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Original Article

HPLC ANALYSIS AND ANTI-INFLAMMATORY PROPERTIES STUDIES OF TRUNK BARKS OF *ACACIA NILOTICA* VAR ADANSONII (GUILL AND PERR) O KTZE (MIMOSACEAE)

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ABSTRACT

Objective: The objective of this study was to evaluate the *in vitro* and *in vivo* anti-inflammatory properties of the aqueous extract and fractions of the trunk bark of *Acacia nilotica*.

Methods: A maceration of the powder of the trunks barks of the plant was realized. Then the aqueous macerate obtained was fractionated with dichloromethane, butanol and ethyl acetate successively. The phenolic compounds of the aqueous extract, butanol and ethyl acetate fractions were identified by HPLC/DAD. Lipoxygenase and phospholipase inhibition tests with the aqueous extract and the butanol and ethyl acetate fractions were carried out. The anti-inflammatory potential of the aqueous extract was assessed *in vivo* by the anti-edema test with carrageenan and the analgesic test with acetic acid at different doses (200 mg/ml; 400 mg/ml; 600 mg/ml). Aspirin (200 mg/ml) and paracetamol (200 mg/ml) were used as a reference.

Results: The HPLC/DAD analysis of the extracts revealed that gallic acid is the most abundant phenol acid in the extracts. The aqueous extract inhibited lipoxygenase (IC50 = 18.32±1.18 µg/ml), phospholipase (11.44±0.32% per 100 µg/ml) and cyclooxygenase (56.48±0.29% for 100 µg/ml) as well as its tested fractions. It also reduced edema and pain in the mice by more than 50% from the 400 mg/ml dose.

Conclusion: Aqueous extract of Acacia nilotica has anti-inflammatory properties. Hence its use in traditional medicine in the treatment of inflammation.

Keywords: *Acacia nilotica,* HPLC, Antiinflammatory, *In vivo*

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INTRODUCTION

Inflammation is a common reaction of the body to restore homeostasis that would be disrupted by infection, contaminant or trauma [1]. This inflammatory reaction leads to a leakage of the plasma proteins from the vascular system through tissue injury or evolves into chronic inflammation.

The resolution of the inflammatory process helps protect the tissues from been damage and prevent them to progress to chronic inflammation. There are steroidal anti-inflammatory drugs (AIS) and nonsteroidal anti-inflammatory drugs (NSAIDs) that are used to treat inflammation. They block the inflammatory process by cyclooxygenase inhibition. It is the most used therapeutic class and often without any prescription [2], which leads to harmful consequences. This excessive use of NSAIDs or AIS causes ulcers [3], kidney disorders [4], cardiovascular disorders, etc.

The search for a new anti-inflammatory molecule with other pathways of action is necessary to reduce or even eliminate the undesirable effects during the treatments.

Medicinal plants remain the richest and most diverse source for the discovery of new active molecules.

This is the example of tramadol painkiller found in 2013, which had a very interesting content in the roots of the plant *Sarcocephalus latifolius*, however, synthesized since the 1970s.

In Burkina faso, rural populations use these medicinal plants to treat inflammation with fewer side effects [5].

The anti-inflammatory efficacy of some of these plants, such as *Pterocarpus erinaceus* Poir. (Fabaceae)[6] and Saba senegalensis (A. DC) Pichon (Apocynaceae) [7] has been demonstrated. Flavonoids such as quercetin [8] or tannins such as epicatechin [9] would confer on to these plants their anti-inflammatory properties. Acacia nilotica var. adansonii is one of those plants used by traditional healers to treat inflammation and gastritis [10].

It is in this context that the present study takes place to evaluate the anti-inflammatory efficacy of Acacia nilotica var adansonii. Its anthelminthic properties [11] and antibacterial [12] have already been proven. The objective of this study was to assess the anti-inflammatory properties of the aqueous macerate of *Acacia nilotica* var adansonii trunk bark from enzymatic test, anti-edema and analgesic *in vivo* after HPLC/DAD analysis.

MATERIALS AND METHODS

Plants

The Acacia nilotica var adansonii plant was harvested 140km from Ouagadougou in the Central-East region [13]. It was identified under number HNBU00210 at the herbarium of the National Center for Scientific and Technological Research (CNRST). The study extract was prepared according to the ethnopharmacological indication.

