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Bioactivity Assessment of Aqueous and Ethanolic Extracts of *Sida rhombifolia*

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Abstract

The aim of this study is to establish a link between the traditional use of *Sida rhombifolia* and its scientific effectiveness by comparing the anti-inflammatory and antioxidant potentials of the aqueous and ethanolic extracts from the stem barks, leaves, and roots of the plant harvested in the central region of Cameroon. Following qualitative and quantitative phytochemical screening, the acute oral toxicity of the extracts was determined using the toxicity class method outlined in the Organization for Economic Co-operation and Development (OECD) guidelines. *In vitro* and *in vivo* anti-inflammatory potentials, along with antioxidant effects, were determined through bovine albumin denaturation assays, measuring edema volume induced by 1% carrageenan in rats' paws using a plethysmometer, and assessing anti-hemolytic and anti-lipoperoxidative activities. The results showed that EER (ethanolic extract of the roots) is the richest in secondary metabolites and that at doses of 2000 and 5000 mg/kg of body weight, no signs of toxicity were noted in rats. Of the anti-inflammatory activity, protein denaturation revealed a maximum percentage of inhibition of 84.18% ± 0.19% at the dose of 10 mg/ml of EEF (ethanolic extract of the leaves), and at the sixth hour, the greatest percentages of inhibition of edema were 95.31% for EER and 95.56% for EET (ethanolic extract of the stem barks) at respective doses of 200 and 400

mg/kg of body weight. Concerning the antioxidant activity, at the dose of 10 mg/ml, the anti-hemolytic and anti-lipoperoxidative activities showed a respective inhibition percentage of 63.08% for EAT (antioxidant extract of the stem barks) and 47.97% for EAR (antioxidant root extract). Based on the research findings, it can be concluded that *Sida rhombifolia* extracts are non-toxic at the tested doses, showcasing significant anti-inflammatory and antioxidant properties. The stem of *Sida rhombifolia* shows great promise as a medicinal treatment for inflammation.

Keywords

Sida rhombifolia, Phytochemical Screening, Acute Oral Toxicity, Anti-Inflammatory, Antioxidant

1. Introduction

In Cameroon, as in many African countries, rural populations use, as part of traditional medicine, the plants they encounter in nature and much more in their neighborhoods to treat numerous illnesses; this is the case for inflammatory diseases [1]. Inflammation is a natural response of the body, involving events responsible for the release of chemical mediators and migrating cells to restore homeostasis [2]. Indeed, it is a pathological condition characterized by pain, heat, redness, and sometimes swelling. To deal with this situation, medications called anti-inflammatories are used. Anti-inflammatories are on the World Health Organization's model list of essential medicines [3] [4], and more than 70 million prescriptions incorporating this pharmacological class are written each year [5]. They are also consumed without prescription to treat joint pain, musculo-tendinous pain, headaches, pain of dental or menstrual origin, colds, trauma, or fever [6].

In traditional African medicine and that of other continents, plants are commonly used by populations to treat various ailments because they possess bioactive secondary metabolites [7] [8]. Several previous studies revealed that secondary metabolites with medicinal properties generally belong to alkaloids, triterpenoids, flavonoids, and coumarins [9] [10]. *Sida rhombifolia*, a plant of the *Malvaceae family* [11] is one of these medicinal plants widely used in traditional medicine thanks to its numerous therapeutic properties [9] [12] [13]. This plant is very present in the tropical regions of Africa, India, Australia, and America. It contains many secondary metabolites such as alkaloids, flavonoids, tannins, saponins, steroids, and triterpenoids [14]-[16]. *Sida rhombifolia* is used to treat abscesses, ulcers, wounds, stomach aches, dysentery, asthma, bronchitis, toothaches, applied to wasp stings, or used as a sedative [1]. Some studies present its antibacterial [17], antiplasmodial [18], and antioxidant activity [19] [20]. *Sida rhombifolia*, in previous studies, has shown by *in vitro* testing its metabolic profile and biological

activity [21], as well as its anti-inflammatory, anticholinergic, and cytotoxic [9], as well as its antibacterial activity [22]. Its acute toxicity of its aqueous-methanol extract has been proven by *in vivo* testing on Albino *Wistar* rats [22] as well as its antioxidant activity [23].

In Cameroon, *Sida rhombifolia* is very frequently found in urban and peri-urban areas, particularly along roadsides and within neighborhood. Although it is traditionally used to treat various ailments, its anti-inflammatory potential remains largely unknown and scientifically unexplored by the local population. Previous studies, such as the one by Mah, The and Ee [9], as well as others, have highlighted the anti-inflammatory and antioxidant properties of *Sida rhombifolia*. However, these studies focused on the whole plant without distinguishing between its different parts, even though the concentration of bioactive secondary metabolites varies depending on the plant organ studied. Additionally, the chemical composition of medicinal plants is influenced by environmental factors such as climate and soil, which can affect their therapeutic efficacy [24] [25]. To date, no study has assessed these potentials in the specific ecological context in Cameroon. With this in mind, the aim of this study is to establish a link between the traditional use of *Sida rhombifolia* and its scientific effectiveness by comparing the anti-inflammatory and antioxidant potentials of the aqueous and ethanolic extracts from the stem barks, leaves, and roots of the plant harvested in the central region of Cameroon. The methodology includes the extraction of secondary metabolites, phytochemical screening, an evaluation of the acute oral toxicity of the extracts, and an analysis of their antioxidant and anti-inflammatory activities. This approach will help identify the most active part of the plant and optimize its use in traditional medicine, while providing a scientific basis for its pharmacological valorization.

2. Materials

2.1. Plant Materials

Sida rhombifolia samples (**Figure 1(a)**) were collected in April 2022 in the central region of Cameroon, near the University of Yaoundé 1 in the Ngoa-ékélé district of the city of Yaoundé. The collected specimens were identified at the National Herbarium of Cameroon by Mr. MOUNMEMI KPOUMIE Hubert, who, by comparing them with patent material n˚169 of the specimen from the herbarium collection n˚9984 SRF Cam, issued a certificate of identification for the botanical reference sample n˚170/IRAD/DG/CRAM/SSRG-HNSF-PV/04/2022. The collected samples were dried at room temperature, protected from sunlight, in the Multidisciplinary Galenic Pharmacy Laboratory of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I (LMDPG/FMSB). The plant raw material used in this study consists of the leaves (**Figure 1(b)**), stems (**Figure 1(c)**), and roots (**Figure 1(d)**) of *Sida rhombifolia* plant (**Figure 1(a)**).

Figure 1. *Sida rhombifolia*; (a) Whole plant, (b) Leaves, (c) Stems, (d) Root.

2.2. Animal Materials

In all our tests, the Wistar strain of *Rattus norvegicus* was exclusively used. For acute oral toxicity testing, we used randomly selected female rats with weights between 100 and 150 g; for the anti-inflammatory test, both male and female rats weighed between 180 and 210 g. These animals were raised in the animal facility of the Multidisciplinary Galenic Pharmacy Laboratory and the Faculty of Medicine and Biomedical Sciences (LMDPG/FMSB) of the University of Yaoundé I under natural lighting and temperature conditions. They had unlimited access to tap water and standard food. Animals were anesthetized with a mixture of ketamine, xylazine, and acepromazine at doses of 60, 7.5, and 2 mg/kg, respectively, intraperitoneally [26]. The animals were properly ventilated, and vital parameters were monitored throughout the experiment. At the end of the procedure, the animals were sacrificed by diethyl ether inhalation [27]. Albino Wistar rats were selected as the animal model for this study due to their many physiological and metabolic similarities to humans, making them preferred choice for pharmacological and toxicological studies. Additionally, these rats are commonly used in biomedical research due to their availability, ease of handling, and low genetic variability, ensuring better reproducibility of results.

2.3. Chemical Materials

Carrageenan was purchased from Sigma-Aldrich (Germany). The reference standard for anti-inflammatory activity, diclofenac (Voltaren®50), and physiological serum were obtained from a local pharmacy in Yaoundé. Dimethyl sulfoxide (DMSO) was obtained from LMDPG/FMSB (University of Yaoundé I), and distilled water was obtained from the Chemistry Laboratory of *École Normale Supérieure de* Yaoundé (University of Yaoundé I).

3. Methods

3.1. Preparation of Powders and Extracts

The extraction of plant material was performed by maceration, a method commonly

used to preserve the integrity of bioactive compounds [12] [28]. After harvesting, the different parts of *Sida rhombifolia* (stem barks, leaves, and roots) were carefully washed with distilled water to remove impurities and then dried in the shade for two weeks at a controlled room temperature (27˚C - 35˚C) to avoid any thermal degradation of secondary metabolites. Once dried, the plant biomass was ground into fine particles using an electric grinder and then sieved to obtain a homogeneous powder. For extraction, 500 g of each powder were subjected to maceration in appropriate solvents (water and ethanol). After the solvent was evaporated using a rotary evaporator under reduced pressure, the dry extracts were stored in airtight glass containers, completely covered with aluminum foil to protect them from light and prevent any photochemical degradation. These measures ensure the stability and quality of the extracts before their use in biological analyses.

3.1.1. Aqueous Extracts

A total of 500 g of powder (roots, leaves, and stem barks) of *Sida rhombifolia* were individually macerated s in 5000 ml of distilled water for 48 hours, with stirring every 6 hours. After double filtration through *Whatman* no. 1 paper, the filtrates were evaporated in an oven at 50˚C until dry crude extracts were obtained. The extracts were coded as follows: EET (ethanolic extract of the stem barks), EAT (aqueous extract of the stem barks), EEF (ethanolic extract of the leaves), EAF (aqueous extract of the leaves), EER (ethanolic extract of the roots), and EAR (aqueous extract of the roots).

