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Phytochemical investigation of three medicinal plants oriented against antioxidant, anti-inflammatory (*in vitro* and *in vivo*) activities and acute toxicity

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Abstract
The aim of this study was to perform phytochemical investigations of three medicinal plants oriented against antioxidant and anti-inflammatory activities. The spectrophotometer was used for quantification tests. The antioxidant activity was evaluated by $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and FRAP method. The acute toxicity (OECD 423) and the anti-edematous activity were performed on NMRI mice. Phytochemical screening allowed the identification of some chemical groups. Ethanolic extracts of *Guiera senegalensis* showed the best total phenolic contents (97.77±0.30 g EAA/100 g extract) and the best antioxidant activity with $ABTS^{\bullet+}$ (IC_{50}: 3.86±0.17 µg/mL), $DPPH^{\bullet}$ (IC_{50}: 12.20±0.02 µg/mL) and FRAP (32.80±0.55 mmol EAA/g) methods. The extracts showed an inhibitory effect on 15-lipoxygenase activity (14.67 to 99.49% at 100 µg/mL). The aqueous decoction (68.10%) and the ethanolic macerate (74.21%) of the recipe showed a good percentage of inhibition of edema from the 5th hour at a dose of 400 mg/kg b.w. These results could constitute a solid scientific basis justifying the traditional uses of these plants.

Keywords: Phytochemical, antioxidant activities, acute toxicity and carrageenan edema test

Introduction
The inflammatory reaction is the response of living and vascularized tissues to an aggression [1]. It can present in two forms: acute and chronic [2]. During the inflammatory process, mediators such as prostaglandins, leukotrienes, cytokines (TNFα, IL1β, IL6) and reactive oxygen species (ROS) are released by cellular activation [3]. Prostaglandins and leukotrienes are produced by the activation of cyclooxygenases (COX) and lipoxygenase (LOX) respectively [3]. In addition, free radicals are produced, creating oxidative stress within the body. This stress is defined as the inability of the body to defend itself against the attack of activated oxygen species, following an imbalance linked either to an increased production of oxygenated species or to a decrease in the antioxidant defense capacity [4]. The consequences of oxidative stress can be observed at several levels, as many constituents of the body are the target of reactive oxygen species (ROS) [5, 6]. It can be the cause of many biological abnormalities such as: mutation, carcinogenesis, fetal malformation, deposition of abnormal proteins, fibrosis, formation of autoantibodies, deposition of oxidized lipids and immunosuppression [7, 8]. The consequences of all these aggressions contribute to aging and the onset of several diseases, namely, cancer, acute respiratory distress syndrome, inflammation, diabetes, ulcers, Alzheimer's disease and cardiovascular diseases [7-10]. The management of inflammatory pathologies is based on steroidal/nonsteroidal anti-inflammatory drugs which induce long-term harmful effects within the body [8, 11, 6]. Medicinal plants used to treat these pathologies could be a natural source for the discovery of anti-inflammatory agents with fewer iatrogenic effects. Indeed, 80% of the world's population uses medicinal plants for their primary health care [11,12]. These therapeutic practices using plants constitute an important health heritage for socio-cultural reasons [13]. These plants contain various secondary metabolites, namely flavonoids, phenolic acids and tannins with antioxidant and anti-inflammatory properties [14]. In Africa, the therapeutic power of plants was empirically known by our ancestors and parents [14]. Thus, we were completely unaware of the chemical composition of the medicines used daily by many populations.

To achieve an improvement in this African medicine, several phytochemical investigations have been carried out, to provide scientific justification for their traditional uses. The objective of our study is to conduct a phytochemical, biological investigation on the species of *Detarium microcarpum* Guill. & Perr, from *Guiera senegalensis* J.F. Gmel. and *Securidaca longepedunculata* Fresen aimed against inflammatory pathologies.

Materials and Methods

Solvents and reagents

They consisted of chloroform, acetic anhydride, sulfuric acid (H_2SO_4), n-Hexane, ethyl acetate, n-Butanol (Sigma-Aldrich-Milan, Italy), Reagent of Folin-Ciocalteu (Sigma Aldrich, Germany), sodium carbonate (20%), gallic acid, aluminum chloride, quercetin, polyvinyl polypyrrolidone (PVPP), potassium persulfate ($K_2S_2O_8$), ascorbic acid, distilled water, ethanol (Merk, analysis), methanol (analytical grade), sodium carbonate, 2,2-diphenyl-1-picryl hydrazyl (DPPH), ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonate)), potassium hexacyanoferrate [$K_3Fe(CN)_6$] (Sigma Aldrich, USA), Trolox (Fluke, France), borate acid, zileuton, linoleic acid, tween 20 and lipoxygenase (type I-B) Sigma® (St Louis, USA).

Plant material

The plant material consisted of leaves of *Detarium microcarpum* Guill. & Perr, from *Guiera senegalensis* J.F. Gmel. and *Securidaca longepedunculata* Fresen collected at the following coordinates: 0354599/1243996; 0354672/1244084 and 0354603/1244007 on November 28, 2021, in the classified forest of Dendérésso (Bobo-Dioulasso) with the assistance of a botanist Dr. Yempambou Hermann OUOBA. A specimen was deposited at the herbarium of the Nazi BONI University after having been previously identified and authenticated by Professor Paulin OUOBA (Plant Ecology Laboratory, Nazi BONI University).