Extraction and fraction

The aqueous maceration was an extract model adopted for evaluation. A test sample of 200 g of plant material (bark powder) was macerated for 24 h. The macerate obtained was concentrated in an oven and then fractionated by the liquid/liquid method. Fractionation of the aqueous extract was carried out using, successively, the solvents dichloromethane, ethyl acetate and butanol. The different fractions obtained were condensed in a rotavapor and then oven-dried.

Substances and pharmacological reagents

The following reference products were required for the various tests. Carrageenan, acetic acid, acetylsalicylic acid, paracetamol were obtained from Sigma-Aldrich. Linoleic acid, lipoxygenase, cyclooxygenase, DTNB, Buffer, Diheptanoythiol-PC, phospholipase (sPLA2) were derived from Cayman.

Identification of phenolic compounds of aqueous extract, ethyl acetate and butanol fraction of *Acacia niloticav*ar adansonii by HPLC/DAD

The analysis of the phenolic compounds was by HPLC/DAD according to the method Castellari *et al.* [14]. It is a model 1100 HPLC system equipped with a photodiode array detector (Agilent Technologies, Palo Alto, California, USA) with Chemstation software (Hewlett-Packard, Waldbronn, Germany). For these, 13 phenolic compounds (Sigma-Aldrich, Milan, Italy) that are gallic acid; protocatechuic acid; p-hydroxybenzoic acid; gentisic acid; m-benzoic acid; vanillic acid; Catechin syringic acid; epicatechin; ferulic acid; sinapinic acid; trihydrate; rutin; quercetin were used as standards. Standard solutions were prepared with an ethanol-water solvent (75: 25 v/v) HPLC grade for a concentration of 5 µg/ml. Extracts and standards were filtered with the 0.22 µm filter glass. After filtration, the solutions were directly injected into the HPLC/DAD system. The elution solvents HPLC grade were methanol-distilled water (2.5: 97.5, v/v) with H_3PO_4 at pH3 and methanol-distilled water (50:50 v/v) with H_3PO_4 at pH 3. The separations were performed on a 100 mm x 4.6 mm (Merck) RP-C_{18} monolithic column at a temperature of 30 ° C±1 using a C-150 furnace (Eldex Laboratories, Napa, CA, USA). The injection volume was 10 µl. The elution rate was 2.1 ml/min for 36 min with a multistage gradient (table 1). The identification of the compounds of each sample, was made by comparing the retention times and spectra (200-450 nm) of their peak chromatograms with those of the standard compounds. Quantification was performed using a standard curve obtained by injecting known amounts of pure compounds as external standards.

Table 1: HPLC solvents A and B gradient elution in relation to time

Time (min)	A%	B%	Elution
0	100	0	Isocratic
10	100	0	Isocratic
15	82	18	Linear gradient
20	75	25	Linear gradient
22	65	35	Linear gradient
30	0	100	Linear gradient
34	0	100	Isocratic
35	100	0	linear gradient
36	100	0	Isocratic

-A: Methanol-water-distilled (2.5:97.5, v/v) à pH 3 avec H_3PO_4;-B: Methanol-water-distilled (50:50, v/v) à pH 3 avec H_3PO_4

Inhibition of lipoxygenase (LOX)

It was carried out according to the method described by Malterud *et al.*, [15]. On a 96-well microplate, the following reaction mixtures were made. Enzyme control mixture consisting of 153.75 µl of borate buffer plus and 146.25 µl of LOX solution (400 U/ml). The mixture of enzyme activity composition was 3.75 µl of the borate buffer plus 146.25 µl of the LOX solution and 150 µl of linoleic acid solution (1.25 mmol). The control of the extract mixture composition was 146.25 µl of the LOX solution, 3.75 µl of the extract and 150 µl borate buffer. The mixture of extract activity consisting of 146.25 µl of the LOX solution, 3.75 µl of extract and 150 µl of linoleic acid solution (substrate).

Each reaction mixture is made in triplecata with the extract at different concentrations (100; 50; 25; 12.5; 6.25; 3.175 µg. ml) in the wells of the microplate. The follow-up of the variation of the reaction mixture is done by photospectrometry (Agilent 8453) at a wavelength of 234 nm, just after the addition of linoleic acid.