3.1.2. Ethanolic Extracts

A total of 500 g of powdered roots, leaves, and stem barks of *Sida rhombifolia* were each macerated separately in 5000 ml of 96% absolute ethanol for 72 hours, with stirring every 6 hours. After double filtration through on *Whatman* no. 1 paper, the filtrates were concentrated under a Heidolph® Hei-VAP Core rotary evaporator under reduced pressure at 50˚C until the crude extracts were obtained.

3.1.3. Extraction Yield

To evaluate the yield of dry extracts obtained, the empty flask and tray were weighed before use and again after solvent evaporation. The difference between the two weights was then calculated [29].

3.2. Phytochemical Screening of *Sida rhombifolia* Extracts

The phytochemical analysis was conducted following the standard protocols of references [10] [30] for qualitative studies. The targeted compound families included saponins, flavonoids, tannins, anthocyanins, alkaloids, phenols, polyphenols, triterpenes, steroids, and anthraquinones. The quantification of these secondary metabolites was carried out using the protocols described in the work of Odoh dan Okoro [31] for quantitative studies.

3.3. Acute Oral Toxicity Study

In this study, a limit test is carried out with a dose of 2000 and then 5000 mg/kg

body weight of the extracts administered orally as a single dose to a group of six animals (three per stage) [32]. A control group of three rats received distilled water at the same time, also administered orally. Based on the chosen test dose, the corresponding weight concentration to be administered was calculated, and the test substance was administered orally to the animals. The animals were weighed individually one hour before extract administration and daily throughout the 14-day study period. From the first to the fourteenth day, weight variations (PV) were calculated using Equation (1), and the results were compared with previous measurements. For this study, 27 female rats were used. This choice is based on the recommendation of the OECD (Organization for Economic Co-Operation and Development), which advocates using females for acute toxicity tests because they are often more sensitive to the toxic effects of substances than males.

$$VP = \frac{P_n - P(n-1)}{P(n-100)} \times 100 \quad (1)$$

where: VP = Percentage weight variation, P_n = nth measurement of body weight, $P(n-1)$ = $(n-1)$th measurement of body weight, n = natural whole number.

On the 14th day, the animals were fasted for 12 hours before being sacrificed, followed by necropsy. Organs such as the liver, spleen, lungs, heart, and kidneys were removed for relative weight calculations. Additionally, arteriovenous blood samples were collected in 5 ml dry tubes. Blood samples were centrifuged at 3000 rpm 15 minutes. The resulting serum was used for biochemical assessments, including transaminases, creatinine, and uric acid levels, measured using UV spectrophotometry. Histological analysis of the organs involved observation, fixation, trimming, dehydration, staining, mounting, embedding, and sectioning [33].

3.4. Evaluation of the Anti-Inflammatory Potential of *Sida rhombifolia* Extracts

The anti-inflammatory effect was evaluated in two stages: first, the *in vivo* evaluation of anti-inflammatory activity through the inhibition of edema of the right hind paw of rats induced by carrageenan [34]-[36]; and second, the *in vitro* evaluation of anti-inflammatory activity using the method described by [37]. For the *in vitro* evaluation, a test solution (0.5 mL) was prepared, consisting of 0.45 ml of a 5% aqueous solution of bovine albumin serum (BSA) and 0.05 mL of aqueous extract at concentrations of 5; 6.25; 7.5; 8.75, and 10 µg/mL. A control test solution (0.5 ml) was also prepared, containing 0.45 ml of a 5% BSA aqueous solution and 0.05 ml of distilled water. Additionally, a blank control solution (0.5 mL) was prepared, containing 0.45 ml of distilled water and 0.05 ml of the aqueous extract at the same concentrations. The standard test solution (0.5 mL) contained 0.45 ml of the 5% BSA aqueous solution and 0.05 ml of an indomethacin standard solution at concentrations of 0.1 and 1 mg/mL. All solutions were adjusted to pH 6.3 using 1 N HCl solution. The samples were incubated at 37˚C for 20 minutes, after which the temperature was increased to 57˚C for 3 minutes. After cooling the tubes, 2.5 mL of the phosphate buffer saline solution (PBS) at pH = 6.3 was added to each

solution. Absorbance was measured using a UV-visible spectrophotometer at 416 nm, and the percentage inhibition of protein denaturation was calculated using equation (2). *In vivo* anti-inflammatory activity was conducted on a total of 70 Witars rats, both males and females. Including both sexes allows for the assessment of potential differences in the inflammatory response and ensures that the observed effects are not biased by hormonal factors.

$$\%\ \text{d'inhibition} = \frac{100 - \left(\text{Optical density of test solution} - \text{Control optical density}\right)}{\text{Control optical density}} \times 100 \quad (2)$$

The control group was considered to have 100% denatured proteins, and the results were compared to those obtained with indomethacin (0.1 and 1 mg/mL). The *in vivo* evaluation of anti-inflammatory activity required 70 male and female rats, divided into 14 groups of 5 rats each. The rats were fasted for 24 hours before the experiment, then weighed and marked one hour before the start of the test [36] [38] [39]. The initial volume (V_0) of the right hind paw of each rat was measured using a plethysmometer before oral administration of the extract via orally a gastric tube. The extract doses administered were 200 and 400 mg/kg of body weight.

The rats were grouped as follows, receiving the respective solutions orally at a dose of 1 mL/100g of body weight:

- Group 1 (negative control): received distilled water.
- Group 2 (positive control): received the diclofenac solution.
- Group 3 and 4: received the aqueous extract of the roots.
- Group 5 and 6: received the aqueous extract of the stem barks.
- Group 7 and 8: received the aqueous extract of the leaves.
- Group 9 and 10: received the ethanolic extract of the roots.
- Group 11 and 12: received the ethanolic extract of the stem barks.
- Group 13 and 14: received the ethanolic extract of the leaves.

One hour after the different treatments, 1% carrageenan was injected under the footpad at a dose of 0.05 mL.

3.4.1. Determination of the Effect of Extracts on Acute Inflammation Induced by 1% Carrageenan in Rats

The volume of paw edema was measured at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, and 6 hours after carrageenan injection using a plethysmometer [39]. The average percentage increase in paw volume (PA) and edema inhibition percentages of edema (PI) were calculated using Equations (3) and (4).

$$\%PA = \frac{V_t - V_0}{V_0} \times 100 \quad (3)$$

$$\%\text{d'Inhibition}\left(\text{PI}\right) = \frac{\left(\text{PATe} - \text{PATr}\right)}{\text{PATe}} \times 100 \quad (4)$$

where: V_0 = Initial paw volume before edema induction; V_t = Paw volume after treatment administration and carrageenan injection; PATe: Percentage increase in paw volume of the control group; PATr: percentage increase in paw volume of the treated group.

Figure 2 below illustrates the steps involved in the anti-inflammatory test conducted on rats.

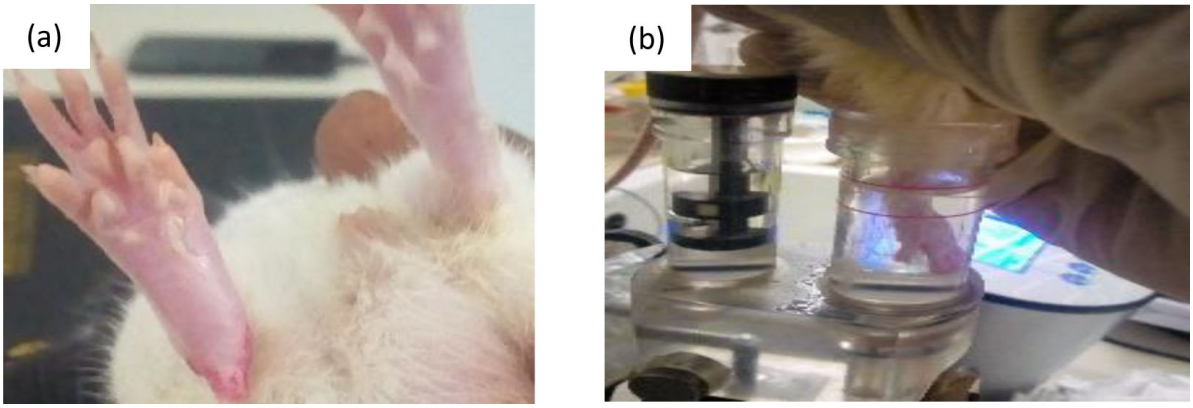

Figure 2. Conduct of the *in vivo* anti-inflammatory test; (a) Rat with paw edema, (b) Measurement of the edema.

3.4.2. Anti-Oxidant Test of *Sida rhombifolia*

To evaluate the antioxidant potential of *Sida rhombifolia*, two activities were measured: antihemolytic activity and antilipoperoxidative activity.

1) Anti-hemolytic activity

This test is based on the reaction of red blood cells subjected to oxidative stress under strictly controlled and standardized conditions. Intra- and extracellular antioxidant defenses act to resist the oxidative attack, maintaining membrane integrity and cellular functions until the membrane is compromised, leading to the leakage of cellular contents. Hemoglobin released into the supernatant is measured by spectrophotometry at 532 nm.

Isolation and preservation of red blood cells

Blood was collected from a rat in a tube containing ethylenediaminetetraacetic (EDTA). The red blood cells were separated from the plasma and white blood cell layer by centrifugation at 1500 rpm for 15 minutes. The obtained red blood cells were washed three times with physiological saline (0.9% NaCl). The final red blood cell suspension (hematocrit at 33%) was gently agitated and stored at 4˚C. The reagents used were $CuSO_4$ (0.1 M) and NaCl (0.09%) are the reagents used.