Animal material and ethics committee approval

Male and female mice (NMRI) were purchased from the animal facility of the Life and Earth Sciences Training and Research Unit at Joseph KI-ZERBO University in Ouagadougou (Burkina Faso). The animals were acclimatized for two weeks at 22 °C with a circadian cycle in the animal facility of the Department of Traditional Medicine and Pharmacopoeia/Pharmacy of the Health Sciences Research Institute (IRSS) in Ouagadougou. Approval was also obtained from the ethics committee within Joseph KI-ZERBO University under the approval code: CE-UJKZ/2024-06.

Preparation and extraction of plant material

After collection, each sample underwent primary treatment. Drying was carried out in the laboratory under ventilation, away from sunlight that could modify the active ingredients of the plants. The dried leaves were then pulverized. The powders thus obtained were used for the various laboratory operations.

A reflux decoction (30 min), an ethanolic and hydro-ethanolic maceration (80:20) were carried out on the individual powders as well as on their mixture. The maceration was carried out over a period of 48 hours. The filtrates from the decoction were freeze-dried and those from the maceration were concentrated in a rotary evaporator (Rotavapor) then dried in an oven (40 °C). At the end of the extractions, 12 extracts including 4 extracts from the aqueous decoction, 4 ethanolic extracts and 4 hydro-ethanolic extracts were obtained for the various laboratory tests.

Phytochemical study

Highlighting chemical groups

General characterization tests were performed according to the method of [15]:

- Iron chloride test $FeCl_3$ for tannins.
- Shibata test for flavonoids.
- Liebermann/Buchard test for triterpenes/steroids.
- Dragendorff test (potassium tetra iodo bismuthate) for alkaloids.
- Foam test for saponins.

Quantification of phenolic compounds

Total phenolic content

The estimation of total extractable phenolic compounds was carried out according to the method described by [16]. To 200 µL of each extract of concentration 1 mg/mL were added 1 mL of Folin-Ciocalteu reagent plus 800 µL of sodium carbonate solution (20%). After 40 minutes of incubation, the optical densities were read at 760 nm using a spectrophotometer (SHIMADU UV Spectrophotometer). The total phenolic contents were determined using a calibration curve (gallic acid). Three analyses were carried out with each extract and the result given is an average of these analyses.

Total flavonoid content

Total flavonoids were determined according to the method adapted by [17]. Briefly, in a test tube, a mixture of 1 mL of 2% aluminum trichloride in methanol with 1 mL of extract of concentration 1 mg/mL plus 2 mL of sodium acetate was made. This mixture was incubated for 40 min and the optical densities were read at 415 nm using the spectrophotometer (Agilent 8453). Quercetin was used as a standard for the plotting of the calibration curve. Three analyses were carried out for each extract and the result given is an average of these analyses.

Total tannin content

The method described by [18] was adopted for the determination of total tannins. This technique is based on the precipitation of tannins with polyvinyl polypyrrolidone (PVPP) by the formation of a complex. The complexion of 2mg of tannins requires 100mg of PVPP. Thus, in a volume of 1 mL of extract with a concentration of 1 mg/mL, enough PVPP was added and then stirred. After 15 minutes of incubation at 4 °C, the mixture was centrifuged at 3000 revolutions for 10 minutes. The tannins having precipitated, the supernatant was recovered for carrying out an assay of total phenolics other than tannins [16]. The difference between the first value of total phenolic compounds (which contained tannins) and the second value of total phenolic compounds (in the absence of tannins) gives the total tannin content.

Biological activities

Evaluation of antioxidant activities

Free Radical Scavenging Activities of 2,2-Diphenyl-1 Picrylhydrazyl (DPPH)

The ability of the extract to inhibit the DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical was determined by the spectrophotometric method described by [19]. The extracts and trolox (reference compound) were prepared at a concentration of 1 mg/mL. 200 µL of each diluted extract were taken to put in test tubes to which 2000 µL of the DPPH solution were

added. The mixture was incubated for 30 nm and the absorbances were read at 490 nm using the spectrophotometer (SHIMADU UV Spectrophotometer).

$$\% \text{ Inhibition} = \frac{\text{Absorbance controlled} - \text{Absorbance of the extract}}{\text{Absorbance controlled}} * 100$$

ABTS Free Radical Scavenging Activity

The anti-ABTS$^{\bullet+}$ activity was carried out according to the method of [20]. All samples were dissolved in ethanol to have a concentration of 1 mg/mL. A cascade dilution of the extracts and Trolox (reference compound) was made. A reaction mixture was made with 200 µL of each dilution with 2000 µL of the ABTS solution. The mixture was incubated for 30 mn and the reading was carried out with cells using the spectrophotometer at 734 nm. Three readings were taken for each sample and the inhibition percentage was calculated according to the following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance controlled} - \text{Absorbance of the extract}}{\text{Absorbance controlled}} * 100$$

Iron reducing activity by FRAP method

The FRAP (Ferric Reducing Antioxidant Power) method was carried out according to the method of [21]. To 200 µL of each extract with a concentration of 1 mg/mL was added 1.25 mL of phosphate buffer (0.2 M, pH 6.6) then 1.25 mL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] 1% in water. The whole was incubated for 30 minutes in a water bath at 50°C. After incubation, 1.25 mL of trichloroacetic acid (10%) was then added and the mixture was centrifuged at 2000 rpm for 10 minutes. After centrifugation, a volume of 0.625 mL of the mixture was diluted in 0.625 mL of distilled water. To this mixture was added 0.125 mL of $FeCl_3$ 0.1% freshly prepared in distilled water for instant reading with a spectrophotometer (SHIMADU UV Spectrophotometer) at 700 nm. From an established ascorbic acid calibration curve, the reducing power of the extract was determined and expressed in mmol Equivalent Ascorbic Acid (EAA)/g of dry extract.

