Open Access Research Journal of
Biology and Pharmacy
Journals home page: https://oarjbp.com/
ISSN: 2782-9979 (Online)

OARJ OPEN ACCESS RESEARCH JOURNALS

(RESEARCH ARTICLE)

Acute toxicity, anti-inflammatory, analgesic and antipyretic effects of aqueous and hydroethanolic extracts of *Brenania brieyi* (Rubiaceae)

Nkundineza JC [1, 2], Nsonde Ntandou GF [1, 2, *], Boumba LS [1, 3], Kibamgou S [1], Motondo E [1] and Abena AA [2]

[1] *Laboratory of Animal Physiology and Pathophysiology, Faculty of Science and Technology, Marien NGOUABI University, Brazzaville, BP 69, Congo.*
[2] *Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences, Marien Ngouabi University, BP 69, Brazzaville, Congo.*
[3] *National Institute of Research in Health Sciences, Scientific City (ex-Orstom), route de l'Auberge-de-Gascogne, Makélékélé, Brazzaville, Congo.*

Open Access Research Journal of Biology and Pharmacy, 2021, 03(01), 019–032

Publication history: Received on 19 October 2021; revised on 20 November 2021; accepted on 22 November 2021

Article DOI: https://doi.org/10.53022/oarjbp.2021.3.1.0050

Abstract

Brenania brieyi (Rubiaceae) is widely used in traditional Congolese medicine in the treatment of many pathologies that are manifested by inflammation, pain and fever. The objective of this study was to study the acute toxicity as well as to evaluate the antipyretic, analgesic and anti-inflammatory effects of the aqueous and hydro-ethanolic extracts of *Brenania brieyi*bark on models of pyrexia, algesia and inflammation induced in rodents. The aqueous extract of *Brenania brieyi*does not cause any mortality up to the dose of 4000 mg/kg, but promotes a slight increase in body weight. From 2000 mg/kg, the signs of toxicity observed were the significant decrease in mobility as well as the loss of alertness. At doses of 100 and 200 mg/kg, aqueous and hydro-ethanolic *Brenania brieyi*extracts showed a very significant anti-inflammatory effect (***p< 0.001) on edemas induced by carrageenin (1%), formaldehyde (2.5%) and histamine (1 mg/mL), greater than that of diclofenac at 10 mg/kg. At 200 mg/kg, both extracts showed a very significant analgesic effect (***p< 0.001), greater than that of paracetamol 100 mg/kg against pain induced by acetic acid 0.6% and formaldehyde 2.5%. *Brenania brieyi*was slightly effective in the tail flick test. Brewer's yeast-induced hyperthermia was reduced by both extracts. However, the hydro-ethanolic extract proves to be more effective than the aqueous extract in all the tests carried out. These pharmacological effects would be related to the presence of alkaloids, tannins, flavonoids, anthraquinones, oses and saponosides.

Keywords: *Brenania brieyi*; Toxicity; Anti-inflammatory; Analgesic; Antipyretic

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely prescribed because of their effectiveness in the treatment of pain, fever and inflammation. However, their long-term therapeutic use is often associated with adverse effects such as gastrointestinal ulcers, renal failure [1] and cardiovascular disorders [2]. Several antipyretic drugs have been shown to be toxic, such as paracetamol which causes liver damage and the fall of glutathione in the liver [3]. Prolonged use of certain central painkillers such as morphine can lead to addiction, mental confusion, respiratory depression, orthostatic hypotension [4]. The use of natural products and more particularly medicinal plants is becoming an important alternative route to explore in order to discover effective drugs well tolerated [5]. Indeed, the flora of the Republic of Congo includes many medicinal plants with therapeutic properties, which can remedy multiple pathological disorders including those related to inflammation, pain and fever [6, 7, 8]. Thus, our study aimed study the acute toxicity and evaluate the anti-inflammatory, analgesic and antipyretic of *Brenania brieyi*bark aqueous and hydro-ethanolic extracts.

* Corresponding author: Nsonde Ntandou GF
Laboratory of Animal Physiology and Pathophysiology, Faculty of Science and Technology, Marien NGOUABI University, Brazzaville, BP 69, Congo.

2. Material and methods

2.1. Material

2.1.1. Plant material

Brenania brieyi bark was recolted in the Plateaux department, in the district of Ngamboma, in the forest of the village of Stou in April 2021. The botanical identification was made by the systematist botanist, Professor Jean-Marie MOUTSAMBOTE of the botany laboratory of the Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN) of Brazzaville Congo. The bark was dried away from the sun and at room temperature. After drying, the bark was finely crushed.

2.1.2. Animal material

Female and male, albinos mince weighing from 18 to 25 g, and Wistar rats weighing from 175 to 200 g were used bred in the animalaria of the Laboratory of Animal Physiology and Pathophysiology, Faculty of Science and Technology, under standard conditions (25±5°C, 40-70 RH), with a cycle of 12 hours of light and 12 hours of darkness. These animals had free access to tap water and standard feed. The ethical rules of animal experimentation published by the International Association for the Study of Pain have been respected [11, 12].

2.1.3. Products used

Carrageenin (Sigma), histamine, formaldehyde (Prolabo, France), acetic acid (Sigma), brewer's yeast (Saccharomyces cerevisiae), paracetamol, tramadol, morphine and diclofenac were used in this study.

2.2. Methods

2.2.1. Preparation of extracts

Aqueous decocté

50 g of powder were put in 500 mL of distilled water, the mixture was brought to a boil (100 ° C) for 30 minutes. After cooling and filtration with hydrophilic cotton, the resulting decocté is then evaporated at reduced pressure to 50-60 ° C. The dry residue was collected and used for the various pharmacological tests.

Hydro-ethanolic macerated

50 g of powder was put in 250 mL of distilled water mixed with 250 mL of 99% ethanol, the whole was put under magnetic stirrer for 72 hours. The macerated obtained after filtration is left under evaporation in the balloon heater at reduced pressure to 70 ° C for 24 hours. The dry concentrate obtained constituted the hydro-ethanolic extract which was used for the various tests.

2.2.2. Study of acute toxicity

The acute toxicity of the aqueous extract of *Brenania brieyi* was evaluated in mice. The observation of the different toxicity parameters was carried out for 14 days. The study was conducted in accordance with OECD (Organisation for Economic Co-operation and Development) Guideline 423 for the testing of chemical solutions described by Boumba et al. in 2018; Nkundineza et al. in 2020. This study determines the lethal dose (LD50) and therapeutic doses of the extracts to be used.

Six (4) lots of the five (5) mice per cage were distributed as follows:

- Lot 1 (control) : mice received 1mL/100 g of orally distilled water per mouse ;
- Lots 2, 3 and 4 : the mice were treated with the aqueous extract of *Brenania brieyi* bark at the respective doses of 500, 2000, and 4000 mg/kg/mouse orally.

After administration of the products, the mice were placed in individual cages for observation for 2 hours. These observations concerned the following toxicity parameters: mobility, alertness and response to external stimuli. The body weight of each animal was measured for 14 days.