The percentage inhibition of lipoxygenase by the extracts is calculated by the following formula:

$$I\,(\%) = 100 \times [(DO.\,\text{enzyme} - DO.\,\text{echantillon})/DO.\,\text{enzyme}]$$

$DO_{enzyme} = DO_{En.\,actif} - DO_{En.\,blanc;}$ $DO_{echantillon} = DO_{actif\,Ech} - DO_{blanc\,Ech}$

The IC50 is determined from a parabolic plot of concentration as a function of the percent inhibition calculated I (%).

Inhibition of phospholipase A2 (sPLA2)

Test was realized according to the manufacture's instruction (Caymans COX Colorimetric, 760111)[16]. In a first well of the multiwell plate (enzyme test), 10 µl of methanol (HPLC) plus 10 µl sPLA 2 and 200 µl of Diheptanoythiol-PC substrate are mixed there. In the second well, 10 µlsPLA2 plus 10 µl of extract (8 mg/ml) and 200 µl of Diheptanoythiol-PC substrate are added thereto. In the third well, it is a mixture of 10 µl of methanol (HPLC) with 10 µl of buffer and 200 µl of Diheptanoythiol-PC substrate. Each mixture is made in triplicate then the entire device is allowed to incubate for 15 min at 25 °C. At the end of the 15 min, a mixing of the plate is carried out for 1 min after the addition of 10 µl of DTNB in each well. After mixing, the reaction mixture is followed by photospectrometry (Agilent 8453) at a wavelength of 405 nm.

The percentage inhibition of sPLA2 is calculated by the following formula:

$$I\,(\%) = 100 \times [(AEA - AIA)/AEA]$$

AEA: Activity enzyme test absorbance; Enzyme test activity: (Enz Test abs–blank abs);

AIA: Activity inhibition test absorbance; Activity Inhibition Test: (Abs Inh Test-blank abs)

Inhibition of cyclooxygenase (COX-1 and COX-2)

The cyclooxygenase inhibition test was evaluated according to the manufacture's instruction (Caymans COX Colorimetric, 760111)[16]. A reaction mixture of 10 µl of an extract with 10 µl of the prepared enzyme, 10 µl of hemin and 150 µl of diluted Buffer buffer is prepared in a well of microplate 96-wells. The same mixture is made without the extract in another well but supplemented with 10 µl of the extract dilution solvent. A blank consisting of 160 µl buffer diluted, 10 µl of hermine and 10 µl of the dilution solvent of the extract is also realized. The whole device is stirred and left in incubation for 5 min. Then 20 µl of arachidonic acid (Substrate) and a colorimetric substrate is added to all wells. The plate is shaken a second time and incubated for 2 min before reading at the 590 nm photo spectrometer (Agilent 8453). The reaction mixtures were in triplicate with indomethacin and salicylic acetic acid as reference. The formula below makes it possible to determine the percentage inhibition of cyclooxygenase by the extract.

$$I\,(\%) = 100 \times [(AEA - AIA)/AEA]$$

AEA: Activity enzyme test absorbance; Enzyme test activity: (Enz Test abs–blank abs);

AIA: Activity inhibition test absorbance; Activity Inhibition Test: (Abs Inh Test-blank abs).

Anti-edematous to the carrageenan testing

This method based on measuring the volume of the mice paw before and after induction of inflammation (edema) to carrageenan [18].

Each group of mice received a dose of the extract (200 mg, 400 mg, and 600 mg), acetylsalicylic acid (200 mg) and water (blank), which was administered per os. After 1h of administration, the initial volume of the right hind paw of the mice is measured. Then in the metatarsal region of this paw, edema was achieved by injection of 0.05 ml of carrageenan (1% suspended in NaCl 0.9%). After 1h of induction of edema, the volume of the paw is measured. Then at 3 and 5 o'clock. From three measurements (difference of less than 4%), the average volume of edema of the paw is calculated.

The percent inhibition of paw edema of the treated mice paws was determined compared to that of the blank control mice. It's determined according to the formula:

$$I\,(\%) = 100 \times [(A - B)/A]$$

A: represents the average difference in the volume of increase of the paw of the mice of the blank control group;

B: represents the average difference in the volume of increase in the paw of the mice treated.

The percentage of inhibition of the extract compared with that of acetylsalicylic acid (reference) makes it possible to assess the anti-inflammatory effect of the extract.

Analgesic activities (Acetic acid test)

The study of the analgesic activity was carried out according to the method described by Sawadogo *et al.*, [19].