Evaluation of anti-inflammatory activity *in vitro*: inhibition of 15-lipoxygenase

Lipoxygenase inhibition was determined according to the method of [22] using linoleic acid as substrate. The inhibitors, namely the extracts as well as Zileuton (reference compound) were prepared at a concentration of 100 µg/mL. A volume of 146.25 mL 15-lipoxygenase solution (820.51 U/mL) prepared with boric acid (pH 9, 0.2 M) was added 3.75 µL of each inhibitor. The mixture was incubated at room temperature for 3 min and 150 µL of linoleic acid was added. The Spectrophotometer (Epoch Biotek Instruments, U.S.A) was used to read the absorbances at 234 nm. The tests were carried out in triplicate and the percentage of lipoxygenase inhibition was calculated according to the following formula:

$$\% \text{ inhibitioN} = \frac{\text{AE} - \text{AI}}{\text{AE}} * 100$$

AE: The absorbance of the enzymatic test – the absorbance of the blank

AI: The absorbance of the inhibition test – the absorbance of the blank

Acute toxicity study of extracts

The acute general oral toxicity test was carried out in accordance with OECD guideline 423 [23] on female mice (NMRI). A single dose of 2000 mg/kg b.w of extract was administered to the test mice orally. The animals were observed individually for 2 hours after administration at the end of which they were fed. They were then observed at least once a day for a period of 14 days. On the 14th day the animals were fasted for a period of 12 hours and then they were sacrificed. The vital organs were removed, observed fresh and then weighed. The test was carried out two (2) times under the same conditions. The relative weight of each organ (PRO) was calculated according to the following formula:

$$\text{PRO } (\%) = \frac{Ab - At}{At} * 100$$

***In vivo* anti-inflammatory activity: Anti-edematous carrageenan test**

The caragenin edema test was performed according to the method described by [24]. All mice were fasted for a period of 18 hours before the experiment. The animals received the different substances (the aqueous decoction and the ethanolic extract from the recipe) at doses of 50, 100, 200 and 400 mg/kg b.w and acetylsalicylic acid (100 mg/kg b.w, reference compound). The control batch received distilled water at a dose of 10 mL/kg b.w. The volume of the initial paw was measured, then one hour after the administration of the anti-inflammatory substances, 50 µL of 1% carrageenan (dissolved in 0.9% NaCl) was injected under the plantar aponeurosis of the paw right hind paw of each mouse. Paw volume measurements were taken 1, 3 and 5 hours after carrageenan injection using a plethysmometer. The variation in the volume of the treated paw made it possible to evaluate the anti-inflammatory potential of the extracts as well as that of the reference substance. Anti-inflammatory activity was evaluated as percentage reduction in edema in treated mice compared to blank controls according to the following formula:

$$\% \text{ inhibitioN} = \frac{Ab - At}{At} * 100$$

Ab: Represents the average difference in the volume of increase in the paw of mice from the white control group

At: Represents the average difference in the volume of increase in the paw of the mice from the treated batches.

Data processing

The various chemical compound dosage and enzymatic inhibition tests were carried out in triplicates. Means and standard deviations were determined using Microsoft Excel. The one-way Anova test (Fisher test) was used for the statistical processing of the different parameters studied. The difference is considered significant when $p<0.05$.

Results and Discussion

Phytochemical study

Highlighting chemical groups: The application of the method of [15] on the extracts made it possible to determine the nature of the different constituents present in the extracts qualitatively. The results of these tests are mentioned in Table 1.

Md_d= aqueous decoction of *Detarium microcarpum*; Sg_d= aqueous decoction of *Guiera senegalensis*; Ls_d= aqueous decoction of *Securidaca longepedunculata* ML_d= aqueous decoction of the recipe. Md_e=ethanolic extracts of *Detarium microcarpum*; Sg_e= ethanolic extracts *Guiera senegalensis*; Ls_e=ethanolic extracts of *Securidaca longepedunculata*; ML_e= ethanolic extracts from the recipe. Md_h=

hydroethanolic extracts of *Detarium microcarpum;* Sg_h= hydroethanolic extracts *Guiera senegalensis*; Ls_h= hydroethanolic extracts of *Securidaca longepedunculata*; ML_h= hydroethanolic extracts from the recipe. (+): presence [25]. They are also known for their pharmacological activities, namely anti-inflammatory, antiviral, antimalarial, antibacterial, antifungal, antimicrobial and antitumor [26]. The presence of these chemical groups in the leaves of the plants studied has been confirmed by several authors [27-31].

Table 1: Highlighting of some chemical groups

Types of extracts	Codes	Chemical groups			
		Flavonoids	Tannins	Saponosides	Triterpenes and steroids
Aqueous decoction	Md_d	+	+	+	+
	Sg_d	+	+	+	+
	Ls_d	+	+	+	+
	ML_d	+	+	+	+
Ethanolic extracts	Md_e	+	+	+	+
	Sg_e	+	+	+	+
	Ls_e	+	+	+	+
	ML_e	+	+	+	+
Hydro-ethanolic extracts	Md_h	+	+	+	+
	Sg_h	+	+	+	+
	Ls_h	+	+	+	+
	ML_h	+	+	+	+

Phenolics, flavonoids and total tannins contents

The results obtained on the dosage of total phenolics, flavonoids and tannins are illustrated in Table 2.

