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Phenolic Content, Antioxidant Activity, 15-Lipoxygenase and Lipid Peroxidase Inhibitory Effects of two Medicinal Plants from Burkina Faso: *Acacia macrostachya* Reich. Ex Benth (Mimosaceae) and *Lepidagathis anobrya* NEES (Acanthaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. Author ACC managed the work, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author WLMEBK contributed to perform the antioxydant, lipid peroxidation and lipoxygenase test. Author MNT contributed to realize the antioxydant and lipid peroxydation tests. Author TKT contributed to manage the ABTS test. Author NN contributed to perform the FRAP test. Author NO contributed to supervise the work. Author MK contributed to analyze the results, read and approved the manuscript. Author RWS designed the idea, supervised the work, analyzed the results, read and approved the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Acacia macrostachya (Mimosaceae) and *Lepidagathis anobrya (*Acanthaceae*)* are two medicinal plants used in Burkina Faso folk medicine for the treatment of inflammatory diseases. The purpose of this study was to assess the phenolic content, the antioxidant, lipoxygenase and lipid

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peroxidaseinhibitory effects of *Acacia macrostachya* and *Lepidagathis anobrya*.The experimental results revealed that *Acacia mascrostachya* and *Lepidagathis anobrya* have respective total phenolic contents varies from 240.13 ± 0.44 and 30.88 ± 0.30 mg GAE / 100 mg extract; and total flavonoids contents of 2.30 ± 0.002 and 4.24 ± 0.28 QE / 100 mg of extract. The two plants demonstrated anti-free radicals (ABTS) capacities of 0.06 and 0.14 TEAC while the FRAP reducing power of 2.24 ± 0.08 and 4.43 ± 0.12 mmol AAE per 100 mg respectively. *Acacia macrostachya* and *Lepidagathis anobrya* showed significant inhibitory effect on lipid oxidation with the inhibition percentage values of 55.45 ± 1.48 and 66.36 ± 0.65 respectively. In the 15-lipoxygenase inhibition, *Acacia macrostachya* demonstrated very important inhibitory effect with the IC_{50} value of 1.32 ± 0.16 while the effect of *Lepidagathis anobrya* is very moderate.The results of this work demonstrated the interest of *Acacia macrostachya* and *Lepidagathis anobrya* in the management of inflammatory diseases.

Keywords: Acacia macrostachya; Lepidagathis anobrya; antioxidant; 15-lipoxygenase; lipid peroxidase.

1. INTRODUCTION

Inflammation is the common symptom of many diseases including cancer, malaria, allergy, asthma, autoimmune diseases, coeliac disease, glomerulonephritis, hepatitis, inflammatory bowel disease, transplant rejection. Among the inflammation mediators, 15-Lipoxygenase (15-LOX) is well known to be expressed in various cells, tissues and organs of the human body where 15-LOX is implicated in a variety of inflammatory diseases, including atherosclerosis, hypertension, diabetes, and neurodegenerative disorders. Indeed, an intradermal injection of 15-LOX metabolite namely 15(S)- HPETE induces inflammatory symptoms like plasma exudation in rabbits [1] and the production of pro-inflammatory cytokine IL-12 in macrophages [2].

In the other side, oxidative stress induces lipid peroxidation leading to a wide range of inflammatory diseases [3]. In conventional medicine, inflammation is treated with steroidal and Non-steroidal anti-inflammatory drugs (SAIDs and NSAIDs) but due to the side effects of these synthetic products, researchers are focused their efforts toward natural products. Despite the development of many molecules of natural origin by many pharmaceutical companies, these products are still inaccessible to African populations for financial reasons. In developing countries, and more particularly in Africa, people frequently resort to traditional medicine for the treatment of various diseases, including inflammation and cancers. This mostly herbal medicine is affordable and accessible, especially for the poorest patients [4].