Mice were fasted for 17 h. Each groups of mice received a corresponding oral dose of the extract (200 mg, 400 mg, 600 mg). One control group received distilled water (blank) and another batch the reference compound paracetamol at a dose of 200 mg.

After 1h of administration of the substances, an injection with acetic acid intraperitoneally on the mice was carried out at a dose of 10 ml/kg. The injection of acetic acid (0.6%) causes acute pain in the mice which is manifested by stretching of the hind legs and contortion of the dorso-abdominal muscles. The contortions of each mouse were counted 5 min after injection for 15 min. The analgesic effect was evaluated according to the following formula:

$$I\,(\%) = 100 \times [(Wb - Wt)/Wb]$$

Wb: an average of the number of contortions of mice in the blank control group; Wt: an average of the number of contortion of the mice in the batch.

Analysis of results

Calculations of percentages of inhibitions performed with the MS Excel software. The results of the pharmacological study expressed as average±Standard Mean Error (E. S. M.). The different fig. were plotted using GraphPad Software Prism version 5.01. The series considered significant when the probability of error (p) is lower than the agreed risk: 0.05 ($p<0.05$).

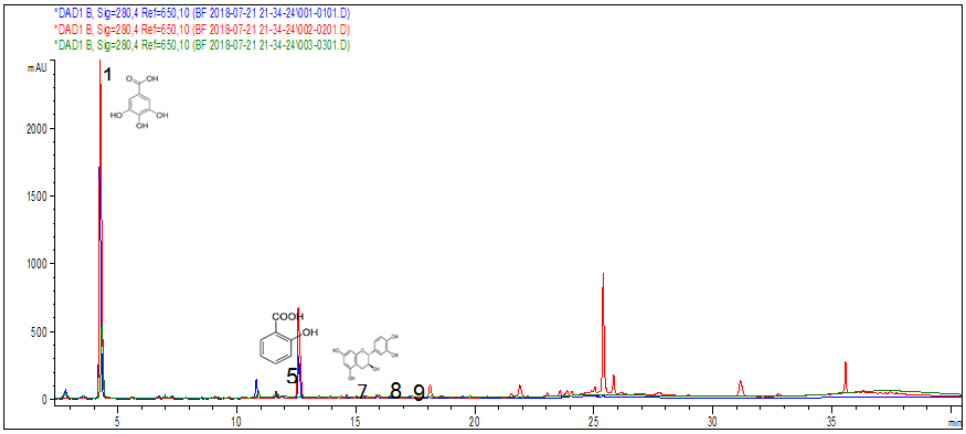

Fig. 1: Chromatogram of extracts at 280 nm, ---DAD1 B: Fraction butanolic; ----DAD1 B: Fraction acetate d'ethyl; ----DAD1 B: Extract aqueous

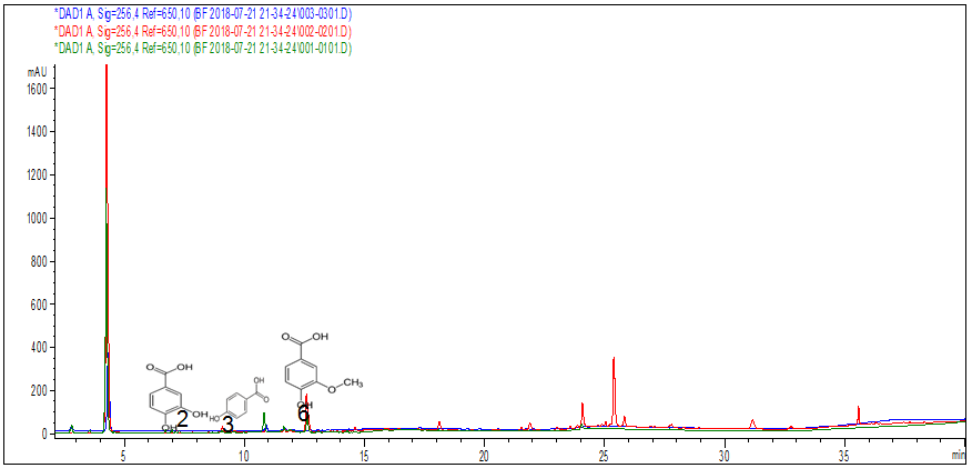

Fig. 2: Chromatogram of extracts at 256 nm, ---DAD1 B: Fraction butanolic; ----DAD1 B: Fraction acetate d'ethyl; ----DAD1 B: Extract aqueous

