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Anti-Inflammatory Activity of Hydro-Ethanolic Extract of *Maytenus senegalensis* Lam (*Celastraceae*) Roots and Leaves and Their Fractions

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Abstract

Maytenus senegalensis (*Celastraceae*) is a plant in the African pharmacopoeia and is widely used in traditional medicine in various pathologies associated to inflammation and pain. The aim of this study was to evaluate the anti-inflammatory activity of hydro-ethanolic extract of *Maytenus senegalensis* roots and leaves and their fractions in rats. Extraction with ethanol and liquid/liquid fractionations were carried out. Characterization reactions were performed to identify the chemical compounds. The anti-inflammatory activity of hydro-ethanolic extract of *Maytenus senegalensis* roots (HEE_R) and leaves (HEE_L) and their ethyl acetate (EAF_R, EAF_L) and dichloromethane (DF_R, DF_L) fractions was investigated on a pharmacological model of carrageenan-induced acute edema of the rat paw in comparison to that of aspirin used as a reference substance. Phytochemical study revealed the presence of tannins, flavonoids, sterols and triterpenes in the extracts. HEE_R, HEE_L and their fractions at doses of 10 and 30 mg/kg *per os* and the reference product (30 mg/kg) significantly prevented rat paw edema associated with carrageenan injection from T1H to T5H. HEE_R at 30 mg/kg was more effective in preventing paw edema in rats than at 10 mg/kg, its fractions EAF_R and DF_R (10 and 30 mg/kg) and aspirin (30 mg/kg). At 10 mg/kg, HEE_L showed a greater preventive effect on inflammatory edema than at 30 mg/kg, but less than its fractions (EAF_L and DF_L). HEE_R exhibits greater anti-inflammatory activity than HEE_F at dose of 30

mg/kg, but at 10 mg/kg, HEE_F is slightly more effective than HEE_R, particularly at T1H and T5H. The results indicate that extracts of *Maytenus senegalensis* roots and leaves contain chemical substances with anti-inflammatory properties and could contribute to the treatment of inflammatory diseases.

Keywords

Maytenus senegalensis, Roots, Leaves, Fractions, Inflammation, Rats

1. Introduction

Inflammation is a complex and important protective response of the body to a stimulus by microorganisms, physical injuries, chemicals, allergic reactions or the presence of endogenous signals due to cellular damage [1]-[3]. Inflammatory processes are involved in the development of a large number of human pathologies including diabetes, asthma, allergies, arthritis, cancer [4] and they constitute a real public health problem. Anti-inflammatory drugs are used for treatment, but their use is associated with numerous adverse effects, particularly gastric and renal [5]-[7] and their accessibility to a large part of the world's population is often limited. The search for molecules with anti-inflammatory properties in medicinal plants with less toxicity is essential.

Maytenus senegalensis, of the Celastraceae family, is a shrub or small tree that grows in semi-desert regions of Asia, India, Africa, Arabia and Afghanistan [8] [9] and it is present throughout Senegal [10]. It is a plant used in traditional medicine for its many properties, including antiplasmodial [11]-[13], antileishmanial [14], antibacterial, anti-inflammatory [15]-[17], analgesic [16], antioxidant [17] [18], antitumor [12] [19], antileukaemic *in vivo* [19], antiviral [20]-[22], anti-sickling [18]. Its roots and bark are traditionally used in folk medicine in certain regions of Africa to treat illnesses including chest pain, rheumatism, snake bites, diarrhea, eye infections, dyspepsia and wounds [15] [23].

Chemical studies have shown the presence of tannins, flavonoids, saponosides, cardiotonic heterosides [17] [24], triterpenoids and alkaloids [17] [24] [25]. Acute and sub-acute toxicity studies have shown that *Maytenus senegalensis* roots are devoid of toxicity [26]. However, very little work on the anti-inflammatory activity of *Maytenus senegalensis* roots and leaves are available.

The aim of the present study was to evaluate the anti-inflammatory activity of hydro-ethanolic extracts of *Maytenus senegalensis* roots and leaves and their fractions in rats.

2. Materials and Methods

The study was conducted at the Pharmacology laboratory of the Faculty of Medicine, Pharmacy and Odonto-Stomatology (FMPOS) at Cheikh Anta Diop University in Dakar (CADU), Senegal.