Table 2: Dosage of phenolic compounds in extracts

Types of extracts	Codes	Total phenolics (g EAG/ 100g extract)	Total flavonoids (g EQ/100g extract)	Total tannins (g EAG/100g extract)
Aqueous decoction	Md_d	85.68±0.52	4.96±0.54	42.51± 0.99
	Sg_d	59.95±0.98	15.31± 0.67	27.44±0.78
	Ls_d	42.97±0.03	20.47±0.79	13.25±0.27
	ML_d	55.80±0.74	20.23±0.87	25.54±0.28
Ethanolic	Md_e	89.99±0.44^{ns}	14.78±0.64***	37.83±0.05*
	Sg_e	97.77±0.30***	23.93±0.15***	43.63±0.19***
	Ls_e	71.18±0.27***	7.54±0.57***	33.85±0.08***
	ML_e	65.21±0.35^{ns}	20.46±0.55^{ns}	35.04±0.33***
Hydro-ethanolic	Md_h	87.85±0.12^{ns}	3.89±0.04^{ns}	44.30±0.64***
	Sg_h	90.76±0.19*	14.07±0.89^{ns}	27.55±0.15^{ns}
	Ls_h	74.09±0.18^{ns}	11.73±0.66***	34.44±0.07***
	ML_h	61.52±0.67^{ns}	19.77±0.64^{ns}	7.41±0.35***

Each result constitutes the average of the three values obtained (N=3). ns: not significant, *: significant with P-value < 0.05; ***: Very significant with P-value < 0.001 compared to the extracts of the aqueous decoction

Md_d= aqueous decoction of *Detarium microcarpum*; Sg_d= aqueous decoction of *Guiera senegalensis*; Ls_d= aqueous decoction of *Securidaca longepedunculata* ML_d= aqueous decoction of the recipe. Md_e=ethanolic extracts of *Detarium microcarpum*; Sg_e= ethanolic extracts *Guiera senegalensis*; Ls_e=ethanolic extracts of *Securidaca longepedunculata*; ML_e= ethanolic extracts from the recipe. Md_h= hydroethanolic extracts of *Detarium microcarpum*; Sg_h= hydroethanolic extracts *Guiera senegalensis*; Ls_h= hydroethanolic extracts of *Securidaca longepedunculata*; ML_h= hydroethanolic extracts from the recipe.

As for the total phenolics of the extracts, the contents generally varied from 42.97±0.03 to 97.77±0.44 g EAG/100g extract. All three (03) types of extracts presented better total phenolic contents, but the highest content was obtained with the ethanolic extracts of *Guiera senegalensis*, i.e. 97.77±0.44 g EAG/100g of extract and the lowest with the aqueous decoction of *Securidaca longepedunculata*, i.e. 42.97±0.03 g EAG/100g of extract. The best total phenolic contents were obtained with ethanolic extracts, followed by hydro-ethanolic extracts and finally the lowest by aqueous decoctions. Water being a more polar solvent than ethanol, normally this solvent should extract more compounds than other solvents (ethanol, water-ethanol). This was not the case in our study. This could show that most of the compounds extracted could be non-polar compounds.

In terms of quantification of total flavonoids, the contents varied from 3.89±0.04 to 23.93±0.15 g EQ/100g of extract. The best content was obtained with the ethanolic extracts of *Guiera senegalensis*, i.e. 23.93±0.15 g EQ/100g of extract, and the lowest with the hydro-ethanolic extracts of *Detarium microcarpum*, i.e. 3.89±0. 15 g EQ/100g extract. At this level we note that the highest contents of total flavonoids were obtained by the ethanolic extracts and the aqueous decoctions and the lowest by the hydro-ethanolic extracts.

Regarding the quantification of total tannins, the contents varied from 7.41±0.35 to 44.30±0.64 g EAG/100g of extract. The highest content was obtained with the hydro-ethanolic extracts of *Detarium microcarpum*, i.e. 44.30±0.64 g EAG/100g of extract and the lowest with the hydro-ethanolic extracts of the recipe (7.41±0.35 g EAG/100g extract). We note a high richness in phenolic compounds in the different extracts of the plants in the study. However, among all the plant species in the study, we mention a high content of these compounds in the ethanolic extracts of *Guiera senegalensis*, i.e. 97.77±0.44g EAG/100g of extract, 23.93±0.15g EQ/100g of extract and 43.63±0.19g EAG/100g of extract for phenolics, flavonoids and total tannins respectively.

Investigations carried out by [29] on the different organs of *Detarium microcarpum* showed better content of phenolics, flavonoids and total tannins in the leaves, i.e. 105.00 mg EAG/g, 23.94 mg EQ/g and 20.56 g/L respectively. The quantification test carried out by [32] and [33] on the methanolic and aqueous extracts of the leaves gave the value of 51.7±0.2 mg of gallic acid equivalent/g and 31.3 mg/g for total phenolics and flavonoids respectively. Our results differ from those obtained by [32]. This difference could be explained either by the nature of the solvents or by edaphic factors.
A large variation in the metabolites studied is observed within the same species. This variation could be explained by the nature of the solvent and the collection site.

