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Phenolic Content, Antioxidant and 15-Lypoxygenase Inhibitory Effects of *Daniellia oliveri* (rolfe) Hutch. & Dalziel (Fabaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. Author MNT investigation, data handling and processing, writing, preparation of original version. Authors ACC, AY and EWLMK investigation, data handling and processing. Authors NO and MK supervision, acquisition of funding. Author RWS idea conception, supervision and acquisition of funding. All authors have read and approved the published version of the manuscript

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Original Research Article

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ABSTRACT

Aims: The aim of this study is to evaluate the content of phenolic compounds, antioxidant and 15-lipoxygenase inhibition effects of methanolic extract and its fractions of *Daniellia. oliveri* stem bark.
Place and Duration of Study: The work was carried out in the Department of Traditional Pharmacopoeia and Pharmacy (MEPHATRA / PH) of the Institute for Research in Health Sciences (IRSS) and LABIOCA from Université Joseph KI-ZERBO in Ouagadougou between February 2018 and June 2019
Methodology: The phytochemical screening and phenolic content were determined by the methods described in the literature. Antioxidant activity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,20-azinobis 3-ethylbenzoline-6-sulphonate), FRAP (ferric reducing

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antioxidant power), and lipid peroxidation (LPO). The anti-inflammatory activities were measured on the ability of the extract to inhibit the activity of enzymes such as 15-lipoxygenase

Results: Phytochemical screening revealed the presence of flavonoids, tannins, saponins, anthocyanosides, triterpenes and sterols. The extract and its fractions showed a good content of phenolic compounds with values ranging from 773.97 ± 22.91 to 505.51 mg EAG / g for total polyphenols; from 24.38 ± 0.34 to 12.04 ± 0.08 mg EQ / g in total flavonoids, 311.65 ± 6.70 to 221 ± 0.52 mg EC / g in condensed tannins; 681.81 ± 3.97 to 421.29 ± 8.73 mg EUA / g in total triterpenes and from 280.29 ± 21.72 to 222.04 ± 20.99 mg EC / g for total sterols. The methanol fraction showed the best antioxidant activity as well as the best inhibitory effect on 15-lipoxygenase activity.

Conclusion: These results showed that *Daniellia. oliveri* possessed an antioxidant power, an inhibitory effect of 15 lipoxygenase. *Daniellia oliveri* could be used in the management of inflammatory pathologies and oxidative stress.

Keywords: Daniellia oliveri; antioxidant; 15-lipoxygenase; anti-inflammatory; phenolic compounds.

1. INTRODUCTION

Medicinal plants have an important role in the life of human populations [1], and are an important source of therapeutic compounds for the improvement of their living conditions [2]. Cultural reasons, the cost of products, and the inaccessibility of health services in remote areas push the population to turn to traditional medicine [2], which is mainly based on medicinal plants. Indeed, many medicinal plants are used for the treatment of chronic diseases [3]. Chronic pathologies are frequently due to oxidative stress [4], which induces the excessive generation of reactive oxygen species (ROS). ROS are directly or indirectly responsible for oxidative damage at the molecular level (DNA, protein, lipid) which can considerably affect the mechanisms for maintaining cellular homeostasis [5]. Lipid peroxidation causes cell membrane dysfunction characterized by a decrease in its fluidity and permeability. Antioxidant deficiency leads to disruption of cell control and redox signaling and / or molecular damage [6]. Cellular damage most of the time increases the inflammatory process, which involves enzymes such as lipoxygenase. This enzyme controls the biosynthesis of leukotrienes from eicosatetraenoic acid. The ROS produced during the biosynthesis of leukotrienes are one of the sources of inflammation involved in pathologies such as cancer, atherosclerosis, diabetes cataracts, Alzheimer's disease, aging, rheumatism, and more [7-8]. Steroidal or non-steroidal anti-inflammatory drugs used to relieve these inflammatory reactions often have secondary side effects [9-10]. Therefore, it would be wise to seek new therapeutic agents with fewer side effects from medicinal plants, which constitute a potential source of natural anti-inflammatory molecules.

