



Phytochemical, Antioxidant and Anti-Inflammatory Effects of Extracts from *Ampelocissus africana* (Lour) Merr (Vitaceae) Rhizomes

**W. Leila Marie Esther Belem-Kabré^{1,2*}, Noufou Ouédraogo^{1,2,3}
Adjaratou Compaoré-Coulibaly^{1,2}, Mariam Nebié-Traoré^{1,2}, Tata K. Traoré^{1,3}
Moumouni Koala², Lazare Belemnaba², Felix B. Kini²
and Martin Kiendrebeogo¹**

¹Laboratoire de Biochimie et Chimie Appliquée (LABIOCA), Ecole Doctorale Sciences et Technologie, Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso.

²Département Médecine et Pharmacopée Traditionnelles – Pharmacie (MEPHATRA-PH), Institut de Recherche en Sciences de la Santé (IRSS/CNRST), 03 BP 7047 Ouagadougou 03, Burkina Faso.

³Laboratoire de Développement du Médicament, Ecole Doctorale de la Santé, Université Joseph KI-ZERBO, 03 BP 7021, Ouagadougou 03, Burkina Faso.

Authors' contributions

This work was carried out in collaboration among all authors. Author WLMEBK carried out the work, performed the statistical analysis, interpreted the results and drafted the manuscript. Author NO conceived the study, design, supervise and coordination and helped to draft the manuscript. Authors ACC and MNT contributed to perform antioxidant and lipoxygenase inhibition tests. Author TKT participated in cyclooxygenases and phospholipase inhibition test. Author MK supervised the phytochemical analysis. Authors LB, FBK and MK contributed to analyze the results, read and approved the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i3130913

Editor(s):

(1) Dr. Syed A. A. Rizvi, Nova Southeastern University, USA.

Reviewers:

(1) M. P. Singh, University of Allahabad, India.

(2) Nasraoui Hajaji Afef, University of Carthage, Tunisia.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/62661>

Original Research Article

Received 01 September 2020

Accepted 05 November 2020

Published 27 November 2020

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.

*Corresponding author: E-mail: leilakabre@gmail.com;

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 antioxidant 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 μ g/ml for the ABTS, 1.71 ± 0.05 μ g/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 μ g/mL to 34.32 ± 1.60 μ g/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; antioxidant; 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 (TNF α , IL1 β , IL6) and NF- κ B 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. MATERIALS 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 benzoic-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 extracts 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, FeCl₃ reagent for tannins and phenolics, anisaldehyde reagent for saponosides, KOH reagent for coumarins and dragendorff 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 AlCl₃ (2% in methanol). After 10 minutes, the absorbance was measured at 415 nm against blank (that

consisted of 100 μ L methanol and 100 μ L 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:

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

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:

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

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 ($\text{K}_2\text{S}_2\text{O}_8$). 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 μ L of each dilution was mixed with 200 μ L 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 μ L of DPPH solution freshly prepared in methanol (4 mg/100 mL) was mixed with 100 μ L 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 (0.2 M, pH 6.6), then 1.25 mL of potassium hexacyanoferrate solution [$\text{K}_3\text{Fe}(\text{CN})_6$] (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 FeCl₃ (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 (FeCl₂)-hydrogen peroxide (H₂O₂) 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 FeCl₂ (0.5 mm) and then 50 µL of H₂O₂ (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 were 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:

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

V_b: Enzymatic activity without inhibitor; V_s 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 (Biotek 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:

% Inhibition = [(AEA - AIA) / AEA] X 100.

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

2.8 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.

3.2 Antioxidant Activity

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 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.

3.3 Inhibition Tests on Pro-inflammatory 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 IC₅₀ compared to Zileuton used as reference compound in LOX inhibition test.

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].

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

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

AAM: *Ampelocissus africana* methanolic extract; AAA: *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 |
| AAM | 471.79 ± 1.71 | 239.21 ± 3.74 | 15.49 ± 0.16 | 24.7 ± 0.64 |
| AAA | 173.88 ± 1.71 | 49.16 ± 0.43 | 7.55 ± 0.44 | 18.8 ± 1.67 |

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

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) |
| AAM | 2.32 ± 0.18 ^{ns} | 1.71 ± 0.05 ^{ns} | 87.44 ± 0.5 ^{****} | 52.21 ± 0.99 ^{****} |
| AAA | 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 followed by Bonferroni multiple comparison). AA M: *Ampelocissus africana* methanolic extract; AA A: *Ampelocissus africana* aqueous extract, AAE: Ascorbic acid equivalent; nd: not determined

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) | | |

| | | | | |
|----------------------|--------------|--------------|--------------|--------------|
| AAM | 26.09 ± 1.83 | 36.07 ± 1.29 | 38.31 ± 1.00 | 26.9 ± 0.004 |
| AAA | 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

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 IC₅₀ values comparable to those of the references used (ascorbic acid, trolox).