Evaluation of biological activities

Antioxidant activities: Removal of DPPH radicals, decolorization of $ABTS^{\bullet+}$ radical cations and reduction of ferric ions were the methods used to evaluate the antioxidant activities of the extracts and the results are reported in Table III. In view of the results obtained, the IC_{50} by the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ method varied considerably. The best scavenging of free radicals was obtained by all the extracts except with the aqueous decoction and the ethanolic extracts of *Securidaca longepedunculata* (84.63±0.32 and 185.80±3.17 µg/mL by the $ABTS^{\bullet+}$ method) and (68.58±0.11 and 226.94±0.16 µg/mL by the $DPPH^{\bullet}$ method). Indeed, according to [25] a relatively very low IC_{50} indicates very high antioxidant activity. In the study, the ethanolic extracts of *Guiera senegalensis* presented an IC_{50} comparable to trolox, i.e. 3.86±0.17 and 2.87±0.01 µg/mL respectively with the $ABTS^{\bullet+}$ method. The antioxidant capacity of the extracts to reduce ferric iron varied from 4.21±0.12 to 36.40±1.42 mmol EAA/g. The highest antioxidant content was obtained with the hydro-ethanolic extracts of *Guiera senegalensis*, i.e. 36.40±1.42 mmol EAA/g, and the lowest with the aqueous decoction and the hydro-ethanolic extracts of *Securidaca longepedunculata*, i.e. 4.21±0.12 and 4.38±1.05 mmol EAA/g respectively.

Table 3: Antioxidant activity of total extracts

Types of extracts	Codes	$ABTS^{\bullet+}$ (IC_{50} (µg/mL))	$DPPH^{\bullet}$ (IC_{50} (µg/mL))	FRAP (mmol EAA/g)
Reference compound	Trolox	2.87±0.01	1.24±0.01	-
Aqueous decoction	Md_d	6.73^{ns}±0.01	25.36^{***}± 0.03	21.52±0.01
	Sg_d	7.39^{ns}±0.21	15.26^{***}±0.02	24.80±0.22
	Ls_d	84.63^{***}±0.32	68.58^{***}±0.11	4.21±0.12
	ML_d	20.01^{*}±0.05	34.16^{***}±0.04	18.265±0.50
Ethanolic	Md_e	7.76^{ns}±1.96	15.82^{***}±0.02	27.85±0.56
	Sg_e	3.86^{ns}± 0.17	12.20^{***}±0.02	32.80±0.55
	Ls_e	185.80^{***}±3.17	226.94^{***}±0.16	5.33± 0.27
	ML_e	10.95^{ns}±0.004	29.01^{***}±0.03	18.37±0.88
Hydro-ethanolic	Md_h	16.77^{ns}±0.40	13.67^{***}±0.03	25.24±0.55
	Sg_h	6.74^{ns}±0.72	7.27^{***}± 0.20	36.40±1.42
	Ls_h	46.71^{***}±6.71	29.03^{***}±0.37	4.38±1.05
	ML_h	17.37^{*}±0.45	22.74^{***}±0.04	21.35±0.79

Values are given as the mean±standard deviation of three independent assays. ns: not significant, *: significant with P-value < 0.05 **: Not very significant with P-value < 0.01; ***: Very significant with P-value < 0.001 compared to Trolox. Md_d= aqueous decoction of *Detarium microcarpum*; Sg_d= aqueous decoction of *Guiera senegalensis*; Ls_d= aqueous decoction of *Securidaca longepedunculata* ML_d= aqueous decoction of the recipe. Md_e=ethanolic extracts of *Detarium microcarpum*; Sg_e= ethanolic extracts *Guiera senegalensis*; Ls_e=ethanolic extracts of *Securidaca longepedunculata*; ML_e= ethanolic extracts from the recipe. Md_h= hydroethanolic extracts of *Detarium microcarpum*; Sg_h= hydroethanolic extracts *Guiera senegalensis;* Ls_h= hydroethanolic extracts of *Securidaca longepedunculata*; ML_h= hydroethanolic extracts from the recipe

The antioxidant activity of our extracts could be due to the presence of phenolic compounds because quantification tests revealed that these extracts contain a panoply of phenolics, flavonoids and total tannins. Indeed, phenolic compounds can act as antioxidants by helping the body strengthen its defense system against diseases linked to oxidative stress such as cardiovascular diseases, cancer and the inflammatory process [34].

Table 4: Effect of extracts on 15-lipoxygenase

Types of extracts		IC_{50} µg/mL	% of inhibition (100µg/mL)
Reference compound	Zileuton	15.88 ± 0.88	99.49 ± 0.72
Aqueous decoctions	Md_d	66.84^{***} ± 1.67	49.6^{***}± 2.85
	Sg_d	44.57^{***} ± 0.73	74.46^{**}± 1.93
	Ls_d	> 100	14.67^{***}± 0.5
	ML_d	> 100	42.56^{***} ± 1.98
Ethanolic	Md_e	44.16^{***} ± 0.95	93.80^{ns} ± 0.35
	Sg_e	30.50^{**} ± 0.95	95.99^{ns} ± 0.02
	Ls_e	> 100	30.49^{***} ± 1.36
	ML_e	> 100	32.63^{***} ± 1.36
Hydroethanolic	Md_h	85.93^{***} ± 2.73	61.63^{**} ± 1.47
	Sg_h	51.95^{***} ± 0.71	84.94^{ns} ± 1.47
	Ls_h	> 100	20.95^{***} ± 1.57
	ML_h	> 100	34.27^{***} ± 1.37

Inhibition of 15-lipoxygenase

The results obtained on the inhibition of 15-lipoxygenase of the extracts are reported in Table IV. The IC_{50} varied from 15.88±0.88 to 85.93±2.73 µg/mL and the inhibition percentages varied from 14.67±0.5 to 99.49±0.72%. The best inhibition percentages were obtained by the ethanolic extracts of *Guiera senegalensis* (93.80±0.35%) and *Detarium microcarpum* (95.99±0.02) which are comparable to the inhibition percentage of zileuton (reference compound) (99.49±0.72%)