Operating mode

A volume of 0.2 ml of extract (5, 6.25, 7.5, 8.75, and 10 µg/ml) prepared in water was mixed with 1 ml of NaCl (0.9%). Then, 0.1 ml of the red blood cell suspension was added. The mixture was incubated at room temperature for 30 minutes before adding 0.1 ml of $CuSO_4$ (0.1 M) to induce hemolysis. After 30 minutes incubation, absorbance was measured at 532 nm against a blank consisting of 1.3 ml of NaCl and 0.1 ml of the red blood cell suspension. Total hemolysis was achieved by adding 1.2 ml of NaCl (0.9%) and 0.1 ml of the suspension, incubating for 30 minutes, and then adding of 0.1 ml of $CuSO_4$ (0.1 M). The hemolysis rate was calculated using Equation (5).

$$\text{Hemolysis rate}(\%) = \frac{(\text{OD total hemolysis} - \text{OD test})}{\text{OD total hemolysis}} \times 100 \quad (5)$$

where: OD total hemolysis: Optical density of the total hemolysis (the positive control); OD test: Optical density of the test sample.

2) Anti-lipoperoxidative activity

Principle of the method

At physiological pH, $FeSO_4$ induces lipid peroxidation in polyunsaturated fatty acids, leading to the production of malondialdehydes (MDA), which absorbs at 532 nm. In the presence of antioxidants, $FeSO_4$'s effect on polyunsaturated fatty acids is inhibited, reducing MDA production.

Presentation of the preparation of liver homogenate:

The liver of a rat was dissected, washed in NaCl solution (0.9%), drained, weighed, and ground in a mortar. The ground material was homogenized in 10% (weight/volume) phosphate buffer (0.1 M, pH = 7.4) and centrifuged at 1500 rpm for 10 min. The supernatant (homogenate) was collected for testing. The reagents used included NaCl (0.9%), phosphate buffer (0.1 M, pH = 7.4), $FeSO_4$ (15 mM), TBA (1%) (Thiobarbiturique), HCl (10%), and ascorbic acid (6 mM).

Presentation of the operating mode

A volume of 300 µL of extract (5, 6.25, 7.5, 8.75 and 10 µg/mL) was added to 500 µL of liver homogenate. Lipid peroxidation was induced by adding 100 µl of iron sulfate. After incubation at 37˚C for 30 minutes, 1000 µL of TBA (Thiobarbituric) (1%) HCl (10%) were added, followed by 1000 µL of ascorbic acid. The mixture was heated to 80˚C for 20 minutes, cooled, and centrifuged at 1500 rpm for 10 min. The OD (Optical Density) was measured at 532 nm. The formula commonly used to calculate the percentage of lipoperoxidation inhibition is given by Equation (6):

$$\%\text{Inhibition} = \frac{(\text{OD control} - \text{OD test})}{\text{OD control}} \times 100 \quad (6)$$

where: OD control is the optical density of the control (without the tested extract or compound), OD test is the optical density in the presence of tested extract or compound.

3.5. Statistical Analysis

In the present study, data were expressed as mean ± standard error of the mean (SEM). Statistical significance between groups was assessed using one-way analysis of variance (ANOVA), followed by Dunnett's pos hoc test for multiple comparisons. Graph Pad Prism 8.0.1 software was used for statistical analysis, and differences were considered statistically significant when p values < 0.05.

4. Results and Discussion

4.1. Extraction Yield

The extraction yields of aqueous and ethanolic extracts from different parts of

Sida rhombifolia are presented in Table 1.

Table 1. Extraction yield results.

Parts of the plant	Roots		Stem barks		Leaves	
Nature of the extract	EER	EAR	EET	EAT	EEF	EAF
Averages (g)	25.375 ± 0.568	36.835 ± 0.425	33.145 ± 0.467	42.675 ± 2.119	53.652 ± 3.54	75.41 ± 0.15
Yield (%)	5.075	7.367	6.629	8.535	10.730	15.082

The results show a significant variation in extraction yields depending on the solvent used and the plant part extracted. The yields of aqueous extracts follow a decreasing order: 15.08% for leaves, 8.53% for stem barks, and 7.36% for roots. Regarding ethanolic extracts, the yields obtained are 10.73%, 6.62%, and 5.07% for leaves, stem barks, and roots, respectively. These findings indicate that aqueous extracts are obtained in greater quantities than ethanolic extracts, suggesting that water (H_2O), due to its polarity, enhances the solubilization of compounds present in the plant. These results are comparable to those reported in the literature. In India, Dhalwal, Deshpande, and Purohit [19] obtained yields of 4.28%, 5.15%, and 6.16% for ethanolic extracts of roots, stem barks, and leaves, respectively. Regarding leaves, Chaves *et al*. [13] in Brazil found a nearly similar yield (10.3613%) for the ethanolic extract. However, these values remain lower than those reported by Youssouf Loutfi, Vavitiana Dhalia Xavier, Rama Michela Stéphanie, and Razafindramanana Jeenath Francelline [40], who recorded an extraction yield of 13% for a hydroalcoholic extract of *Sida rhombifolia* leaves. This difference could be attributed to the hydroalcoholic nature of the extract, as water contributes to more efficient extraction due to its higher polarity. The yields from leaves are consistently higher than those from stems and roots, regardless of the solvent used. This observation could be related to the presence of chlorophyll and other polar compounds in the leaves, which dissolve more easily in water. Ferro *et al*. [14] demonstrated that the extraction yield of secondary metabolites, particularly flavonoids and phenols, depends on the extraction method and the polarity of the solvents used. Thus, extraction efficiency is influenced not only by the chemical nature of the solvent but also by the biochemical composition of each plant part. Using water as an extraction solvent results in higher yields, particularly for leaves. This trend could be explained by the increased solubility of bioactive compounds in water compared to ethanol. However, ethanol, although less efficient in terms of overall yield, may facilitate the extraction of specific compounds that are less soluble in water, potentially influencing the composition and bioactivity of the extracts obtained.

4.2. Phytochemical Study

4.2.1. Qualitative Phytochemical Screening

The qualitative phytochemical Screening of aqueous and ethanolic extracts of *Sida*

rhombifolia was carried out in triplicate. To assess the secondary metabolite content, the following codes were adopted: high content: (+++), medium content: (++), low content: (+), and absence of content: (−) [41]-[44]. The results are presented in Table 2.

Table 2. Results of qualitative phytochemical screening.

	Roots		Stem barks		Leaves	
	EER	EAR	EET	EAT	EEF	EAF
Alkaloids	+++	++	++	+++	+	+
Phenols	+++	++	+++	++	+	+
Polyphenols	++	++	+	++	+	+
Tannins	+++	++	++	++	+	+
Saponins	+++	+	+	+	±	+
Flavonoids	++	++	+	++	+	+
Triterpenes	+	+	+	+	+	++
Steroids	+	+	+	+	+	++
Anthocyanins	−	−	−	−	−	−
Anthraquinones	++	−	+	−	−	−

The analysis confirms the richness of aqueous and ethanolic extracts of *Sida rhombifolia,* harvested in the central region of Cameroon, in secondary metabolites. Identified compounds include phenolic compounds (phenols, polyphenols, tannins, flavonoids, and anthraquinones), terpenes (steroids, triterpenes, saponins), and nitrogen compounds (alkaloids). Qualitative phytochemical screening confirms the richness of *Sida rhombifolia* in secondary metabolites. This chemical diversity highlights the bioactive potential of the studied species. To assess the consistency of these results, a comparison with previous studies was conducted. Table 3 presents a summary of the main similarities and differences observed.

Table 3. Comparison of qualitative phytochemical screening results.

Secondary metabolites of *Sida rhombifolia*	
Metabolites	References
Alkaloids, Flavonoids, Tannins, Saponins, Steroids, Triterpenes, Glycosides, Resins,	[13] [14]
Alkaloids, Flavonoids Tannins, Saponins, Steroids, Triterpenes, Glycosides Antraquinones, Phenols, Polyphenols,	Present study

These findings align with previous studies on the chemical composition of *Sida rhombifolia*. According to [13] isolated steroids, alkaloids, and flavonoids from the aerial parts of the plant, confirming the presence of these metabolite classes. Similarly, [22] identified tannins, flavonoids, saponins, alkaloids, and steroids in *Sida rhombifolia* leaves. However, anthraquinones were absent in their study, whereas in the present research, this metabolite was detected only in ethanolic extracts. Furthermore, the work of [40] reported the presence of tannins, flavonoids, alkaloids, and anthocyanins in *Sida rhombifolia* leaves. However, in this

study, anthocyanins were absent. This difference may be due to environmental variations influencing secondary metabolite biosynthesis. Factors such as climate, soil composition, altitude, and growth conditions can significantly impact the chemical composition of plants [14]. The observed variations in the presence or absence of certain secondary metabolites suggest an influence of environmental conditions on the biosynthesis of bioactive compounds. The detection of anthraquinones exclusively in ethanolic extracts in this study, contrary to some previous reports, could be attributed to differences in abiotic and biotic factors. Similarly, the absence of anthocyanins, which were identified in other studies, highlights the possible impact of local environmental conditions on *Sida rhombifolia*'s chemical profile.

4.2.2. Quantitative Phytochemical Screening

The test was carried out in triplicate, and the values were expressed as mean ± standard deviation. The results of the quantitative phytochemical screening of the aqueous and ethanolic extracts of *Sida rhombifolia* are presented in **Table 4**.

Table 4. Quantities of secondary metabolite of *Sida rhombifolia* in µg/mg.