Daniellia oliveri is a savanna plant that is widely spread in West and Central Africa. In some African countries like Burkina Faso, this plant is used in traditional medicine for the treatment of old wounds, infections and inflammatory diseases. *Daniellia oliveri* stem bark is used in the treatment of tuberculosis, neuralgia, hemiplegia, pneumonia, and jaundice [11]. Some previous studies demonstrated the antibacterial, analgesic and anti-inflammatory properties of *Daniellia oliveri* [12]. This study aims to evaluate the phytochemical characteristics, antioxidant, and lipoxygenase inhibitory activities of the methanolic extract of *Daniellia. oliveri* stem bark and its fractions

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 plant collection

Daniellia oliveri stem bark was harvested in February 2018 at Ziniaré (Burkina Faso). The plant was identified by professor Amadé OUEDRAOGO from the ecology laboratory of the university Pr Joseph KI-ZERBO (Burkina Faso). A Voucher specimen was deposited in the OUA herbarium of the biodiversity information center (CIB) under the identification number 17251. The plant material was dried in laboratory conditions. After drying, the stem bark was crushed and packaged for experimentation.

2.1.2 Chemicals

All chemicals used were of analytical grade. Folin Ciocalteu Reagent, sodium carbonate, ABTS [2,2'-azinobis(3-ethylbenzoin-6-sulfonate)], DPPH (2,2-diphenyl-1- picrylhydrazyl), trichloroacetic acid, ferric chloride, hydrochloric acid, potassium

persulfate, ascorbic acid, potassium hexacyanoferrate, gallic acid, quercetin, 2-thiobarbituric acid, sodium tetraborate, boric acid, zileuton, linoleic acid and the enzyme lipoxygenase (Sigma Aldrich) were purchased from Sigma® (St Louis, USA).

2.2 Methods

2.2.1 Extraction

A methanolic maceration of the stem bark powder of *Daniellia oliveri* was carried out at room temperature for 48 hours. The macerated product was filtered and then concentrated in a rotavapor, then frozen and lyophilized for 48 hours. The extracts were hermetically sealed and kept at 4°C for the various tests.

The fractionation of the crude extract was done using hexane, dichloromethane, ethyl acetate, methanol 50% (methanol- water v/v) and butanol respectively. At the end of the fractionation, all the organic phases were dried and conditioned for phytochemical and biological tests.

2.2.2 Phytochemical screening

The crude methanolic extract of *Daniellia oliveri* was used for the various chemical characterization tests in tubes according to the method described by Ciulei [13]

2.2.3 Phenolic compounds

The various assays were carried out with the crude extract and the fractions (ethyl acetate; methanol 50%; butanolic).

2.2.3.1 Determination of total phenolics

The determination of total phenolics was carried out according to Singleton et al. [14]. This method is based on reducing FCR (Folin Ciocalteu Reagent) in an alkaline medium by phenolic compounds, resulting in the formation of a blue complex. A volume of 25 µL of extracts (0.1 mg / mL) was mixed with 125 µL of Folin Ciocalteu reagent (0.2 N FCR). After 5 minutes at room temperature, 100 µL of sodium carbonate solution (7.5%) was added. After 1 h incubation of the mixture at room temperature, the absorbance was read at 760 nm (UV spectrophotometer, Epoch Biotek Instruments, USA) against a blank. A standard calibration curve was plotted using gallic acid as a reference (R^2 = 0.9995). The tests were carried out in triplicate, and the results were expressed in mg gallic acid equivalent (mg/ EGA).

2.2.3.2 Dosage of condensed tannins

The condensed tannins were determined according to Swain T et al. [15]. 1 ml of extract (5 mg / ml) and 2 ml of 1% vanillin (1 g of vanillin was dissolved in 100 ml of 70% sulfuric acid) were mixed. The absorbance of the mixture was read (UV spectrophotometer, Epoch Biotek Instruments, USA) at 500 nm after incubation for 15 min in a water bath at 20 ° C. The content of condensed tannins T (%) was determined according to the following formula:

T (%) = 5.10-2 x (A x V / P)

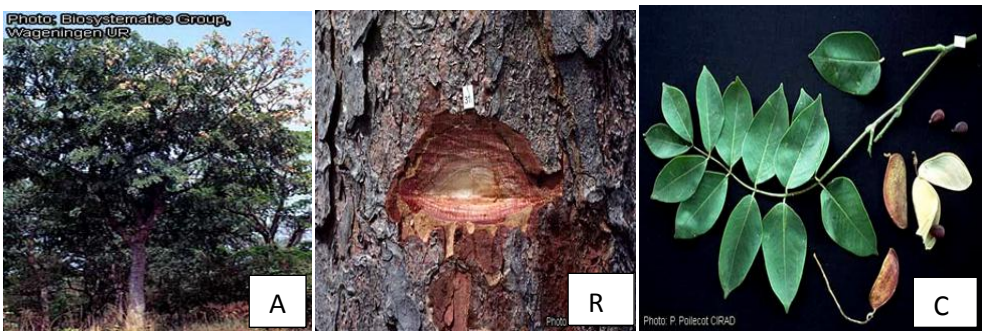
5, 2. 10-2 = Constant in cyanidin equivalence, A = Absorbance, V = extraction volume, P = weight of the sample.

