Original Research Article

Phytochemical, antioxidant and anti-inflammatory effects of extracts from *Ampelocissus africana* (Lour) Merr (Vitaceae) rhizomes

ABSTRACT

Aims: To determine the phytochemical composition and evaluate the antioxidant and anti-inflammatory properties of methanolic and aqueous extracts from *Ampelocissus africana* (Lour) Merr rhizomes
Study design: Phytochemical content and screening, *in vitro* antioxidant and anti-inflammatory assays
Place and Duration of Study: Department of Traditional Pharmacopoeia and Pharmacy (MEPHATRA / PH) of the Institute for Research in Health Sciences (IRSS) and Laboratory of Applied Biochemistry and Chemistry (LABIOCA), University Joseph KI-ZERBO in Ouagadougou between April and August 2020
Methodology: The antioxidative activity of both extracts of the plant was assessed using DPPH radical scavenging, ABTS+ radical cation decolorization, ferric ion reduction and lipid peroxidation inhibition in rat liver assays. The anti-inflammatory activities *in vitro* were measured on the ability of the extract to inhibit the activity of enzymes such as 15-lipoxygenase, phospholipase A2 (PLA2) and cyclooxygenases (COX-1 and COX-2).
Results: This study revealed that the total phenolic contents of the extracts varied from 471.79 ± 1.71 mg GAE/g to 173.88 ± 1.71 mg GAE/g for methanolic and aqueous extract respectively. The extracts were also rich in flavonoids and tannins. The methanolic extract possessed better antioxidant activity with an IC_{50} of 2.32 ± 0.18 µg / mL for the ABTS, 1.71 ± 0.05 µg/mL for the DPPH, a reducing power agent of 87.44 ± 0.5 mmol AAE /100 g and a percentage inhibition of lipid peroxidation of 52.21%. The methanolic and aqueous extract of *A. africana* has an inhibitory action on activity of lipoxygenase with IC_{50} values of 26.09 ± 1.83 µg/mL to 34.32 ± 1.60 µg/mL, respectively. The methanolic extract caused COX-1 inhibition of 36.07%, COX-2 inhibition of 38.31% and PLA2 inhibition of 26.9%.
Conclusion: These results showed that the methanolic and aqueous extract from the rhizomes of Ampelocissus africana possessed antioxidant power, inhibitor effect against proinflammatory enzymes.

Keywords: Ampelocissus africana, phytochemical, antioxydant, proinflammatory enzymes

1. INTRODUCTION

Oxidative stress is a significant underlying factor to dysfunctional of immune system and inflammatory responses [1]. It plays an important role in the pathogenesis of chronic diseases including chronic inflammation, cancer, cardiovascular disease, diabetes. These diseases can result from the increased expression of redox regulated pro-inflammatory factors such as eicosanoids and cytokines [2]. In fact, reactive oxygen species (ROS) or oxygen free radicals are derived from normal cellular metabolism. They may play a beneficial role in defense against infectious agents and in the function of many cellular signaling systems when at low or moderate concentrations [3]. However, when there is an excess of ROS caused by an imbalance of prooxidant / antioxidant reactions in response to the entry of a toxin in living organisms, it can damage DNA, proteins or cellular lipids and lead to the introduction of a number of human diseases and increase the inflammatory process [4, 5].

ROS plays a major role in inflammation inducing inflammatory mediators' productions such as prostaglandins, leukotrienes, chemokines, cytokines (TNFα, IL1β, IL6) and NF-κB activation [6]. Prostaglandins and leukotrienes are synthesized respectively through cyclooxygenases (COX) and lipoxygenase (LOX) activation. The substrate of these pro-inflammatory enzymes is produced by phospholipase activation. These mediators work by recruiting more inflammatory cells to the site of damage and producing more reactive species [7]. The maintaining of the balance between the beneficial and harmful effects of free radicals is a very important aspect and is achieved by redox regulatory mechanisms. The process of redox regulation protects living organisms from various oxidative stresses and verifies redox homeostasis by controlling the redox status in vivo [8].