RESULTS

Identification of phenolic compounds of aqueous extract, ethyl acetate and butanol fraction of *Acacia nilotica* var adansonii by HPLC/DAD

Analysis of aqueous extract, ethyl acetate and butanol fraction by HPLC revealed the presence of a certain number of phenolic acids indicated in table 2 and fig. (1; 2; 3; 4). Gallic acid had the highest content in the aqueous extract (843.56 µg, g-1), in the ethyl acetate fraction (1500.20 µg. g-1) and in the butanol fraction (365.18 µg. g-1). Only ethyl acetate fraction revealed high m-hydroxybenzoic acid content (996.18 µg. g-1). It is in this fraction the quercetin was identified.

This Chromatogram show the molecule which were identified at 280 nm; (1) Gallic acid; (5) m-hydroxybenzoic acid; (7) Catechin; (8) Syringic acid; (9) epi-catechin.

The pics of this chromatogram, which were identified, are: (2) protocatechic acid; (3) p-hydroxybenzoic acid; (6) vanillic acid.

The molecules, which were, identify at 324 nm; (4) gentisicacid; (10) acid ferulic;

The molecules pics which identified on this spectrum at were 365 nm: (11) acid Sinapinic; (12) trihydrate rutin; (13) Quercetin.

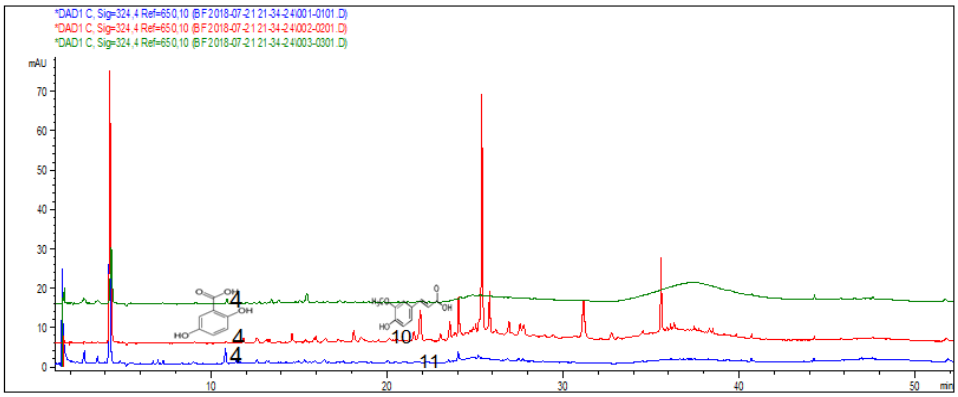

Fig. 3: Chromatogram of extracts at 324 nm, ---DAD1 B: Fraction butanolic; ----DAD1 B: Fraction acetate d'ethyl; ----DAD1 B: Extract aqueous

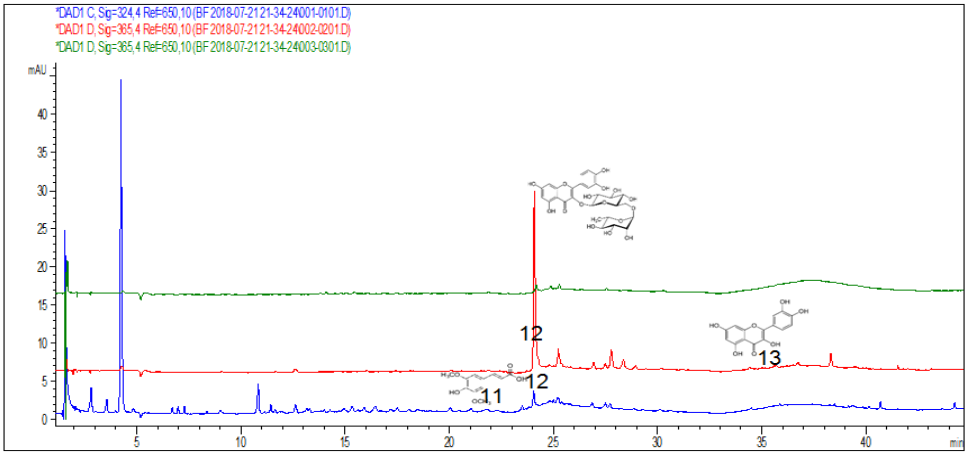

Fig. 4: Chromatogram of extracts at 365 nm, ---DAD1 B: Fraction butanolic; ----DAD1 B: Fraction acetate d'ethyl; ----DAD1 B: Extract aqueous