2.2.3.3 Total flavonoids content

The determination of the flavonoids content was carried out according to Arvouet-Grandet et al., [16]. 100 µL of extracts at 1 mg/ml were mixed with 100 µL of aluminium trichloride (2% $AlCl_3$) in methanol. After 10 min of incubation, the absorbance was read at 415 nm using UV spectrophotometer, Epoch Biotek Instruments, USA. The blank consisted of 200 µL of methanol. The absorbance of quercetin (used as a reference compound) is measured under the same conditions and allows one to obtain the correlation coefficient of the calibration (R2 = 0.9998). The tests were carried out in triplicate, and the total flavonoid content was expressed in mg of quercetin equivalent (QE) / g of extract.

2.2.3.4 Triterpenoid and steroid content

The total content of triterpenes and sterols was determined by colorimetry using the method described by Chang et al. [17]. 30 µL of vanillin - 5% glacial acetic acid solution was mixed with 20 µL of the extracts (5 mg / mL) dissolved in methanol. 100 µL of perchloric acid was then added. The mixture was placed in a water bath at 60 ° C for 45 min, then cooled in an ice-water bath for a few minutes. After adding 450 µL of glacial acetic acid, the absorbance of each sample solution was measured using a UV spectrophotometer (Epoch Biotek Instruments, USA). One was measured at 548 nm against a curve of ursolic acid (y = 0.1259x + 0.0683, R^2 = 0.99) for the triterpenes, and the other was measured at 640 nm against a cholesterol calibration curve (y = 0.0744x - 0.0056; R^2 = 0.99) for sterols.



Pic 1. Pictures of *Daniellia oliveri* (Rolfe) Hutch. & Dalziel (Fabaceae): (A) whole plant, (B) stem bark, (C) leaves, flower and fruit

2.2.4 Antioxidant activity

2.2.4.1 DPPH method

The antiradical activity of the extract and its fractions was evaluated using DPPH method according to Velázquez et al., [18]. 100 µL of the different concentrations of the methanolic extract, fractions, and reference substances were mixed in 96-well microplate, with 200 µL of a solution of DPPH prepared in methanol (4 mg / 100 mL). The blank consisted of 100 µL of methanol and 200 µL of DPPH solution. The absorbances were read at 490 nm with the spectrophotometer (Epoch, BioTeck instruments, USA) after 30 min incubation at room temperature. Each test was performed in triplicate. Ascorbic acid and Trolox were used as reference compounds.

2.2.4.2 ABTS method

The anti-free radical activity ABTS was evaluated according to the procedure described by Re et al., [19]. A cascade dilution of the methanolic extract, fractions, and reference substances (Trolox, ascorbic acid) was carried out starting from a concentration of 1 mg / mL. On a 96-well microplate, a reaction mixture was made with 20 µL of each dilution and 200 µL of ABTS $^{\bullet+}$ solution diluted in triplicate. The blank consisted of 20 µL of ethanol and 200 µL of ABTS $^{\bullet+}$ solution. The reaction mixture was then incubated at room temperature for 30 min in the dark, and the absorbances were read using a spectrophotometer at 415 nm (Epoch, BioTeck instruments, USA).

2.2.4.3 FRAP method

The FRAP method is based on reducing the ferric ion (Fe3+) to ferrous ion (Fe2+) by reducing compounds. The ability of *D. oliveri* extracts to reduce the ferric ion (Fe3+) to ferrous ion (Fe2+) was evaluated according to the method described by Hinneburg et al., [20]. In test tubes containing 0.5 mL of the methanolic extract and fractions (1 mg / mL), 1.25 mL of phosphate buffer (0.2 M, pH 6.6), then 1.25 mL of potassium hexacyanoferrate [$K_3Fe\ (CN)_6$] (1% in distilled water) were added. After 30 min of incubation at 50 ° C in a water bath, 1.25 mL of trichloroacetic acid (10%) was added, and the mixture was centrifuged at 2000 rpm for 10 min. Three aliquots were made, each containing 625 µL of the supernatant, 625 µL of distilled water and then 125 µL of freshly prepared 1% $FeCl_3$ in water. A blank was prepared under the same conditions. Absorbances were read by a spectrophotometer (Epoch Biotek Instruments, USA) at 700 nm against a standard curve of ascorbic acid ($y = 12.514\ x + 0.2567$; $R^2 = 0.99$)

