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Phytochemical screening, antioxidant and anti-edematous activities of aqueous extract of *Sarcocephalus latilolius* (sm.) Bruce ripe fruits in rat

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Abstract

Background: The vegetative system of *Sarcocephalus latifolius* represents a natural source of active substances, as the species is used in traditional african medicine to treat several diseases involving inflammation and oxidation.

Objective: The aim of the study is to determine the phytochemical composition and evaluate the antioxidant and anti-inflammatory properties of the aqueous extract of *Sarcocephalus latifolius* ripe fruits in rats.

Material and method: DPPH radical scavenging and iron reduction were used to assess antioxidant activity *in vitro*. Rat paw edema induced by carrageenan, dextran and formalin was used to assess acute and subacute anti-inflammatory activity *in vivo*.

Results: Extract at 10 mg/mL yielded 92.95±11.44 mg EAG/g total polyphenols, 0.082±0.005 mg ERu/g total flavonoids and 0.14 ± 0.003 mg EAT/g condensed tannins. Aqueous extract of *Sarcocephalus latifolius* ripe fruits showed DPPH radical inhibition with an IC_{50} greater than 25 µg/mL. DPPH inhibition by gallic acid was 92.91% at 25 µg/mL. IC_{50} of ascorbic acid and aqueous extract of *Sarcocephalus latifolius* on Fe^{3+}reducing antioxidant power (FRAP) were 18.9 µg/mL and 111.6 µg/mL, respectively. For carrageenan, the aqueous extract of *Sarcocephalus latifolius* ripe fruits showed a maximal anti-inflammatory effect at five hours, with inhibitions ($p<0.05$) of 48.49% at the dose of 200 mg/kg and 56.16% at 300 mg/kg. For dextran, the inhibitions ($p<0.01$) observed at the thirtieth minute were 34.55% at the dose of 200 mg/kg and 44.44% at 300 mg/kg. For formalin, significant inhibitions ($p<0.05$) on the first day were 29.19% at 200 mg/kg and 30.60% at 300 mg/kg.

Conclusion: These results confirm the use of *Sarcocephalus latifolius* ripe fruits in traditional medicine for the treatment of diseases with an inflammatory component and involving oxidative stress.

Keywords: Antioxidant; Anti-inflammatory; *Sarcocephalus latifolius* fruits; Rats

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1. Introduction

Sarcocephalus latifolius is included in the Rubiaceae family with 500 genera and over 1,000 species. Fruits are generally red and fleshy when ripe and yellow when unripe [1]. The tree flowers from April to June, while the fruit ripens from July to September. *Sarcocephalus latifolius* is a species with many therapeutic virtues. In many African countries, it is used as a remedy for diarrhea, pain and diabetes [2]. It is also used to treat malaria, leprosy, fertility, hypertension, gastrointestinal disorders, prolonged menstrual flow and sleeping sickness [3]. The leaves and fruits of *Sarcocephalus latifolius* were shown to possess antioxidant activity [4]. The fruits of *Sarcocephalus latifolius* possesses hypocholesterolemic potential [5]. Previous studies have shown that the aqueous extract of *Sarcocephalus latifolius* fruit is non-toxic [6] and provides renal protection [7], hepatic protection [8] and arthritic protection [9]. There is less scientific literature on the analgesic and anti-inflammatory properties of *Sarcocephalus latifolius* fruit. Inflammation is a defense response that occurs after cell damage caused by microbes, physical agents (radiation, trauma, burns), chemicals (toxins, caustic substances), tissue necrosis, or immune reactions whose purpose is to promote healing or tissue repair. It is a complex process associated with pain and involves phenomena such as increased vascular permeability, protein denaturation and membrane destruction. It is a process regulated by pro and anti-inflammatory factors [10],[11]. Inflammation, as the most important response of a damaged tissue which is observed in many inflammatory disorders, is characterized by redness, edema, heat, and pain at the site of injury [12]. Inflammation is characterized by heat, redness, swelling and pain. The aim of this study is to determine the phytochemical compounds and evaluate the antioxidant and anti-oedematous effects of the aqueous extract of *Sarcocephalus latifolius* ripe fruits using several models of acute and chronic inflammation in rats. Because existing synthetic molecules like nonsteroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors that increase the incidence of adverse cardiovascular thrombotic effects [13].

2. Material and methods

2.1. Experimental animals

Wistar rats of both sexes were obtained from University Joseph KI-ZERBO. They were housed under automatically controlled temperature conditions (temperature: 22±3 ° C; relative humidity: 50 ±10 % and 12 h light:12 h dark). Their weight was between 100 and 150 g. Animals had access to water and pellets *ad libitum*. The experiments were authorized by the ethical committee for University Joseph KI-ZERBO.

2.2. Plant samples and extraction

Sarcocephalus latifolius ripe fruits were harvested between 11 and 12 a.m. in Gaoua, southwestern part of Burkina Faso, in July 2023. The authenticity of the plant was verified by the ''Laboratoire de Biologie et Ecologie Végétale'' of the University Joseph KI-ZERBO. A specimen of the plant has been registered there under number 18028. One hundred grams of *Sarcocephalus latifolius* ripe fruits powder (1g) were macerated with distilled water (10 mL) for 24 hours, at room temperature with constant agitation. The macerate was filtered through Watman paper and the filtrate was divided into Petri dishes and placed in the oven at 60 degrees. The yield (w/w) of the extract was 17.52 %. The aqueous extract of *Sarcocephalus latifolius* ripe fruits was designated *EASL*.

2.3. Phytochemical analysis

The qualitative phytochemical analysis of the aqueous extract of *Sarcocephalus latifolius* was performed according to the method described by [14]. Determination of total polyphenols in extracts from the plant studied was performed by the method of [15] using the commercial Folin Ciocalteu reagent applied by [16]. Quantification of flavonoids in the plant extracts studied was performed by a method adapted from [17],[18] using aluminum trichloride ($AlCl_3$) as reagent. Condensed tannin content was determined by the vanillin method described by [19].

