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Phytochemical Study and Evaluation of the Anti-Inflammatory Activity of the Ethanolic Extract of the Leaves and Stems of *Melothria maderaspatana* (Cucurbitaceae)

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Abstract

Melothria maderaspatana is a plant used in traditional African medicine to treat several ailments. The aim of this study was to determine the anti-inflammatory activity of the ethanolic extract of the leaves and stems of this plant. A phytochemical screening test was first performed to identify the presence or absence of certain families of secondary metabolites, which are generally responsible for the biological activity of plants. The rat paw inflammatory edema model, induced by injection of 1% carrageenan into the footpad, was used to assess inflammatory activity. Ethanolic extracts of the leaves and stems of *Melothria maderaspatana* were used at doses of 30 and 100 mg/kg, respectively. The phytochemical screening revealed that this plant is rich in the tested secondary metabolites, such as polyphenols, flavonoids, and alkaloids. After oral administration, the results showed that the different extracts significantly prevented ($p < 0.05$) carrageenan-induced rat leg edema. Indeed, a significant variation in the percentage of inflammatory edema of the leg was observed: 40.87% for the leaf extract and 23.43% for the stem extract (compared to 97.78% ± 4.29% when carrageenan alone was applied), after 5 hours of carrageenan injection at a dose of 30 mg/kg. For the 100 mg/kg dose, this percentage was estimated at 54.62% for the leaves and 33.2% for the stem extract after 5 hours of carrageenan injection. These results demonstrate that the plant possesses significant anti-inflammatory activity, which would justify its use in traditional African medicine for the prevention and treatment of inflammation.

Keywords

Melothria maderaspatana, Inflammatory, Carrageenan

1. Introduction

Inflammation is a non-specific defense mechanism that responds to aggression, whether endogenous, such as damaged cells, immune reactions, etc., or exogenous, such as physical agents (burns, frostbite, radiation), chemical agents (cosmetic products), or microbial agents (bacterial exotoxins, viruses). It can be local or systemic, and in all cases, it aims to maintain the body's integrity. In both endogenous and exogenous circumstances, the defense systems involved are the same, but the intensity and duration of inflammation modify and determine the type of inflammation, distinguishing between acute and chronic inflammation [1]. Their treatment is often based on the use of non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids. These molecules have harmful side effects, especially with long-term use, particularly in the treatment of chronic inflammation [2]. Taking anti-inflammatory drugs often presents gastrointestinal risks, renal risks such as acute renal failure, and sometimes cardiac complications [3]. The search for new molecules with therapeutic potential and a lower risk of side effects is essential for treating these patients. Consequently, increasing emphasis is being placed on the search for new molecules with anti-inflammatory activity extracted from medicinal plants.

Melothria maderaspatana is an annual or perennial herbaceous plant with a creeping or climbing habit and a stem equipped with tendrils. This species, which has a very wide distribution in Africa, is present in the Republic of South Africa as far north as the Senegal-Sudan border. In Asia, it is found west of Pakistan, through India, Sri Lanka, and Nepal [4]. This plant is widely used in traditional medicine to treat diabetes mellitus, scabies, cancer, flatulence, inflammation, and high blood pressure [5] [6]. This study is part of a broader effort to promote the use of plants from the African flora. Therefore, the anti-inflammatory activity of ethanolic extracts from the leaves and stems is being evaluated in order to obtain solid scientific information on the biological activity of the plant's organs.

2. Materials and Methods

2.1. Plant Material

The plant was collected in the commune of Taïba Ndiaye, a town located in the Thiès region of western Senegal, at geographic coordinates 15˚3'0"N and 16˚52'60"W. Identification was carried out at the Fundamental Institute of Black Africa (IFAN) using the LEBRUN & STORK database.

The plant material consists of the leaves and stems of *Melothria maderaspatana*. After harvesting, these parts were dried in the dark at room temperature (approximately 25˚C) for three weeks at the Bioactive Substances Research Group (GRSB)

laboratory of Cheikh Anta Diop University in Dakar. They were then ground into a fine powder using an electric grinder (Silver Crest Powder Grinder SC-200) and carefully stored in jars to prevent contamination.

2.2. Animal Material

Adult Wistar rats weighing between 120 g and 150 g were used for this study. These rats were kept in the animal facility of the Pharmacology and Pharmacodynamics Laboratory with free access to food and water. They were maintained at a constant temperature of 22˚C - 28˚C and illuminated according to a 12-hour light-dark cycle. They were acclimated one week before the start of the experiments.