Table 2: Retention time, wavelength and mass characteristic of phenolic compounds detected

S. No.	Compounds	tr (min)	λ (nm)	Extract aqueous (µg. g^{-1})	Fraction acetate ethyl (µg. g^{-1})	Fraction butanol (µg. g^{-1})
1	Gallic acid	4,3	280	843,56	1500,20	365,18
2	Protocatechic acid	7,2	256	0,46	0,93	0,40
3	p-hydroxybenzoic acid	9,7	256	0,80	1,65	0,31
4	gentisicacid	11,3	324	9,95	2,42	3,26
5	m-hydroxybenzoicacid	12,7	280	2,17	996,18	20,60
6	vanillic acid	12,6	256	9,30	25,99	0,67
7	Catechin	15,9	280	4,41	18,51	4,56
8	syringicacid	16,6	280	4,02	12,07	3,14
9	epi-catechin	17,8	280	2,99	7,92	1,55
10	acid ferulic	21,2	324		0,04	
11	acid Sinapinic	22,6	324	3,74		
12	trihydrate rutin	24,9	365	1,39	5,60	
13	Quercetin	36,8	365		0,59	

Enzyme inhibition: lipoxygenase, phospholipase and cyclooxygenases

The aqueous extract and its two fractions showed a percentage inhibition of COX-2 cyclooxygenase greater than 50% (table 3). But on cyclooxygenase COX-1, the percentage inhibition was less than 50% for the aqueous extract and the butanol fraction. Only the fraction of ethyl acetate had a percentage higher than 50% (52.04±0.99) against COX-1.

The highest percentage inhibition (88.46±0.21) of the phospholipase was also obtained with the ethyl acetate fraction at 100 µg/ml. Nevertheless, its aqueous extract showed the best inhibition (IC_{50} = 18.32±1.18 µg/ml) of lipoxygenase.

Table 3: Enzyme inhibition (lipoxygenase, phospholipase and cyclooxygenases) of A. nilotica bark extract

Extract (n=3)	FB	FAE	AE	AAS
%Inh COX-2 (at 100 µg/ml)	55.07±0.69	59.19±1.43	56.48±0.29	16.58±1.45
%Inh COX-1 (at 100 µg/ml)	45.34±0.35	52.04±0.99	36.05±2.89	0
%Inh sPLA2 (at 100 µg/ml)	61.50±0.13	88.46±0.21	11.44±0.33	21±0.1
LOX (IC_{50}µg/ml)	31.30±0.14	23.91±2.64	18.33±1.9	-

The values are expressed on average±SEM. EA: aqueous extract; FAE: ethyl acetate fraction; FB: butanol fraction; AAS: acetylsalicylic acid

Anti-edematous to the carrageenan testing

The results of the test of inhibition of edema of mice paw by the aqueous *Acacia nilotica* extract are shown in the table 4. These results show an inhibitory effect concentration dependent on the extract and function of time (fig. 5). The strong inhibition of edema was obtained at 600 mg/ml (68.73%±0.01) at 5th h.

Analgesic activities

The mice contortion inhibition test by the aqueous *Acacia nilotica* extract revealed the results summarized in the table 5. The analgesic effect of the extract is dependent on concentration. The dose of 600 mg (42.76%) indicated the strongest inhibition close to that of paracetamol at 200 mg (63.82%).