2.4. *In vitro* antioxidant activity

2.4.1. Ferric reducing antioxidant power

Ferric reducing antioxidant power was evaluated according to the protocols of [20],[21]. For this purpose, 500 µL of extract at different concentrations (0.1 to 100 µg/mL) were mixed with phosphate buffer, 0.2 M; pH 6.6 (1.25 mL) and potassium ferricyanide solution 1% (1.25 ml) in dry tubes. Preparations were incubated at 50 °C for 20 minutes, then cooled to room temperature. Trichloroacetic acid 10% (1.25 ml) was added to the preparations. The tubes were then centrifuged at 3000 rpm for 10 minutes. To 2 ml of the supernatant were added 2 ml of distilled water and 400 µL of fresh iron chloride ($FeCl_3$) solution (0.1%). Absorbance was read against a blank at 700 nm. Ascorbic acid used as a

positive control underwent the same experimental conditions as the extract. An increase in absorbance indicates greater reducing power. The antioxidant activity of the extract was expressed as IC_{50}, which was defined as the concentration (in µg /mL) of extract required to inhibit the formation of DPPH radicals by 50%. The antioxidant activity related to the reducing power of the extracts is expressed as Reducing Power (RP) using the following formula:

$$\mathbf{PR} = \frac{\mathbf{Absorbance\ control - Absorbance\ extrait}}{\mathbf{Absorbance\ control}} \mathbf{X\ 100}$$

2.4.2. Determination of scavenging effect on DPPH radicals

The ability of extract to scavenge free radicals was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical reduction assays according to the method described by [22] with some modifications. Six concentrations of the extract between 0.1 and 100 µg/mL obtained by dilution were used for determining inhibitory concentrations 50. For the assays, 700 µL of each dilution was mixed with 1400 µL of DPPH solution (0.025 g/L). The mixtures were incubated for 30 min at room temperature, protected from light, and the absorbance was measured at 517 nm. Gallic acid was used as a positive control. The percentage reduction of the DPPH radical was calculated using the following formula:

$$\mathbf{\%\ Reduction} = \frac{\mathbf{Absorbance\ control - Absorbance\ sample}}{\mathbf{Absorbance\ control}} \mathbf{X\ 100}$$

2.5. Anti-inflammatory studies

2.5.1. Carrageenan-induced paw edema in rats

Wistar rats were randomly divided into five groups of five animals each. Animals were treated orally with extract (200 and 300 mg/kg), diclofenac (20 mg/kg) and distilled water (10 mL/kg). Animals were treated one hour before injection of 0.1 mL of 1% carrageenan into the subplantar tissue of the right hind paw [23],[24]. Paw thickness was measured by the immersion method (water column dislocation) with the Ugo Basile plethysmometer at 0, 1, 2, 3, 4 and 5 hours after this administration. After each post-treatment paw volume measurement, the difference (df) between treated (inflamed) and basal paw volumes was calculated as df = volume of the inflamed paw mL– basal volume of the same paw mL. The percentage of antiedematous activity (%AE) was calculated as according to [25].

$$\mathbf{\%\ AE = [\ (control\ group\ mean\ df - test\ group\ mean\ df)/(control\ group\ mean\ df)\ ]\ x\ 100}$$

2.5.2. Dextran-induced paw edema in rats

The effect of the aqueous extract on acute inflammation was assessed using dextran-induced paw edema [26]. Twenty rats were divided equally into four groups and treated. Rats received (*p.o.*) normal saline (control), diclofenac 20 mg/kg (standard), extract 200 mg/kg, 300 mg/kg (tests). Animals were pretreated with control/diclofenac/extract 30 min before injection of 0.1 mL 1% dextran into the subplantar region of the right hind paw of rats. Paw volumes were measured and compared between groups as in the carrageenan study.

2.5.3. Formalin-induced chronic inflammation

The effect of aqueous extract on chronic inflammation was tested using formalin-induced paw edema for seven days [27]. In this model, chronic inflammation was induced by injecting 0.1 mL of 2% formalin into the subplantar area of the right hind paw of rats. For substance administration, five groups of five animals were formed:

Group I: Normal saline (*p.o.*); Group II: Formalin (s.*p*) + normal saline (*p.o.*); Group III: Formalin (*s.p.*) + diclofenac 20 mg/kg (*p.o.*); Group IV: Formalin (*s.p.*) + aqueous extract 200 mg/kg (*p.o.*); Group V: Formalin (*s.p.*) + aqueous extract 300 mg/kg (*p.o.*).

Paw thickness was measured by the immersion method (water column dislocation) with the Ugo Basile plethysmometer at 1, 3, 5 and 7 days after this administration. Paw volumes were measured and compared between groups as in the carrageenan study.

2.6. Relative organ weight determination

After treatment, the animals were weighed, anesthetized and autopsied. Organs such as kidneys, liver and spleen were removed, and relative organ weights were calculated according to the method described by [28].

2.7. Evaluation of hematological and biochemical parameters

Blood samples were collected in EDTA (ethylenediaminetetraacetic acid) vacutainers. Hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC) and platelets were counted using an automated hematology analyzer (URIT 3000 Plus). Biochemical parameters of urea, creatinine, aspartate aminotransferase (AST) and alanine transaminase (ALT) were determined by an automated chemistry analyzer (URIT 880), following the kit manufacturer's protocol.

2.8. Statistical analysis

The results were expressed as mean ± ESM where each value represents a minimum of 5 rats (n = 5). All data were tested for one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests using Graph pad software version GraphPad Prism© version 5.03 for Windows (GraphPad Software, USA). The results were compared with that of control rats and were considered statistically significant at P values below 0.05.

3. Results

3.1. Phytochemical analysis

The aqueous extract of the ripe fruits of *Sarcocephalus latifolius* revealed the presence of a few phenolic compounds such as tannins, flavonoids, coumarins and steroids and terpenoids as saponosides. The absence of compounds nitrogenous as alkaloids was noted (Table 1). Extract at 10 mg/mL yielded 92.95±11.44 mg EAG/g total polyphenols, 0.082±0.005 mgERu/g total flavonoids and 0.14 ± 0.003 mg EAT/g condensed tannins (Table 2).

Table 1 Qualitative and quantitative phytochemistry of the aqueous extract of *Sarcocephalus latifolius* ripe fruits

Plant	Crude extract	Phytochemical content							
		Saponin	Alkaloid	Phenolic	Flavonoid	Terpenoid	Tanin	Coumarin	Anthracenic
Fruits	17.52 %	+	-	+	+	-	+	+	+

+: Presence of compound; -: No compound

Table 2 Quantitative phytochemistry of the aqueous extract of *Sarcocephalus latifolius* ripe fruits

Phytochemical groups	*Sarcocephalus latifolius* ripe fruits
Polyphenolics	929.47±114.36 mgEAG/100g
Flavonoids	81.65±5.32 mgERu/g
Tannins	137.03 ± 2.86 mgEAT/g

Each experiment was performed at least three times and data are expressed as mean ± esm

3.2. Antioxidant effects of the aqueous extract of *Sarcocephalus latifolius* ripe fruits

3.2.1. Effect of the aqueous extract on DPPH radical scavenging

Extract at 0.1 µg/mL inhibited the DPPH radical to around 30%. This inhibition was around 50% for extract at 25 µg/mL. Concentrations of 100 µg/mL extract inhibited around 63% of the radical. Inhibition of the radical by gallic acid was 21.07, 92.91 and 93.18% respectively at 0.1, 25 and 100 µg/mL (Figure 1).