Family of Compounds	Roots		Stem barks		Leaves	
	EER	EAR	EET	EAT	EEF	EAF
Alkaloids	161.42 ± 0.75	96.32 ± 2.73	79.31 ± 3.54	145.13 ± 9.36	32.63 ± 0.54	23.15 ± 1.18
Tannins CA	157.06 ± 3.53	104.25 ± 5.43	84.25 ± 2.69	71.74 ± 1.05	13.56 ± 1.91	27.28 ± 0.61
Saponins	161.63 ± 5.63	27.52 ± 2.29	30.25 ± 0.03	31.04 ± 1.30	0.45 ± 0.33	25.01 ± 3.42
Flavonoids	80.26 ± 3.14	89.25 ± 6.02	35.43 ± 2.85	69.36 ± 3.04	25.79 ± 2.74	31.45 ± 0.92
Triterpenes	35.64 ± 6.23	36.15 ± 0.16	26.01 ± 0.98	18.53 ± 1.02	27.42 ± 0.92	72.83 ± 1.41
Steroids	43.09 ± 3.61	15.97 ± 0.74	41.24 ± 0.34	23.48 ± 0.29	16.73 ± 1.67	14.86 ± 0.09

These findings align with previous studies, such as those by Youssouf Loutfi, Vavitiana Dhalia Xavier, Rama Michela Stéphanie, and Razafindramanana Jeenath Francelline [40], who reported that the aerial parts of *Sida rhombifolia* contained significant levels of tannins, with average amounts of alkaloids and leucoanthocyanins, but lower levels of flavonoids, anthocyanins, and polysaccharides. Although most studies in the literature do not specify the exact quantities of each secondary metabolite family, they generally confirm that the composition of *Sida rhombifolia* extracts follows similar trends. Furthermore, a study by Assam *et al.* [22] found that the leaves of *Sida rhombifolia* contained significant amounts of tannins, flavonoids, saponins, alkaloids, and steroids. However, they did not report the presence of saponins in the roots, which contrasts with our results where saponins were found in the ethanolic extracts of the roots. This discrepancy could be due to differences in extraction techniques, solvent types, or environmental conditions. The extraction of secondary metabolites is highly influenced by the solvent used and the part of the plant analyzed. In the present study, alkaloids, tannins, saponins, and flavonoids were found in higher quantities compared to other me-

tabolites. Specifically, the ethanolic extract of the roots exhibited the highest levels, with 161.42 µg/mg of alkaloids, 157.06 µg/mg of tannins, 161.63 µg/mg of saponins, and 80.26 µg/mg of flavonoids. These results suggest that ethanol is a more efficient solvent for extracting these metabolites from the roots, likely due to its ability to dissolve a wide range of bioactive compounds. This is consistent with findings from several studies. For instance, Chaves *et al*. [13] found that ethanol extracts of *Sida rhombifolia* also yielded higher concentrations of tannins and alkaloids compared to aqueous extracts. The decreasing order of secondary metabolite concentrations in this study was as follows: roots > stem barks > leaves. This distribution could be linked to the physiological role of these compounds in the plant. The roots, in direct contact with the soil and exposed to various microbial and environmental stresses, may produce and accumulate higher amounts of secondary metabolites for protective purposes. Similarly, studies reported that roots tend to accumulate more bioactive compounds compared to stems and leaves. The stems, which play an intermediary role in transporting nutrients and water, have moderate levels of secondary metabolites, while the leaves, primarily responsible for photosynthesis, generally accumulate fewer defense-related compounds. The differences observed in the content of secondary metabolites between studies could be attributed to several factors, including environmental conditions, harvest season, and the extraction methods used. The chemical composition of medicinal plants is strongly influenced by abiotic factors such as soil composition, climate, and altitude, as well as biotic factors such as stress from pests or disease and interactions with microorganisms. For instance, Dhalwal *et al*. [19] found that ethanol generally yields higher amounts of secondary metabolites, especially alkaloids and flavonoids, compared to aqueous extracts. The effectiveness of ethanol as an extraction solvent, due to its ability to extract both polar and non-polar compounds, may explain the higher yields found in this study compared to aqueous extraction methods. However, the specific differences in the results of various studies may be due to variations in plant material, including the geographic origin, harvest time, and plant age, all of which can significantly impact the concentration of secondary metabolites.

4.3. Study of the Acute Oral Toxicity of *Sida rhombifolia* Extracts

During this test, no deaths were recorded among the animals throughout the duration of the study. Additionally, the behavior of the animals remained unchanged. The results of the acute oral toxicity study of the aqueous and ethanolic extracts of *Sida rhombifolia* are presented through the kinetics of the weight evolution of the treated rats, as well as the analysis of their liver parameters.

4.3.1. Evolution of the Weight of the Rats during the Test

It was observed that the extracts of *Sida rhombifolia,* at doses of 2000 and 5000 mg/kg body weight, had no significant effect on the weight of the animals. **Table 5** presents the kinetics of the weight changes in the rats during the test.

Table 5. Kinetics of weight change in rats during the test.

Days	EAR		EER		EAT		EET		EEF		EAF		Witness
	2000 mg	5000 mg	2000 mg	5000 mg	2000 mg	5000 mg	2000 mg	5000 mg	2000 mg	5000 mg	2000 mg	5000 mg	
1	6.66	2.66	6.33	8	4.33	3	6.33	7.33	6	5.66	5.66	4	3.33
2	9	5.33	8	9	6	6	9.33	10	7	6.33	7.33	5	7.66
3	11	7	9	12	8.33	7.66	13	12.66	8	7.66	9	7	9
4	13.66	9	9.66	12.66	12	9.33	14	14.66	8.66	9	11	9.33	10.66
5	15	11	11.33	13.33	12.33	10.33	15.33	16.66	10.33	11	13.33	11.66	12
6	16.33	12.66	13	14.33	13.33	12	17	18.33	11	13	15	13	14
7	18.66	14	16.33	4	11	12.66	20.33	20.33	13	14.66	18	15	14.33
8	24	18.33	19	20.66	15	17.33	25.33	23	15	17	21.33	18.33	20.66
9	26.33	21.33	22.33	24	17	20	27	25.33	18.33	19	23	21.33	25.66
10	28.33	23	24	25.33	19.33	21.66	29	28	20	22.66	25.33	23	27.66
11	30	25.66	25.33	26.66	21.33	22.66	30	30	23	24.66	27	25	28.66
12	28.66	25.66	25.66	28.33	23.33	23	32.33	30.66	25	26	28.33	27	27
13	28.66	26.33	27.66	29.66	23	23.66	34.66	31.66	26.66	27.33	30	29	28
14	30.66	30	27.33	31	25.33	27	37.66	32.66	28.33	29	32	31.66	28.66

Statistical analysis showed no significant difference in the weight change between the treated rats and the control group. Figure 3 illustrates the kinetic curves of the weight changes in the rats throughout the 14-day duration of the test.

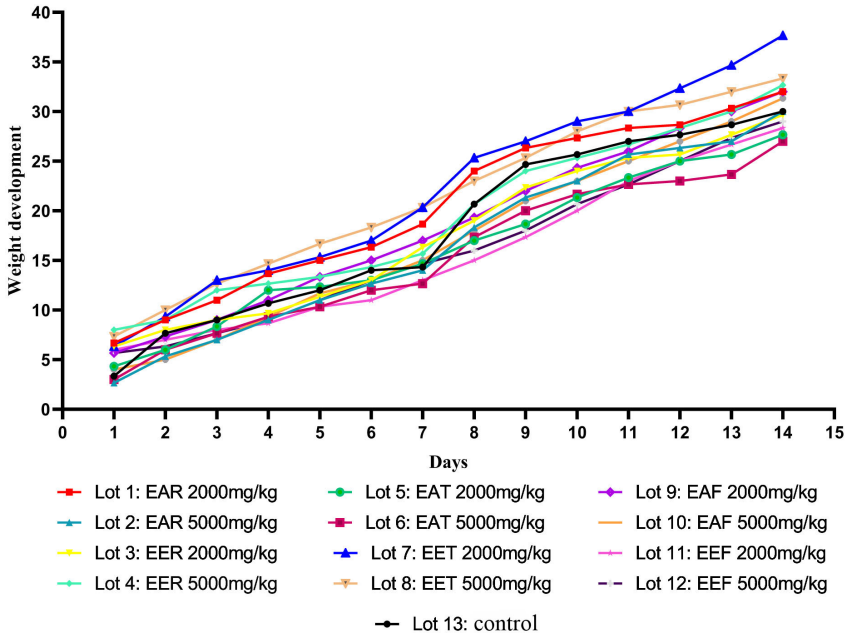

Figure 3. Kinetics of weight change in rats during the test.

The administration of the different *Sida rhombifolia* extracts at the maximum doses of 2000 mg/kg and 5000 mg/kg of body weight showed no visible sign of toxicity in the treated animals during the 14 days of observation and monitoring following treatment. Additionally, no animal deaths were recorded. It appears

from this study that the animals continued their normal growth after the administration of *Sida rhombifolia* extracts. For acute oral toxicity, the administration of the different extracts at the maximum doses of 2000 mg/kg and 5000 mg/kg body weight showed no visible signs of toxicity in the treated animals during the 14 days of observation and monitoring after treatment. No deaths were recorded in any of the animals. These results are consistent with those in the literature, which suggest that *Sida rhombifolia* extracts are not toxic to the body [22] [45]. In this study, the histological sections of the animals' organs were normal, with no signs of organ toxicity observed in the treated rats. An increase in the weight of the rats and their organs was observed in all groups, with no significant difference. In terms of blood parameters, no significant differences were recorded between the data from the control groups and those from the treated animals. Tumanggor, Bintang, and Priosoeryanto [45] demonstrated in their studies that *Sida rhombifolia* leaf extracts are safe for normal cells in the body.