The extracts also showed significant reducing capacity of the ferric ion Fe³⁺. 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.

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors are grateful to the COMSTECH-TWAS project 17-319 RG/PHA/AF/AC_C – FR3240300079 (Granted to Dr N. OUEDRAOGO), the Laboratory of Applied Biochemistry and Chemistry (LABIOCA), University Joseph KI-ZERBO and the Department of traditional medicine of Research / Institute of Health Sciences (IRSS).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sordillo LM, and Aitken SL. Impact of oxidative stress on the health and immune function of dairy cattle. *Veterinary immunology and immunopathology*. 2009;128(1-3):104–109.
2. Aitken SL, Karcher EL, Rezamand P, Gandy JC, VandeHaar MJ, Capuco AV et al. Evaluation of antioxidant and proinflammatory gene expression in bovine mammary tissue during the periparturient period. *Journal of Dairy Science*. 2009; 92(2):589–598.
3. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*. 2007;39(1):44–84.
4. Joshua Loke WS, Lim MY, Lewis CR, Thomas PS. Oxidative stress in lung cancer. *Cancer*. 2014;23–32.
5. Zhang X, Chen Y, Cai G, Li X, Wang D. Carnosic acid induces apoptosis of hepatocellular carcinoma cells via ROS-mediated mitochondrial pathway. *Chemico-Biological Interactions*. 2017;277: 91–100.
6. Sahib AS, Al-Anbari HH, Salih M, Abdullah F. Effects of Oral antioxidants on lesion counts associated with oxidative stress and inflammation in patients with papulopustular acne. *J Clin Exp Dermatol Res*. 2012;3:163.
7. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: How are they linked? *Free Radical Biology and Medicine*. 2010;49(11):1603–1616.
8. Dröge W. Free radicals in the physiological control of cell function. *Physiological Reviews*. 2002;82(1):47–95.
9. Moualek I, Iratni Aiche G, Mestar Guechaoui N, Lahcene S, Houali K. Antioxidant and anti-inflammatory activities of *Arbutus unedo* aqueous extract. *Asian Pacific Journal of Tropical Biomedicine*. 2016;6(11):937–944.
10. Oliveira AM, De Conserva LM, De Souza Ferro JN, Brito F de A, Lemos RPL, Barreto E. Antinociceptive and anti-inflammatory effects of octacosanol from the leaves of *sabicea grisea* var. *grisea* in mice. *International Journal of Molecular Sciences*. 2012;13(2):1598–1611.
11. Nacoulma OG. Plantes médicinales et pratiques médicinales traditionnelles au Burkina Faso - Cas du plateau central medicinal plants and traditional medical practices in Burkina Faso: The case of the central plateau), Thèse de Doctorat d'Etat: Tome II. Université de Ouagadougou; 1996.
12. Ladigina EY, Safronich LN, Otriacheva VE, Balandina IA, Grinkevich NI, Sorokina AA et al. Analyse chimique des plantes