2.1. Plant Material

Maytenus senegalensis roots and leaves were collected from Hann Forest Park in Dakar, Senegal. They were identified at the Pharmacognosy and Botany laboratory of FMPOS, CADU where samples was deposited for future reference. The roots and leaves were then air dried in shade at room temperature before being pulverized.

2.2. Animals

Adult male and female Wistar rats weighing between 120 and 180 grams (g) were used in the study. The animals were procured and stored at the Pharmacology Laboratory's animal house, FMPOS, CADU. They were housed in cages under standard conditions at 25˚C ± 2˚C and 12 h dark/light cycle. Animals were provided with standard rodent pellet and had free access to water. The experimental protocols were conducted in accordance with the guidelines of the Institutional Ethics Committee (Research Ethics Committee of Cheikh Anta Diop University: approval n˚ 0372/2019/CER/UCAD).

2.3. Chemicals and Instruments

A digital plethysmometer (APELEX 05-7150, Allende Bagneux France) measured the volume of rat paw edema. Carrageenan and acetyl salicylic acid were from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol, ethyl acetate and dichloromethane were provided from Sigma/BES (Dakar, Senegal).

2.4. Experimental Procedures

2.4.1. Extraction and Fractionation

An amount of 50 g of powdered roots or leaves of *Maytenus senegalensis* were decocted for 15 minutes in 800 mL of ethanol and 200 mL of boiling water. After filtration through Whatman No. 1 filter paper, the hydro-ethanolic extracts of *Maytenus senegalensis* roots (HEE_R) and leaves (HEE_F) obtained was evaporated to dryness using a rotary evaporator.

For liquid-liquid fractionation with a non-polar solvent and a polar solvent, 1 g of dried roots or leaves extract was dissolved in a distilled water/dichloromethane mixture (500 ml/500 ml). After decantation, the aqueous solution obtained was extracted again twice with dichloromethane. The collected dichloromethane phase was evaporated to give the dichloromethane fraction (DF_R or DF_L). The residual aqueous phase was again subjected to liquid-liquid extraction under the same conditions as above with ethyl acetate. The collected ethyl acetate phase was evaporated to give the ethyl acetate fraction (EAF_R or EAF_L). Dry extracts dissolved in distilled water were used for the pharmacological tests.

2.4.2. Phytochemical Characterizations

Standard characterization reactions were carried out on the HEE_R and HEE_F and their fractions in order to identify the presence of phytochemical constituents. The classic methods of characterization were used on these samples for the detec-

tion of condensed and hydrolysable tannins (Stiasny test followed by a ferric chloride test), flavonoids (Shibata's test), alkaloids (Bouchardat, Valser-Mayer and Dragendorff's reagents tests), steroids and triterpenoids (Liebermann-Buchard test), cardiac glycosides (Baljet, Kedde and Raymond-Marthoud reagents tests) [27] [28].

2.4.3. Anti-Inflammatory Activity

Anti-inflammatory activity study was carried out using carrageenan-induced rat paw edema method described by Winter *et al.* [29]. Prior to the experiment, the rats were weighed, divided into 14 groups of 5 and then fasted for 12 hours. The initial volume (V_0) of the right hind paw of each rat was measured using digital plethysmometer.

The rat paw edema was induced by injection of 1% carrageenan suspension in 0.9% NaCl solution (0,1 ml) into the sub-plantar tissue of the right hind paw of each rat, 1 h after administration of the different treatments by gavage through a gastric tube (**Table 1**). The plethysmometric measurements were carried out at T1h, T3h and T5h after carrageenan injection.

Table 1. Products tested and doses administered.

Groups	Treatment	Doses
1	Physiological water	10 ml/kg
2	Acetyl salicylic acid (Aspirin)	30 mg/kg
3	Hydro-ethanolic extract roots (HEE_R)	10 mg/kg
4		30 mg/kg
5	Dichloromethane fraction (DF_R)	10 mg/kg
6		30 mg/kg
7	Ethyl acetate fraction (EAF_R)	10 mg/kg
8		30 mg/kg
9	Hydro-ethanolic extract leaves (HEE_L)	10 mg/kg
10		30 mg/kg
11	Dichloromethane fraction (DF_L)	10 mg/kg
12		30 mg/kg
13	Ethyl acetate fraction (EAF_L)	10 mg/kg
14		30 mg/kg

The importance of edema was assessed by determining the mean percentage increase (% INC) of volume of rat paw according to the following formula:

$$\%\ \mathrm{INC} = \frac{V_t - V_0}{V_0} \times 100$$

V_t = Paw volume at t time;

V_0 = Initial paw volume.