2.3. Extract Preparation

The extracts were prepared by cold maceration with magnetic stirring in ethanol. Specifically, 100 g of finely powdered leaves or stems of the plant were placed in an Erlenmeyer flask with 500 mL of ethanol. The mixture was covered with aluminum foil after 48 hours of magnetic stirring in the dark at room temperature. Filtration was performed using a funnel and Whatman® brand filter paper.

The operation is repeated twice, in the presence of 300 mL of solvent, with the pomace obtained from the filtration. The resulting filtrates are combined, and the solvent is evaporated using a Buchi R-80 rotary evaporator at 60˚C, yielding the ethanolic extract [7].

2.4. Phytochemical Screening

Phytochemical screening is a qualitative analysis based on precipitation or color reactions. These reactions allow for the detection of secondary metabolites that may be present in a plant sample. In this work, the screening focuses on the detection of alkaloids, polyphenols, tannins, flavonoids, saponins, sterols and polyterpenes, leucoanthocyanins, catechols, and mucilage. The presence of these different chemical groups is demonstrated by referring to the techniques described in the work of Bedie *et al.* [8].

Polyphenols and tannins are identified by the $FeCl_3$ test and Stiasny's reagent; flavonoids, leucoanthocyanins, and catechols by the cyanidin reaction; saponins by the foam test; sterols and polyterpenes by the Liebermann-Burchard test; mucilage by the absolute ethanol test; and alkaloids by the Mayer test [9].

2.5. Evaluation of the Anti-Inflammatory Activity of Ethanolic Extracts of *Melothria maderaspatana*

Anti-inflammatory activity is evaluated using the carrageenan-induced rat paw edema method [10]. Rats, divided into six groups of four, are weighed and then fasted for 12 hours before the experiment. For each rat, the initial thickness (E0) of the left hind leg is measured using stainless-hardened calipers before treatment administration. The different treatments are administered by gavage.

- Batch 1: Physiological saline at a concentration of 10 mL/kg (control);
- Batch 2: Acetylsalicylic acid solution at a concentration of 200 mg/kg;

- Batch 3: Extract of *Melothria maderaspatana* leaves with ethanol at a concentration of 30 mg/kg;

- Batch 4: Extract of *Melothria maderaspatana* leaves with ethanol at a concentration of 100 mg/kg;

- Batch 5: Extract of *Melothria maderaspatana* stems with ethanol at a concentration of 30 mg/kg;

- Batch 6: Extract of *Melothria maderaspatana* stems with ethanol at a concentration of 100 mg/kg.

One hour after oral administration of the different solutions, each rat received an injection of 100 µl (0.1 mL) of 1% carrageenan solution under the footpad of its left hind paw. The thickness of the injected paws was measured at 1 h, 3 h, and 5 h (T1 h, T3 h, and T5 h) after carrageenan injection, again using stainless-hardened calipers. The degree of edema was assessed by determining the mean percentage increase (% Aug) in paw thickness using the following formula:

$$\%\ \text{Increase in leg thickness} = \frac{Ef - Ei}{Ei} \times 100$$

with:

Ef: final thickness of the leg; *Ei*: initial thickness of the leg.

2.6. Statistical Analysis of Results

Results are expressed as means with standard errors (Mean ± SEM). Data were graphically represented using GraphPad Prism 7.0 software (Microsoft USA). Statistical analysis was performed using analysis of variance (one-way ANOVA). Differences between means were determined using Dunnett's test. A statistically significant difference was defined as $p < 0.05$.

3. Results

3.1. Phytochemical Screening

Table 1. Phytochemical study of the ethanol extract of *Melothria maderaspatana* leaves and stems.

Researched compounds		Results	
		Leaves	Stems
Polyphenols		+	+
Tannins	Catechics	+	+
	Gallics	−	−
Sterols et polyterpenes		+	+
Flavonoids		−	+
Leucoanthocyanins et catechols		+	+
Alcaloids		+	+
Saponins		−	−
Coumarins		+	+

− = Missing; + = Present.

The phytochemical study of the ethanolic extract of *Melothria maderaspatana* leaves and stems yielded the results presented in Table 1. This table shows that the ethanolic extract of *Melothria maderaspatana* leaves and stems contains a significant quantity of polyphenols, catecholic tannins, sterols and polyterpenes, leucoanthocyanins and catechols, and coumarins, which are indicated by intense coloration. Alkaloids are present with moderate coloration in both extracts, as determined by the Dragendorff test. The Shibata reaction revealed an absence of flavonoids in the ethanolic extract of the leaves.