2.2.3. Evaluation of effects

All animals subjected to the various tests were deprived of food (but no water) for 18 hours before the test.

Anti-inflammatory activities

Subcutaneous injection of an edematogenic agent subcutaneously into the plantar region of the right hind leg (PPD) causes inflammation characterized by the appearance of edema of the affected paw 30 minutes later. Oral administration of an anti-inflammatory substance in an animal one hour before the injection of the edematogenic agent reduces the volume of edema.

After the injection of the edematogenic agent, the course of edema is controlled by measuring the volume of PPD after 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours and 24 hours using the Plethysmometer. The percentages of inhibition of edema (PI) are calculated according to the following formula:

$$\%\mathrm{PI} = \frac{[(\mathrm{Vt} - \mathrm{Vo})\mathrm{t} - (\mathrm{Vt} - \mathrm{Vo})\mathrm{tr}]}{(\mathrm{Vt} - \mathrm{Vo})\mathrm{t}} \times 100$$

PI: percentage of inhibition; Vt: volume of the paw after induction of inflammation at time t; Vo: volume of the paw before induction of inflammation; (Vt-Vo) t: volume of the witness's edema; (Vt-Vo)tr: volume of treaty edema.

For each test of anti-inflammatory activity, thirty (30) mice were selected and divided into six (6) groups of five (5) mice each:

- group1 (control): received distilled water (0.5 mL/100g body weight/mouse per bone) ;
- group 2 (reference): received diclofenac (50 mg) at a dose of 10 mg/kg/mouse per bone ;
- groups 3 and 4 (test groups): received the aqueous extract of *Brenania brieyi* bark at doses of 100 and 200 mg/kg/mouse per os respectively.
- groups 5 and 6 (test groups): received the hydroethanolic extract of *Brenania brieyi* bark at doses of 100 and 200 mg/kg/mouse per os respectively.

Acute inflammation induced by carrageenn

Inflammation was induced by subcutaneous injection of 0.1 mL of 1% carrageenin into the plantar region of the right hind leg of the mouse one hour after oral administration of the various products. The study was conducted using the method reported by Borgi W et al. in 2007; as well as Nsonde Ntandou GF et al. in 2020 [13,14].

Acute formaldehyde-induced inflammation

The study was conducted using the method described by Itou R. D. G. E et al. in 2014 [6]. Inflammatory leg edema was induced by subcutaneous administration of 0.1 mL of formaldehyde to 2.5% one hour after oral administration of the various products.

Histamine-induced acute inflammation

The study was conducted according to the method described by Iwueke A. V et al. in 1929; and reported by Nsonde Ntandou GF et al. in 2020 [14,15]. Inflammatory edema on ppd in mice was induced with 0.1mL of histamine (1mg/mL) one hour after administration of the various test products. The volume of the PPD at the insert t (Vt) is taken in order to calculate the IP as mentioned above.

2.2.4. Study of analgesic activity

Acetic acid test

Administration of acetic acid 0.6% intraperitoneally to mice causes pain syndrome. The appearance of pain is characterized by stretching movements of the hind legs and twisting of the dorso-abdominal musculature. An analgesic would work by reducing the number of abdominal cramps compared to the control group. The study was conducted using the method described by Iwueke A. V et al. in 1929; and reported by Boumba LS et *al*. in 2018 [9,15].

One hour before induction of abdominal cramps by intraperitoneal injection of acetic acid 0.6% at a dose of 10 ml/kg, the mice were treated as follows:

- the control group received distilled water,
- the reference group received paracetamol (100 mg/kg),
- four test groups received the aqueous and hydro-ethanolic extracts of *Brenania brieyi* at 100 and 200 mg/kg.

Five minutes after the acetic acid injection, the number of contortions was counted in each mouse for 20 minutes. The analgesic effect was evaluated according to the following formula:

$$\% \text{ inhibition} = \left(1 - \frac{Wt}{Wo}\right) * 100$$

Wo: represents the average contortions number of the mice in the control group ; Wt is the average abdominal cramps number of mice in the treated group.

Formaldehyde test

The study was conducted using the method described by Wibool R et *al*. in 2008, and SantaCecília FV et *al*. in 2011 [17,18]. Plantar administration of a 5% formaldehyde solution induces neurogenic pain and inflammatory pain. Pain syndrome is characterized by licking or biting of the paw. A central analgesic would inhibit both phases equally, but a peripheral analgesic would only inhibit the second phase. The control batch received distilled water (0.5 mL/100g body weight) while the other groups were treated with the aqueous and hydroethanolic extracts of *Brenania brieyi* (200, 100 and 200 mg/kg) and tramadol (10 mg/kg). One hour before injecting 20 µl of the formaldehyde solution (2.5%) into PPD, the mice received the test products orally. Immediately after the injection of the formaldehyde solution, the licking time of the treated paw was counted for the first ten minutes, and then the last 20 minutes. The analgesic effect was determined according to the following formula:

$$\% \text{ inhibition} = \left(1 - \frac{Tt}{To}\right) * 100$$

Tt is the time (in seconds) of licking of mice treated with extracts and reference substances ; To is the time (in seconds) for licking unprocessed mice.

Tail flick test

Study was done with the D'Amour & Smith test, modified by Nsonde Ntandou GF et *al* in 2020 [14, 19]. This test makes it possible to assess the analgesic effect of the central type. It consists of inducing pain by immersing the tail of the animal in a hot water bath at a temperature of 57 ± 2 ° C.

The reaction time (in seconds) that the animal takes to remove its tail from the water was noted 30, 60, 90 and 120 minutes after administration of the product. The maximum amount of time the animal must pass through hot water during this test is 30 seconds.

Exceed this time, the animal is not taken into account. The sensation of pain is characterized by the rapid removal of the animal tail. An analgesic would increase the reaction time of the animal. Five (7) lots of five (5) Rats each were formed. The rats were treated as follows:

- the 1st group (control) received 0.5 mL/100kg/rat of distilled water per rat;
- the 2nd group received 100 mg/kg/rat of paracetamol, used as the reference molecule;
- the 3rd group received 10 mg/kg/rat of morphine, used as a reference molecule per rat ;
- the 4th and 5th groups received respectively 100 and 200 mg/kg/rat of the aqueous extract of *Brenania brieyi* bark;
- the 6th and 7th groups received respectively 100 and 200 mg/kg/rat of the hydroethanolique extract of *Brenania brieyi* bark.

2.2.5. Study of antipyretic activity

The study was carried out according to the method described by Sawadogo WR et *al*. in 2006 [20]. After the initial rectal temperature measurement, the rats were given a 20% aqueous suspension of brewer's yeast (*Saccharomyces cerevisiae*) subcutaneously in the dorsolateral region due to 1 mL per 100 g body weight. Then the animals were put on an empty stomach. Seventeen (17) hours later, rectal temperature was taken again in each rat, and groups of six rats were formed

(as in the acetic acid test) with those with a temperature increase greater than or equal to 0.5°C. One hour after the administration of the extracts, the temperature was taken every hour for five hours.