Medicinal plants are considered as an important source of molecules can be used against diseases such as inflammation and oxidative stress [9]. Thus, identifying bioactive compounds from plants became an active field of pharmaceutical research [10].

Ampelocissus africana (Lour) Merr from Vitaceae family is a woody liana with tuberous perennial strain, climbing or crawling stem with sturdy branches from the Sudano-Guinean and Sudano-Sahelian regions of intertropical Africa. It is already used in traditional medicine in Burkina Faso against inflammatory diseases, rheumatism, infections, circulation disorders, drops, edema, abscess, hemorrhoids, healing, antiseptic, old wounds [11].

To the best of our knowledge no report is available on the effects of this plant rhizomes acting as antiinflammatory agent. Hence, the aim of this study was to determine the phytochemical composition and evaluate *in vitro* antioxidant (by four methods) and anti-inflammatory properties of methanolic and aqueous extracts from *Ampelocissus africana* (Lour) Merr rhizomes.

2. MATERIAL AND METHODS

2.1 Plant collection

The rhizomes of *Ampelocissus africana* (Lour) Merr were harvested in September 2019 in the region of the Mouhoun loop around Dedougou located to 250 km from Ouagadougou, the capital city of Burkina Faso. A sample was authenticated by the National Herbarium (HNBU) located at the National center for Scientific and Technological Research (CNRST) where the voucher specimen was deposited under N° 8754.

2.2 Chemicals

All chemicals used were analytical grade. ABTS [2,2'-azinobis (3-ethyl benzoin-6- sulphonate)], DPPH (2, 2-diphenyl-1-picrylhydrazyl), trichloroacetic acid, ferric chloride, hydrochloric acid, potassium persulfate, ascorbic acid, potassium hexacyanoferrate, gallic acid, quercetin, Folin Ciocalteu reagent, 2-thiobarbituric acid, Sodium tetraborate, boric acid, Zileuton, linoleic acid, and lipoxygenase (type I-B) enzyme were purchased were purchased from Sigma® (St Louis, USA) Trolox was purchased from (Fluke, France) and Indomethacin, iron dichloride, hydrogen peroxide trichloroacetic acid, aluminum trichloride, ammonia, hydrochloric acid by Prolabo (Paris, France).
COX-1 and human COX-2, Screening Kit (Item No. 560131) and sPLA2 (Item No. 765001) manufactured by Cayman Chemical Co. (MI, USA). All solvents used were of analytical grade.

2.3 Extraction

Rhizomes were washed, cut, then dried under ventilation in the shelter of light and dust. The dried rhizomes were then crushed to obtain a fine powder. About 100 grams of powder were extracted using maceration with 1 L of methanol. After 24 hours, the mixture was filtrated with whatman's filter paper, concentrated using rotary evaporator and kept in an oven until completely evaporation of solvent. The same proportions were used for the aqueous maceration. However, the mixture of this maceration after filtration was centrifuged, frozen and lyophilized. The extracts were stored at 4 °C for the investigations.

2.4 Phytochemical screening

The phytochemical screening of the methanolic extract and its fractions by thin layer chromatography (TLC) was carried out on chromatoplates (60 F254, 10 x 5 cm, 10 x 20 cm glass support, Merck) in accordance with the methods studied in

the literature [12,13]. Each dry extract was solubilized in its extraction solvent and 5 µL were offered on the plate for the evolution of the chromatogram. Polar and nonpolar solvent systems were used to develop the path chromatography to screen for large chemical groups by thin layer chromatography (TLC). These were steroids, terpenes and phenolics. For this purpose, specific reagents were used to reveal these groups of compounds: Neu's reagent for flavonoids, Sulfuric vanillin reagent for terpenes and sterols, $FeCl_3$ reagent for tannins and phenolics, anysaldehyde reagent for saponosides, KOH reagent for coumarins and dragendor'ff reagent for alkaloids.