The anti-inflammatory activity of the tested products was evaluated by calculating the percentage of edema inhibition (% INH):

$$\% \text{ INH} = \frac{P_0 - P_t}{P} \times 100$$

P_0 = average percentage increase in pat volume in the control group;
P_t = average increase in pat volume in the treated group at time *t*.

2.5. Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM) and analyzed by GraphPad 6.0 software. A one-way analysis of variance (ANOVA) followed by Dunett's test was performed separately at T1H, T3H and T5H and applied to determine the significance of the difference between the control group and the groups treated with the products. A significant difference was indicated by a p-value < 0.05 (n = 5 represents the number of animals in each group).

3. Results

3.1. Extraction and Fractionation

From 50 g of dried powdered roots or leaves of *Maytenus senegalensis*, 8 g of dried HEE_R or HEE_L were obtained corresponding to a yield of 16%. Respectively, the EAF_R and DF_R represented 12.5% and 7.5% of the dried HEE_R and EAF_L and DF_L 5.25% and 3.5% of the dried HEE_L.

3.2. Phytochemical Characterizations

Phytochemical characterizations showed the presence of tannins, flavonoids, alkaloids, sterols and triterpenoids in HEE_R and HEE_L. The EAF_R and EAF_L revealed the presence of condensed tannins and flavonoids. Sterols and triterpenes were detected in the DF and EAF fractions of HEE_R and HEE_L, and alkaloids only in the DF and EAF fractions of HEE_R (Table 2).

Table 2. Phytochemical groups identified in hydro-ethanolic extracts of roots and leaves of *Maytenus senegalensis* and in their fractions.

Phytochemical groups	HEE_R	DF_R	EAF_R	HEE_L	DF_L	EAF_L
Condensed tannins	+	-	+	+	-	+
Hydrolyzables tannins	----	-	-	….	-	….
Flavonoids	+	-	+	+	-	+
Alkaloids	+	+	+	+	-	-
Sterols and triterpenoids	+	+	+	+	+	+
Cardiac glycosides	-	-	-	-	-	-
Saponosides	+	-	-	-	-	-

+: presence, -: absence, ----: traces.

3.3. Anti-Inflammatory Activity

Before experimentation, comparison of the average initial volumes of rat paws showed no significant difference between the different batches.

3.3.1. Increase in Paw Rat Edema in the Control Group

Following carrageenan injection into the plantar pad, rats in the control group showed a significant increase in paw volume as compared to initial volume. The percentages of increase in paw edema at T_{1H}, T_{3H} and T_{5H} were 41.16 ± 3.9, 62.39 ± 8.5, 84.65 ± 8.8% respectively (n = 5, $p < 0.05$ vs baseline).

3.3.2. Inhibitory Effect of HEE_R and Its Fractions on Carrageenan Induced Inflammatory Edema in Rats

Prior oral administration of HEE_R (10 and 30 mg/kg) was associated with significant inhibition ($p < 0.05$; $p < 0.01$; $p < 0.001$ vs. control, n = 5) of inflammatory edema from T1H to T5H. At 30 mg/kg, HEE_R was more effective at preventing inflammatory edema than at 10 mg/kg. The maximum preventive effects were noted at T1H for the 30 mg/kg dose and at T5H for the 10 mg/kg dose, with percentage increases of 4.98 ± 2.0 (87.90%) and 38 ± 8.6 (55.11%), respectively (table). The results showed, at the same dose of 30 mg/kg, a better anti-inflammatory activity of HEE_R compared to aspirin used as a reference product, which showed % INH of 14.7 ± 3.1 (64.28%), 36.13 ± 3.5 (42.09%) and 33.8 ± 5.8 (60.07%) at T1H, T3H and T5H respectively (Figure 1, Figure 2).