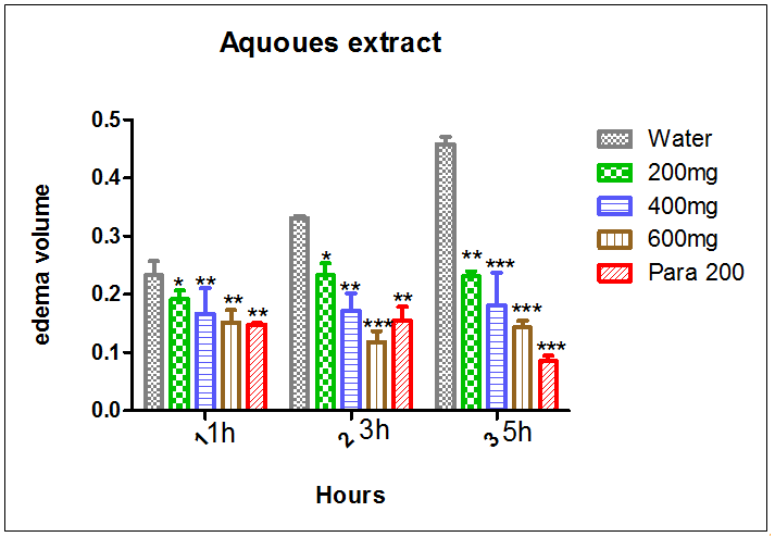

Fig. 5: Variation of edema volume of mice paw at 1h, 3h and 5h, The values are expressed on average±SEM, *p<0.05; **p<0.01; ***p<0.001 is considered significant compared to the control (Water)

Table 4: The percentage inhibition of the volume of the paw at different concentrations of the aqueous extract after 1 h; 3h; 5h of time after injection of carrageen an

Extract (n=6)	AAS 200 mg	EA 200 mg	EA 400 mg	EA 600 mg
Temps	% in h	% in h	% in h	% in h
1H	27.62±0.02	17.89±0.01	28.57±0.04	35.24±0.02
3H	48.66±0.02	29.71±0.02	47.98±0.03	64.43±0.02
5H	84.73±0.01	49.69±0.01	60.48±0.56	68.73±0.01

The values are expressed on average±SEM. EA: aqueous extract; AAS: acetylsalicylic acid

Table 5: Percent inhibition of contortions at different concentrations of the extract

eExtract (n=6)	Water	EA 200 mg	EA 400 mg	EA 600 mg	Para 200 mg
Contortion number	50.67±1.15	34.33±1.53*	31±2*	29±1.73*	18.33±0.58**
%Inhibition	0	32.24	38.81	42.76	63.82

The values are expressed on average±SEM, *p<0.05; **p<0.01 is considered significant compared to the control. EA: aqueous extract; Para: Paracetamol

DISCUSSION

HPLC analysis made possible to identify 13 phenols molecules in the aqueous extract of Acacia nilotica and in its two fractions. Of these polyphenols identified, gallic acid had the highest content in the aqueous extract (843.56 µg, g-1), in the ethyl acetate fraction (1500.20 µg. g-1) and in the butanolic fraction (365.18 µg. g-1). Gallic acid content of aqueous extract is better than gallic acid content (544 µg. g-1) in ethanolic extract of Acacia nilotica var nilotica [20]. The catechin, quercetin and rutin, which identified in aqueous extract, were also reveal in the ethanol extract of Acacia nilotica var nilotica [20]. Singh *et al.*, revealed the presence of ferric acid, hydroxybenzoic acid and epicatechin in the methanolic extract of pod of Acacia nilotica var nilotica by mass spectrometry (MS/MS) [21]. These molecules were also identified in the aqueous extract of barks Acacia nilotica var adansonii.

The reduced number of standards did not allow the identification of other phytochemicals, whose peaks of high intensity appeared on the chromatograms. For example, at the length of 365 nm and the retention time of fewer than 5 min, two unidentified major peaks were observed on the chromatogram of the aqueous extract.

The anti-edema, analgesic, inhibition of lipoxygenase, phospholipase and cyclooxygenase tests allowed to appreciate anti-inflammatory properties of the extracts.

Indeed, the aqueous extract and its fractions strongly inhibited the activity of lipoxygenase. The best inhibition was obtained with the aqueous extract (IC50 = 18.32±1.18 µg/ml) followed butanol fraction and ethyl acetate. This shows a decrease of the inhibitory potential of the aqueous extract by the fractionation effect. The inhibitory action of LOX would be by synergistic action of molecules groups such as the catechinand epicatechin that make up the aqueous extract. It was demonstrated epicatechin inhibiting 5-lipoxygenase-1 [22] and catechin inhibiting mackerel muscle LOX [23]. The absence of one or more molecules in the extract would reduce this inhibitory power. The inhibitory effect of the aqueous extract of LOX is better than that of *V. colorata* (42.76±2.45% at 100 µg/ml) [24].