4.3.2. Analysis of the Liver Parameters of the Rats after the Test

Table 6 presents the relative mass of the organs.

Table 6. Relative mass of organs in mg.

		Heart	Lungs	Liver	Kidneys	Missed
Lot 9 witness		0.30 ± 0.030	0.753 ± 0.289	3.028 ± 0.255	0.542 ± 0.023	0.355 ± 0.143
EAR	2000 mg/kg	0.312 ± 0.027	0.797 ± 0.278	3.263 ± 0.455	0.575 ± 0.027	0.686 ± 0.228
	5000 mg/kg	0.326 ± 0.056	0.912 ± 0.278	3.528 ± 0.524	0.575 ± 0.096	0.668 ± 0.232
EER	2000 mg/kg	0.306 ± 0.032	0.755 ± 0.191	4.069 ± 0.820**	0.557 ± 0.008	0.748 ± 0.399
	5000 mg/kg	0.326 ± 0.025	0.889 ± 0.233	4.080 ± 0.670**	0.772 ± 0.313	0.572 ± 0.099
EAT	2000 mg/kg	0.264 ± 0.007	0.656 ± 0.125	3.526 ± 0.560	0.548 ± 0.057	0.699 ± 0.247
	5000 mg/kg	0.301 ± 0.019	0.725 ± 0.141	4.174 ± 1.094***	0.532 ± 0.038	0.371 ± 0.037
EET	2000 mg/kg	0.310 ± 0.009	0.739 ± 0.201	3.613 ± 0.233	0.558 ± 0.033	0.557 ± 0.146
	5000 mg/kg	0.323 ± 0.027	0.818 ± 0.276	3.571 ± 0.726	0.565 ± 0.045	0.586 ± 0.122
EEF	2000 mg/kg	0.320 ± 0.005	0.660 ± 0.251	3.285 ± 0.454	0.512 ± 0.076	0.312 ± 0.025
	5000 mg/kg	0.313 ± 0.015	0.752 ± 0.114	3.501 ± 0.365	0.521 ± 0.053	0.566 ± 0.064
EAF	2000 mg/kg	0.311 ± 0.030	0.655 ± 0.111	3.280 ± 0.305	0.547 ± 0.080	0.512 ± 0.019
	5000 mg/kg	0.302 ± 0.051	0.780 ± 0.223	3.236 ± 0.505	0.572 ± 0.011	0.601 ± 0.117

** = significant difference at $p < 0.01$; *** = very significant difference at $p < 0.001$.

The results of analysis of liver parameters for the aqueous and ethanolic extracts of *Sida rhombifolia* are presented in Table 7.

Table 7. Analysis of liver parameters.

		EAR		EER		EAT		EET		EEF		EAF	
	Control	2000	5000	2000	5000	2000	5000	2000	5000	2000	5000	2000	5000
ASAT	94.04	95.19	104.59	91.14	109.81	99.37	89.52	91.87	100.60	91.78	97.51	96.71	98.55
ALAT	45.99	53.71	53.79	36.55	50.92	44.21	57.82	52.00	39.43	50.10	47.33	48.23	57.82
Creatinine	8.19	9.00	9.37	8.21	8.24	9.14	8.33	7.89	8.48	8.69	8.54	8.25	8.37
Uric acid	21.92	21.31	21.83	19.13	25.60	26.21	26.53	21.91	22.00	21.86	22.31	21.16	22.35

[22] reported that the blood and liver parameters of rats that received extracts at a dose of 8000 mg/kg showed a significant increase ($p < 0.05$) compared to the control group. In the present study, no significant differences were observed between the control group and the treated animals. This discrepancy may be attributed to the higher dose of 8000 mg/kg used in the study by [22] studies. Therefore, based on the present study, the LD_{50} (lethal dose) of *Sida rhombifolia* extracts is greater than 5000 mg/kg of body weight. The analysis of the relative organ mass (**Table 6**) reveals that most organs do not show significant variations compared to the control group, except for the liver. A significant increase in relative liver mass is observed with 5000 mg/kg of EER (4.069 ± 0.820; $p < 0.01$) and 5000 mg/kg of EET (4.174 ± 1.094; $p < 0.001$), suggesting hepatic hypertrophy. This increase could be related to the accumulation of metabolites or an inflammatory reaction induced by the extracts. In contrast, the relative masses of the kidneys, heart, and lungs do not show significant variations, indicating that these organs do not undergo notable effects from the extracts, even at high doses. The biochemical parameters (**Table 7**) reveal an increase in hepatic transaminases (ASAT and ALAT) for certain extract doses. The highest values are recorded for EER at 5000 mg/kg (ASAT: 109.81 U/L; ALAT: 58.52 U/L) and EET at 5000 mg/kg (ASAT: 91.87 U/L; ALAT: 39.43 U/L). This elevation in transaminases indicates hepatic cytolysis, confirming the signs of liver damage already observed in the increased relative liver mass. Regarding renal parameters, creatinine and uric acid levels do not show significant variations between treated groups and the control group, suggesting the absence of notable renal impairment. The results suggest that the tested extracts have a dose-dependent effect on liver function, with signs of toxicity appearing mainly at high doses (5000 mg/kg). The increase in relative liver mass, accompanied by elevated transaminases, indicates hepatic distress, which may be attributed to an increased metabolic load or cellular damage. In contrast, the kidneys and other organs appear less affected by the extracts, even at high doses, indicating a selective toxic effect on the live.

4.4. Anti-Inflammatory Potential of *Sida rhombifolia* Extracts

The results of the *in vitro* and *in vivo* evaluation of the anti-inflammatory potential of *Sida rhombifolia* extracts are presented in **Table 8** and **Table 9**.

4.4.1. *In Vitro* Evaluation: Protein Denaturation Test (BSA)

The protein denaturation test (BSA) results of *Sida rhombifolia* leaf, root and stem extracts are shown in **Table 8**.

Table 8. Effect of *Sida rhombifolia* extract on protein denaturation (BSA).

	Protein denaturation (BSA)			
Standard concentration (µg/mL)	**0.1**	**0.4**	**0.7**	**1**
% inhibition Indomethacin (standard)	16.22 ± 1.89	18.66 ± 4.22	25.93 ± 3.26	28.51 ± 2.60
Concentration (mg/mL)	**6.25**	**7.5**	**8.75**	**10**
% inhibition EET	74.64 ± 0.53	78.75 ± 0.39	80.09 ± 0.12	84.18 ± 0.19

Continued

% inhibition EAT	34.28 ± 1.21	44.32 ± 0.36	56.15 ± 0.65	60.94 ± 0.65
% inhibition EER	61.06 ± 8.29	67.31 ± 10.29	71.51 ± 4.52	72.93 ± 6.63
% inhibition EAR	27.95 ± 2.03	40.72 ± 1.14	53.91 ± 0.87	60.52 ± 1.05
% inhibition EEF	43.94 ± 4.37	66.70 ± 2.15	73.98 ± 2.98	77.80 ± 1.24
% inhibition EAF	59.26 ± 5.36	60.76 ± 12.13	68.47 ± 3.82	70.79 ± 3.03

Protein denaturation plays a key role in inflammation, as denatured proteins can trigger immune responses and excessive production of inflammatory mediators. The results suggest that ethanolic extracts exhibit greater activity than aqueous extracts, indicating that bioactive anti-inflammatory compounds are more effectively extracted in ethanol than in water. The high inhibition rate observed in EET suggests that the stem bark of *Sida rhombifolia* is particularly rich in bioactive compounds such as flavonoids and polyphenols, which are known for their anti-inflammatory properties. These findings are consistent with previous studies showing that ethanolic extracts of medicinal plants often have superior anti-inflammatory activity due to their ability to extract non-polar bioactive compounds. The dose-dependent effect observed in this study suggests that increasing extract concentrations enhances anti-inflammatory potential, which could be relevant for the development of natural anti-inflammatory treatments. However, while these *in vitro* results are promising, further in vivo studies and clinical trials are needed to confirm the efficacy and safety of *Sida rhombifolia* extracts for therapeutic applications. The strong inhibitory activity observed, particularly in EET, highlights the potential of this plant as a valuable source of natural anti-inflammatory agents.

4.4.2. *In Vivo* Assessment: Test of Evolution of Paw Edema

The *in vivo test* conducted on a sample of 70 rats showed almost similar significant activity for all extracts. The results of the test on the evolution of edema in the right paw of the rats that received different treatments are presented in Table 9.

Table 9. Effect of *Sida rhombifolia* extracts on the evolution of rat paw edema.