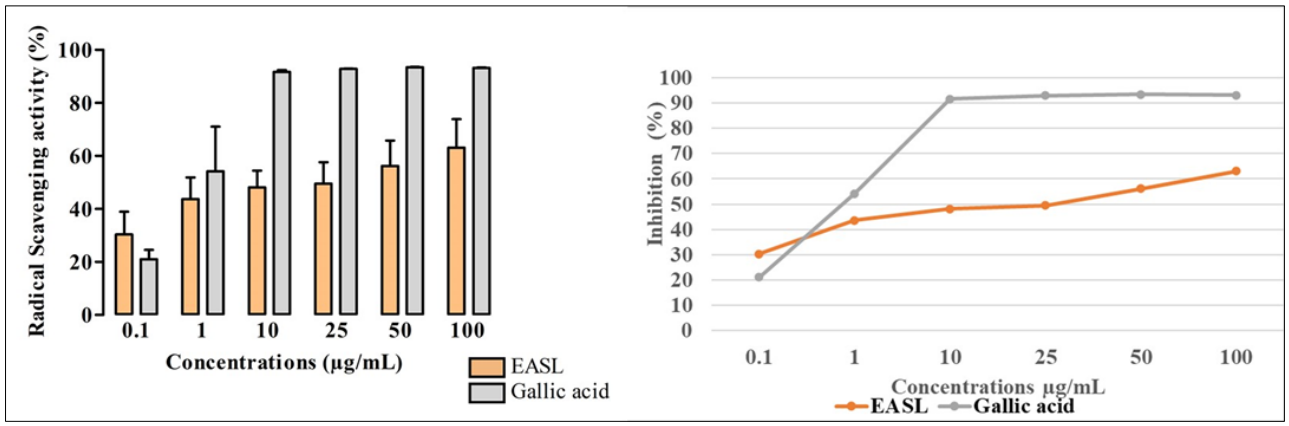


Figure 1 DPPH radical scavenging activity of the aqueous extract of *Sarcocephalus latifolius* ripe fruits and gallic acid. Each experiment was performed at least three times and data are expressed as mean ± esm versus the control gallic acid

3.2.2. Effect of aqueous extract on ferric ion reduction power

Figure 2 shows the concentration-response curves for the reducing powers of *Sarcocephalus latifolius* aqueous extract using the ferric ion reduction method. The amount of Fe^{2+} complex was then measured at 700 nm. Absorbance was minimal at 0.1 µg/mL, and the reducing power of the extract and ascorbic acid increased with concentration. The inhibiting concentration for 50% of complexed Fe^{2+} is 18.9 µg/mL for ascorbic acid and 111.6 µg/mL for the aqueous extract of *Sarcocephalus latifolius*.

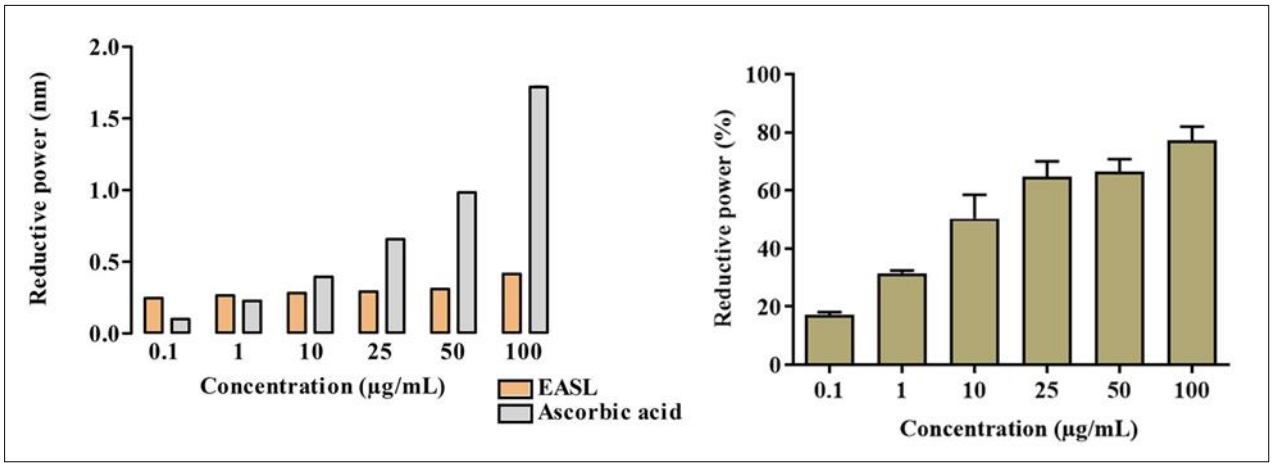


Figure 2 Ferric ion-reducing capacity of the aqueous extract of *Sarcocephalus latifolius* ripe fruits and ascorbic acid. Each experiment was performed at least three times and data are expressed as mean ± esm versus the control ascorbic acid