- médicinales, édition Moskva vischaya chkola. 1983;172.
13. Lalrinzuali K, Vabeiryureilai M, Jagetia GC. Phytochemical and TLC profiling of *oroxylum indicum* and *milletia pachycarpa*. *J Plant Biochem Physiol*. 2015;3:152.
 14. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology*. 1999; 299:152–178
 15. Arvouet-Grand A, Vennat B, Pourrat A, Legret P. Standardisation d'un extrait de Propolis et identification des principaux constituants. *Journal de Pharmacie de Belgique*, 1994;49(6):462-468.
 16. Mole S, Waterman PG. A critical analysis of techniques for measuring tannins in ecological studies II, Techniques for biochemically defining tannins. *Oecologia*. 1987;72:148-156.
 17. Swain T, Hillis WE. The phenolics constituents of *Prunus domestica* -I- the quantitative analysis of phenolics constituents. *J. Sci. Food Agric*. 1959;10: 63-81.
 18. Re R, Pellegrini N, Poteggent A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and medicine*. 1999;26(9/10): 1231-1237.
 19. Velázquez E, Tournier HA, Mordujovich de Buschiazzo P, Saavedra G, Schinella GR. Antioxidant activity of Paraguayan plant extracts. *Fitoterapia*. 2003;74(1–2):91–97.
 20. Hinneburg I, Damien Dorman HJ, Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chemistry*. 2006;97:122–129.
 21. Sombié PEAD, Hilou A, Coulibaly AY, Tibiri A, Kiendrebeogo M, Nacoulma OG. Brain protective and erythrocytes hemolysis inhibition potentials from galls of *Guiera senegalensis* JF Gmel (Combretaceae). *Journal of pharmacology and toxicology*. 2011;6(4):361-70.
 22. Malterud KE, Rydland KM. Inhibitors of 15-lipoxygenase from orange peel. *Journal of Agricultural and Food Chemistry*. 2000;48: 5576-5580.
 23. Sadat A, Hore M, Chakraborty K, Roy S. Phytochemical analysis and antioxidant activity of methanolic extract of leaves of *corchorus olitorius*. *International Journal of Current Pharmaceutical Research*. 2017; 59–63.
 24. Zongo C, Savadogo A, Ouattara L, Bassole IHN, Ouattara CAT, Ouattara AS et al. Polyphenols content, antioxidant and antimicrobial activities of *Ampelocissus grantii* (Baker) Planch. (Vitaceae), a Medicinal Plant from Burkina Faso. *Inter j Pharmacol*. 2010;6(6):880-887.
 25. Mohi U. Environmental factors on secondary metabolism of medicinal plants. *Acta Scientific Pharmaceutical Sciences*. 2019;3(8):34-46.
 26. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. *Int Pharm Sci*. 2011; 1:98-106
 27. Lapornik B, Prosek M, Wondra AG. Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of Food Engineering*. 2005;71:214–222.
 28. Ijeoma UF, Aderonke SO, Ogbonna O, Augustina MA, Ifeyinwa CN. Antinociceptive and anti-inflammatory activities of crude extracts of *Ipomoea involucreata* leaves in mice and rats. *Asian Pacific Journal of Tropical Medicine*. 2011; 4:121-124.
 29. Albarello N, Simões-Gurgel C, Carvalho de Castro T, Gayer CRM, Coelho MGP, Soares de Moura R et al. Anti-inflammatory and antinociceptive activity of fieldgrowth plants and tissue culture of *Cleome spinosa* (Jacq.) in mice. *Journal of Medicinal Plants Research*. 25 April, 2013; 7(16):1043-1049.
 30. Hafsé M, Farah A, Mouktadir J E, Fikri-Benbrahim, K. Antioxidant and anti-inflammatory activities evaluation of *Coriaria myrtifolia* from the North of Morocco. *International Food Research Journal*. 2017;24(2):498-502.
 31. Prior RL, Cao G. In vivo total antioxidant capacity: Comparison of different analytical methods. *Free Radical Biol Med*. 1999;27: 1173–1181.
 32. Nidyaletchmy SR, Suerialoasan N, Saravana KS, Norhanom AW, Kae SS. Phenolic content, antioxidant effect and cytotoxic activity of *Leea indica* leaves. *BMC Complementary and Alternative Medicine*. 2012;12:128.
 33. Ouédraogo N, Hay AE, Ouédraogo JCW, Sawadogo WR, Tibiri A, Lompo M et al. Biological and phytochemical

- investigations of extracts from *pterotheca erinaceus* (fabaceae) root barks. Afr J Tradit Complement Altern Med. 2017; 14(1):187-195.
34. Yougbare-Ziebrou MN, Lompo M, Ouedraogo N, Yaro B, Guissoun IP. Antioxidant, analgesic and anti-inflammatory activities of the leafy stems of *Waltheria indica* L. (Sterculiaceae). J App Pharm Sci. 2016;6:124-9.
35. Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. African Journal of Microbiology Research. 2009;3(13):981-996.
36. Thiombiano EAM, Bangou MJ, Couliati TH, Sombié PAED, Kiendrébéogo M. Comparative study of *in vitro* antioxidant and anti-inflammatory potentials of two malvaceae used in folk medicine in Burkina Faso. Int J Complement Alt Med. 2019;12(5):225–230.
37. D'Almeida RE, Isla M, Vildoza E, Quispe C, Schmeda-Hirschmann G, Alberto M. Inhibition of arachidonic acid metabolism by the Andean crude drug *Parastrephia lucida* (Meyen) Cabrera. Journal of Ethnopharmacology. 2013;150:1080–1086.
38. Shah SMM, Sadiq A, Shah SMH, Ullah F. Antioxidant, total phenolic contents and antinociceptive potential of *Teucrium stocksianum* methanolic extract in different animal models. BMC Complement Altern Med. 2014;14:1–7.
39. Khanum SA, Murari SK, Vishwanth BS, Shashikanth S. Synthesis of benzoyl phenyl benzoates as effective inhibitors for phospholipase A2 and hyaluronidase enzymes. Bioorganic and Medicinal Chemistry letters. 2005;15(18):4100-4104.
40. González-Gallego J, Sánchez-Campos S, Tuñón M J. Anti-inflammatory properties of dietary flavonoids. Nutrición Hospitalaria. 2007;22(3):287-93.
41. Wink M. modes of action of herbal medicines and plant secondary metabolites. Medicines. 2015;2(3):251–286.

© 2020 Belem-Kabré et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/62661>