	Evolution of rat paw edema at time T = n						
	V1/2 - V0	V1 - V0	V2 - V0	V3 - V0	V4 - V0	V5 - V0	V6 - V0
Distilled water	0.97 ± 0.18	1.20 ± 0.17	1.388 ± 0.183	1.59 ± 0.113	1.33 ± 0.08	1.21 ± 0.14	1.036 ± 0.1
Diclofenac	0.81 ± 0.22 16.50%	0.81 ± 0.10 32.39%*	0.648 ± 0.133 53.31%****	0.476 ± 0.194 70.10%****	0.21 ± 0.07 84.56%****	0.74 ± 0.08 88.51%****	0.60 ± 0.07 94.51%****
EER 200	0.72 ± 0.09 25.36%**	0.64 ± 0.09 46.35%****	0.37 ± 0.17 72.91%****	0.19 ± 0.07 87.56%****	0.15 ± 0.06 88.6%****	0.11 ± 0.06 90.74%****	0.06 ± 0.01 94.4%****
EER 400	0.54 ± 0.18 44.33%*	0.57 ± 0.30 53%****	0.35 ± 0.19 74.78%****	0.28 ± 0.19 82.41%****	0.16 ± 0.07 88%****	0.10 ± 0.09 91.57%****	0.06 ± 0.09 93.82%****
EAR 200	0.73 ± 0.23 24.95%	0.54 ± 0.36 55.31%****	0.75 ± 0.25 45.96%****	0.26 ± 0.14 83.67%****	0.19 ± 0.14 85.46%****	0.12 ± 0.07 89.92%****	0.05 ± 0.07 95.31%****
EAR 400	0.78 ± 0.22 20%	0.804 ± 0.31 39.22%**	1.03 ± 0.29 25.65%*	0.45 ± 0.23 71.49%****	0.32 ± 0.29 76.01%****	0.28 ± 0.31 76.85%****	0.142 ± 0.25 86.29%****
EET 200	0.58 ± 0.14 40.2%	0.61 ± 0.09 49.66%****	0.44 ± 0.14 68.15%****	0.29 ± 0.06 81.9%****	0.23 ± 0.10 82.6%****	0.18 ± 0.09 84.79%****	0.13 ± 0.09 87.45****

Continued

EET 400	0.88 ± 0.13 9.27%	0.69 ± 0.08 42.86%***	0.34 ± 0.16 75.36%****	0.33 ± .0.17 79.4%****	0.21 ± 0.01 84.56%****	0.15 ± 0.01 87.93%****	0.05 ± 0.04 95.56%****
EAT 200	0.78 ± 0.24 19.38%	0.7 ± 0.322 42.03%***	0.76 ± 0.17 45.10%****	0.38 ± 0.20 76.25%****	0.24 ± 0.120 82%****	0.15 ± 0.08 87.28%****	0.13 ± 0.09 87.45%****
EAT 400	0.84 ± 0.17 13.4%	0.89 ± 0.13 26.08%	0.48 ± 0.24 65.27%****	0.32 ± 0.19 80.02%****	0.276 ± 0.19 79.31%****	0.19 ± 0.18 84.13%****	0.136 ± 0.14 86.87%****
EEF 200	0.58 ± 0.14 34.12%	0.61 ± 0.09 39.45%****	0.44 ± 0.14 48.52%****	0.29 ± 0.06 61.39%****	0.23 ± 0.10 73.26%****	0.18 ± 0.09 79.88%****	0.13 ± 0.09 89.57****
EEF 400	0.88 ± 0.13 27.84%	0.68 ± 0.07 39.16%***	0.34 ± 0.16 61.63%****	0.33 ± 0.17 73.24%****	0.21 ± 0.013 79.63%****	0.15 ± 0.01 85.63%****	0.046 ± 0.04 90.35%****
EAF 200	0.75 ± 0.19 24.95%	0.75 ± 0.25 45.96%****	0.54 ± 0.36 56.31%****	0.26 ± 0.14 71.75%****	0.194 ± 0.14 80.54%****	0.12 ± 0.07 88.21%****	0.05 ± 0.06 85.63%****
EAF 400	0.77 ± 0.22 21.46%	1.03 ± 0.29 24.63%*	0.80 ± 0.30 37.82%**	0.45 ± 0.23 76.93%****	0.32 ± 0.29 83.29%****	0.28 ± 0.31 89.21%****	0.142 ± 0.24 91.39%****

* = difference not very significant at $p < 0.05$; ** = significant difference at $p < 0.01$; *** = very significant difference at $p < 0.001$; **** = extremely significant difference at $p < 0.0001$, compared to the negative control.

The anti-inflammatory potential was evaluated by calculating the percentage reduction in paw edema of the treated rats compared to the negative control group. Figure 4 presents the curves depicting the kinetics of rat paw edema evolution over time.

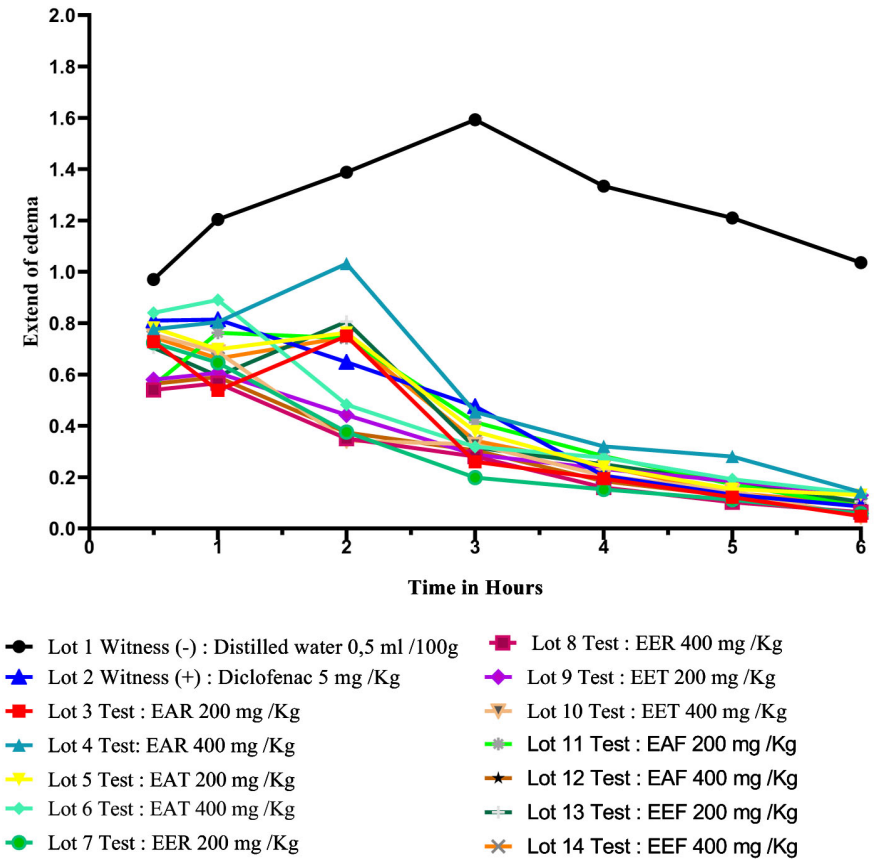

Figure 4. Kinetics of progression of rat paw edema.

The presence of statistical annotations ($p < 0.05$ to $p < 0.0001$) reinforces the reliability of the results. The very significant (*) and extremely significant (**) differences between treated groups and the negative control confirm the effectiveness of the tested extracts. This eliminates the possibility of a placebo effect and validates the hypothesis that *Sida rhombifolia* possesses genuine anti-inflammatory properties. The *in vivo* anti-inflammatory test demonstrates that the aqueous and ethanolic extracts of *Sida rhombifolia* significantly reduced rat paw edema. By the 3rd hour, inhibition was already very significant at the dose of 200 mg/kg, with 87.56% for the ethanolic extract of the roots (EER) and 83.67% for the aqueous extract of the roots (EAR). The highest inhibition percentage reached 95.56% for the ethanolic extract of the twigs (EET) at 400 mg/kg in the 6th hour. At this same time point, EER at 200 mg/kg exhibited an inhibition rate of 95.31%, while diclofenac, a standard anti-inflammatory drug, showed 94.51%. Notably, from the 3rd hour onward, inhibition results exceeded 70%, indicating strong anti-inflammatory activity. *In vitro* anti-inflammatory activity, assessed through the protein denaturation test (BSA), revealed that the ethanolic extract of the stem exhibited the highest inhibition percentage, reaching 84.18% at a concentration of 10 mg/mL. These results align with findings from Mah, Teh, and Ee [9], which highlighted the anti-inflammatory potential of this plant. Their study showed that the hexane extract exhibited nitric oxide (NO) inhibition with respective IC50 values of 52.16 and 58.57 µg/mL, as well as inhibition of protein denaturation at 146.03 µg/mL [9] [12] [40]. These findings collectively support the strong anti-inflammatory potential of *Sida rhombifolia*, reinforcing its effectiveness as a natural anti-inflammatory agent.

4.5. Evaluation of the Sida *rhombifolia* Antioxidant Test

4.5.1. Anti-Hemolytic Activity

The results of the anti-hemolytic activity of the *Sida rhombifolia extracts* are presented in Table 10.

Table 10. Effect of *Sida rhombifolia* extract on anti-hemolytic activity.