Acacia macrostachya (Mimosaceae) and *Lepidagathis anobrya (*Acanthaceae*)* (Fig.1) are two medicinal plants used in Burkina Faso folk medicine for the treatment of inflammatory diseases [5-6]. Our previous research has proved that the anti-inflammatory, analgesic, antipyretic, and antioxidant properties of *L. anobrya* while *A macrostachya* demonstrated antioxidant and no genotoxic effects in the human organism [7].

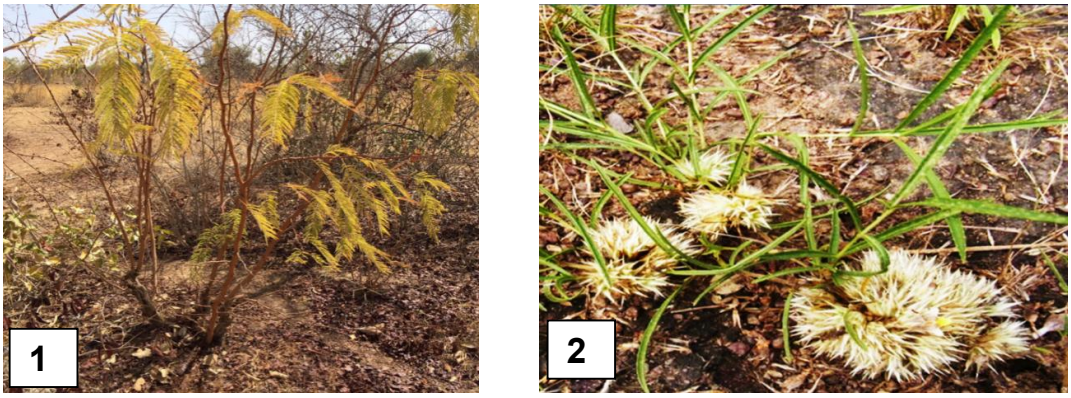

Fig. 1. Photo of *Acacia macrostachya* Reich. exBenth (Mimosaceae) [1] and *Lepidagathisanobrya* NEES (Acanthaceae) [2]

The purpose of this work is to evaluate the phenolic content and the antioxidant properties, the lipid peroxidation and 15-lipoxygenase inhibition effects of *Acacia macrostachya* (Mimosaceae) and *Lepidagathis anobrya (*Acanthaceae*)* in order to promote traditional medicine in Burkina Faso and to find new molecules for inflammation management.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material consists of dried *Lepidagathis anobrya* NEES whole plant and the root's barks of *Acacia macrostachya*. These plant parts are harvested in February 2018 at Laongo, 25 miles from Ouagadougou, capital of Burkina Faso and identified by Prof Amadé OUEDRAOGO from the Laboratory of Ecology (University Joseph KI ZERBO of Ouagadougou). Voucher specimens has been deposited in the herbarium of University Joseph KI/ZERBO with identification number 17252 for *Acacia macrostachya* and R03 for *Lepidagathis* anobrya.

2.2Extraction

Methanolic and dichloromethane macerations were performed with each plant at room temperature for 24 hours under magnetic rotation. The macerates were filtered, concentrated in Rotavapor and lyophilized. The dried extracts after lyophilization have been used for biological tests. Some preliminary tests were performed to select the most active extracts to be used in this study. Thus, the methanol extract of *Lepidagathis anobrya* and the dichloromethane extract of *Acacia macrostachya* was selected for the antioxidant and the inhibitory effect on 15-LOX and lipid peroxidase.

2.3 Phenolic Content

2.3.1Total polyphenols

The total polyphenols of the extracts were determined according to the method described by Singleton et al., 1999 [8] which is based on the oxidability of phenolic compounds. The reagent used was a mixture of phosphomolybdate and sodium tungstate which is reduced during the oxidation of phenols in an alkaline medium into a mixture of tungsten blue and molybdenum. There is then a change in the colorimetric properties of the Folin-Ciocalteu reagent, which reacts with the OH function of the phenols. For the present study, we used 96-well microplates. In each well 25 µl of extract solution (0.1 mg / mL) and 125 µL of FCR solution (0.2N) were put successively. The mixture incubated for 5 minutes in the dark and then 100 µL of a sodium carbonate solution (75 g / L) was added. Leave the mixture to stand for one hour in the dark, then read the absorbance at 760nm. Gallic acid (200 mg / L) is used as standard. The results are expressed in mg Equivalent Gallic Acid per 100 mg of dry.