2.2.4.4 Inhibition of lipid peroxidation (LPO)

The inhibitory effect of the extract and its fractions on the activity of lipid peroxidation (LPO) was evaluated according to the method of Ohkawa et al. [21], adapted by Sombié et al. [22]. Hydrated iron sulfate ($FeSO_4$, $7H_2O$) and hydrogen peroxide (H_2O_2) were used to induce lipid peroxidation of the hepatic homogenate. A volume of 0.2 mL of each extract (1.5 mg / mL) was mixed with 1.0 mL of the liver homogenate in 1% Tris-HCl buffer (50 mM, pH 7.40). Then 50 µL of $FeSO_4$ (0.5 mM) and 50 µL of H_2O_2 (0.5 mM) were added. The mixture was incubated at 37 ° C for 60 minutes. After this step, 1.0 ml of trichloroacetic acid (15%) and 1.0 ml of 2-thiobarbituric acid (0.67%) were added. The mixture was heated in boiling water for 15 minutes, and the absorbances were read at 532 nm. Ascorbic acid was used as a reference compound.

2.2.5 15-lipoxygenase inhibitory effect

The evaluation of the inhibitory effect of the crude extract of *D. oliveri* and its factions on 15-lipoxygenase (15-LOX) was carried out by the spectrophotometric method described by Maltherud and Rydland [23]. Volumes of 146.25 µL of 15-lipoxygenase (400 Units / mL) and 3.75 µL of the extracts were mixed and incubated for 2 minutes at 25 ° C. A volume of 150 µL of linoleic acid (124mM) solution was added to the mixture to initiate the reaction. The absorbance is monitored at 234 nm for 2 minutes using a UV spectrophotometer, Epoch Biotek Instruments, USA. All tests were performed in triplicate. Zileuton was used as a reference compound

2.3 Statistical Analyses

Graph Pad Prism version 5 software was used for data analysis. ANOVA followed by Dunnett's test was used as a statistical treatment The differences were considered significant when $p \leq 0.05$ when compared to the control and between treated groups.Experiments were performed in triplicate and results were expressed as mean ± standard deviation.

3.3 RESULTS AND DISCUSSION

3.1 Results

3.1. 1 Phytochemical screening

Chemical characterization tests have shown the presence of different bioactive substances in the raw extract of *Daniellia. oliveri* stem bark such as flavonoids, tannins, saponins, anthocyanosides, triterpenes and sterols.

3 .1.2 Content of phenolic compounds

The levels of compounds of interest contained in the methanolic extract of the stem bark of *Daniellia oliveri* and its fractions, namely polyphenols, flavonoids, condensed tannins, triterpenes and sterol are grouped together (Fig. 1).