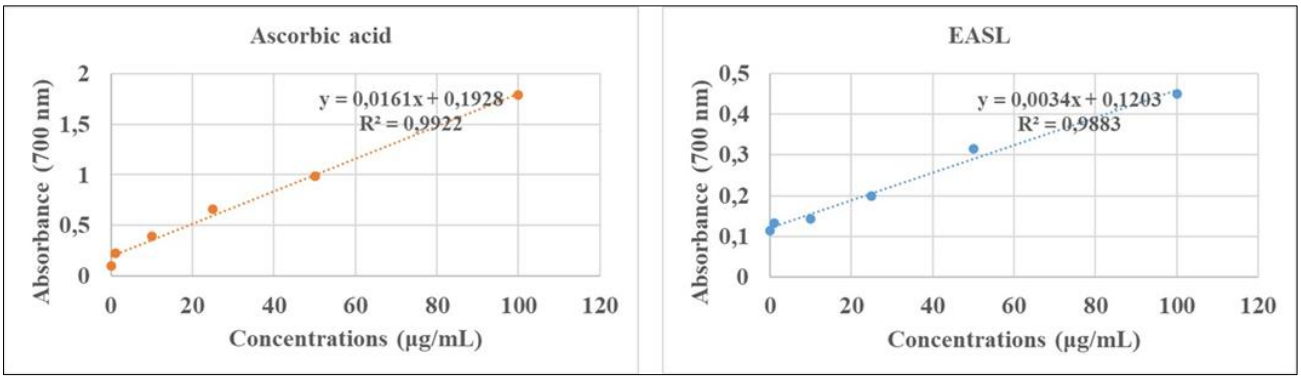
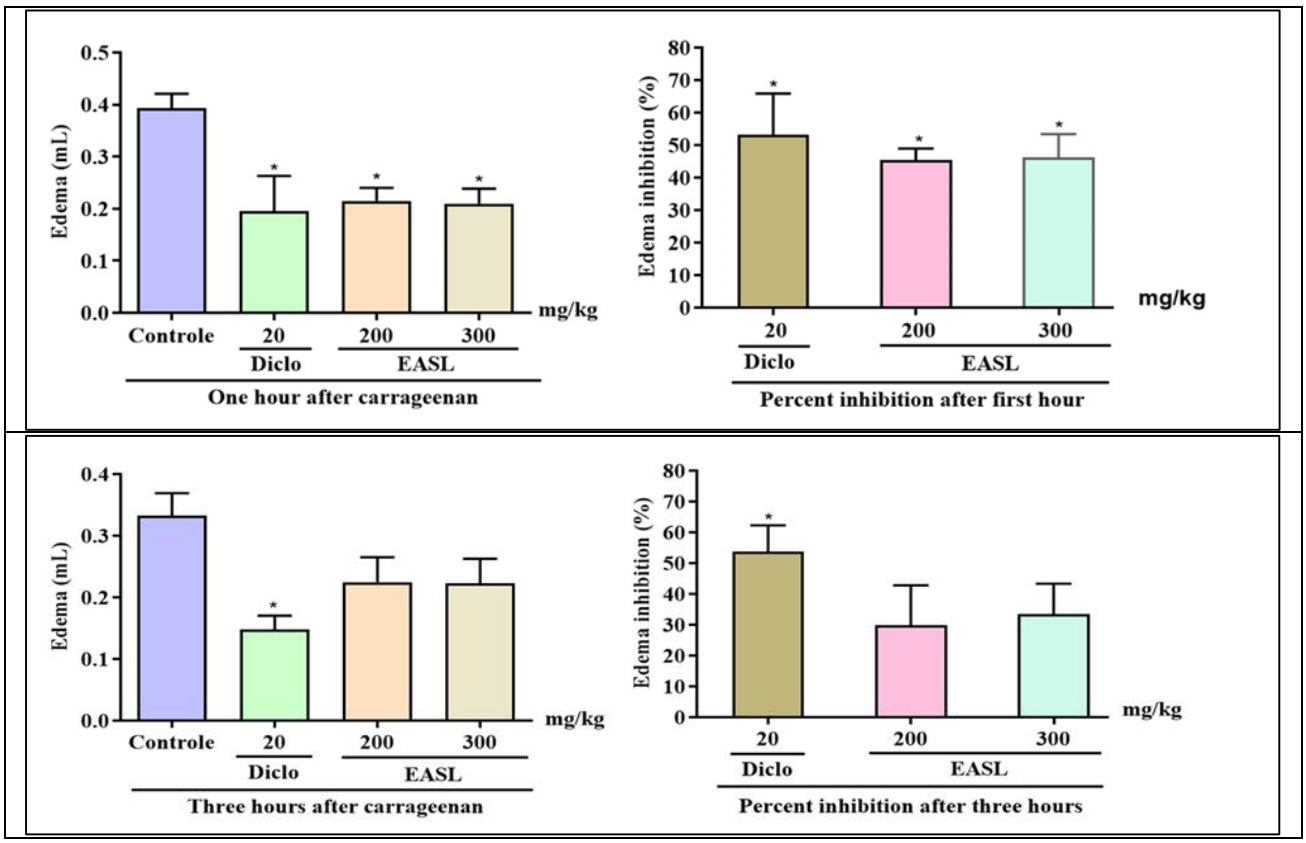


Figure 3 Ascorbic acid and aqueous extract of *Sarcocephalus latifolius* absorbance against concentrations. IC_{50} (µg/mL) of Ascorbic acid and EASL on FRAP was calculated using X= (y-b)/a with y = 0.5. IC_{50} were 18.9 µg/mL for ascorbic acid and 111.6 µg/mL for the aqueous extract of *Sarcocephalus latifolius*

3.3. Effects of the aqueous extract on *carrageenan* and dextran induced inflammation

3.3.1. Effects of aqueous extract on carrageenan-induced inflammation

At the first and fifth hour, all doses of extract produced a significant inhibition ($p<0.05$) of paw edema volume. However, maximum inhibition was observed at the fifth hour. They were respectively 48.49% and 56.16% at 200 and 300 mg/kg. At the third hour, the different doses of extract showed a non-significant inhibition ($p>0.05$) of edema (Figure 4).