Values are given as the mean±standard deviation of three independent assays. ns: not significant, *: significant with P-value < 0.05 **: Not very significant with P-value < 0.01; ***: Very significant with P-value < 0.001 compared to Zileuton. Md_d= aqueous decoction of *Detarium microcarpum*; Sg_d= aqueous decoction of *Guiera senegalensis*; Ls_d= aqueous decoction of *Securidaca longepedunculata* ML_d= aqueous decoction of the recipe. Md_e=ethanolic extracts of *Detarium microcarpum*; Sg_e= ethanolic extracts *Guiera senegalensis*; Ls_e=ethanolic extracts of *Securidaca longepedunculata*; ML_e= ethanolic extracts from the recipe. Md_h= hydroethanolic extracts of *Detarium microcarpum*; Sg_h= hydroethanolic extracts *Guiera senegalensis*; Ls_h= hydroethanolic extracts of *Securidaca longepedunculata*; ML_h= hydroethanolic extracts from the recipe

Overall, the extracts showed good inhibition of 15-lipoxygenase. These results confirm the anti-inflammatory properties of the leaves of the different species studied [35-38]. The flavonoids and sterols/triterpenes contained in the extracts are known for their ability to inhibit pro-inflammatory enzymes [39,40].

The acute toxicity of extracts

Survival and general observation

This activity was carried out on the ethanolic and hydro-ethanolic decoctions and macerations of the recipe. During the 14 days of observation, no signs of toxicity or mortality were observed in the mice receiving the extracts orally during the 1st and 2nd tests (Table 5).

Relative organ weight

The relative weights of the organs are recorded in Table VI. When we compare the different relative weights of the organs from the control and treated batches with the recipe extracts, we notice that there is no significant difference. The same observation was made by [41]. The latter in their investigations showed that the aqueous extract of *Tetracera potatoria* Ex. G. Don does not significantly influence the relative weights of the noble organs (heart, spleen, lungs, kidneys).

Table 5: General observation of mice after administration of extracts (2000 mg/kg)

Extracts of the recipe[1] and observed parameters		1e test	2e test
Witness and extracts	Witness	3	3
	Aqueous decoction	3	3
	Ethanolic maceration	3	3
	Hydro-ethanolic maceration	3	3
Observed parameters[2]	Mortality	A	A
	Mobility	N	N
	Aggressiveness	A	A
	Condition of stools	N	N
	Tremor	A	A
	Sleep	N	N
	Pain sensitivity	N	N
	Vomiting	A	A

A: absent; N: normal; n=3 repeated 2 times; [1]: recipe (mixture of the three powders); [2]: The same observations were made with all the extracts.

Table 6: Relative weights of organs of control and treated animals

Organs	Control	Extracts of the recipe[1]		
		Aqueous decoction	Ethanolic maceration	Hydro-ethanolic maceration
Heart	0.52±0,07	0.56^{ns}±0,08	0.52^{ns}±0,05	$0.52^{ns\pm}$0,06
Stomach	2.07±0,31	1.98^{ns}±0,49	2.09^{ns}±0,3	2.35^{ns}±0,52
Liver	5.72±1,5	5.57^{ns}±0,24	5.39^{ns}±0,24	$5.46^{ns\pm}$0,48
Missed	0.42±0,07	0.64^{ns}±0,26	0.59^{ns}±0,21	0.58^{ns}±0,16
Kidneys	1.11±0,12	1.15^{ns}±0,13	1.08^{ns}±0,1	1.09^{ns}±0,14
Lungs	0.85±0,21	0.72^{ns}±0,14	0.73^{ns}±0,16	0.65^{ns}±0,12

NS: not significant compared to the control; N=3. The test was carried out in duplicate; [1]: recipe (mixture of the three powders)

Anti-inflammatory activity *in vivo*

The mouse right paw edema inhibition test was performed on the aqueous decoction and the ethanolic maceration of the recipe. The results are reported in Table VII.

Table 7: Effect of treatments on the volume (mL) of plantar edema in mice

Extracts of the recipe[1]	Dose (mg/kg)	Edema volume (mL)			Inhibition percentage		
		1 hour	3 hours	5 hours	1 hour	3 hours	5 hours
Control	-	0.47 ± 0.02	0.53 ± 0.01	0.69 ± 0.02	-	-	-
Salicylic Acid	100	0.25^{***} ± 0.01	0.16^{***} ± 0.01	0.10^{***}± 0.01	47.64 ± 0.47	67.96 ± 0.86	85.03 ± 1.01
Aqueous decoction	50	0.42^{**} ± 0.03	0.40^{***} ± 0,02	0.38^{***}± 0.02	15.83^{***} ± 1.17	25.53^{***} ±1.96	32.11^{***} ± 0.53
	100	0.38^{***}± 0.03	0.32^{***} ± 0.03	0.29^{***}± 0.03	25.67^{***} ±2.64	40.92^{***} ±2.00	57.68^{***} ± 0.26
	200	0.28^{***}± 0.04	0.26^{***} ± 0.02	0.24^{***}± 0.05	37.46^{***} ±4.87	52.78^{***} ±4.80	63.54^{***} ± 3.78
	400	0.24^{***}± 0.02	0.22^{***} ± 0.03	0.19^{***} ± 0.04	43.84^{***} ±3.34	55.00^{***} ±1.12	68.10^{***} ± 1.11
Ethanolic maceration	50	0.34^{***} ± 0.04	0.29^{***} ± 0.06	0.25^{***} ± 0.08	28.45^{***} ±4.17	38.26^{***} ± 3.4	46.59^{***} ± 2.3
	100	0.30^{***}± 0.05	0.26^{***} ± 0.02	0.23^{***} ± 0.01	36.72^{***} ±3.43	50.31^{***} ±3.00	67.12^{***} ± 1.26
	200	0.28^{***} ± 0.02	0.24^{***} ± 0.06	0.20^{***} ± 0.04	39.94^{***} ±5.87	55.65^{***} ±2.80	70.42^{***} ± 5.78
	400	0.27^{***} ± 0,01	0.21^{***} ± 0,03	0.18^{***} ± 0,03	42.4^{***} ± 2.34	60.04^{***} ±3.23	74.21^{***} ± 2.36