2.3. Phytochemical study

The identification of the different chemical groups or secondary metabolites (alkaloids, flavonoids, tannins, free anthraquinones, saponosides, steroids, oses and Mucilage), we carried out a chemical screening of the dry bark of *Brenania brieyi*, by useing conventional phytochemical tests based on staining and precipitation reactions [20, 21].

2.4. Statistical analysis

The results expressed as averages assigned to the standard error were subjected to a one-factor analysis of variance followed by the Student-Fischer t-test. The observed difference was significant when the calculated t-value is, in absolute terms, greater than the t-value read in Student's table t for the degree of freedom. The values are expressed as an average ± ESM, n = 5, *** $p < 0.001$; * $p < 0.05$; ** $p < 0.01$ significant difference from control (Distilled water) and NS : non-significant difference from control (Distilled water).

3. Results and discussion

3.1. Acute toxicity

3.1.1. Effects of aqueous extract of Brenania brieyi on behaviour, general condition and mortality

Results on behaviour, general condition and mortality were reported in Table 1.

The study of acute toxicity in mice shows that the aqueous extract of *Brenania brieyi* bark was well tolerated, no mortality was observed up to the dose of 4000 mg / kg. From 500 mg/kg, the aqueous extract of *Brenania brieyi* bark causes a decrease in spontaneous mobility and sensitivity to pain induced by tail pinching. At 2000 mg/kg, in addition to the sedative effects observed at the previous dose, *Brenania brieyi* causes a loss of alertness compared to control mice (distilled water 1 mL / 100g, per bone).

Table 1 General state of animals after administration of aqueous extracts of *Brenania brieyi*

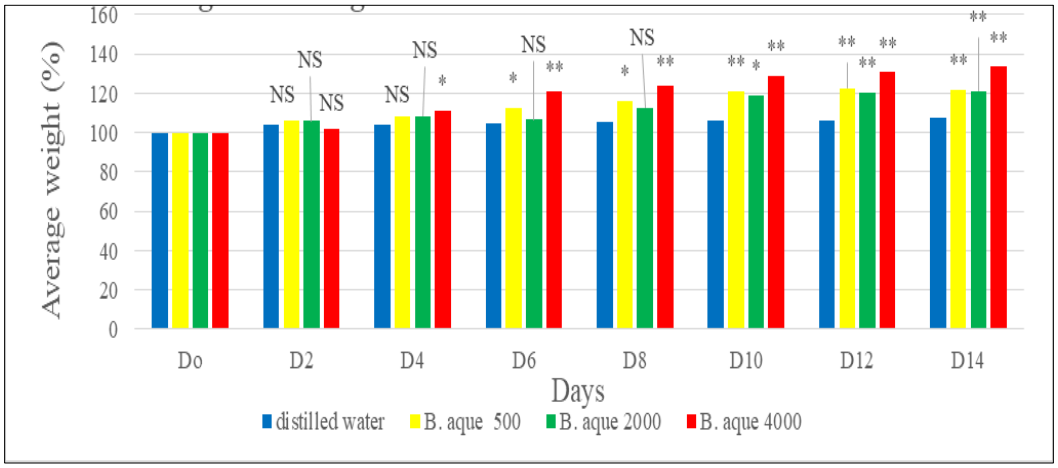
Parameter	**Treatments**			
	Distilled Water	***Brenania brieyi* aqueous extract**		
	10 mL/kg	**500 mg/kg**	**2000 mg/kg**	**4000 mg/kg**
Mobility	N	D	D	D
Aggressiveness	A	A	A	A
State of salts	N	N	N	N
Pain sensitivity	N	D	D	-
Vomiting	A	A	A	A
Vocalization	A	A	A	A
Erection pilot	A	A	A	A
Falling asleep	A	A	A	+
Vigilance	+	+	-	-
Deaths number	A	A	A	A

A : Absent ; N : Normal ; + : yes ; -very weak; -- : no reaction ; D :decrease ; n=5 mice

3.2. Effect of aqueous extract of *Brenania brieyi* on the evolution of mouse weight

Results of *Brenania brieyi* aqueous extract effect on the evolution of the weight of mice are reported in Figure 1.

The aqueous extract of *Brenania brieyi*, at 500, 2000.et 4000 mg/kg, promotes a slight increase in body weight up to 14 days after administration compared to those who received distilled water.



The values are expressed as an average ± ESM, n = 5, *** p < 0.001; * p < 0.05; ** p < 0.01 significant difference from control (Distilled water) and NS : non-significant difference from control (Distilled water)

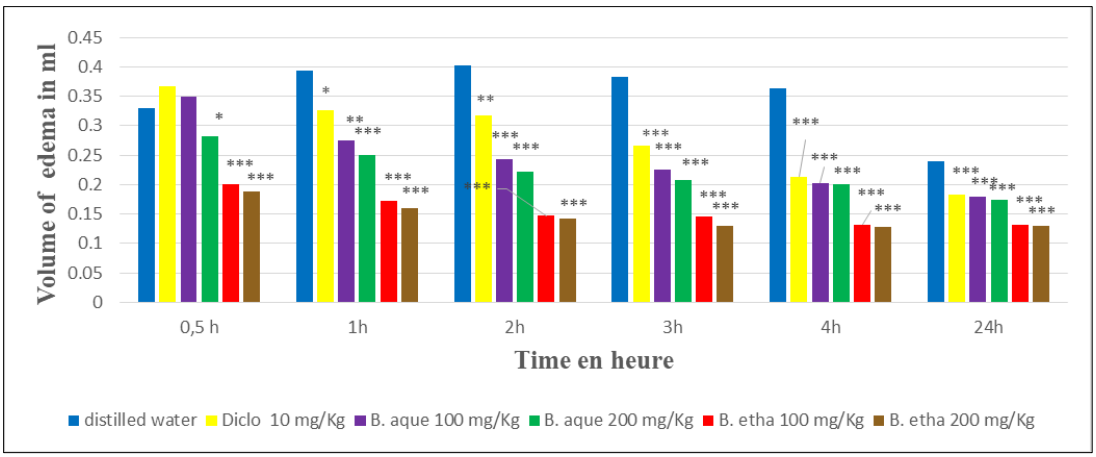
Figure 1 Weight evolution of mice as a function of time

3.3. Anti-inflammatory activities

3.3.1. Acute inflammation induced by carrageenan

The results of the study of acute inflammation induced by carrageenn are shown in Figure 2 and Table 2.

The aqueous and hydro-ethanolic bark of *Brenania brieyi* has an anti-inflammatory effect against carrageenan-induced edema. This effect is very significant from 30 minutes after administration of carrageenn for the hydroethanolic extract (B. etha) at doses of 100 and 200 mg/kg. While for the aqueous extract (B. aque), the effect is very significant from one hour at the dose of 200 mg / kg and after 2 hours at the dose of 100 mg / kg. This effect remains significant up to 24 hours of observation.