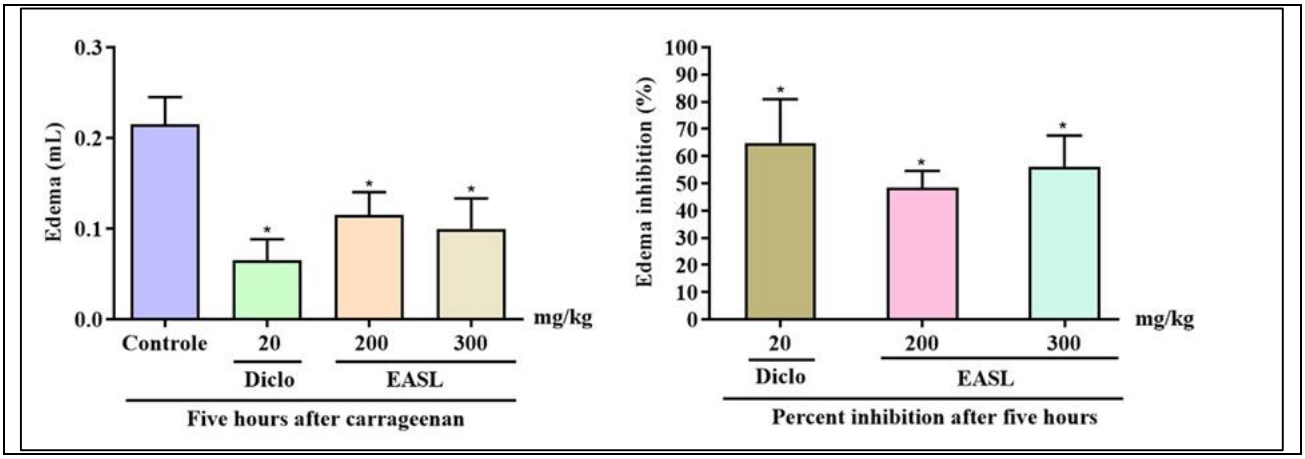


Figure 4 Anti-inflammatory effects of aqueous extract of *Sarcocephalus latifolius* ripe fruits on carrageenan-induced acute inflammation in rats. Each bar represents the mean ± esm, (n =5). *: p<0.05 significant difference from control

*3.3.2. Effects of the aqueous extract on **dextran**-induced inflammation*

After thirty minutes of induction, the extract at all doses inhibited paw edema highly significantly (p<0.01). They were 34.55% at 200 mg/kg and 44.44% at 300 mg/kg. At one and two hours, the different doses of extract showed a non-significant inhibition (p>0.05) of oedema (Table 3).

Table 3 Effects of aqueous extract of *Sarcocephalus latifolius* ripe fruits on dextran-induced acute inflammation in rats

Time	Treatments (mg/kg)			
	Control	**Diclofenac 20**	**EASL 200**	**EASL 300**
0.5 hour	0.42±0.03	0.19±0.02 (54.10±3.21) ***	0.27±0.02 (34.55±7.43) **	0.23±0.03 (44.44±3.3) **
1 hour	0.34 ±0.05	0.14 ±0.02 (57.46±6.04) *	0.2±0.05 (35.85±18.07) ns	0.18±0.02 (45.39±5.5) ns
2 hours	0.31±0.03	0.15±0.03 (51.28±6.39) ns	0.2±0.02 (34.36±4.17) ns	0.17±0.06 (46.69±15.25) ns

Each value represents the mean ± esm, (n=5). *: p < 0.05; ***: p < 0.001; significant difference from control. ns: not significant. Values in parentheses represent percent inhibition of paw edema.

3.4. Effects of the aqueous extract on formalin-induced subacute inflammation

3.4.1. Effects of the aqueous extract on relative organ weights

Relative kidney and liver weights showed no significant variation (p>0.05) between rat groups (Table 4). For the spleen, this variation was significant (p<0.05) at 200 and 300 mg/kg compared with the control.

Table 4 Effects of aqueous extract of *Sarcocephalus latifolius* ripe fruits on relative weights of liver, kidney and spleen

	Treatments (mg/kg)			
	Formalin 2%	**EASL 200**	**EASL 300**	**Diclofenac 20**
Weight gain (%)	3.21 ± 1.4	6.02 ± 0.1	7.08 ± 0.5	6.29 ± 4.65
Relative weight Liver (g)	3.23 ± 0.2	3.37 ± 0.2	3.23 ± 0.1	3.51 ± 0.16
Relative weight Kidneys (g)	0.68 ± 0.01	0.57 ± 0.03	0.62 ± 0.08	0.78 ± 0.1
Relative weight Spleen (g)	0.42 ± 0.04	0.25 ± 0.04 *	0.27 ± 0.01 *	0.35 ± 0.02

Each value represents the mean ± esm, (n=5). *: p < 0.05 significant difference from control.

*3.4.2. Effects of the aqueous extract on formalin-induced **inflammation***

The extract at all doses inhibited (p>0.05) edema from day one to day seven. However, these inhibitions were significant (p<0.05) on the first day. They were 29.19% at 200 mg/kg and 30.60% at 300 mg/kg (Table 5).

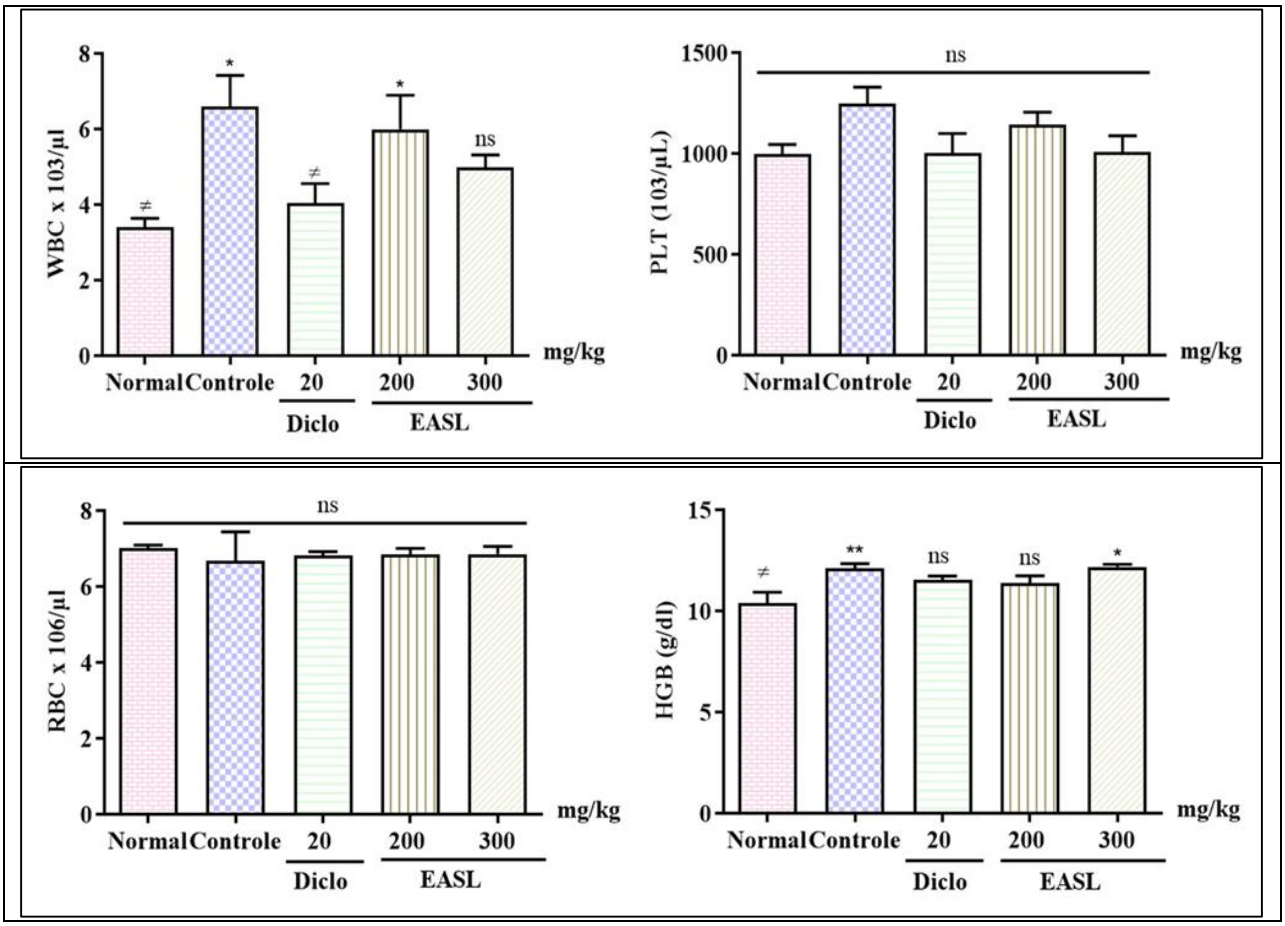
Table 5 Effects of aqueous extract of *Sarcocephalus latifolius* ripe fruits on formalin-induced inflammation in rats