N=6; **: Not very significant with P-value < 0.01; ***: Very significant $p<0.001$ compared to the control; [1]: recipe (mixture of the three powders)

Paw volume of the mice was measured before and after the injection for five hours. The effect of the extracts and that of the reference compound was compared to a control batch (blank) which had not received treatment at time intervals (1 hour, 3 hours and 5 hours). We see that the inflammation caused by carrageenan increases over time in the untreated batch (control). The volume of the paw of these mice increased from 0.47±0.02 at t = 1 hour to 0.69±0.02 at t = 3 hours. On the other hand, the batches having been treated either with the extracts or with the reference compound, at t=1

hour, we notice an increase in the volume of the paw, then a significant decrease compared to the untreated batch, at t=3 hours and 5 hours.

Considering the inhibition percentages, we see that these results show a dose-dependent inhibitory effect of the extracts as a function of time. Strong inhibition of edema of more than 50% was obtained at a dose of 400 mg/kg at the 5th hour with the extracts and with the reference compound. At this same dose at the 5th hour, 85% inhibition was obtained with salicylic acid (reference compound), 68.10% with the aqueous decoction of the recipe and 74.21% with the ethanolic maceration of the recipe. Which could confirm the anti-inflammatory potential of our extracts.

According to [42], the development of edema in the mouse paw after carrageenan injection is described as a biphasic event. Indeed, the early phase of 0-2 hours just after the injection of carrageenan involves histamine, serotonin and bradykinin. At 1 hour, the edema reaches its maximum and then begins to decrease. The late phase of the inflammatory response has been demonstrated due to the potentiating effect of bradykinin on the release of prostaglandins, responsible for increased vascular permeability [35]. Nitrous oxide (NO), which is a powerful vasodilator, is also involved in the inflammatory process induced by carrageenan. Its action is linked to its ability to increase vascular permeability through changes in local flow [42,43]. Anti-inflammatory drugs generally intervene by opposing the effect of these chemical mediators which are histamine, serotonin, kinins and prostaglandins. The significant inhibition of swelling of the right paw by the extracts from the 3rd and 5th hours shows that they influence both phases and therefore on mediators such as histamine, serotonin, kinins and prostaglandins.

The aqueous decoction and ethanolic maceration of the recipe significantly reduced ($p < 0.001$) the edema induced by carrageenan, whose anti-edematous activity was greater at the dose of 400 mg/kg b.w. These results suggest that these extracts have an effect which opposes the action of endogenous pro-inflammatory mediators. This action would be exerted more on cyclo-oxygenase, the enzyme responsible for the synthesis of prostaglandins [44].

Relationship between biological activities and phytochemicals composition

A correlation study was carried out between polyphenols, flavonoids and tannins on the inhibition of $ABTS^{\bullet+}$ (Table VIII). As for the aqueous decoction extracts, an average correlation was observed with total tannins (0.69) followed by total phenolics (0.55) and a low contribution to the level of total flavonoids. Concerning the ethanolic extracts, a low contribution of phenolic compounds was obtained. Because all the correlations were less than 0.50. Regarding the hydro-ethanolic extracts, a weak correlation was observed. Previous studies have shown the contribution of polyphenols, flavonoids and tannins to $ABTS^{\bullet+}$ radical inhibitions. According to the investigations of [45] it was 0.73; 0.77 and 0.16 respectively for polyphenols, tannins and flavonoids in *Combretum sericeum* G. Don extracts. Comparative studies carried out by [46] obtained 0.68 (polyphenols) on extracts of *Stereospermum kunthianum* Chan and *Bauhinia rufescens* Lam. In our study, the contribution of flavonoids was low on the extracts of the aqueous decoction and those ethanolic and very low 0.0086 on the hydro-ethanolic extracts. This correlation shows that the inhibition of the $ABTS^{\bullet+}$ radical in our extracts couldn't be attributable to flavonoids.

A good correlation was obtained only with total tannins regarding the aqueous decoction extracts. At the level of the ethanolic extracts, a weak contribution of the metabolites studied on the inhibition of the $DPPH^{\bullet}$ radical was observed. Regarding the hydro-ethanolic extracts, a good contribution was observed only at the level of total phenolics (0.60). In summary we can say that the inhibition of the $DPPH^{\bullet}$ radical could be attributed to the contribution of tannins and total phenolics on the extracts of the aqueous decoction and those hydro-ethanolic. In the study, the flavonoids did not show a good correlation. Also, a strong correlation between the anti-radical activity of the $DPPH^{\bullet}$ radical and the content of total phenolics in various plants and fruits was reported by [47,48]. According to [49], phenolic acids, flavonoids, tannins, stilbenoids, coumarins, lignans and quinones are endowed with DPPH inhibitory power in decreasing order.