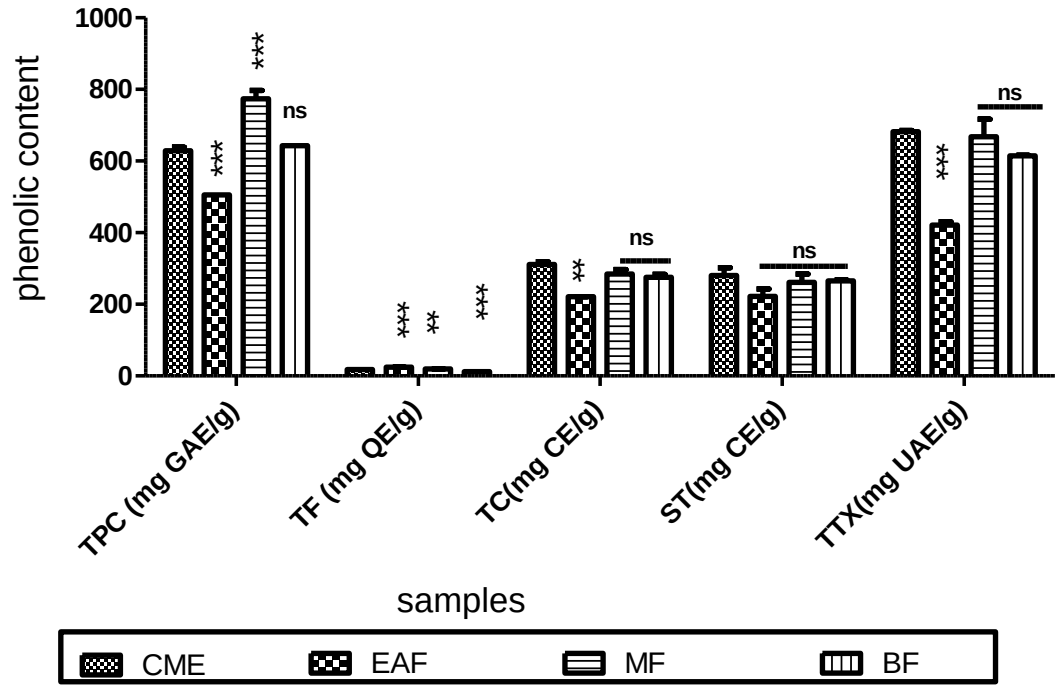


Fig. 1. GAE: Gallic acid equivalent (TPC mgGAE/g: Total phenolic content), QE: Quercetin equivalen (TF mgQE/g: total flavonoid content), UAE: ursolic acid equivalent (TTx mgUAE/g: Triterpene content), CE: cholesterol equivalent (ST mgCE/g: Sterol content), CE: cyanidin equivalent (TC mgCE/g: total condensed tannin) CME (crude extract); EAF (ethyl acetate fraction); MF (methanolic fraction50%); BF (butanolic fraction). Mean values ± standard deviation were presented (n = 3); ; P <0.05 is considered significant compared to the CME. (**) = p <0.01, and (***) = p <0.0001 ns: not significant P> 0.05

3.1.3 Antioxidant activity

The results of the antioxidant activities of *Daniellia oliveri* extract and its fractions by ABTS, DPPH, FRAP and LPO methods are shown (Table 1).

The values are expressed as mean ± standard deviation (n = 3). P <0.05 is considered significant compared to the control. (*) = p < 0.05, (**) = p <0.01, and (***) = p <0.001 Plant extracts vs ascorbic acid for DPPH and ABTS, crude extract (EM) vs fractions for FRAP, plant extracts vs ascorbic acid for lipid peroxidation. ns: not significant; Nd: not determined. CME (crude extract); EAF (ethyl acetate fraction); MF (methanolic fraction); BF (butanolic fraction).

3.1.4 15-lipoxygenase inhibition effect

The inhibitory effect of the crude extract of *Daniellia oliveri* and its fractions on the activity of 15-lipoxygenase was determined and the results are presented (Fig. 2.).

3.2 Discussion

The phytochemical screening showed the presence of secondary metabolites such as triterpenes, tannins, flavonoids, sterols, anthocyanosides, saponins, and reducing compounds in the methanolic extract of *D. oliveri*. The presence of these compounds could justify the pharmacological properties and the traditional uses of this plant. Indeed, some previous studies demonstrated the antioxidant, anticancer, anti-inflammatory, hypoglycemic, and other properties of these compounds [24]. The works of Muanda Francois [25] showed similar results with additional alkaloids in these extracts. The time and location of harvest could explain this difference. Environmental factors influence plant development and secondary metabolite biosynthesis [26].

Table 1. Antioxidant activities of *D. oliveri* and its fractions

Extracts	DPPH (IC_{50} µg / ml)	ABTS (IC_{50} µg / mL)	FRAP (mmol EAA/g)	LPO (%)
CME	4.76 ± 0.01 ***	1.24 ± 0.11 ***	0.8 ± 0.04	56.7 ± 0.66 ***
EAF	13.61 ± 0.12***	1.41 ± 0.11 ***	0.8 ± 0.06 ns	62.6 ± 1.53 ***
MF	2.67 ± 0.0 ***	1.24 ± 0.11 ***	0.82 ± 0.07 ns	62.7 ± 0.64 ***
BF	7.10 ± 0.25 ***	4.10 ± 0.07 ***	1.04 ± 0.02 *	63.4 ± 1.08 ***
Trolox (control)	1.79 ± 0.1	2.04 ± 0.12 ***	Nd	Nd
Ascorbic acid (control)	1.82 ± 0.02	0.94 ± 0.08	Nd	97.1 ± 0.26