The values are expressed as an average ± ESM, n = 5, *** p < 0.001; * p < 0.05; ** p < 0.01 significant difference from control (Distilled water) and NS: non-significant difference from control (Distilled water)

Figure 2 *Brenania brieyi* aqueous and hydroethanolic extract anti-inflammatory activity on carrageenan-induced mouse paw edema

Table 2 Evolution of the PI in function of time

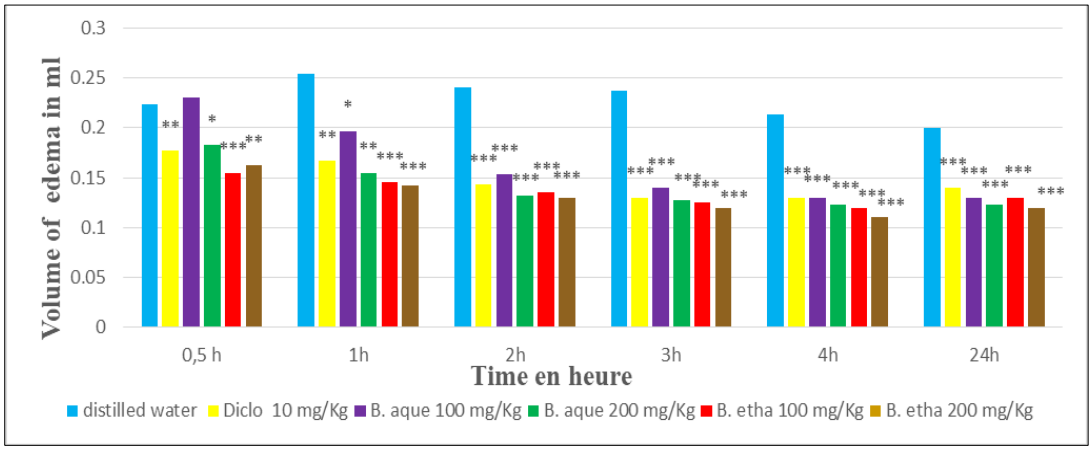
Product tested	PI $_1$	PI $_2$	PI $_3$	PI $_4$	PI $_{24}$
Diclo 10 mg/Kg	26,984127	34,848485	53,333333	77,777778	82,352941
B. aque 100 mg/Kg	51,190476	68,181818	73,75	83,333333	86,764706
B. aque 200 mg/Kg	61,904762	76,136364	81,25	83,333333	91,176471
B. etha 100 mg/Kg	78,571429	90,909091	91,25	97,222222	82,352941
B. etha 200 mg/Kg	84,52381	93,181818	98,75	100	95,588235

Legend: B. aque (aqueous extract of *Brenania brieyi* bark);B. etha (hydro-ethanolic extract of *Brenania brieyi* bark).

3.4. Acute formaldehyde-induced inflammation

Figure 3 and Table 3 represent the results of the study of formaldehyde-induced acute inflammation.

The aqueous and hydro-ethanolic extracts of *Brenania brieyi* bark exhibit an anti-inflammatory effect against formaldehyde-induced edema. The aqueous and hydroethanolic extracts exhibit an anti-inflammatory effect that is very significant with both doses after 2 hours up to 24 hours after formaldehyde administration.



The values are expressed as an average ± ESM, n = 5, *** p < 0.001; * p < 0.05; ** p < 0.01 significant difference from control (Distilled water) and NS: non-significant difference from control (Distilled water)

Figure 3 *Brenania brieyi* aqueous and hydroethanolic extract anti-inflammatory activity on formaldehyde-induced mouse paw edema

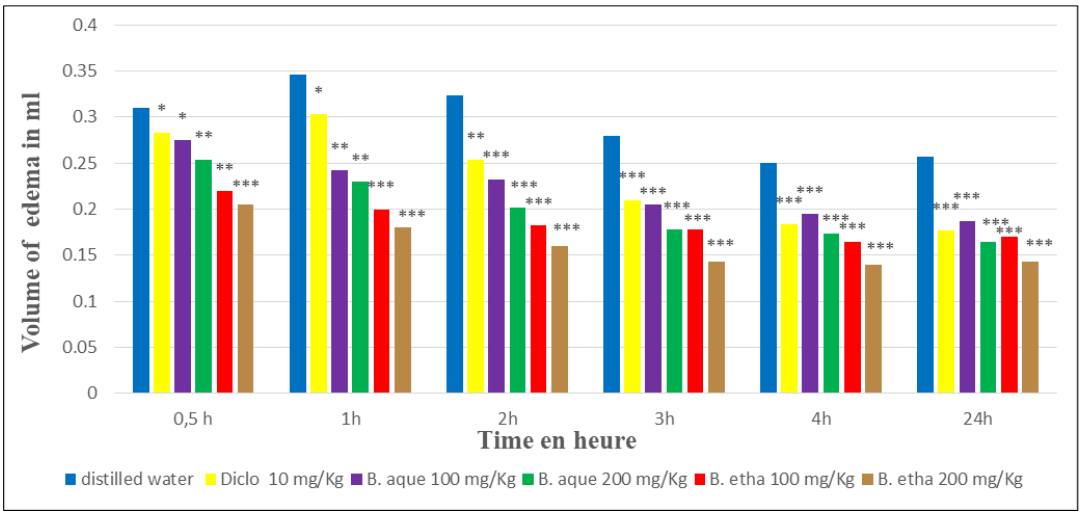
Table 3 Evolution of the PI in function of time

Product tested	PI $_1$	PI $_2$	PI $_3$	PI $_4$	PI $_{24}$
Diclo 10 mg/Kg	63,157895	79,411765	90,909091	88,461538	72,727273
B. aque 100 mg/Kg	39,473684	70,588235	81,818182	88,461538	86,363636
B. aque 200 mg/Kg	64,473684	80,147059	84,090909	85,576923	82,954545
B. etha 100 mg/Kg	72,368421	77,941176	86,363636	88,461538	72,727273
B. etha 200 mg/Kg	74,342105	82,352941	90,909091	100	86,363636

3.5. .Histamine-induced acute inflammation

Figure 4 and Table 4 represent the results of the study of histamine-induced acute inflammation.

The aqueous and hydro-ethanolic extracts of *Brenania brieyi*bark significantly reduce histamine-induced edema from 30 minutes for the hydroethanolic extract at both doses. The aqueous extract is very significant from one hour at the dose of 200 mg / kg and after 2 hours at the dose of 100 mg / kg. These effects remain significant up to 24 hours after formaldehyde administration. The percentages of inhibition are shown in Table 4.