Concerning the FRAP reducing power, the highest correlation was obtained by the total tannins, i.e. 0.58, with the extracts of the aqueous decoction. At the level of ethanolic extracts, a very good correlation was obtained by phenolics and total tannins, i.e. 0.76 and 0.67 respectively. Concerning a weak correlation was obtained with total phenolics (0.30) and a zero correlation R^2=0.0002 and 0.0074 was obtained at the level of flavonoids and tannins. Many authors have carried out investigations in this direction, but which have obtained good correlation. As an example, we have [47] and [48] who reported a contribution of total phenolics of 0.89 and 0.90 respectively. According to [48] and [46], these good capacities are due to their electron donor capacities, in particular tannins and phenolic acids. In our study we observed a poor correlation of flavonoids in the extracts of the aqueous decoction (0.29) and those hydro-ethanolic (0.0002). Studies carried out by [50] have shown the difficulty of establishing a very clear link between the level of flavonoids and the reducing power. According to the same author, sometimes a good correlation was demonstrated and sometimes a poor correlation as in the case of our work. These observations could be explained by several possibilities: (i) the compounds existing in the extracts have large molecular weights or are heterosidic, (ii) many flavonoids are non-antioxidants, (iii) the underestimation of flavonoids by the $AlCl_3$ method [51].

Table 8: Relationship between antioxidants activities, total phenolic, flavonoid and tannin content

R^2	$ABTS^{\bullet+}$	$DPPH^{\bullet}$	FRAP
Aqueous decoction			
Total phenolics	0.55	0.43	0.45
Total flavonoids	0.34	0.29	0.29
Total tannins	0.69	0.55	0.58
Ethanol extracts			
Total phenolics	0.32	0.24	0.67
Total flavonoids	0.34	0.27	0.39
Total tannins	0.44	0.38	0.76
Hydro-ethanolic extracts			
Total phenolics	0.12	0.60	0.30
Total flavonoids	0.0086	0.060	0.0002
Total tannins	0.048	0.04	0.0074

The results of the contribution of secondary metabolites to the level of inhibition of 15-lipoxygenase are recorded in Table IX. At the level of the aqueous decoction extracts, we observe a low contribution of phenolics, flavonoids and tannins. Concerning the ethanolic extracts, a strong contribution of the secondary metabolites of the study was observed on the inhibition of lipoxygenase. Regarding the hydroethanolic

extracts, a good contribution of total phenolics was noted on the inhibition of lipoxygenase. The correlation coefficients obtained between the contents of polyphenolic compounds in the extracts and the inhibition percentages show that total phenolics could be good inhibitors of 15-lipoxygenase. When we compare the inhibition percentages obtained with the three types of extracts in terms of the results obtained on the inhibition of 15-lipoxygenase, we also notice that the best percentages were obtained with the ethanolic extracts. For example, we obtained: 93.80% and 95.99% respectively with the ethanolic extracts of *Detarium microcarpum* and *Guiera senegalensis* which confirms the good contribution of phenolics, flavonoids and total tannins observed with the ethanolic extracts.

Table 9: Relationship between 15-lipoxygenase, total phenolic, flavonoid and tannin content

R^2	Inhibition of 15-lipoxygenase		
Extracts	**Aqueous decoction**	**Ethanol extracts**	**Hydro-ethanolic extracts**
Total phenolics	0.24	0.93	0.65
Total flavonoids	0.16	0.64	0.06
Total tannins	0.33	0.72	0.054

Concerning the contribution of secondary metabolites to anti-inflammatory activities *in vivo*, correlation studies were also carried out. These results are recorded in Table X. Concerning the correlations carried out, we observe a good correlation with the extracts from the two recipes. But the strongest correlation was obtained with the aqueous decoction of the recipe, i.e. 0.69. Indeed, the carrageenan edema test made it possible to obtain 68.10±1.11 and 74.10±0.14 as percentage of inhibition respectively for the aqueous decoction of the recipe and the ethanolic extracts of the recipe from the 5th hour of carrageenan injection. These high inhibition percentages obtained could be explained by the good contribution of phenolic compounds in this activity. We can therefore say that phenolic compounds could be at the origin of the anti-inflammatory activity.

Table 10: Relationship between anti-edematous activity and phenolic compound content

R^2	Anti-edematous activity	
Extracts of the recipe	**Aqueous decoction**	**Ethanol extracts**
Phenolic compounds	0.69	0.39

Conclusion

This study was initiated with a view to carrying out phytochemical investigations of *Detarium microcarpum*, *Guiera senegalensis* and *Securidaca longepeduncultata* used in traditional medicine against inflammatory pathologies. At the end of the study, the tube tests allowed the identification of flavonoids, tannins, saponins, sterols and triterpenes. The quantification tests showed that these extracts contain high contents of phenolics, flavonoids and total tannins but the highest contents of phenolics, flavonoids and total tannins were obtained with the ethanolic macerated of *Guiera senegalensis* 97.77±0.30 g EAG/100g extract, 23.93±0.15 g EQ/100 g extract and 43.63±0.19 g EAG/100 g extract respectively. Antioxidant activities, the ethanolic macerated *Guiera senegalensis* presented good antioxidant activity with the $ABTS^{\bullet+}$ method (IC_{50}: 3.86±0.17 µg/mL), $DPPH^{\bullet}$ (IC_{50}: 12.20±0.02 µg /mL) and FRAP (32.80±0.55 mmol EAA/g). In terms of the carrageenan edema test which was based on the extracts of the aqueous and ethanol decoction of the recipe, a good percentage of inhibition was obtained with its extracts from the 5th hour of administration at the dose of 400 mg/kg or 68.10% and 74.21% respectively. However, additional studies are needed to further support the antioxidant and anti-inflammatory effects of the study plant extracts.

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Conflict of Interest

There are No potential conflicts of Interest.

Authors contributions

This work was carried out in collaboration among all authors.

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