	Anti-hemolytic activity				
Concentration (mg/ml)	**5**	**6.25**	**7.5**	**8.75**	**10**
% EET inhibition	6.37 ± 3.87	6.98 ± 4.74	16.60 ± 0.54	11.6 ± 15.62	37.07 ± 2.89
% EAT inhibition	52.33 ± 0.05	58.33 ± 12.86	57.22 ± 13.44	62.64 ± 2.64	63.08 ± 0.69
% EER inhibition	48.72 ± 19.51	47.84 ± 18.53	44.25 ± 4.62	56.53 ± 2.77	58.67 ± 1.24
% EAR inhibition	35.13 ± 6.22	39.48 ± 82.35	42.33 ± 14.39	47.36 ± 16.89	51.45 ± 31.13
% EEF inhibition	37.78 ± 5.12	38.01 ± 2.97	45.72 ± 4.49	58.04 ± 2.45	59.26 ± 0.90
% EAF inhibition	24.99 ± 6.51	40.21 ± 2.71	54.26 ± 7.45	54.35 ± 4.60	57.62 ± 1.17

Table 10 presents the effect of *Sida rhombifolia* extracts on anti-hemolytic activity at different concentrations (5 to 10 mg/mL). The results show that hemolysis inhibition increases with extract concentration, indicating a dose-dependent effect. Ethanolic extracts (EAT, EER, EEF) are generally more effective than aque-

ous extracts (EAF, EAR), with maximum inhibition reaching 63.08% ± 0.69% at 10 mg/mL for the ethanolic extract of aerial parts (EAT). The ethanolic extract of twigs (EET) shows relatively low inhibition at lower concentrations (6.37% ± 3.87% at 5 mg/mL) but significantly increases at 10 mg/mL (37.07% ± 2.89%). These findings suggest that *Sida rhombifolia* possesses anti-hemolytic properties, likely due to the presence of bioactive compounds such as flavonoids and polyphenols, known for their ability to stabilize cell membranes and reduce oxidative stress. These observations align with literature findings, particularly those of Mah, Teh, and Ee [9], who demonstrated that the hexane extract of *Sida rhombifolia* inhibits NO production with IC50 values of 52.16 and 58.57 µg/mL, confirming an anti-inflammatory potential linked to membrane stabilization. Similarly, Tanumihardja *et al*. [12] reported protein denaturation inhibition at 146.03 µg/mL, supporting the hypothesis that this plant's extracts protect cell membranes from oxidative damage. Compared to other medicinal plants such as *Curcuma longa* and *Moringa oleifera*, whose hemolysis inhibition often exceeds 60% at 10 mg/mL, *Sida rhombifolia* ranks among the plants with strong anti-hemolytic activity. Thus, these results reinforce the idea that *Sida rhombifolia* has genuine therapeutic potential as an anti-inflammatory and membrane-protective agent. Identifying the specific active compounds responsible for this activity and comparing these extracts to reference anti-inflammatory drugs such as diclofenac would be valuable. Furthermore, validation using *in vivo* oxidative stress models would provide a deeper understanding of the mechanism of action of these extracts.

4.5.2. Anti-Lipoperoxidative Activity

The results of the anti-lipoperoxidative activity of extracts from the roots and stem of *Sida rhombifolia* are presented in Table 11.

Table 11. *Sida rhombifolia* extract on anti-lipoperoxidative activity.

	Anti-lipoperoxidative activity				
Concentration (mg/ml)	**5**	**6.25**	**7.5**	**8.75**	**10**
% EET inhibition	32.27 ± 4.32	35.76 ± 1.68	32.83 ± 0.17	36.07 ± 0.87	35.70 ± 0.17
% EAT inhibition	30.90 ± 0.97	28.72 ± 1.22	18.63 ± 4.19	16.13 ± 3.04	19.28 ± 0.59
% EAR inhibition	38.44 ± 0.89	41.75 ± 1.09	47.041 ± 0.74	36.63 ± 0.09	47.97 ± 1.98
% EER inhibition	19.04 ± 4.75	35.06 ± 19.92	30.07 ± 5.71	42.42 ± 5.97	41.58 ± 4.58
% EEF inhibition	13.36 ±3.89	23.55 ±3.15	38.60 ±3.43	48.91 ±1.37	51.54 ±4.90
% EAF inhibition	21.39 ±3.74	32.62 ±4.11	54.77 ±3.70	60.18 ±3.18	63.41 ±1.48

At a concentration of 10 mg/mL, the anti-lipoperoxidative test showed an inhibition of 63.41% for EAF and 51.54% for EEF, compared to 47.97% ± 1.98% for EAR and 41.58% ± 4.58% for EER. These results confirm the antioxidant potential of *Sida rhombifolia*. In the anti-hemolytic test, the ethanolic extract of aerial parts (EAT) at the same concentration exhibited an inhibition of 63.08%, compared to 59.26% for EEF and 58.67% for EER. Furthermore, at this concentration, the anti-lipoperoxidative test showed an inhibition of 61.41% for EAF, 47.97% for EAR, and 35.70% for EET. These findings further support the antioxidant potential of

Sida rhombifolia, as demonstrated in previous studies. Mah, Teh, and Ee [9] demonstrated in their studies that the ethyl acetate extract of *Sida rhombifolia* exhibited antioxidant activity by scavenging DPPH (2,2-Diphenyl-1-picrylhydrazyl) radicals and ferrous ions, with EC50 (effective concentration) values of 380.5 µg/mL and 263.4 µg/mL, respectively. Similarly, research conducted in India determined that ethanolic extracts from the roots of *Sida rhombifolia* exhibit significantly higher antioxidant activity compared to extracts from stems or leaves [19].

5. Conclusion

The results of this study confirm the therapeutic potential of *Sida rhombifolia*, particularly its anti-inflammatory and antioxidant activities. Phytochemical analysis revealed a high concentration of bioactive secondary metabolites, especially in ethanolic extracts from the stem bark. Toxicity studies demonstrated that the extracts were safe at the tested doses, reinforcing their relevance for traditional medicine and potential pharmaceutical applications. The anti-inflammatory evaluation showed significant inhibition of edema, with efficacy comparable to diclofenac. Regarding antioxidant activity, aqueous extracts from the stem barks and leaves exhibited the strongest ability to reduce oxidative stress. *Sida rhombifolia* appears to be a promising source of bioactive compounds for the development of natural treatments against inflammation and oxidative stress. However, further research, including clinical and mechanistic studies, is necessary to validate its therapeutic potential and optimize its medicinal use.

Author's Contribution

Teclaire Ngoup: Interpretation of results, drafting and reading of manuscript; **Martin Nyangono Ndongo:** Interpretation of results, drafting and reading of manuscript; **Moïse Henri Julien Nko'o:** Interpretation of results, drafting and reading of manuscript; **Jonas Peequeur Essome Mbang:** Interpretation of results , drafting and reading of manuscript; **Efeze Nkemaja Dydimus:** Supervision of work, validation of tests and reading of manuscript; **Thomas Kanaa:** Methodology, reading of manuscript; **Nnanga Nga:** Reading of manuscript; **Ebenezer Njeugna:** Reading of manuscript and supervision of work.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that might appear to influence the work reported in this article.

References

[1] Brink, M. and Achigan-Dako, E.G. (2012) Ressources végétales de l'Afrique tropicale

16. Plantes à fibres.

[2] Netea, M.G., Balkwill, F., Chonchol, M., Cominelli, F., Donath, M.Y., Giamarellos-Bourboulis, E.J., *et al*. (2017) A Guiding Map for Inflammation. *Nature Immunology*, **18**, 826-831. https://doi.org/10.1038/ni.3790

[3] Médecins Sans Frontières (2019) Médicaments essentiels.

[4] OMS (2011) Liste modèle de l'OMS des médicaments essentiels 17e liste (Révision mars 2011).

[5] Medscap (2017) Toxicité des anti-inflammatoires non stéroïdiens (AINS). Medscap.

[6] Cooney, N., Pollack, C. and Butkerait, P. (2015) Adverse Drug Reactions and Drug-Drug Interactions with Over-the-Counter NSAIDs. *Therapeutics and Clinical Risk Management*, **11**, 1061-1075. https://doi.org/10.2147/tcrm.s79135

[7] Edoga, H.O. and Okwu, D.E. (2005) Constituants phytochimiques de certaines plantes médicinales nigérianes. *African Journal of Biotechnology*, **4**, 685-688.

[8] Parekh, J.C.S. (2008) Criblage phytochimique de certaines plantes de la région occidentale de l'Inde. *Arches végétalisées*, **8**, 657-662.

[9] Mah, S.H., Teh, S.S. and Ee, G.C.L. (2017) Anti-Inflammatory, Anti-Cholinergic and Cytotoxic Effects of *Sida rhombifolia*. *Pharmaceutical Biology*, **55**, 920-928. https://doi.org/10.1080/13880209.2017.1285322

[10] Trease, G.E. (1989) Pharmacognosy. Bailliere Tinndall.

[11] Ngoup, T., Efeze, N.D., Kanaa, T., Mbang, J.P.E., Segovia, C., Nga, N., *et al*. (2024) Physical, Chemical and Mechanical Characterization of *Sida rhombifolia* Fibers from the Center Region of Cameroon for Their Potential Use in Textiles and Composites. *Journal of Natural Fibers*, **21**, Article ID: 2294478. https://doi.org/10.1080/15440478.2023.2294478

[12] Tanumihadja, M., Mattulada, I.K., Natsir, N., Subehan, S., Mandey, F. and Muslimin, L. (2019) Structural Assessment of Chemical Constituent of Sidaguri (*Sida rhombifolia* Linn) and Its Ability to Inhibit Cyclooxygenase. *Pesquisa Brasileira em Odontopediatria e Clínica Integrada*, **19**, 1-7. https://doi.org/10.4034/pboci.2019.191.96

[13] Chaves, O., Gomes, R., Tomaz, A., Fernandes, M., Das Graças Mendes Junior, L., De Fátima Agra, M., *et al*. (2013) Secondary Metabolites from *Sida rhombifolia* L. (Malvaceae) and the Vasorelaxant Activity of Cryptolepinone. *Molecules*, **18**, 2769-2777. https://doi.org/10.3390/molecules18032769

[14] Ferro, D.M., Mazzutti, S., Vitali, L., Oliveira Müller, C.M. and Ferreira, S.R.S. (2019) Integrated Extraction Approach to Increase the Recovery of Antioxidant Compounds from *Sida rhombifolia* Leaves. *The Journal of Supercritical Fluids*, **149**, 10-19. https://doi.org/10.1016/j.supflu.2019.03.013

[15] Lenny, S., Barus, T. and Sitopu, E.Y. (2010) Isolasi Senyawa Alkaloid Dari Daun Sidaguri (*Sida rhombifolia* L.). *Jurnal Kimia Mulawarman*, **8**, 40-43.