2.5 Phytochemical composition

2.5.1 Total phenolics content

The determination of total phenolics to aqueous and methanolic extracts was carried out according to the method described by [14]. 25 µL of extracts (0.1 mg/mL) was mixed to 125 µL of Folin Ciocalteu reagent (FCR 0.2 N). After 5 min at room temperature, 100 µL of sodium carbonate solution (7.5 %) were added. After 1 h incubation of the mixture at room temperature, the absorbance at 760 nm was measured (Spectrophotometer UV, Epoch Biotek Instruments, U.S.A.) against blank. A standard calibration curve was plotted using Gallic acid ($y = 0.0062x + 0.078$, $R^2 = 0.9995$). The mixture made in triplicate and the results were expressed as mg of Gallic acid equivalent per g of extract (mg GAE/g).

2.5.2 Flavonoids content

The total flavonoids of extract were determined by the colorimetric method using aluminium chloride described by [15]. 100 µL of plant extract (1 mg/mL) were mixed to 100 µL of aluminium trichloride solution $AlCl_3$ (2% in methanol). After 10 minutes, the absorbance was measured at 415 nm against blank (that consisted of 100 µL methanol and 100 µL of extract without $AlCl_3$), using spectrophotometer (Spectrophotometer UV, Epoch Biotek Instruments, U.S.A.). Quercetin was used as a reference compound to produce the standard curve ($y = 0.01x + 0.0128$, $R^2 = 0.9998$). The tests were performed in triplicate and the total flavonoid content was expressed as mg of quercetin equivalent (QE)/g of extract.

2.5.3 Tannins content

2.5.3.1 Hydrolyzable tannins

The method described by [16] was adopted for the determination of hydrolyzable tannins. 1 mL of each extract (5 mg/mL) was added to 3.5 mL of the reagent (ferric chloride $FeCl_3$ 10^{-2} M in hydrochloric acid HCl 10^{-3} M). After 15 s, the absorbance of the mixture was measured (spectrophotometer UV, Shimadzu) at 660 nm. The hydrolysable tannins content T (%) was determined using the following formula:

$$T (\%) = (A \times PM \times V \times FD) / \varepsilon \text{ mole} \times P$$

A = absorbance, ε mole = 2169 (for gallic acid), PM = weight of gallic acid (170.12 g/mol), V = volume of extract, P = sample weight and FD = dilution factor

2.5.3.2 Condensed tannins

The condensed tannins were determined to [17] method. 1 mL of extract (5 mg/mL) and 2 mL of vanillin 1% (1 g of vanillin was dissolved in 100 mL of 70% sulfuric acid) were mixed. The absorbance of the mixture was measured (spectrophotometer UV, Shimadzu) at 500 nm after incubation of 15 min in a water bath at 20 °C. The condensed tannins content T (%) was determined according to the following formula:

$$T (\%) = 5,2. 10^{-2} \times (A \times V / P)$$

5,2. 10^{-2} = constant in equivalence of cyanidine, A = absorbance, V = extract volume and P = sample weight.

2.6 Antioxidant activities

2.6.1 ABTS (2, 2'-azinobis- [3-ethylbenzothiazoline-6-sulfonic acid]) test

The capacity of extracts to scavenge the ABTS radical cation was determined according to the procedure described by [18]. A stock solution of ABTS (7 mM) was mixed with 2.45 mM of potassium persulfate ($K_2S_2O_8$). The mixture was stored out of the light at the room temperature for 12-16 h before use. A cascade dilution ranges of the extracts and trolox (reference substance) was performed from a concentration of 1 mg/mL in order to determine the inhibitory concentration at 50% (IC_{50}). To do this, 20 µL of each dilution was mixed with 200 µL of the ABTS solution diluted in ethanol in the 96-wells microplate. The absorbances were read against blank (ethanol) at 734 nm on a spectrophotometer (Epoch Biotek Instruments, U.S.A.) after 30 min of incubation in dark at room temperature. The test was performed in triplicate and the percentage inhibition was determined by the following formula:

$$\% \text{ Inhibition} = (A_b - A_s) / A_b \times 100$$

A_b: absorbance of blank; A_S: absorbance of sample/reference compound

2.6.2 DPPH (2,2-diphényl-1-picrylhydrazyl) test

DPPH radical scavenging ability of extracts was assayed as described by [19]. A cascade dilution of the extract and trolox (reference substance) was performed from a concentration of 1 mg/mL. For this purpose, 200 µL of DPPH solution freshly prepared in methanol (4mg / 100 mL) was mixed with 100 µL of each dilution in the 96-wells microplate. The mixture was incubated for 30 min at ambient temperature and the absorbances were measured at 517 nm against a blank (methanol) with a spectrophotometer (Epoch Biotek Instruments, U.S.A.). The DPPH radical inhibition was calculated as follows:

$$\% \text{ Inhibition} = (A_b - A_s) / A_b \times 100$$

A_b: absorbance of blank; A_s: absorbance of sample/reference compound

2.6.3 FRAP (ferric reducing antioxidant power) test

The method described by [20] evaluates the ability of compounds to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). To 0.5 mL of sample solution (1mg/mL) is added 1.25 mL of phosphate buffer (0.2M, pH 6.6), then 1.25 mL of potassium hexacyanoferrate solution [$K_3Fe(CN)_6$] (1% in water). After 30 min 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. 0.625 mL of the supernatant was mixed with 0.625 mL of distilled water and then 0.125 mL of freshly prepared $FeCl_3$ (0.1%) in water. A blank without sample is prepared under the same conditions. The absorbances were read by a spectrophotometer (Epoch Biotek Instruments, U.S.A.) at 700 nm against a standard curve of ascorbic acid (y = 12.514x + 0.2567; R^2 = 0.9921). The potential of extracts to reduce iron (III) to iron (II) was expressed in millimole Ascorbic Acid Equivalent per gram of dry extract (mmol AAE/g).

2.6.4 Lipid peroxidation inhibitory test (LPO)

The method described by [21] was used to evaluate the inhibitory capacity of lipid peroxidation activity in rat liver. Iron dichloride ($FeCl_2$)-hydrogen peroxide (H_2O_2) reaction was used to induce peroxidation of rat liver homogenate. The liver was taken from an anesthetized Wistar rat with ketamine. Briefly, 0.2 mL of a concentration 1.5 mg / mL to extracts or trolox (positive control) was mixed with 1 mL of liver homogenate in 10% phosphate buffered saline (PBS) buffer (pH 7.40), 50 µL of $FeCl_2$ (0.5 mM) and then 50 µL of H_2O_2 (0.5 mM). After incubation at 37 °C for 60 min, 1 mL of trichloroacetic acid (15%) and 1 mL of 2-thiobarbituric acid (0.67%) were added. The mixture was incubated for 15 min at 100 °C and centrifuged (2000 rpm for 10 min). The absorbances was read at 532 nm against control (without extract) with a spectrophotometer (Epoch Biotek Instruments, U.S.A.). All of these measurements were carried out in triplicate. The percentage of inhibition induced by 100 µg/mL was calculated as follows:

$$\% \text{Inhibition} = (A_b - A_s) / A_b \times 100$$

A_b: absorbance of control; A_s: absorbance of sample/ reference compound

2.7 Anti-inflammatory activities *in vitro*

2.7.1 15-Lipoxygenase inhibition assay

The inhibitory activity of lipoxygenase was determined according to the spectrophotometric method described by [22] with slight modifications. 146.25 µL of lipoxygenase solution (820.51 U/mL) prepared in boric acid buffer (0.2 M, pH 9.0) was mixed with 3.75 µL of extracts (8 mg/mL) in the 96-wells microplate and then incubated at room temperature for 3 min. the reaction was initiated by the addition of 150 µL of the substrate (1.25 mM of linoleic acid) and the absorbances was recorded for 3 min at 234 nm with a spectrophotometer (Epoch Biotek Instruments, U.S.A.). All tests were performed in triplicate and Zileuton was used as reference compound. The Percentage of lipoxygenase inhibition was calculated using the formula:

$$\% \text{ Inhibition} = (V_b - V_s) / V_b \times 100$$

Vb: Enzymatic activity without inhibitor; Vs Sample: Enzymatic activity with sample/reference compound