EAF_R at 10 mg/kg showed significant ($p < 0.05$; $p< 0.01$ vs. control) and important inhibition of edema from T1H onwards, but this effect decreased from T3H. At 30 mg/kg, the anti-inflammatory effect was weaker and not significant from T1H to T5H. Similarly, administration of DF_R at doses of 10 and 30 mg/kg was not associated with significant inhibition of edema for almost the entire duration of the experiment, except at T5H where the 30 mg/kg dose induced significant inhibition. Comparison between the two fractions shows that the anti-inflammatory effect of EAF_R at 10 mg/kg is superior to that of DF_R (Figure 1, Figure 2).

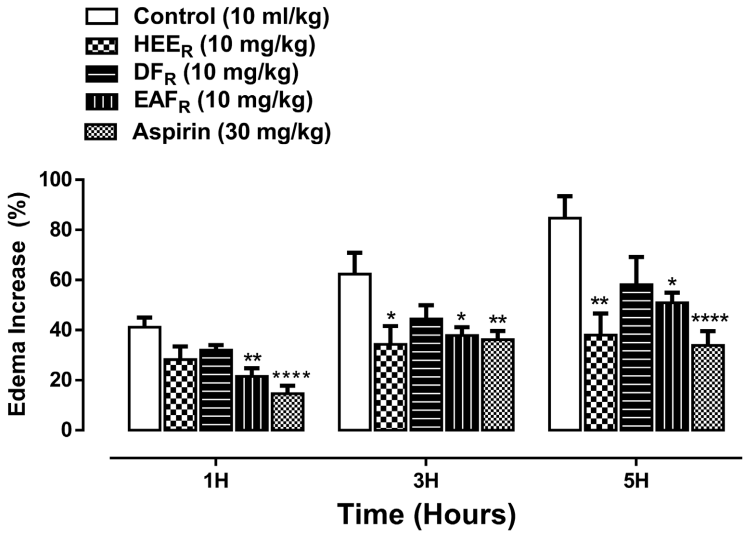

Figure 1. Effect of HEE_R, DF_R, EAF_R (10 mg/kg) and Aspirin (30 mg/kg) on carrageenan-induced inflammatory edema in rats. Results are expressed as means ± SEM. * = $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, ****= $p < 0.0001$ vs. control group. n = 5.

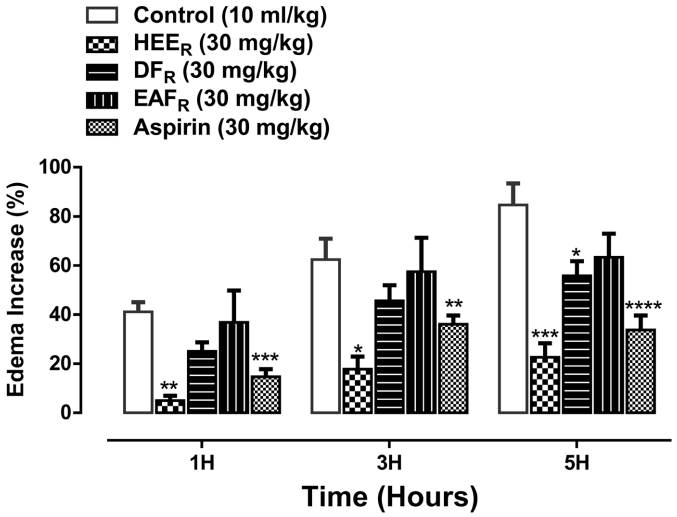

Figure 2. Effect of HEE$_R$, DF$_R$, EAF$_R$ and Aspirin (30 mg/kg) on carrageenan-induced inflammatory edema in rats. Results are expressed as means ± SEM. * = $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, ****= $p < 0.0001$ vs. control group. n = 5.

3.3.3. Inhibitory Effect of HEE$_L$ and Its Fractions on Carrageenan Induced Inflammatory Edema in Rats

With HEEL, the greatest anti-inflammatory effect was observed with the 10 mg/kg dose compared to the 30 mg/kg dose. The prevention of paw edema is maximal at T5H for the 10 mg/kg dose and at T3H for the 30 mg/kg dose with respective % INC of 31 ± 8 (63.51%) and 41.4 ± 7.1 (33.64%) (**Figure 3**, **Figure 4**).