2.3.2 Total flavonoid assays

The total flavonoid content of extracts was determined by the Dowd colorimetric method adapted by Arvouet-Grandet al., 1994 [9]. A volume of 100 µl of 2% $AlCl_3$ in methanol was mixed with a volume of 100 µl of extract (10 mg / ml) diluted in methanol; blank is a mixture of extract and methanol. Absorbance was measured at 415 nm after 10 min incubation in the dark. Quercetin is used as a standard for setting up the standard curve. The results are expressed in mg Equivalent Quercetin (EQ) per 100 mg of dry extract.

2.4 Antioxidant Activities

2.4.1 ABTS Method

The Trolox Equivalent Antioxidant Capacity (TEAC) test was determined by the ABTS test [10]. This test is based on the oxidation-reduction mechanism of ABTS (2, 2'-azino bis (3-ethylbenzothiazoline-6-sulfonic acid) ammonium salt). During this test, the ABTS salt loses an electron to form a dark cation radical (ABTS • +) in solution. In the presence of the antioxidant, the radical thus formed is reduced to give the cation ABTS +, which causes discoloration of the solution. Trolox was used as the reference substance and the absorbances are read at 734 nm.

2.4.2 FRAP method

The Ferric Reducing Antioxidant Power (FRAP) method is based on the reduction of ferric ion (Fe^{3+}) to ferrous (Fe^{2+}) ion by reducing compounds. This method evaluates the reducing power of the compounds as described by Hinneburg et al., 2006 [11].

For this, 1.25 ml of phosphate buffer (0.2 M, pH 6.6) then 1.25 ml of potassium hexacyanoferrate [K is added to a test tube containing 0.5 ml of sample solution (1 mg / ml) [$3Fe(CN)_6$] 1% in

water. The whole was heated to 50°C in a water bath for 30 minutes. 1.25 ml of trichloroacetic acid (10%) was then added and the mixture was centrifuged at 3,000 rpm for 10 minutes. Three 0.625 mL aliquots are made in 3 tubes to which 0.625 mL of distilled water is added followed by 0.125 mL of 1% $FeCl_3$ freshly prepared in water. A blank without sample is prepared under the same conditions. The reading was taken at 700 nm against a standard curve of ascorbic acid.

The concentration of reducing compound (anti-free radical) in the extract is expressed in mmol Ascorbic Acid Equivalent (EAA) / mg of dry extract.

2.5 Inhibitory Effect on 15-lypoxygenase

The principle of this test consists of inhibiting lipoxygenase *in vitro* to produce leukotrienes and lipoxin in the presence of an inhibiting substance. The spectrophotometric method developed by Malterud and Rydland, 2000 [12] was used to assess the inhibitory activity of the extract on lipoxygenase. On a 96 microplate 146.25 µl of a lipoxygenase solution (820.51 U / ml), 3.75 µl of an extract solution were mixed and incubated for 2 min at 25°C. The reaction was initiated by adding 150 µl of a solution of linoleic acid substrate (1.25 mM) and the absorbance's change was followed at 234 nm for 90 seconds. All tests have been carried out in triplicate. The 50% inhibiting concentration (IC_{50}) was determined. Zileuton has been used as a reference substance.