2.7.2 Cyclooxygenases (COX-1 and COX-2) inhibition assay

The inhibition of the methanolic extract on Cox-1 (ovine) and Cox-2 (ovine recombinant) was carried out using a commercial colorimetric inhibitor test kit (Catalog No. 560131, Cayman Chemical Company, U.S.A). Inhibition tests against COX 1 and 2 were carried out following manufacture's intructions. Spectrophotometer Epoch (Bioteck Instruments, U.S.A) was used to record absorbance at 590 nm. The percentage of inhibition induced by 100 µg/mL was calculated according to the formula:

$$\% \text{ Inhibition} = [(AEA - AIA) / AEA] \times 100$$

AEA: Activity enzyme test absorbance, AIA: Activity inhibition test Absorbance

2.7.3 Phospholipase A2 inhibition assay

Type V sPLA2 (phospholipase A2) is involved in the formation of eicosanoids in inflammatory cells such as macrophages and mast cells. The sPLA2 inhibition test from Cayman Laboratories allows the screening of sPLA2 inhibitors (Type V). Thus, the assay of this enzyme was carried out using 1,2-diheptanoylthio-glycerophosphocholine (1,2dHGPC) and the method described by Cayman Chemical Co. (MI, USA) in the catalog No. 765001. For this purpose, extracts and reference compounds were used so that their final concentrations in the wells were 100 µg/mL. The reaction was initiated with the addition of diheptanoythiol-PC substrate and stopped by DTNB (5,5′ dithiobis-2-nitrobenzoic acid). The assay was done in triplicate and 96-well microplates were used. Analysis was performed spectrophotometrically (Agilent 8453) at the wavelength of 405 nm against a blank that had not received the enzyme. The percentage inhibition of sPLA2 per 100 µg/mL was given by the formula:

$$\% \text{ Inhibition} = [(AEA - AIA) / AEA] \times 100$$

AEA: Activity enzyme test absorbance; AIA: Activity inhibition test Absorbance

2.7 Statistical analysis

The data were expressed as Mean ± Standard Error of Mean (SEM). The statistical analysis was carried out using one-way ANOVA followed by the Bonferroni multiple comparison test on Graph Pad Prism software version 6.0. The level of significance was accepted at $p < 0.05$ compared to the control and between treated groups.

3. RESULTS

3.1 Phytochemical analysis

The qualitative phytochemical screening by TLC of the extracts of the tubers of *A. africana* revealed the presence of flavonoids, saponins, tannins and phenolics compounds and absence of alkaloids in both extracts. The results were recorded in Table 1.

The total phenolics, tannins and flavonoids contents of methanolic and aqueous extracts were showed in Table 2. The methanolic extract exhibited higher levels of phenolics, total flavonoids and tannins.

Table 1. **Phytochemical screening of methanolic and aqueous extracts of *A. africana* rhizomes by TLC**

	Extracts	
Chemical groups	**AA M**	**AAA**
Flavonoids	+	+
Coumarins	+	-
Alkaloids	-	-
Terpenes and sterols	+	+
Tannins and phenolics	+	+
Saponins	+	+

AA M: Ampelocissus africana methanolic extract; AA A: Ampelocissus africana aqueous extract; -: not detected; +: detected

Table 2. **Total phenolics, condensed tannins, hydrolyzable tannins and flavonoids contents of methanolic and aqueous extracts of *A. africana* rhizomes**

Extracts	Phenolics	Condensed Tannins	Hydrolyzable Tannins	Flavonoids
	mg GAE/g	mg/g	mg/g	mg QE/g
AA M	471.79 ± 1.71	239.21 ± 3.74	15.49 ± 0.16	24.7 ± 0.64
AA A	173.88 ± 1.71	49.16 ± 0.43	7.55 ± 0.44	18.8 ± 1.67

Mean values ± standard deviation were presented (n = 3); AA M: Ampelocissus africana methanolic extract; AA A: Ampelocissus africana aqueous extract, GAE: Gallic acid equivalent; QE: quercetin equivalent