The values are expressed as an average ± ESM, n = 5, *** p < 0.001; * p < 0.05; ** p < 0.01 significant difference from control (Distilled water) and NS: non-significant difference from control (Distilled water)

Figure 4 *Brenania brieyi* aqueous and hydroethanolc extract anti-inflammatory activity on histamne-induced mouse paw edema

Table 4 Evolution of the PI in function of time

Product tested	PI ½	PI $_1$	PI $_2$	PI $_3$	PI $_4$	PI $_{24}$
Diclo 10 mg/Kg	18,181818	23,636364	43,75	60	70	85,714286
B. aque 100 mg/Kg	31,818182	63,181818	64,0625	74,285714	70	86,607143
B. aque 200 mg/Kg	37,272727	62,909091	75	86,285714	82	97,857143
B. etha 100 mg/Kg	53,977273	74,090909	81,25	78,571429	81,25	81,25
B. etha 200 mg/Kg	53,977273	76,818182	85,9375	95,714286	96,25	94,642857

3.6. Analgesic activity

3.6.1. Acetic acid test

Table 5 Analgesic effect of aqueous and hydroethanolic extracts of *Brenania brieyi* bark on abdominal contractions induced in mice by injection of acetic acid

Product tested	Doses	Number of abdominal cramps	Inhibition (%)
distilled water	0.5 ml / 100g	41,8 ± 1.2	
Paracetamol	100 mg / kg	22,6 ± 0,6 ***	45,93
Brenania brieyi aqueous extract	100 mg / kg	34.6 ± 0,67 ***	17,22
	200 mg / kg	20.2 ± 1,01 ***	51,67
Brenania brieyi hydroethanolic extract	100 mg / kg	23,5 ± 1,17 ***	43,77
	200 mg / kg	16,4 ± 0.85 ***	60,76

Table 5 shows the effects of the different substances tested on abdominal cramps induced by the injection of acetic acid (0.6%). The reduction of abdominal cramps number was dose-dependent for both *Brenania brieyi* extracts and very significant with inhibition percentages of 51.67% for the aqueous extract at 200 mg/kg, 43.77% and 60.76% for the hydroethanolic extract at doses of 100 and 200 mg/kg respectively. The values are expressed as an average ± ESM, n = 5, *** p < 0.001; * p < 0.05; ** p < 0.01 significant difference from control (Distilled water) and NS : non-significant difference from control (Distilled water)

3.6.2. Formaldehyde test

The aqueous and hydro-ethanolic extracts of *Brenania brieyi* bark exhibit a significant dose-dependent analgesic effect in both phases of formaldehyde-induced pain 2.5%. These effects are recorded in Table 6. The values are expressed as an average ± ESM, n = 5, *** p < 0.001; * p < 0.05; ** p < 0.01 significant difference from control (Distilled water) and NS : non-significant difference from control (Distilled water)

Table 6 Effect of aqueous and hydroethanolic extracts of *Brenania brieyi* bark on the frequency of leg licking and the percentage reduction in pain

Product tested	**Dose**	**Neurogenic pain (0-10)**		**Inflammatory pain (0-10Min)**	
		Licking frequency	**Inhibition (%)**	**Licking frequency**	**Inhibition (%)**
Distilled water	0,5 mL/100g	41,6 ± 3,35		57,0 ± 8,40	
Tramadol	10 mg/kg	15,8 ± 1,98***	62,01 %	23,4 ± 0,81***	58,94 %
Brenania brieyi aqueous extract	100 mg/kg	22.2 ± 0.73***	46,63 %	10,8 ± 1,39***	81,05 %
	200 mg/kg	16,8 ± 0,48***	59,61 %	6,6 ± 1,28***	88,42 %
Brenania brieyi hydroethanolic extraxt	100 mg/kg	15,4 ± 1,02***	62,98 %	6,8 ± 1,20***	88,07 %
	200 mg/kg	13,8 ± 0,37***	66,82 %	2,2 ± 0,80***	96,14 %

3.6.3. Tail flick test

Figure 5 shows the effects of the different substances tested on pain induced by hot water. The aqueous extract of *Brenania brieyi* bark has a slightly significant effect against pain induced by hot water at 57 ± 2 ° C compared to the control who received the distilled water. While the hydro-ethanolic extract is very significant compared to the control and superior to paracetamol at 100 mg / kg, but lower than morphine at 10 mg / kg. The values are expressed as an average ± ESM, n = 5, *** p < 0.001; * p < 0.05; ** p < 0.01 significant difference from control (Distilled water) and NS : non-significant difference from control (Distilled water).

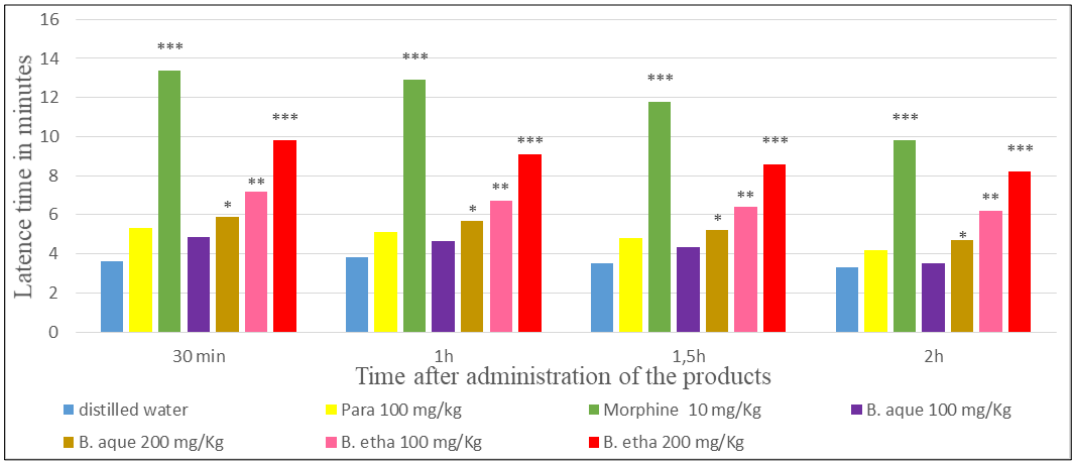


Figure 5 Analgesic activity of *Brenania brieyi* aqueous and and hydroethanolic extracts on tail flick method

3.7. Antipyretic activity

Table 7 shows the results of the study of the antipyretic effect of aqueous and hydroethanolic extracts of *Brenania brieyi* bark on hyperthermia induced by injection of a solution of brewer's yeast (20%). The aqueous extract of *Brenania brieyi* bark significantly decreases fever from the third hour while the decrease in fever caused by the hydroethanolic extract is very significant from one hour after administration. The values are expressed as an average ± ESM, n = 5, *** $p < 0.001$; * $p < 0.05$; ** $p < 0.01$ significant difference from control (Distilled water) and NS: non-significant difference from control (Distilled water).

