

<table>
<thead>
<tr>
<th>Extracts</th>
<th>SC_{50} en mg/ml</th>
<th>EC_{50}</th>
<th>PA</th>
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<tbody>
<tr>
<td>Aqueous extract (Ac1+Ak1)</td>
<td>0.47±0.2725</td>
<td>1.175</td>
<td>0.851*</td>
</tr>
<tr>
<td>Hydroethanolic extract (Ac2+Ak2)</td>
<td>0.048±0.8143</td>
<td>1.2</td>
<td>0.83*</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>0.015±0.000</td>
<td>0.375</td>
<td>2.66</td>
</tr>
</tbody>
</table>

Legend: (Ac1+Ak1): aqueous extracts *Alchornea cordifolia* and *Antrocaryon klaineanum* (v/v); (Ac2+Ak2) hydroethanolic extracts *Alchornea cordifolia* and *Antrocaryon klaineanum* (v/v). Values that have the same symbols are statistically identical (*p >0.05*).
The two extracts had substantially the same antiradical activity with statistically identical power of 0.81 and 0.851 respectively for the aqueous and hydroethanolic extracts of *Alchornea cordifolia* and *Antrocaryon klaineanum*, but Ascorbic acid with antiradical power of 2.66 had a antiradical activity greater than that of the two extract plants.

**β-carotene bleaching assay**

The fig. 4 represents the reducing capacity of the aqueous and hydroethanolic extracts by the β-carotene method. This curve made it possible to obtain the IC₅₀ and reducing power values and the values are recorded in the following table 3:

**Table 3: Reductive capacity of in vitro extracts by the β-carotene test**

<table>
<thead>
<tr>
<th>Extracts</th>
<th>IC₅₀ (µg/ml)</th>
<th>PR (mg EAA/g dw)</th>
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<tbody>
<tr>
<td>Aqueous (Ac1+Ak1)</td>
<td>2.64±0.11*</td>
<td>94.2±2.03*</td>
</tr>
<tr>
<td>Hydroethanolic (Ac2+Ak2)</td>
<td>2.86±0.0655*</td>
<td>97.4±4.16*</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>0.53±0.0053</td>
<td></td>
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</table>

Legend: (Ac1+Ak1): aqueous extracts *Alchornea cordifolia* and *Antrocaryon klaineanum* (v/v); (Ac2+Ak2) hydroethanolic extracts *Alchornea cordifolia* and *Antrocaryon klaineanum* (v/v). Values that have the same symbols are statistically identical (*p* >0.05).

Extracts and Ascorbic Acid have statistically different reducing capacities at *p* = 0.0001 < 0.005. Indeed, the reducing capacity of Ascorbic Acid (0.53±0.0053 µg/ml) is higher than that of the two recipes of aqueous and hydroethanolic extracts (between 2.64±0.11 and 2.86±0.0655 µg/ml) respectively. In addition, the reducing capacity of the recipe composed of same proportions of aqueous extracts of *Alchornea cordifolia* and *Antrocaryon klaineanum* (2.64±0.11 µg/ml) is greater than that of the hydroethanolic extracts of the same plants (2.86±0.0655 µg/ml).

**Anti-inflammatory assay**

The fig. 5 below shows the curve of the percent inhibition as a function of the concentration of the aqueous (*Alchornea cordifolia* and *Antrocaryon klaineanum*, v/v) and the hydroethanolic (*Alchornea cordifolia* and *Antrocaryon klaineanum*, v/v) extracts compared to the anti-inflammatory reference, Diclofenac®.

The IC₅₀ was determined from the regression line *Y* = *ax+b*.

The denaturation test of BSA reveals that the two extracts studied have substantially the same activity as Diclofenac at *p*<0.05 with an IC₅₀ of 50.21 µg/ml.