3.2 Antioxidant activities

The results of the antioxidant activities of extracts through four methods (ABTS, DPPH, FRAP, LPO) are indicated in table 3. Both extracts exhibited antioxidant activities. Methanolic extract showed the best activity.
The methanolic extracts was demonstrated interesting results in ABTS and DPPH assays comparable to the reference substances used (Trolox, ascorbic acid).
The percentage inhibition inhibitory effect on lipid peroxidation in rat liver homogenates *in vitro* at 100 µg/mL of the methanolic extract was greater than 50%. However, the percentage of ascorbic acid was better than both of extracts.

Table 3. **Summary of antioxidant activity values ABTS, DPPH, FRAP, lipid peroxidation of methanolic and aqueous extracts of *A. africana* rhizomes**

Extracts	ABTS	DPPH	FRAP	LPO
	IC_{50} (µg/mL)	IC_{50} (µg/mL)	mmol AAE/100 g	(% Inhibition at 100 µg/mL)
AA M	2.32 ± 0.18 [ns]	1.71 ± 0.05 [ns]	87.44 ± 0.5 [****]	52.21 ± 0.99 [****]
AA A	13.26 ± 0.33 [****]	34.53 ± 1.29 [****]	24.74 ± 0.7 [****]	42.17 ± 2.18 [****]
Trolox	2.04 ± 0.12 [ns]	1.74 ± 0.002 [ns]	nd	nd
Ascorbic acid	0.94 ± 0.08 [ns]	1.82 ± 0.02 [ns]	nd	94.95 ± 0.94

Values are expressed as mean ± SEM, n = 3 (****) = p < 0.0001 is considered significant compared to the control, ns: no significance P > 0.05. Plant extracts vs Trolox and ascorbic acid for ABTS, DPPH, LPO and AA M vs AA A for FRAP (one-way ANOVA analysis

3.3 Inhibition tests on proinflammatory enzymes

The results of the enzymatic inhibition tests were presented in table 4. From these results, it appears that the methanolic extract of *A. africana* presented the high inhibition activity. Both extracts had lower IC_{50} compared to Zileuton used as reference compound in LOX inhibition test.

Table 4. **Summary of inflammation enzyme activity values 15-lipoxygenase (15-LOX), cyclooxygenases (COX-1 & COX-2), Phospholipase A2 (PLA2) of methanolic and aqueous extracts of *A. africana* rhizomes**

Extracts	15-LOX	COX-1	COX-2	PLA2
	IC_{50} (µg/mL)	(% inhibition at 100 µg/mL)		
AA M	26.09 ± 1.83	36.07 ± 1.29	38.31 ± 1.00	26.9 ± 0.004
AA A	34.32 ± 1.60	nd	nd	nd
Zileuton	2.74 ± 0,02	nd	nd	nd
Ascorbic acid	nd	38.56 ± 0.73	22.82 ± 0.40	20.95 ± 0.00
Indomethacin	nd	44.93 ± 0.83	53.94 ± 1.43	nd

Mean values ± standard deviation standard deviation were presented (n = 3); AA M: Ampelocissus africana methanolic extract; AA A: Ampelocissus africana aqueous extract, nd: not determined

4. DISCUSSION

Phytochemical screening showed that the rhizomes of *A. africana* contain secondary metabolites, in particular triterpenes, tannins, flavonoids, saponins, phenolic compounds (Table 1). These phytochemicals possess therapeutic activity that could justify its uses as traditional medicine [23].
The results corroborate those of the literature which had shown flavonoids, and saponins in the aqueous extracts of the plant [24]. However, the triterpenes and sterols found in our study (table 1) were absent in their study.

The extracts from *A. africana* rhizomes presented high concentrations of polyphenols, flavonoids and tannins (Table 2).
[24] found values 74.25 mg GAE/100 g of extract and 6.32 mg QE/100g of extract total phenolics and flavonoids respectively of aqueous extract respectively from the same plant collected in Sapone/Burkina Faso, different to our results (173.88 ± 1.71 mg GAE/g of extract and 18.8 ± 1.67 mg QE/g) presented in table 2. This difference could be explained by the location of the harvest. Indeed, environmental factors completely influence plant development and the biosynthesis of secondary metabolites [25].