Table 7 the antipyretic effect of *Brenania brieyi* on hyperthermia induced by the injection of brewer's yeast

Product tested	dose	Initial T	Rectal température (°C)					
			Pyretic	1 h	2 h	3 h	4 h	5 h
Distilled water	1ml/100g	36,08± 0,03	36,70± 0,12	37,38±0,17	37,22±0,58	37,18±0,54	37,16±0,50	37,10±0,52
Paracetamol	100 mg/kg	35,96± 0,26	36,68± 0,19	36,34±0,15***	36,12±0,16***	35,90±0,12***	35,82±0,17***	35,82±0,15***
B. Aque	100 mg/kg	35,90± 0,21	36,76± 0,29	36,50±0,26 **	36,38±0,17**	36,32±0,19***	36,16±0,11***	36,32±0,12***
	200 mg/kg	36,46± 0,04	36,98± 0,05	36,98±0,08NS	36,70±0,13**	36,66±0,13 **	36,14±0,21***	36,38±0,12***
B. Etha	100 mg/kg	36,32± 0,15	37,12± 0,24	36,87±0,03**	36,65±0,15***	36,44±0,02***	36,31±0,16***	36,44±0,05***
	200 mg/kg	36,61± 0,03	37,38± 0,19	36,89±0,11***	36,62±0,23***	36,60±0,16***	36,51±0,04***	36,64±0,21***

3.8. Chemical screening

The phytochemical study highlighted the presence of anthraquinones, flavonoids, alkaloids, tannins, saponosides, oses and steroids (Table 8).

Table 8 Results of the chemical screening of *Brenania brieyi* aqueous extract

Brenania brieyi	Chemical families							
	Alkaloids	Tannins	Flavonoids	Anthraquinones	Stéroïds	dares	Saponosides	Mucilage
Tested part : bark	+++	++	++	++	++	++	+++	-

- : negative reaction; + : present ; ++ : abundant , +++ : very abondant

4. Discussion

Some Congo plants belonging to the Rubiaceae family have already demonstrated their anti-inflammatory and analgesic effects [9]. This study aimed to establish a scientific basis for the use of *Brenania brieyi* widely used in traditional Congolese medicine in various pathologies characterized by inflammatory, pain and fever.

4.1. Acute toxicity of aqueous extract of *Brenania brieyi* bark

The purpose of the acute toxicity study was to determine LD50 and therapeutic doses [8 ; 21]. It follows that the bark of *Brenania brieyi* is not toxic up to the dose of 4000 mg / kg because no mortality has been observed. According to the GLOBALLY HARMONISED OECD Classification System [23], aqueous extract from *Brenania brieyi* bark can be classified as Category 5 and is considered a low-toxic substance with LD50 greater than 4000 mg/kg [22]. Sedation has been observed and is manifested by a decrease in spontaneous mobility and pain sensitivity induced by tail pinching at a dose of 500 mg/kg. To this is added a significant loss of vigilance at doses 2000 and 4000 mg / kg, which suggests that at doses greater than 4000 mg / kg, the plant could be toxic. These results are consistent with those obtained by Boumba

and *al*. (2018) which demonstrated that another plant of the Rubiaceae family, Heinsia crinitia., does not exhibit toxicity up to the dose of 2000 mg/kg.

In clinical practice, the therapeutic interest of NSAIDs is based on three major properties: anti-inflammatory, antipyretic, minor analgesic [36]. This work aimed to evaluate the anti-inflammatory, analgesic and antipyretic effects of aqueous and hydro-ethanolic extracts of *Brenania brieyi* bark.

4.2. Anti-inflammatory effect of aqueous and hydro-ethanolic extracts of *Brenania brieyi* bark

Measuring edema is an excellent tool for quantifying acute skin inflammation induced by agents like carrageenan 1%, formaldehyde 2.5% and histamine 1%. Acute inflammation is manifested by the very rapid but not very prolonged onset of edema caused either by carrageenan or formaldehyde [6; 9; 24; 25]. Carrageenin edema is an excellent representative model of the induction of acute inflammation. Carrageenin is a sulfated muco-polysaccharide from a Rhodophyceae, it causes inflammation typically related to the activation of cyclooxygenase. This inflammation is biphasic [9; 14]. In this research model, the first phase (1/2 to 1 h) is mainly modulated by the release of amino-vasoactive mediators (histamine and serotonin) in the tissue surrounding the injection site while bradykinin and leukotrienes are released during the second phase (1.5–3 hours) and the biosynthesis of prostaglandins by macrophages occurs beyond the third hour [27]. This last step is sensitive to PGs synthesis antagonists and natural or synthetic anti-inflammatories such as glucocorticoids [28]. Diclofenac 10 mg/kg as well as aqueous and hydro-ethanolic extracts of *Brenania brieyi* bark significantly inhibit edema caused by injection of 1% carrageenan. At the selected experimental doses, the aqueous extract and diclofenac show better inhibition in the second phase with PIs of 81.25% and 83.33% at 200 mg/kg respectively at the 3rd and 4th hour, while the hydroethanolic extract acts on both the first (PI of 84.52% after 30 minutes at 200mg/kg) and the second phase. These results suggest that the aqueous extract inhibits the synthesis of prostaglandin while the hydro-ethanolic extract would present an inhibition on both the synthesis of PG and that of histamine and serotonin. Carrageenin-induced edema is sensitive to cyclooxygenase inhibitors (COX 1 and COX 2) that inhibit the transformation of arachidonic acid into prostaglandins [30].

The same trend is observed with acute inflammation induced by formaldehyde whose mode of induction of edema involves the release of the same vasoactive amines [6].

The preliminary mechanisms of action of *Brenania brieyi* bark at doses of 100 and 200 mg/kg were studied with histamine-induced inflammation. Indeed, inflammatory edema leads to the local accumulation of blood cells by plasma leakage [28]. Histamine is the cause of increased vascular permeability after tissue aggression [29]. Prostaglandins can be produced under the influence of vasoactive amines including histamine. This suggests that the inhibitory action of the extracts would be exerted more on cyclooxygenases. *Brenania brieyi* significantly inhibits histamine-induced inflammation. The high significance of *Brenania brieyi* compared to diclofenac could be explained by the difference in doses of the two products. These results are consistent with those obtained by Chukwuma et al. in 2021 in Nigeria who demonstrated an inhibitory effect of the activities of phospholipase A2 and prostaglandin synthase of methanol and chloroform fractions of *Brenania brieyi* bark against egg albumin-induced edema and granuloma induced by cotton pellets [10]. The particularity of our study is that it focuses on aqueous and hydro-ethanolic extracts. The other difference lies in the estrogenic agents which are carrageenan, formaldehyde and histamine. However, additional cellular and molecular mechanisms of action, including cytokine release, should be investigated. These results also correspond to those obtained by Amouzoum et *al*. (2008) [4] who demonstrated the anti-inflammatory effect of hydro-alcoholic extracts of the roots and leaves of *Nauclea latifolia* (Rubiaceae) in the Wister rat.

4.3. Analgesic effect of aqueous and hydro-ethanolic extracts of *Brenania brieyi* bark

The analgesic effect of *Brenania brieyi* bark is evaluated with three experimental protocols. The acetic acid test highlights the analgesic property of peripheral type. The tail flick test made it possible to highlight the analgesic property of central type. The neurogenic phase of the formaldehyde test. A central-type analgesic inhibits both phases of formaldehyde-induced pain, while a peripheral-type analgesic inhibits only the second phase.