The effects of the two fractions EAF$_L$ and DF$_L$ at a dose of 10 mg/kg on edema inhibition were similar, particularly between T1H and T3H. At 30 mg/kg, the effect of DF$_L$ appears to be greater than that of EAF$_L$ except at T5H (**Figure 3**, **Figure 4**).

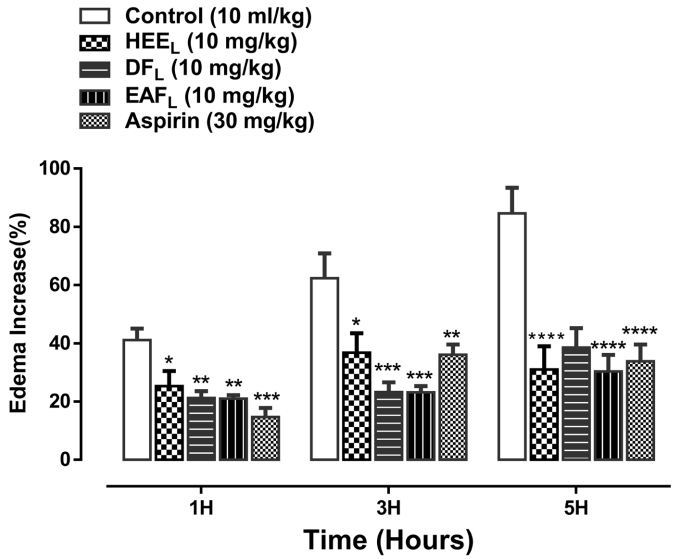

Figure 3. Effect of HEE$_L$, DF$_L$, EAF$_L$ (10 mg/kg) and Aspirin (30 mg/kg) on carrageenan-induced inflammatory edema in rats. Results are expressed as means ± SEM. * = $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, ****= $p < 0.0001$ vs. control group. n = 5.

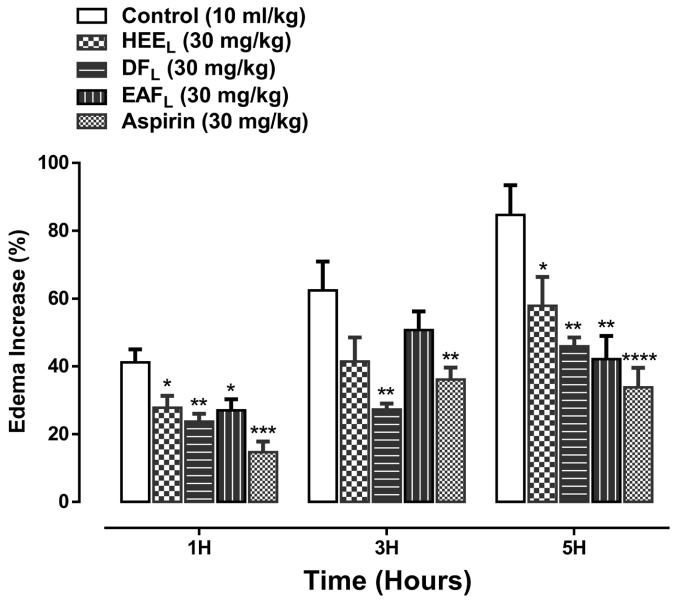

Figure 4. Effect of HEE$_L$, DF$_L$, EAF$_L$ and Aspirin (30 mg/kg) on carrageenan-induced inflammatory edema in rats. Results are expressed as means ± SEM. * = $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, ****= $p < 0.0001$ vs. control group. n = 5.

4. Discussion

Maytenus senegalensis is a plant widely used in traditional African medicine. This study, which contributes to the valorization of medicinal plants in the Senegalese pharmacopoeia, was designed to assess the anti-inflammatory activity of hydroethanolic extracts from the roots and leaves of this plant and their fractions, as well as that of aspirin used as a reference product, on the carrageenan-induced inflammatory paw edema model in rats.

Indeed, treatment with HEE$_R$ at 30 mg/kg showed a maximal inhibitory effect on rat paw edema at T1H, with an INH of 87.90%. Although a decrease in activity was observed at T3H (71.45%), it remained significant until T5H (73.21%). For the 10 mg/kg dose of HEE$_R$, the prevention of edema was greatest at T5H, with an INH of 55.11%, compared to 31.20% and 44.98% at T1H and T3H, respectively. These results suggest that HEE$_R$ prevents inflammatory edema in a dose-dependent manner.