The higher activity of the methanolic extract compared to the aqueous extract presented in table 2 can be attributed to his higher value in phenolic compounds [26]. Indeed, methanol is an efficient solvent in the degradation of cell walls having a non-polar character and therefore cause a better extraction of phenolic compounds [27].

Several researches suggest that polyphenols, flavonoids and tannins are responsible for a wide set of pharmacological properties including antioxidant, anti-inflammatory, etc. [28, 29]. They play an important role in prevention and treatment of chronic diseases including inflammatory processes. It has been known that plant steroid, flavonoids, tannins, and phenols are antioxidants [23, 30].

There are several methods in the literature that can measure antioxidant capacity *in vitro*, but a single test is not capable of determine the total antioxidant capacity of a sample studied. [31, 32] have indicated that more than one the measurement of antioxidant capacity is necessary for take into account the different antioxidant modes of action.

Thus, the antioxidant activity of extracts of *A. africana* rhizomes was evaluated according to four methods. The results showed that the extracts of *A. africana* exhibited capacities for trapping ABTS and DPPH radicals with IC_{50} values comparable to those of the references used (ascorbic acid, trolox).

The extracts also showed significant reducing capacity of the ferric ion Fe^{3+}. Likewise, the extracts had an inhibitory effect on lipid peroxidation.

In fact, free radicals such as reactive oxygen species (ROS) damage the functionality of the cell membrane by peroxidized lipids [33]. Lipid peroxidation has been reported to complicate the inflammatory process [34].

The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers and they are found in all parts of plants [35].

[33] also report that antioxidant substances can prevent and delay the oxidation of lipids by acting on ROS reduction and the production of other free radicals. Thus, the inhibition lipid peroxidation contributes to the protection of the cell membrane against free radicals. All four methods used in this study allowed a better assessment of the antioxidant potential of the plant extracts (table 3).

The treatment of several diseases with an inflammatory component is based on the inhibition of the enzymes involved in the production of mediators initiating or enhancing the inflammatory process [36]. The synthesis of arachidonic acid from cell membrane phospholipids results from the activation of phospholipases A2 (PLA2) by cell stimulation, which is a limiting step in the production of pro-inflammatory lipid mediators namely prostaglandins (PG), leukotrienes (LT), lipoxins and plaque activating factor (PAF) [37].

But, considering the consequences and side effects caused by current anti-inflammatory drugs [38], reports have been published about the principle on « dual inhibitors », consisted in inhibition of cyclooxygenases but also lipoxygenase-mediated metabolism of arachidonic acid [39]. Thus, the present study showed that *A. africana* rhizomes extracts effectively inhibited the activity COX 1, COX 2, 15-LOX, and sPLA2, key enzymes in the formation of pro-inflammatory eicosanoids mediators from arachidonic acid (table 4).

Flavonoids also have anti-inflammatory properties due to their inhibitory effects on pro-inflammatory enzymes [40]. These constituents have been reported to exert potent analgesic and anti-inflammatory properties. It has also been reported that flavonoids directly inhibit prostaglandins which can cause pain and inflammation [41].

Tannins have both protection and potential therapeutic in pathologies linked to oxidative damage and inhibit the actions of cyclooxygenase, phospholipase A2, lipoxygenase and arachidonic acid [30].

5. CONCLUSION

According to this study *Ampelocissus africana* (Lour) Merr rhizomes contained secondary metabolites (flavonoids, saponins, tannins, triterpenes) and demonstrated antioxidant activity through scavenging, chelating, reducing, and lipid peroxidation inhibition. The rhizomes also exhibited inhibitor effect against lipoxygenase, cyclooxygenases and phospholipase A2 activities.

Ampelocissus africana (Lour) Merr rhizomes could be a source of research for new molecules and formulation of phytomedicines to treat diseases with an inflammatory component.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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

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