The pain caused by the administration of acetic acid is due to the release of chemical mediators such as serotonin, histamine, bradykinin, substance P and prostaglandins (PGE2α and PGF2α) [28; 29]. Local peritoneal receptors may be the cause of abdominal contractions [30]. A decrease in carrageenan-induced abdominal cramps was observed with PIs of 51.67% and 60.76%, at 200 mg/kg, respectively, for hydro-ethanolic and aqueous extracts of *Brenania brieyi* bark. The peripheral analgesic effect of *Brenania brieyi bark* is explained by their inhibitory effect on the synthesis of GPs [8]. The central type analgesic effect of the hydro-ethanolic and aqueous extracts of *Brenania brieyi bark* is confirmed by the inhibition of pain in both phases of pain caused by formaldehyde and the test of immersion of the tail in hot water. However, in both tests, the aqueous extract had a less central type analgesic effect compared to the hydroethanolic

extract which had a better PI than tramadol at 10 mg/kg in the formaldehyde test ; but slightly lower than morphine at 10 mg/kg in the tail flick test. In subsequent studies, it would be interesting to highlight the different molecular targets (specific receptors, enzymes, or proteins) to which *Brenania brieyi* would attach themselves at the level of the central nervous system. These results are consistent with those obtained by Boumba et *al*. in 2018 which demonstrated the anti-inflammatory and analgesic effect of a Rubiaceae, Heinsia crinita.

4.4. Antipyretic effect of aqueous and hydro-ethanolic extracts of *Brenania brieyi* bark

The hydro-ethanolic and aqueous extracts of *Brenania brieyi* bark reduce hyperthermia induced by the injection of brewer's yeast (20%). Hyperthermia induced by yeast injection is related to the release of cytokines (TNFα, IL 1β, IL6) that have reached the blood vessels stimulate the biosynthesis of prostaglandins (PGE2) around the thermoregulatory hypothalamic center [31; 32]. The antipyretic effect of these extracts may be due to their effect on reducing prostaglandin biosynthesis [8]. These results also correspond to those obtained by Amouzoum et al. (2008) [4] who demonstrated the antipyretic effect of hydro-alcoholic extracts of the roots and leaves of *Nauclea latifolia* (Rubiaceae) in the Wister rat.

4.5. Screening chimique

The flavonoids, tannins, anthraquinones, steroids and the large amount of saponosides found in *Brenania brieyi* bark could justify the anti-inflammatory, analgesic and antipyretic effects observed. Flavonoids have been shown to be able to alter arachidonic acid metabolism by blocking both cyclooxygenase and lipooxygenase pathways [35]. Alkaloids, saponosides and flavonoids have been shown to be responsible for anti-inflammatory, analgesic and antipyretic activities [34, 36, 37].

5. Conclusion

The toxicity study demonstrated that *Brenania brieyi* bark is not toxic up to the dose of 4000 mg/kg. The hydro-ethanolic and aqueous extracts of *Brenania brieyi* bark, at 100 and 200 mg/kg, exhibit dose-dependent anti-inflammatory, analgesic and antipyretic effects. These pharmacological effects are believed to be due to the presence of alkaloids, flavonoids, tannins, anthraquinones, steroids and saponosides identified in the bark of *Brenania brieyi.*

Compliance with ethical standards

Acknowledgments

The authors express their deep gratitude to Dr. Jean Akiana for his support in the search for funding and its involvement in collecting the plant sample. The authors are grateful to the botanists of the Botany Laboratory of the National Institute for Research in Exact and Natural Sciences (IRSEN) of Brazzaville-Congo for the botanical identification of plants. We are also grateful to the traditional healers for their time to accompany us through the forest during the harvest.

Disclosure of conflict of interest

The people carrying this work, me, my thesis supervisor and my supervisor, are not involved in any conflict of interest whatsoever.

Statement of ethical approval

This work has been carried out in compliance with the ethical standards advocated by the World Health Organization and the Organization for Economic Co-operation and Development. Anyone involved in this work will be according to their will. The various biological and chemical tests will be carried out in accordance with laboratory safety standards.

References

[1] Corrado B, Marco T, Colucci R, et al. Role of coxibs in the strategies for gastrointestinal protection in patients requiring chronic non-steroidal anti-infllammatory therapy. Pharm Res. 2009 59: 90–100.

[2] Zeilhofer HU. Prostanoids in nociception and pain. Bioch Pharmacol. 2007; 73: 165-74.

[3] AndréE M McLean, Pauline A Day. L'effet de l'alimentation sur la toxicité du paracétamol et la sécurité des mélanges paracétamol-méthionine. Pharmacologie biochimie. 1975; 24(1): 37-42.

[4] LA-L Amouzoum, A Agbonon, K Eklu-Gadegbeku, K Aklikokou, M Gbéassor. Activités antipyrétique et anti-inflammatire d'extraits hydr-alcooliques des racines et feuilles de *Nauclea latifolia* Smith (Rubiaceae) chez le rat Wister. Phytothérapie. 2008; 6(4): 228.

[5] Chebaibil A, Filali FR, Amine A, Zerhouni M. Effet bactéricide (in vitro) des extraits aqueux des feuilles du grenadier marocain (Punica granatum L.) sur des bactéries multirésistantes aux antibiotiques. Phytothérapie. 2011.

[6] Itou RDGE, Sanogo, R, Ossibi AWE, Ntandou FGN, Ondelé R, Pénemé BM, Abena AA. Anti-inflammatory and analgesic effects of aqueous extract of stem bark of Ceiba pentandra Gaertn. Pharmacology & Pharmacy, 2014; 5(12): 1113.

[7] Nsonde Ntandou GF, Banzouzi JT, Mbatchi B, Elion-Itou RDG, Etou-Ossibi AW, Ramos S, Ouamba JM. Analgesic and antiinflammatory effects of Cassia siamea Lam. stem bark extracts. Journal of ethnopharmacology. 2010; 127(1): 108-111.

[8] Nsonde Ntandou GF, Boumba LS, Gouollaly T, Makambila MC, Ouamba JM. Abena Acute toxicity, anti-inflammatory and analgesic effects of aqueous extract of Tetracera alnifolia Willd. (Dilleniaceae). Research Journal of Pharmacology and Pharmacy. 2017; 1(2): 1-10.

[9] LS Boumba, Nsonde Ntandou GF, AB Loufoua, MC Makambila, AA Abena. Toxicité aigüe, effets anti-inflammatoire et analgésique de l'extrait aqueux de *Heinsia crinitia* (Afzel.) G. Taylor (Rubiaceae). Phytothérapie. 2018; 16(S1): S8-S16.