[16] Prakash, A., Varma, R. and Ghosal, S. (1981) Alkaloid Constituents of *Sida acuta*, *S. humilis*, *S. rhombifolia* and *S. spinosa*. *Planta Medica*, **43**, 384-388. https://doi.org/10.1055/s-2007-971529

[17] Aminah, N.S., Laili, E.R., Rafi, M., Rochman, A., Insanu, M. and Tun, K.N.W. (2021) Secondary Metabolite Compounds from Sida Genus and Their Bioactivity. *Heliyon*, **7**, e06682. https://doi.org/10.1016/j.heliyon.2021.e06682

[18] Kamdoum, B.C., Simo, I., Wouamba, S.C.N., Tchatat Tali, B.M., Ngameni, B., Fotso, G.W., *et al*. (2022) Chemical Constituents of Two Cameroonian Medicinal Plants: *Sida rhombifolia* L. and *Sida acuta* Burm. f. (Malvaceae) and Their Antiplasmodial

Activity. *Natural Product Research*, **36**, 5311-5318. https://doi.org/10.1080/14786419.2021.1937156

[19] Dhalwal, K., Deshpande, Y.S. and Purohit, A.P. (2007) Evaluation of *in Vitro* Antioxidant Activity of *Sida rhombifolia* (L.) ssp. *Retusa* (L.). *Journal of Medicinal Food*, **10**, 683-688. https://doi.org/10.1089/jmf.2006.129

[20] Arciniegas, A., Pérez-Castorena, A.L., Reyes, S., Contreras, J.L. and De Vivar, A.R. (2003) New Oplopane and Eremophilane Derivatives from *Robinsonecio gerberifolius*. *Journal of Natural Products*, **66**, 225-229. https://doi.org/10.1021/np0203739

[21] Laili, E.R., Aminah, N.S., Kristanti, A.N., Wardana, A.P., Rafi, M., Rohman, A., *et al*. (2022) Comparative Study of *Sida rhombifolia* from Two Different Locations. *Rasayan Journal of Chemistry*, **15**, 642-650. https://doi.org/10.31788/rjc.2022.1516588

[22] Assam, J.P.A., Dzoyem, J., Pieme, C. and Penlap, V. (2010) *In Vitro* Antibacterial Activity and Acute Toxicity Studies of Aqueous-Methanol Extract of *Sida rhombifolia* Linn. (Malvaceae). *BMC Complementary and Alternative Medicine*, **10**, Article No. 40. https://doi.org/10.1186/1472-6882-10-40

[23] Narendhirakannan, R.T. and Limmy, T.P. (2011) Anti-inflammatory and Anti-Oxidant Properties of *Sida rhombifolia* Stems and Roots in Adjuvant Induced Arthritic Rats. *Immunopharmacology and Immunotoxicology*, **34**, 326-336. https://doi.org/10.3109/08923973.2011.605142

[24] Foumane Maniepi, J., Soppo Lobe, V., Nga, N., Metogo Ntsama, J., Mbenga Mekoulou, F., Ngolsou, F., Diboué, B.P., Obono, P., Ndongo, M.N. and Ze Minkande, J. (2022) Analyse phytochimique des extraits aqueux de Sida acuta et Triumfetta cordifolia, deux plantes utilisées pour faciliter l'accouchement en médecine traditionnelle au Cameroun. *Health Sciences and Disease*, **23**, 14-18.

[25] Brinckmann, J.A. (2013) Emerging Importance of Geographical Indications and Designations of Origin-Authenticating Geo-Authentic Botanicals and Implications for Phytotherapy. *Phytotherapy Research*, **27**, 1581-1587. https://doi.org/10.1002/ptr.4912

[26] Alemán-Laporte, J., Bandini, L.A., Garcia-Gomes, M.S., Zanatto, D.A., Fantoni, D.T., Amador Pereira, M.A., *et al*. (2019) Combination of Ketamine and Xylazine with Opioids and Acepromazine in Rats: Physiological Changes and Their Analgesic Effect Analysed by Ultrasonic Vocalization. *Laboratory Animals*, **54**, 171-182. https://doi.org/10.1177/0023677219850211

[27] Aguwa, U.S., Eze, C.E., Obinwa, B.N., Okeke, S.N., Onwuelingo, S.F., Okonkwo, D.I., *et al*. (2020) Comparing the Effect of Methods of Rat Euthanasia on the Brain of Wistar Rats: Cervical Dislocation, Chloroform Inhalation, Diethyl Ether Inhalation and Formalin Inhalation. *Journal of Advances in Medicine and Medical Research*, **32**, 8-16. https://doi.org/10.9734/jammr/2020/v32i1730636

[28] Adedapo, A.A., Jimoh, F.O., Koduru, S., Afolayan, A.J. and Masika, P.J. (2008) Antibacterial and Antioxidant Properties of the Methanol Extracts of the Leaves and Stems of *Calpurnia aurea*. *BMC Complementary and Alternative Medicine*, **8**, Article No. 53. https://doi.org/10.1186/1472-6882-8-53

[29] Lakache, Z., Tigrine, C., Aliboudhar, H. and Kameli, A. (2019) Composition chimique, activités anti-inflammatoire, antalgique et cytotoxique *in vivo* de l'extrait méthanolique des feuilles d'*Olea europaea*. *Phytothérapie*, **19**, 83-92. https://doi.org/10.3166/phyto-2019-0195

[30] Harborne, J.B. (1998) Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edition, Chapman & Hall, 58.

[31] Odoh, U., Ezugwu, C. and Okoro, E. (2012) Quantitative Phytochemical, Proximate/

Nutritive Composition Analysis of β Vulgaris Linnaeus (Chenopodiceae). *Planta Medica*, **78**, PI116. https://doi.org/10.1055/s-0032-1320803

[32] OCDE (2009) Lignes Directrices de L'OCDE Pour Les Essais. 1-13.

[33] Bancroft, J.D., Suvarna, K. and Layton, C. (2019) Bancroft's Theory and Practice of Histological Techniques. Elsevier, 557.

[34] Djikem, T.R.N., *et al*. (2022) Activités analgésiques et anti-inflammatoires du tengho: Une boisson à base de quelques épices du Cameroun. *Biology and Medicine*, **14**, 1-6.

[35] Gunathilake, K.D.P.P., Ranaweera, K.K.D.S. and Rupasinghe, H.P.V. (2018) *In Vitro* Anti-Inflammatory Properties of Selected Green Leafy Vegetables. *Biomedicines*, **6**, Article 107. https://doi.org/10.3390/biomedicines6040107

[36] Diatta, W., Sy, G., Manga, C., Diatta, K., Fall, A. and Bassene, E. (2014) Recherche des activités anti-inflammatoire et analgésique des extraits de feuilles de *Zanthoxylum zanthoxyloides* (Lam) zepernick et timler (*Rutaceae*). *International Journal of Biological and Chemical Sciences*, **8**, 128-133. https://doi.org/10.4314/ijbcs.v8i1.12

[37] Chandra, S., Chatterjee, P., Dey, P. and Bhattacharya, S. (2012) Evaluation of *in Vitro* Anti-Inflammatory Activity of Coffee against the Denaturation of Protein. *Asian Pacific Journal of Tropical Biomedicine*, **2**, S178-S180. https://doi.org/10.1016/s2221-1691(12)60154-3

[38] Lee, Y.Y., Saba, E., Irfan, M., Kim, M., Chan, J.Y., Jeon, B.S., *et al*. (2019) The Anti-Inflammatory and Anti-Nociceptive Effects of Korean Black Ginseng. *Phytomedicine*, **54**, 169-181. https://doi.org/10.1016/j.phymed.2018.09.186

[39] Epa, C., Elion Itou, R., Etou Ossibi, A., Attibayeba, O.P.R. and Abena, A.A. (2015) Effet anti-inflammatoire et cicatrisant des extraits aqueux et éthanolique des écorces du tronc de *Buchholzia coriacea* Engl. (Capparidaceae). *Journal of Applied Biosciences*, **94**, 8858-8868. https://doi.org/10.4314/jab.v94i1.9

[40] Loutfi, Y., Xavier, V.D., Stephanie, R.M. and Francelline, R.J. (2020) Évaluation de l'effet cicatrisant de *Sida rhombifolia* (malvaceae) sur les plaies cutanées chez la souris. *Revue des Sciences, de Technologies et de l'Environnement*, **2**, 122-129.

[41] Anthony, O.E. (2013) Preliminary Phytochemical Screening and Antidiarrheal Properties of Manniophyton Fulvum. *IOSR Journal of Dental and Medical Sciences*, **10**, 46-52. https://doi.org/10.9790/0853-01024652

[42] Koudoro, D.V., Wotto, R.C., Konfo, T.C.P., Agbangnan, D. and So-hounhloue, C.D. (2015) Phytochemical Screening, Antibacterial and Anti-Radical Activities of *Daniellia oliveri* Trunk Bark Extracts Used in Veterinary Medicine against Gastrointestinal Diseases in Benin. *International Journal of Advanced Research*, **3**, 1190-1198.

[43] Bekro, Y., Mamyrbekova, J., Boua, B., Tra Bi, F. and Ehile, E. (2008) Étude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (Caesalpiniaceae). *Sciences & Nature*, **4**, 217-225. https://doi.org/10.4314/scinat.v4i2.42146

[44] N'Guessan, K., Kadja, B., Zirihi, G., Traoré, D. and Aké-Assi, L. (2009) Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire). *Sciences & Nature*, **6**, 1-15. https://doi.org/10.4314/scinat.v6i1.48575

[45] Tumanggor, L., Bintang, M. and Priosoeryanto, B.P. (2019) Assessing Cytotoxicity and Antiproliferation Effects of *Sida rhombifolia* against MCA-B1 and A549 Cancer Cells. *Journal of Applied Biology and Biotechnology*, **7**, 63-68. https://doi.org/10.7324/JABB.2019.70610