2.6 Inhibitory Effect on Lipid Peroxidation

The lipid peroxidation inhibitory activity's in rat liver (LPO) was determined by the 2-thiobarbituric acid method of Ohkawa et al., 1979 [13] adapted by Sombié et al., 2011 [14]. Iron dichloride ($FeCl_2$) with hydrogen peroxide H_2O_2have been used to induce peroxidation of rat liver homogenate. In this process 0.2 ml of the extracts (1.5 mg / ml) has been mixed with 1.0 ml of liver homogenate in 1% PBS buffer (50 mm, pH 7.40), then 50 µl of $FeCl_2$ (0.5 mM) and 50 µl of H_2O_2 (0.5 mM) were added. The mixture was incubated at 37°C for 60 min, then 1 ml of trichloroacetic acid (15%) and 1 ml of 2-thiobarbituric acid (0.67%) were added and the mixture heated in boiling water for 15 min. Absorbances was read at 532 nm using the spectrophotometer (Epoch Spectrophotometer 251465, Biotek Instruments, U.S.A.). The percentage of inhibition has been calculated according to the following formula:

$$\% \text{ Inhibition} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 the inhibition percentage of the negative control, A_1 the sample absorbance. Quercetin has been used as a positive control. All measurements are carried out in triplicate.

2.7 Statistical Analysis

For statistical analysis, One-way ANOVA followed by the Dunett test have been used to plot the graphs and to measure the degree of statistical significance of the results using the Graph Pad module. A significant difference is considered for $p < 0.05$. Values are expressed as mean ± SD, $p < 0.05$ (*) is considered significant compared to the control. (**) = $p < 0.01$, (***) = $p < 0.001$, and (****) = $p < 0.0001$ vs control.

3. RESULTS

3.1 Phenolic Content

The extracts of *Acacia macrostachya* and *Lepidagathis anobrya* have polyphenol contents of 240.13 ± 0.44 and 30.88 ± 030 mg EAG / 100 mg, respectively. The extracts of *Acacia macrostachya* and *Lepidagathis anobrya* had total flavanoids contents of 2.30 ± 0.02 and 4.24 ± 0.28 mg EQ / 100 mg of extract. *Acacia macrostachya* extract has a better total poplyphenol content but remains less rich in total flavonoids.

Table 1. Antioxidant activities of *Acacia macrostachya* and *Lepidagathis anobrya*

Samples	ABTS / IC_{50} (mg/mL)	FRAP/TEAC (mmol AAE/100 mg)
A. macrostachya	64.76 ± 1.14***	2.24 ± 0.08 ns
L. anobrya	19.44 ± 0.01***	4.43 ± 0.12 ns
Trolox	2.04 ± 0.12 ns	Nd
Ascorbic acid	0.94 ± 0.08 ns	Nd

Values are expressed as mean ± SD, p <0.05 (*) is considered significant compared to the control. (**) = p < 0.01, and (***) = p < 0.0001, ns: no significant P> 0.05. Plant extracts vs ascorbic acid for ABTS and A. macrostachya vs L. anobrya for FRAP

3.2 Antioxidant Activities

The results of the antioxidant activities through the ABTS and FRAP methods of the two plants are indicated in Table 1.

Acacia macrostachya and *Lepidagathis anobrya* have interesting antioxidant power. The methanolic extract of *Lepidagathis anobrya* had a more significant antioxidant power than the dichloromethane extract of *Acacia macrostachya* but all of them were less effective compared to the reference substances (Trolox and Ascorbic acid).

3.3 Inhibitory Effect of *Acacia macrostachya* and *Lepidagathis anobrya* on Lipoxygenase Activityand Lipid peroxidation

The inhibitory effect of the plant extracts on lipoxygenase activity and lipid peroxidation are indicated in Table 2.

The inhibition percentage of the plant extracts at the concentration of 150 µg/ml on lipid peroxidation were lower than the reference substances (ascorbic acid and trolox). However, statistical analysis shows that there was no significant difference between *A. macrostachya* extract and trolox.

The enzymatic activity of 15-lipoxygenase was highly inhibited by the dichloromethane extract of *Acacia macrostachya* while the methanol extract of *Lepidagathis anobrya* exhibited moderate inhibitory effect. The dichloromethane extract of *Acacia macrostachya* revealed a better activity than Zileuton used as reference substance.