Time	Treatments (mg/kg)			
	NaCl	**Diclo 20**	**EASL 200**	**EASL 300**
Day 1	0.57±0.04	0.27±0.03 (53.60±2.62) ***	0.4±0.04 (29.19±6.79) *	0.39±0.03 (30.60±1.59) *
Day 3	0.34±0.05	0.16±0.03 (55.95±2.9) *	0.23±0.04 (32.86±2.73) ns	0.23±0.04 (35.24±2.44) ns
Day 5	0.27±0.06	0.11±0.007 (53.60±8.25) ns	0.17±0.02 (34.10±6.36) ns	0.15±0.05 (46.28±8.64) ns
Day 7	0.27±0.06	0.11±0.03 (61.09±2.56) *	0.18±0.05 (34.89±4.52) ns	0.17±0.03 (36.13±4.46) ns

Each value represents the mean ± esm, (n=5). *: p < 0.05; ***: p < 0.001; significant difference from control. ns: not significant. Values in parentheses represent percent inhibition of paw edema.

3.4.3. Effects of aqueous extracts on blood constituents

Figure 5 shows the results obtained on selected blood cells from experimental and normal rats. A significant increase (p<0.05) in leukocytes and hemoglobin was observed in control rats. In rats treated with the extract at all doses, a non-significant (p>0.05) decrease in leukocytes and hemoglobin was observed compared to normal rats. Changes in platelet and red blood cell counts were not significant (p>0.05) in test rats compared with normal and control rats.



Each bar represents the mean ± esm, (n=5). *: p<0.05; **: p < 0.01 significant difference from normal rats. #: p<0.05 significant difference from control rats (formalin). ns: not significant. WBC: White blood cell, RBC: Red blood cell, HGB: Hemoglobin, PLT: Platelet

Figure 5 Effects of aqueous extract of *Sarcocephalus latifolius* ripe fruits on hematologic parameters in rats.

3.4.4. Effects of aqueous extract on renal and hepatic biomarkers

Figure 6 shows the results of selected biochemical parameters in experimental and normal rats. A highly significant (p<0.001) increase in ALT, AST and creatinine levels was observed in control rats. In test rats, a highly significant

($p<0.001$) decrease in ALT levels at all extract doses and in AST levels at 300 mg/kg was observed compared to normal rats. This decrease was significant ($p<0.05$) for creatinine at all extract doses and for AST at 200 mg/kg.