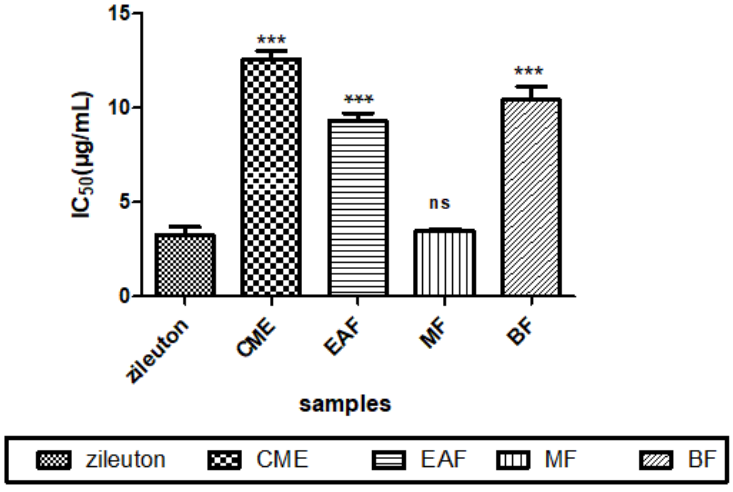


Fig. 2. Inhibitory effect of *Daniellia oliveri* and its fractions on the activity of 15-lipoxygenase. Included are CME (crude extract; EAF (ethyl acetate fraction); MF (methanolic fraction); BF (butanolic fraction). The values are considered significant compared to the zileuton; (***) = p <0.0001. ns: not significant. Values are expressed as mean ± SEM

The dosages of the compounds of interest showed a high content of total polyphenols in *D. oliveri* extracts (Fig. 1) with the highest in the methanolic fraction 50%. The difference in the contents could be explained by the fact that methanol is an effective solvent in the degradation of cell walls with a non-polar character, causing a better extraction of phenolic compounds [27].

The antioxidant activity of the extract and its different fractions through the DPPH, ABTS, FRAP, and LPO methods did not show a significant difference. The methanolic fraction 50% demonstrated interesting results in ABTS and DPPH assays comparable to the reference substances used (Trolox, ascorbic acid). In contrast, the dosage of phenolic compounds shows more richness in tannins, triterpenes and steroids in the stem bark of *D. oliveri*. The antioxidant power of the extract and its fractions could be due to the tannins, anthocyanosides, flavonoids, steroids and triterpenes, and triterpenes known for their antioxidant activities in the extracts [24]. These results could justify the traditional uses of *D. oliveri* stem bark in the treatment of inflammatory pathologies such as cancer. Indeed, antioxidants are used in the treatment of cancer because of their free radical scavenging capacity [24]. Plants with high antioxidant potential constitute a natural source of preventive and curative drugs against diseases with an inflammatory component [28-29].

Lipoperoxidation of membranes results in impaired functionality [30]. The methanolic extract of *Daniellia. oliveri* and its fractions showed a lipid peroxidation inhibition greater than 50%. These extracts demonstrated a moderate effect, but this effect is appreciable compared to ascorbic acid used as reference compound. *Daniellia. oliveri* extracts, through their inhibitory effect on lipid peroxidation, could contribute to treatment of neurodegenerative diseases (Alzheimer's, Parkinson's), diabetes, cancers, and inflammatory diseases. This inhibitory effect on lipid peroxidation can be attributed to flavonoids. Takahama (1983) showed that flavonoids can complete the chain reaction of lipid peroxidation by trapping the peroxide radical LOH [31].

The methanolic extract of *Daniellia oliveri* and its fractions showed a significant inhibitory effect on the activity of 15-LOX. The best inhibitory activity was obtained with the methanolic fraction. The presence of tannins and flavonoids in the stem bark could explain this inhibitory activity of the crude methanolic extract and its fractions. Tannins are recognized for their inhibitory effect on lipoxygenase due to their antioxidant power [32-34]. Likewise, tannins are endowed with anti-inflammatory and analgesic properties [34]. Flavonoids are potential inhibitors of enzymes such as aldose reductase, cyclooxygenase, Ca2+ 2-ATP, xanthine oxidase phosphodiesterase and lipoxygenase [35]. Further studies will focus on the methanolic and ethyl acetate fraction, which showed the best biological activities in order to develop new anti-inflammatory drugs through clinical translational research.

4. CONCLUSION

The methanolic extract of the stem bark of *Daniellia oliveri* and its fractions exhibited some interesting biological properties for the treatment of inflammatory pathologies. The best activity for the various tests is obtained with the methanolic fraction and the ethyl acetate fraction. These results provide a scientific basis for the uses of *Daniellia oliveri* and perspective for further research.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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