On the other hand, on the phospholipase, the inhibitory action of the aqueous extract was weak compared to those of the fractions. The ethyl acetate fraction showed the highest percent inhibition (88.46±0.21%) followed by the butanol fraction and the aqueous extract. This percentage inhibition was higher than that of aspirin (21±0.1% to 100 µg/ml). Tannins would be responsible for this phospholipase inhibitory activity. This was showed with gallic acid and hydroxybenzoïc acid, which inhibited phospholipase activity [25]. Moreover, there is a strong correlation between the tannin content and the inhibitory activity of phospholipase [26].

Aqueous extract and its fractions showed a strong inhibitory effect on COX-2 less than on COX1. This inhibitory power of the extracts was better compared to that of aspirin, which had no effect on COX-1. The results obtained by Elgorashi *et al.*, [27] was indicated an inhibitory effect equal to 44.1±3.4% on COX-2 and 50.9±2.4% on COX-1 by the methanol extract of bark of Acacia nilotica var tomentosa at 250 µg/ml. This shows a better efficiency of the aqueous extract of the Acacia nilotica var adansonii on COX-2 (56.48±0.29%) and COX-1 (36.05±2.89%) at 100 µg/ml. Indomethacin, however, remains better on COX-1 (95.0±48%) compared to the aqueous extract and its fractions [28]. Flavonoids and tannins would be responsible for this cyclooxygenase inhibiting capacity by extracts. Some of their molecules, flavonols and flavones, have been identified as cyclooxygenases inhibitors [29]. The affinity of extracts for COX-2 may be related to the presence of apigenin and catechin, which are COX-2 inhibitors [30].

The results of the anti-edema test shows that the aqueous extract is endowed with anti-edema property. It inhibited edema development by more than 50% compared to white at 400 mg/kg (60.48±0.56) at the 5th hour and at 600 mg/kg (64.43±0.02) from the 3rd hour. The effect of the extract is more significant from the second phase of inflammation. Leukotrienes and prostaglandins would continue the inflammatory process after the first few hours [31]. This means that the extract would have acted by blocking the production of leukotrienes or prostaglandins by inhibition of lipoxygenase or cyclooxygenase. The percentage value of inhibition of lipoxygenase (IC50 = 18.32 µg/ml) and cyclooxygenase (56.48±0.29% for 100µg/ml) could corroborate this mechanism of action of the extract *in vivo*. The effect of aspirin, however, remains greater with more than 80% inhibition of the development of edema (84.73±0.01). The methanolic extract of the fruit of the Acacia nilotica var nilotica has also a significantly higher effect (68.86%) than of the aqueous extract at a dose of 300 mg/kg [32]. However, the effect at a dose of 600 mg is greater than of aspirin at the dose of 200 mg/kg at the 1st and 3rd hour (table 1). This could also reflect an inhibition of histamine and serotonin to a lesser extent, or even phospholipase by the extract. This weak action on phospholipase is confirmed by *in vitro* inhibition of 11.44% at 100 µg/ml of aqueous extract.

The analgesic effect of the aqueous extract was significant, nearly 40% reduction in pain sensation at 400 mg/kg (38.81 %) dose. It is a concentration-dependent effect. But less high than of paracetamol (63.82 %) and of Metamizole [33]. The effect of the 400 mg/kg dose of the aqueous extract of the bark is not significantly different than of the methanolic fruit extract (41.31%) at of 300 mg/kg dose [34]. The effect is also less than of plant *Solanum Surattense* [35]. The pain caused by acetic acid is indirect by release of prostaglandin-leukotrienes in the peritoneum that induce the stimulation of nociceptive neurons. The extract could therefore have inhibited the pain by preventing the production of prostaglandin-leukotrienes, with regard to its cyclooxygenase, lipoxygenase and phospholipase inhibiting properties.

This anti-inflammatory property of the aqueous extract is due to the phytochemical groups that it contains including flavonoids and tannins. These phytochemical groups and many others are recognized to have very valuable anti-inflammatory properties [36].

CONCLUSION

The aqueous extract of Acacia nilotica has demonstrated an anti-edematous and analgesic effect very significant in terms of the results obtained. It is therefore endowed with anti-inflammatory property. It would acts by inhibition of lipoxygenase, phospholipase and cyclooxygenase to a lesser extent. Its use by traditional healers to treat inflammation and gastritis is justified.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest

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