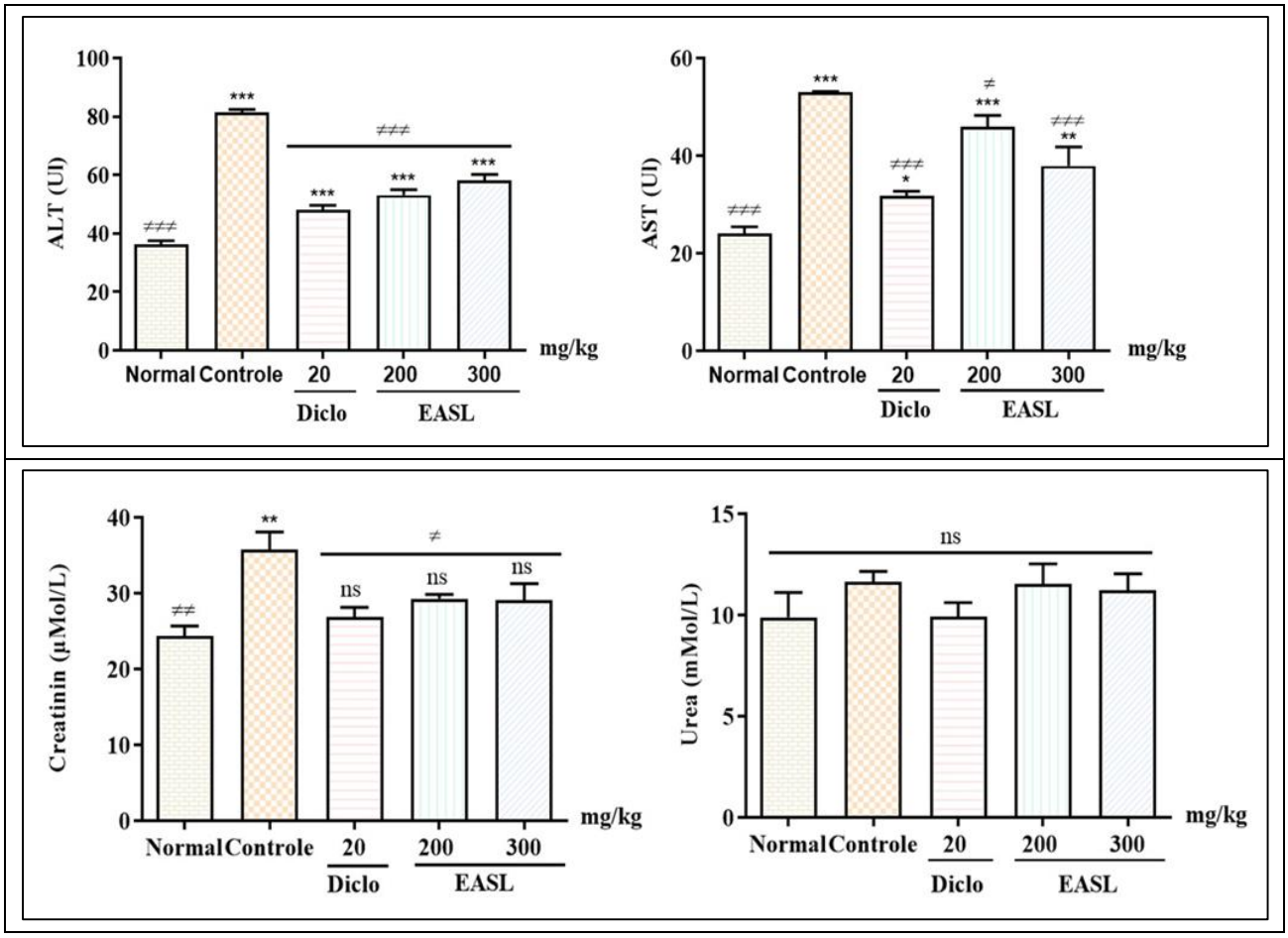


Figure 6 Effects of aqueous extract of *Sarcocephalus latifolius* ripe fruits on biochemical parameters in rats. Each bar represents the mean ± esm, (n=5). **: $p<0.01$ significant difference from normal rats. #: $p<0.05$ significant difference from control rats

4. Discussion

Preliminary phytochemistry indicates the presence of saponins, flavonoids, tannins, glycosides and phenols in the ripe fruits. In addition to these metabolites, [29] revealed alkaloids, steroids and terpenoids. Tannins were not found. Except for sterols and terpenes, [30] revealed the presence of the other metabolites mentioned in the hydroethanolic extract of *Sarcocephalus latifolius* leaves. The differences in compounds observed could be explained by environmental factors that vary from one region to another. They could be explained by edaphic factors linked to the hygrometric gradient, but also by the sampling period. This difference could also come from the type of extraction.

The reducing power of DPPH may be due to the ability to give electrons or hydrogen [31],[32] and is generally associated to the presence of reductones [33]. The antioxidant activity of the aqueous extract of *Sarcocephalus latifolius* ripe fruits could be attributed to its flavonoid and phenol content. Flavonoids act as scavengers of various oxidative species, namely superoxide anion, hydroxyl radical or peroxy radicals. They also act as absorbers of singlet oxygen [34]. Flavonoids do not necessarily act as antioxidants. Some have the ability to stimulate deoxyribose degradation as a result of Fe^{3+} reduction. These flavonoids have pro-oxidant properties, probably through iron redox [35],[36]. The hydroxyl groups of phenols have the ability to scavenge radicals [37]. Phenolic compounds have also been reported to be efficient hydrogen donors. As such, they are excellent antioxidants [38]. Free radicals released by phagocytic cells are important in inflammatory processes. Indeed, they are involved in the activation of nuclear factor kappa Beta, which induces the transcription of inflammatory cytokines and cyclooxygenase 2 [39],[40]. The anti-inflammatory activity of the aqueous extract of *Sarcocephalus latifolius* ripe fruits may be due to the synergistic effect of pro-inflammatory enzyme inhibitors or free radical scavenging activities. The anti-inflammatory activities of extract could be explained, at least in part, by

their antioxidant properties. Our results are comparable to those of [4] who showed that *Nauclea latifolia* leaves had a high antioxidant potential.

Carrageenan-induced paw edema is a well-known experimental model for acute inflammation. Carrageenan is the most frequently used phlogogen for testing the anti-inflammatory properties of plants. It is not antigenic and has no apparent systemic effect [41]. Carrageenan-induced paw edema as an *in vivo* model of inflammation is a screening procedure in which the involvement of cyclooxygenase products of arachidonic acid metabolism and the production of reactive oxygen species are well established [42]. Carrageenan edema presents three distinct phases. The first phase occurs an hour after inflammation and is attributed to the release of cytoplasmic enzymes, histamine and serotonin by the mast cells [43],[44]. The second phase, occurring after one hour, is mediated essentially by the release of kinin [43],[44],[45]. The second phase of edema formation is sensitive to both steroidal and non-steroidal agents [46]. The third phase (around 5 hours of edema) in which the mediator is suspected to be prostaglandin [47]. In the carrageenan-induced edema model, the aqueous extract of *Sarcocephalus latifolius* ripe fruits at all doses reduced paw edema. This anti-oedematous effect was significant in the first phase. This suggests that the extract could inhibit the release of histamine and prostaglandins. The extract could have an antihistaminic property, as it neutralizes histamine directly by mast cell degranulation. In addition, the anti-edema effect of the extract was significantly maintained in the second phase of edema formation. The extract is thought to inhibit cyclooxygenase enzymes involved in prostaglandin formation. Our results corroborate those of [48] who showed that methanolic extract of *Sarcocephalus latifolius* root effectively inhibits carrageenan-induced paw edema by reducing the action of inflammation mediators. Our results are also comparable to those of [49], who demonstrated the anti-nociceptive, anti-inflammatory and anti-pyretic effects of the aqueous extract of *Nauclea latifolia* root bark.

Injection of dextran into the paw of rats induces an anaphylactic reaction. This reaction is characterized by extravasation and edema formation due to the release of histamine and serotonin by mast cells [50]. Dextran is known to be a potent osmotic agent, which causes a significant increase in vascular permeability and blood flow to the inflammatory site [51]. Histamine is the principal mediator involved in the initial phase of inflammation. It increases vascular permeability by acting on microcirculation [52]. It is a powerful vasodilator that increases vascular permeability [53],[54]. Serotonin, released mainly by mast cells and platelets, is also involved in this process. It induces edema and plasma extravasation in the acute inflammation [55]. In contrast to carrageenan, dextran-induced fluid accumulation consists of few proteins and few neutrophils [51]. Dextran-induced inflammation was significantly reduced by the aqueous extract of *Sarcocephalus latifolius* ripe fruits. The extract revealed an antihistaminic effect, thought to be due to inhibition of mast cell degranulation. At all doses, the extract effectively suppressed histamine-induced edema. The extract contains compounds capable of inhibiting histamine release. The extract also suppressed serotonin-induced inflammation. It could exert its anti-inflammatory action by inhibiting the synthesis or release of mediators involved in inflammation, such as serotonin, histamine and prostaglandins. The extract has antihistaminic and antiserotoninergic properties.