[10] IF Chukwuma, VO Apeh, F Nkechi Nworah, CC Nkwocha, J Emaimo, V Nwadiogo Ogugua, LUD Ezeanyika. Inhibition des activités de la phospholipase A_2 et de la prostaglandine synthase entant qu'aperçu mécanistique possible de l'activité antiinflammatoire des fractions de méthanol et de chloroforme des écorces *Brenania brieyi*. Journal thaïlandais des sciences pharmaceutiques (TJPS). 2021.

[11] Canadian council on Animal care. Guide to the Care use of experimental animals. 1984; Vol 2 Canadian council on Animal care, Ottawa, Ontario, Canada, 208 pp.

[12] Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983; 16(2): 109-110.

[13] Borgi W, Ghedira K, Chouchane, N. Antiinflammatory and analgesic activities of Zizyphus lotus root barks. Fitoterapia. 2007; 78(1): 1619.

[14] Nsonde Ntandou GF , Dianzitoukoulou Matsima LD, Ingabire J, Makemba Nkounkou GS2, Nkundineza JC, ABENA AA. Anti-Inflammatory and Central Analgesic Effect of the Aqueous Extract of the Leaves of Vitex Madiensis Oliv (Lamiaceae-Viticoïdeae). Sch Int J Tradit Complement Med. Jan 2020; 3(1): 12-17.

[15] Iwueke AV, Nwodo OFC, Okoli CO. Evaluation of the anti-inflammatory and analgesic activities of Vitex doniana leaves. African Journal of Biotechnology. 2006; 5(20): 1929.

[16] Sawadogo WR, Boly R, Lompo M, et al. Anti-inflammatory, analgesic and antipyretic activities of Dicliptera verticillata. Int J Pharmacol. 2006; 2(4): 435–8.

[17] Santa-Cecília FV, Vilela FC, Rocha CQ, et al. Anti-inflammatory and antinociceptive effects of Garcinia brasiliensis. J Ethnopharmacol. 2011; 133: 467–73.

[18] Wibool R, Sae-wonga C, Reanmongkol W, Wongnawaa M. Antinociceptive Activity of the methanolic extract of Kaempferia galangal Linn. in experimental animals. J Ethnopharmacol. 2008; 118: 225–30.

[19] D'amour FE, Smith DL. Journal of Pharmacology and Experimental. Therapeutics. 1941; 72(1): 74-79.

[20] Sawadogo WR, Boly R, Lompo M, et al. Anti-inflammatory, analgesic and antipyretic activities of Dicliptera verticillata. Int J Pharmacol. 2006; 2(4): 435–8.

[21] Nkundineza JC, Nsonde Ntandou GF, Bassoueka D'AJ, Boumba LS., Makambila MC, Abena AA. Galore International Journal of Health Sciences and Research. 1 Jan.-March 2020; 5: 2456-9321.

[22] Zeilhofer HU. Prostanoids in nociception and pain. Bioch Pharmacol. 2007; 73: 165-74.

[23] OCDE. Toxicité orale aigue-Méthode par classe de toxicité aiguë. Ligne directrice de l'OCDE pour les essais de produits chimiques. OCDE. 2001; 423: 14.

[24] Ochieng CO, Ishola IO, Opiyo SA, Manguro LAO, Owuor PO, Wong KC. Phytoecdysteroids from the Stem Bark of Vitex doniana and Their AntiInflammatory Effects. Planta Med. 2013; 79: 52–59.

[25] Lee YY, Saba E, Irfan M, Kim M, Chan JY, Jeon BS, Choi SK. The antiinflammatory and anti-nociceptive effects of Korean black ginseng. Phymed. 2018; 54: 169-181.

[26] Dharmasiri MG, Jayakody JRAC, Galhena G, Liyanage SSP, Ratnasooriya WD. Anti-inflammatory and analgesic activities of mature fresh leaves of Vitex negundo. J. Ethnopharmacol. 2003; 87: 199– 206.

[27] Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008; 454: 436–444.

[28] Okoli CO, Akah PA, Nwafor SV, Anisiobi AI, Ibegbunam IN, Erojikwe O. Anti-inflammatory activity of hexane leaf extract of Aspilia Africana C.D. Adams, Journal of Ethnopharmacology. 2007; 109: 219-225.

[29] Dhara AK,Suba V,Sen T,Pal S. Preliminary studies on the anti-inflammatory and analgesic activity of the methanolic fraction of the root extract of the Tragia involucrata Linn,Journal of ethnopharmacology. 2000; 72: 265-268.

[30] Diatta W, Sy GY, Manga CI, Diatta K, Dior A, Bassene E. Recherche des activités anti-inflammatoire et analgésique des extraits de feuilles de Zanthoxylum zanthoxyloides (Lam) zepernick et timler (Rutaceae). Int. J. Biol. Chem. Sci. 2014; 8: 128–133.

[31] Tijjani MA, Abdurahaman FI, Khan IZ, Sandabe UK. The Effects of ethanolic extract of Vitex doniana stem bark on peripheral and central nervous system of laborotory animals. J. Appl. Pharm. Sci. 2012; 02: 74–79.

[32] Krishnaraju AV, Rao CBM, Sundararaju D, Sengupta K, Trimurtulu G. Antiinflammatory activity of Vitex leucoxylon l. Bark extracts against freund's complete adjuvant induced arthritis in sprague dawley rat. Am. J. Infect. Dis. 2009; 5: 68–73.

[33] Ribeiro RV, Matos da Silva R, Corsino da Silva JL, Tabajara de Oliveira MD. Antiinflammatory, antinociceptive and antipyretic effects of hydroethanolic extract from Macrosiphonia velame (A. St.-Hil.) M. Arg. in animal models, Brazil. J Pharmaceut Sci. 2010; 46: 515–23.

[34] Sajeli B, Bhagawati S, Goyal M, et al. Study of anti-inflammatory, analgesic and antipyretic activities of seeds of Hyoscyamus niger and isolation of a new coumarinolignan. Fitot. 2010; 81: 178–84.

[35] Sofowora A. Plantes médicinales et médecines traditionnelles d'Afrique. Karthala, Paris. 1996; 378.

[36] N Ouédraogo, M Lompo, RW Sawadogo, A Tibiri1, A-E Hay, J Koudou, M-G Dijoux, IP Guissou. Étude des activités anti-inflammatoire, analgésique et antipyrétique des décoctés aqueux des feuilles et des racines de Pterocarpus erinaceus Poir. (Fabaceae). Phytothérapie © Springer-Verlag France. 2012.

[37] Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar Kohli K. Mechanism ofAction of Flavonoids as Anti-inflammatory Agents: A Review. Inflammation & Allergy Drug Targets. 2009; 8: 229-235.

[38] Wahida BORGI, Nabil CHOUCHANE. Activité anti-inflammatoire des saponosides et des flavonoides des écorces et des racines de *Zizyphus lotus*. Revue des régions arides. 2007; 283-286.

[39] SE Khamtache, Chahinez Raad. Evaluation des activités antiinflammatoireet analgésique des alcaloïdes isoquinoléiques de *Fumaria officinalis*. Université de Béjaia. 2013.