4. DISCUSSION

The results of this study demonstrated the richness of *Acacia macrostachya* and *Lepidagathis anobrya* extracts in phenolic compounds which could justify their antioxidant properties. Indeed, these phytochemical compounds possess antioxidant and anticancer properties [15,16,17,18]. According to Ghedira, 2005 [19], flavonoids could exercise a multitude of biological activities, in particular antioxidant, vasculoprotective, antihepatotoxic, antiallergic, anti-inflammatory, antiulcerous and even significant anti-tumor properties. The ABTS test was used to quantify the anti-free radical potential of the H• donor compounds contained in the extracts while the FRAP method allow to assess the reducing power of ferric ions in the extracts. Thus the dichloromethane extract of *A. macrostachya* and the methanol extract of *L. anobrya* demonstrated real power of chelation of the ferric ion (Fe3 +).

The anti-radical assay (ABTS) revealed that the extracts are endowed with the capacity to trap the ABTS+ radicals and this capacity results in the elimination of the free radicals formed in the organism.

The presence of phenolic compounds, especially flavonoids, may explain the antioxidant activity of these extracts; because flavonoids are scavengers of free radicals, which gives them antioxidant properties [20,21].

Free radicals oxidize the unsaturated lipids of the cell membrane leading to the formation of lipid peroxides. Lipid peroxides are potentially toxic and have the ability to destroy cells. Lipid peroxidation is involved in several pathological conditions including inflammation, cancer, hepatotoxicity and cardiovascular disease [22]. These results suggest that the reducing effect of the lipid peroxidation of the liver homogenate induced by the extracts gives them a hepatoprotective power [23]. During the inflammation process, the excessive production of free radicals can lead to cell and tissue damage and lipid peroxidation, which contribute to the maintenance of inflammation [24]. Consequently, the reduction in the formation of lipid peroxides by the extracts could be linked to its antioxidant and anti-inflammatory power.

The tested extracts have an inhibitory effect on the activity of 15-lipoxygenase (15-LOX). LOX catalyzes the transformation first stages of arachidonic acid into leukotrienes which are mediators of inflammatory and allergic reactions including rheumatism, rhinitis, asthma, cancer and cardiovascular diseases [25]. The Phenolic compounds and more specifically flavonoids are enzyme inhibitors of phospholipase A2 [26] and some other inflammation enzyme's including cyclooxygenase and lipoxygenase [27]. The flavonoids could modulate the activity of certain enzymes and modify the behavior of several cellular systems [19] in order to reduce or eliminate inflammation process.

Table 2. Inhibitory effect of *Lepidagathisanobrya* and *Acacia macrostachya* on lipoxygenase activity and lipid peroxidation

Samples	Lipid peroxidation inhibition (%)	Lipoxygenase Inhibition IC_{50} (µg/ml)
A. macrostachya	66.36 ± 0.65***	1.32 ± 0.16*
I. anobrya	55.45 ± 1.48*** #	100± 0.05***
Zileuton	Nd	3.11 ± 0.45
Ascorbic acid	94.10 ± 0.61	Nd
Trolox	76.23 ± 0.47	Nd

Values are expressed as mean ± SD, p <0.05 is considered significant compared to the control. Plant extracts vs ascorbic acid and vs zileuton: (*) = p < 0.05, (**) = p < 0.01, and (***) = p < 0.001. Plant extracts vs trolox: (#) = p < 0.05

Acacia macrostachya demonstrated better 15-LOX inhibitory effect which could be justified by the presence of tannins in this plant. Indeed, tannins are well known to possess LOX inhibition effect [28] and antioxidant [29], anti-inflammatory and analgesic [30] properties. Thus, the phenolic content of these plants and their antioxidant power, their inhibitory effect on 15-lipoxygenase and lipid peroxidation demonstrated by this study could justify the traditional uses of these plants for the treatment of inflammatory pathologies. Further studies will focus on the isolation and identification of natural anti-inflammatory molecules from these two plants, especially from *Acacia macrostachya*.

5. CONCLUSION

The present study has made it possible to demonstrate by scientific tests for the interest of *A*cacia *macrostachya* and *Lepidagathis anobrya* in the management of inflammatory diseases. The results obtained constitute a scientific basis for the uses of these plants in traditional medicine and for the isolation of natural anti-inflammatory molecules.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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