Some results suggest that various plant extracts have appetite and digestion stimulating properties [56]. Most of them stimulate the secretion of saliva, bile and enzyme activity. Herbal extracts accelerate digestion and shorten the time it takes for food to pass through the digestive tract [57]. The non-significant weight gain of rats in all groups could be explained by the phenols and flavonoids found in the aqueous extract of *Sarcocephalus latifolius* ripe fruits. The spleen is the main filter for blood-borne pathogens and antigens, as well as a key organ for iron metabolism and erythrocyte homeostasis. Furthermore, immune and hematopoietic functions have recently been unveiled for the mouse spleen, suggesting additional roles for this secondary lymphoid organ [58]. The significant reduction in relative spleen weight in the test groups was due to the non-toxic effects of the aqueous extract of *Sarcocephalus latifolius* ripe fruits [6]. The extract improves spleen function thanks to active biomolecules such as flavonoids, tannins and phenols.

Histamine, serotonin, bradykinin and other inflammatory mediators do not play a role in formalin-induced edema. In fact, it is the active globulins that seem to be involved in this model [59],[60]. However, the inhibitory effect of formalin-induced edema by the extract was significant only on the first day, so we presume that formalin causes inflammation of peripheral tissues. Acute inflammation lasts from a few minutes to a few days, whereas chronic inflammation is longer in duration. Formalin-induced acute inflammation results from cell damage. These lesions trigger the production of endogenous mediators. These mediators, such as histamine, serotonin, prostaglandins and bradykinin, lead to the exudation of fluid and plasma proteins, and the migration of leukocytes, particularly neutrophils. Neutrophil stimulation also leads to an increase in vascular permeability, producing the edema that causes inflammation [61]. The anti-inflammatory activity of the aqueous extract of *Sarcocephalus latifolius* ripe fruits may be attributed to inhibition of the arachidonic acid pathway by cyclooxygenase. In addition, the aqueous extract of *Sarcocephalus latifolius* ripe fruits may inhibit the production of prostaglandins from arachidonic acid. Prostaglandins tend to stimulate the nerves that transmit pain to the brain. As a result of stimulation, blood vessels in the injured site dilate, creating space in the capillary walls for white blood cells to penetrate. The reduction in prostaglandin levels may be due to the phenolic

compounds present in the aqueous extract of *Sarcocephalus latifolius* ripe fruits, which can reduce paw edema. The presence of phenolic compounds in the aqueous extract of *Sarcocephalus latifolius* ripe fruits may be responsible for anti-inflammatory activities in models of acute and chronic inflammation.

Formalin caused an elevated leukocyte count in the control group of rats. Indeed, the high fractional volume of leukocytes results in increased blood viscosity. As a result, blasts occlude microvessels and reduce vessel flow. The other approach stipulates that blast-activated endothelial cells secrete the cytokines TNF-α and IL-1β. Blast-endothelial cell interaction is mediated by specific adhesion receptors such as selectins and VCAM-1. These interactions play an important role in promoting blast cell recruitment [62],[63]. These mechanisms lead to vascular obstruction, which induces tissue hypoxia. Extract at 300 mg/kg reduced formalin-induced hyperleukocytosis. This reduction was not significant. The extract may interfere with specific adhesion receptors to block cytokine production by endothelial cells. Anti-inflammatory activities such as the non-significant reduction in white blood cells observed indicate that the aqueous extract of *Sarcocephalus latifolius* may enhance rat immunity through immunomodulatory properties. The phenols, flavonoids and total tannins contained in the aqueous extract of *Sarcocephalus latifolius* ripe fruits could boost the immune system of rats. Our results are comparable to those of [64] who showed that aqueous and methanolic extracts of *Nauclea pobeguinii* were able to significantly inhibit formalin-induced edema.

Elevated transaminases may indicate inflammation or damage to the liver. Transaminases are the most active enzymes in cells. They exist in mitochondrial and cytosolic variants. Transaminases escape in large quantities from dead or dying tissue and enter the bloodstream. In addition to liver cells, AST is also abundantly expressed in various tissues such as heart, skeletal muscle, blood, kidney, pancreas, spleen, lung and erythrocytes. In contrast, high concentrations of ALT are found in the liver, and relatively low concentrations in the heart, muscles and other tissues [65],[66]. Formalin may enhance ALT and AST activity. ALT and AST catalyze the transfer of alanine to α-cetoglutarate and aspartate to α-cetoglutarate respectively. Specifically, pyruvate is formed for ALT and oxaloacetate for AST. These compounds then complex to pyruvate hydrazone or oxaloacetate hydrazone in the presence of 2,4-dinitrophenylhydrazil (DNPH). Aqueous extracts of *Sarcocephalus latifolius* ripe fruits at 200 and 300 mg/kg significantly reduced ALT and AST levels. Aqueous extract of *Sarcocephalus latifolius* ripe fruits may exert its action by blocking or inhibiting the transfer of alanine and aspartate to their respective sites. Thanks to its secondary metabolites, the aqueous extract of *Sarcocephalus latifolius* ripe fruits could have cytoprotective properties on all cells producing its enzymes. The action of the aqueous extract of *Sarcocephalus latifolius* ripe fruits could involve stabilizing cell membranes by preventing membrane lipid peroxidation.

5. Conclusion

The aim of this study is to determine the phytochemical compounds and evaluate the antioxidant and anti-oedematous effects of the aqueous extract of *Sarcocephalus latifolius* ripe fruits. According to the results obtained, the ripe fruits of *Sarcocephalus latifolius* contain tannins gallic, flavonoids, derivates anthracenic, coumarine and saponosides. This study suggests that the aqueous extract of *Sarcocephalus latifolius* ripe fruits possesses antioxidant activity. Aqueous extract of the ripe fruits of *Sarcocephalus latifolius* showed more interesting anti-oedematous effects than diclofenac, which is synthetic product used in this case. These results confirm the use of *Sarcocephalus latifolius* ripe fruits in traditional medicine for the treatment of diseases with an inflammatory component and involving oxidative stress.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare an absence of conflict for this work.

Statement of ethical approval

The authors declare that the protocol has been examined and validated by the members of the ethical committee for animal experimentation of the University of Joseph KI ZERBO (UJKZ).

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