The fig. 6 shows percentage inhibition of proteinase extracts and acetylsaliclic acid versus concentration. This curve made it possible to determine the IC₅₀ and the results recorded in the following table 4:

![Fig. 4: Curve of the percentages of entrapments as a function of the concentration of the extracts](image)

**Fig. 5: Calibration curve of the extracts and Diclofenac and capacity to denature the BSA**

Legend: (Ac1+Ak1): aqueous extracts *Alchornea cordifolia* and *Antrocaryon klaineanum* (v/v); (Ac2+Ak2) hydroethanolic extracts *Alchornea cordifolia* and *Antrocaryon klaineanum* (v/v)
justify the use of these plants in the preventive treatment of diseases caused by oxidative stress and would add that both extracts had statistically the same amount of primary antioxidants on the one hand at

Alchornea cordifolia (270 mg AA/g d.w) composed extracts of same proportions of Alchornea cordifolia and Antrocaryon klaineanum, contained more total antioxidants than the hydroethanolic extract (26.24 mg AAE/g d.w) composed of extracts of same proportions of the two plants. In addition, both extracts had statistically the same amount of primary antioxidants on the one hand at p < 0.05. On the other hand, the aqueous extract (77±1 mg AAE/g d.w) was richer than the hydroethanolic extract (9.9±1.59 mg AAE/g d.w) as antioxidants secondary to the threshold of significance (p = 0.0008<0.05).

The preponderance of secondary antioxidants compared with the primary of one of the two extracts studied refers to a preventive role in the treatment of diseases caused by oxidative stress and would justify the use of these plants in the preventive treatment of a pathology that can degenerate into cancer. In several authors have substantially the same activity (p >0.05), although different from that of acetylsalicylic acid at the p<0.05 threshold.

Chemical screening
Table 5 below shows the families of bioactive compounds present in the extracts studied:

### Table 5: Chemical screening

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<td>A. cord.</td>
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<td>+</td>
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<td>He</td>
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Thus, the compound mainly revealed and present in all the extracts are alkaloids, flavonoids, carbohydrates, phenols and tannins.

**DISCUSSION**

The determination of the amount of total phenols content of the two extracts recipes previously made showed that the aqueous extract (270 mg AAE/g d.w) composed extracts of same proportions of Alchornea cordifolia and Antrocaryon klaineanum, contained more total antioxidants than the hydroethanolic extract (26.24 mg AAE/g d.w) composed of extracts of same proportions of the two plants. In addition, both extracts had statistically the same amount of primary antioxidants on the one hand at p < 0.05. On the other hand, the aqueous extract (77±1 mg AAE/g d.w) was richer than the hydroethanolic extract (9.9±1.59 mg AAE/g d.w) as antioxidants secondary to the threshold of significance (p = 0.0008<0.05).

The preponderance of secondary antioxidants compared with the primary of one of the two extracts studied refers to a preventive role in the treatment of diseases caused by oxidative stress and would justify the use of these plants in the preventive treatment of a pathology that can degenerate into cancer. In several authors have substantially the same activity (p >0.05), although different from that of acetylsalicylic acid at the p<0.05 threshold.

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Thus, the compound mainly revealed and present in all the extracts are alkaloids, flavonoids, carbohydrates, phenols and tannins.
plants in traditional medicine has not always the potentiating effect sought for a specific activity.