With the HEE$_R$ fractions (EAF$_R$ and DF$_R$), a significant inhibition of rat paw edema was observed with EAF$_R$ at 10 mg/kg, which was greater than that of EAF$_R$ at 30 mg/kg and that of DF$_R$ at 10 and 30 mg/kg. This suggests that the chemical compounds responsible for the anti-inflammatory activity of *Maytenus senegalensis* roots would be more polar in nature, such as EAF$_R$. In addition, the limited ability of dichloromethane to extract polar phytochemicals such as polyphenols could explain the lower anti-inflammatory activity of DF$_R$ compared to that of EAF$_R$.

As shown in Figure 1 and Figure 2, HEE$_R$ at 30 mg/kg prevented edema better than the 10 mg/kg dose, its two fractions and aspirin at 30 mg/kg. At 10 mg/kg,

the effect was similar to that of the reference product, particularly from the 3rd hour onward. In contrast, the HEE_R fractions exhibited a weaker inhibitory effect than aspirin. The reduced anti-inflammatory activity of the fractions on rat paw edema could result from HEE_R fractionation leading to a polarity-dependent distribution of the bioactive chemical compounds between the different sub-fractions.

Unlike the roots, HEE_L at 10 mg/kg produced a greater inhibitory effect on edema than the 30 mg/kg dose from T1H to T5H. The same anti-inflammatory profile was observed with the tested fractions (EAF_L and DF_L) and it appears that they are more active than the HEE_R extract in the prevention of edema. According to this study, EAF_L at 10 mg/kg was found to be slightly more active than DF_L in preventing the increase of inflammatory edema, although similar activity was observed between them at T1H and T3H. At 30 mg/kg, edema inhibition was greater with DF_L than with EAF_L, particularly at the 1st and 3rd hours. These results suggest, overall, that the anti-inflammatory effect of HEE_L and its fractions is not dose-dependent and that both polar and nonpolar chemical compounds are involved in the anti-inflammatory activity of *Maytenus senegalensis* leaves. It was also observed that HEE_L, EAF_L and DF_L at a dose of 10 mg/kg exhibited greater anti-inflammatory activity than the reference product, particularly from the 3rd hour onward.

Comparing the roots and leaves extracts, HEE_R was found to have superior anti-inflammatory activity to HEE_L at 30 mg/kg throughout the experiment. At 10 mg/kg, HEE_L produced a slightly greater inhibition of rat paw edema than HEE_R, particularly during the 5th hours (Figure 5, Figure 6). This could be explained by the chemical diversity of compounds found in the two parts of the plant.

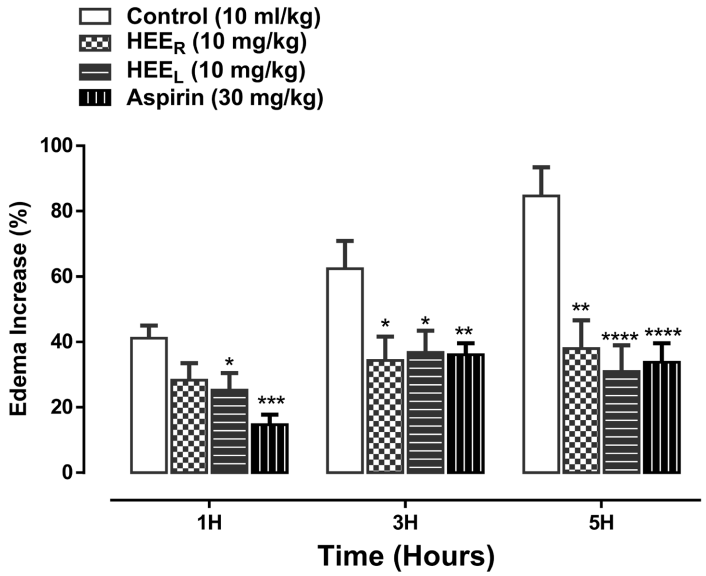

Figure 5. Comparison of the effect of HEE_L and HEE_L (10 mg/kg) and Aspirin (30 mg/kg) on carrageenan-induced inflammatory edema in rats. Results are expressed as means ± SEM. * = $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, ****= $p < 0.0001$ vs. control group. n = 5.