The reducing power expressed in mg of Ascorbic acid equivalent per g of dry weight of extract was statistically higher in the aqueous extract composed in the same proportions of the aqueous extract of Alchornea cordifolia and Antrocaryon klaineanum (70.75±0.34), compared to the reducing power of the hydroethanolic extract composed of hydroethanolic extracts of the same plant species (62.67±0.46) at the significance level p=0.05. Both extracts studied demonstrated a good reducing activity of ferric iron. Nevertheless, the extracts and Ascorbic acid have statistically different reducing capacities at p = 0.0001. Indeed, the reducing capacity of Ascorbic Acid (0.53±0.0053 μg/ml) was higher than that of both extracts. Furthermore, the reductive capacity of the aqueous extract (2.64±0.11 μg/ml) composed in same proportions of the aqueous extracts of Alchornea cordifolia and Antrocaryon klaineanum was greater than that of the hydroethanolic extract (2.86±0.0655 μg/ml) composed in 50:50 proportions of hydroethanolic extracts of Alchornea cordifolia and Antrocaryon klaineanum. An ethno botanical, phytochemical and pharmacological review of Alchornea cordifolia has also highlighted its antioxidant properties both in vitro and in vivo [35], reinforcing its therapeutic anti-inflammatory activity. Agbo et al. [36] report that the extracts of this plant have a high antioxidant activity because of their ability to reduce iron and to trap free radicals. These results are in harmony with those found in the literature that phenolic compounds, most especially flavonoids, possess a strong reducing power [36].

The denaturation test of Bovine Serum Albumin (BSA) revealed that the two extracts had substantially the same activity as Diclofenac® with an IC50 of 59.21 μg/ml. With respect to the protease assay, the IC50 values obtained ranged from 10±0.57 μg/ml for acetylsalicylic acid to 60±1.52 μg/ml for the aqueous extract composed of 50:50 proportions of extracts aqueous solution of Alchornea cordifolia and Antrocaryon klaineanum and 75±2.08 for the hydroethanolic extract compound with same proportions of hydroethanolic extracts of the same plant. Both extracts had substantially the same activity, although it was different from that of Acetylsalicylic acid at p=0.05. The methanolic extract of Alchornea cordifolia has, in the past, demonstrated anti-inflammatory activity [34].

Inflammation is the main producer of reactive forms of oxygen, so it may be interesting to combine anti-inflammatory treatment with antioxidant treatment [37]. Vitamin A, like β-carotene, is a lipophilic antioxidant whose role is to protect the body against reactive forms of oxygen, and many studies tend to demonstrate their involvement in the regulation of inflammation genes [38]. In humans, the addition of antioxidants seems, to a certain extent, to have preventive or beneficial effects on the evolution of several pathologies [39]. Few studies report the effect of antioxidants in equine medicine; however, it appears that antioxidants can be used beneficially in inflammatory conditions [40]. In addition, as part of the prevention of degenerative diseases of the nervous system in horses, it seems that vitamin A such as β-carotene are unavoidable elements.

The chemical screening carried out on the 5 types of plant extracts gave an insight into the different groups of metabolites found there. Indeed, the compounds present in the aqueous extracts were alkaloids, flavonoids, carbohydrates, phenols and tannins. The extracts of Alchornea cordifolia are those containing all ten groups of secondary metabolites sought. This result is the same as those obtained by a large number of studies where Alchornea cordifolia, thanks to its wide range of active ingredients, would be used as a panacea in several African countries and fo the treatment of recurrent tropical diseases. In this study, they were associated with the same concentrations and their estimated antioxidant and anti-inflammatory potential. Thus, prepared in two ways, the extracts of the same plant revealed the presence of the same bioactive compounds. The antioxidant potential evaluated was found to be important, although lower than that of the standard molecule. Regarding the anti-inflammatory potential of the extracts studied, it was identical to that of Diclofenac® thus enhancing their use in the management of inflammatory diseases. The anti-inflammatory activity of these extracts, which is substantially the same as that of Diclofenac®, supplemented with their antioxidant potential, would be of therapeutic importance for the management of diseases related to oxidative stress.

ACKNOWLEDGMENT
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Nil

AUTHORS CONTRIBUTIONS
Ndjib Rosette Christelle, Djova Steve Valdi, Kom Wayoué Christelle and Amina Manam carried out the experiments. D’bong Siefried Didier and Agbor Gabriel Agbor designed the study. Nyegue Maximilienne Assens supervised the work and provided the facilities for the study. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST
The authors declare that there are no conflicts of interest regarding this paper.

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