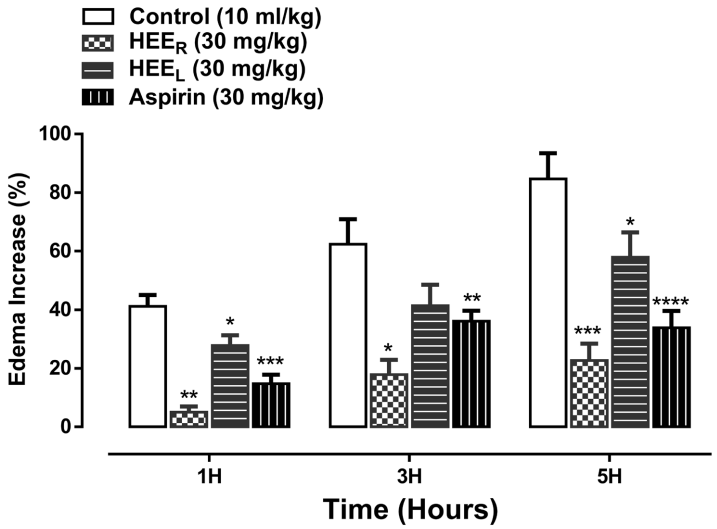

Figure 6. Comparison of the effect of HEE$_L$ and HEE$_L$ and Aspirin (30 mg/kg) on carrageenan-induced inflammatory edema in rats. Results are expressed as means ± SEM. * = $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, ****= $p < 0.0001$ vs. control group. n = 5.

Carrageenan paw edema is a test often used to investigate anti-inflammatory medicines, both steroidal and nonsteroidal because it involves numerous mediators. A sub-plantar injection of 1% carrageenan in the hind paw causes inflammation in two phases: the first initial phase, which lasts 1 to 1.5 hours after the injection is triggered by histamine, serotonin, bradykinin and platelet activating factor, while the late second lasting from 2 to more than 5 hours in which the edema reaches its peak, is characterized mainly by cellular infiltration of the inflammatory site and the release of prostaglandins into the tissues [30]-[36]. The results of this study showed that HEE$_R$, HEE$_L$ and their fractions, like aspirin, act in both the initial and late phase of inflammation. This late phase is also attributed to the induction of type II cyclooxygenase in rat paw edema [37] and anti-inflammatory drugs such as aspirin act by blocking this enzyme in the arachidonic cascade [38].

Studies have shown the important role of phytochemical components, highlighted in the present work, in mechanisms for reducing inflammation. Flavonoid-like compounds and tannins have been shown to be potent inhibitors of cyclooxygenase, an enzyme involved in prostaglandin synthesis [39]-[41]. It has also been revealed that many plant species owe their properties to the presence of flavonoids and saponins [42] [43], and that plants containing saponins, terpenoids, and alkaloids exhibit anti-inflammatory and analgesic properties [44]-[47].

In view of these results, it is suggested that the anti-inflammatory activity found in this work could be attributed to the presence of flavonoids, tannins, saponins, but also to that of other chemical compounds contained in *Maytenus senegalensis* roots and leaves.

5. Conclusion

In our study, hydro-ethanolic extracts of *Maytenus senegalensis* roots and leaves

and their ethyl acetate and dichloromethane fractions at doses of 10 and 30 mg/kg, exhibit anti-inflammatory properties in a carrageenan-induced inflammatory edema model. The anti-inflammatory activity of these extracts may be linked to the action of the bioactive chemical compounds identified in the phytochemical study. These results support the traditional use of *Maytenus senegalensis* roots and leaves to treat symptoms associated with inflammatory diseases. Further chemical and pharmacological studies would be needed to identify the chemical compounds responsible for the anti-inflammatory activity of the plant extracts.

6. Limitations

A study examining other models of inflammation induction should help to support the acute model. Quantification of the chemical compounds will complement the qualitative study in order to differentially distribute the chemical families among the extracts and their fractions.

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Conflicts of Interest

The authors declare that there is no conflicts of interest regarding the publication of this paper.

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