3.2. Induction of Edema

Injection of the 1% carrageenan solution into the left hind leg of control rats causes edema with percentage increases of 40.30% ± 8.34%, 72.70% ± 6.03% and 97.78% ± 4.29% after 1 h, 3 h and 5 h, respectively.

3.3. Leaves

Oral administration of the ethanolic extract of *Melothria maderaspatana* leaves is associated with a blockage of the increase in edema induced by carrageenan. In the presence of the ethanolic extract of *Melothria maderaspatana* leaves, the percentage increases in inflammatory edema of the leg are 23.69% ± 6.45%; 34.65% ± 13.93%; 40.87% ± 7.36% for the 30 mg/kg dose and 34.95% ± 5.32%; 48.85% ± 7.28%; 54.62% ± 4.37% for the 100 mg/kg dose. Compared to the controls, these results are highly significant for the ethanolic extract of the leaves at a dose of 30 mg/kg at 3 h ($p < 0.001$) and 5 h ($p < 0.0001$). These results are superior to those obtained with the dose of 100 mg/kg ($p < 0.001$ at 5 h), but identical to those observed with acetylsalicylic acid administered at a dose of 200 mg/kg as illustrated in Figure 1.

From the outset, these results showed that the leaf extract has a more pronounced anti-inflammatory effect at 30 mg/kg than at 100 mg/kg, suggesting an atypical dose-response relationship. This non-linear profile could reflect a biphasic effect, a phenomenon described for several plant extracts, some of whose constituents exhibit optimal activity at low doses, while at higher doses, saturation of the target molecules or the expression of compounds with an antagonistic effect may occur. The chemical composition of the extract may also contribute to this reversal: active but minor molecules could become sufficiently concentrated at high doses to diminish the overall effect. Thus, the maximum effect observed at low doses is an important observation, suggesting that the optimal dose may lie within a submaximal range. Further investigations will be necessary to clarify the mechanisms involved and confirm the biphasic nature of this response.

3.4. Stems

The results of the anti-inflammatory activity of the ethanolic extract of *Melothria maderaspatana* stems are shown in Figure 2, which presents the changes in the kinetics of left hind paw edema (LHP) increase in rats, induced by the injection of a 1% carrageenan solution into the aponeurosis of the paw (foot pad). These re-

sults are compared to those of acetylsalicylic acid, a non-steroidal anti-inflammatory drug, and to those of the oral administration of the ethanolic extract of *Melothria maderaspatana* stems, which is associated with a highly significant blockade of the increase in edema induced by carrageenan. The anti-inflammatory activity is assessed by the percentage increase (% Aug) in edema. Indeed, oral administration of the *Melothria maderaspatana* stem extract resulted in the following percentage increases: 16.08% ± 5.27%; 23.43% ± 8.21%, 22.15% ± 7.00% at a dose of 30 mg/kg (batch 5) and 19.64% ± 7.61%; 33.2% ± 8.40%, 19.61% ± 7.57% at a dose of 100 mg/kg (batch 6). The results obtained, presented in Figure 2, show that the anti-inflammatory profile appears to be better than that of the ethanolic extract of the leaves, with also better activity at the dose of 30 mg/kg, identical to that of aspirin at the dose of 200 mg/kg.

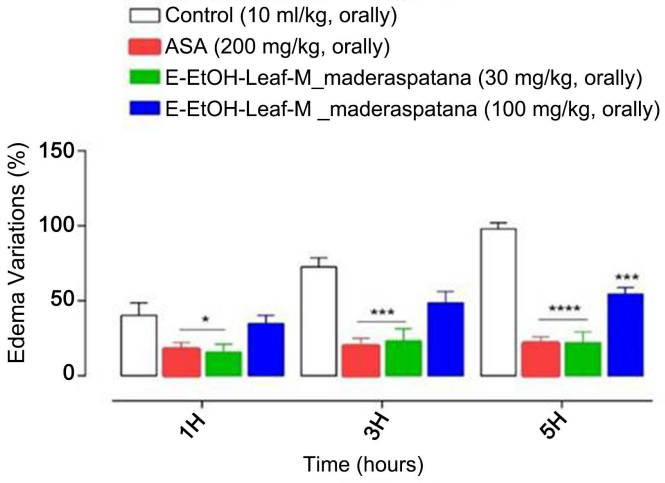

Figure 1. Mean percentage increase in edema after administration of crude ethanolic extract of *Melothria maderaspatana* leaves at doses of 30 and 100 mg/kg and aspirin at a dose of 200 mg/kg to Wistar rats.

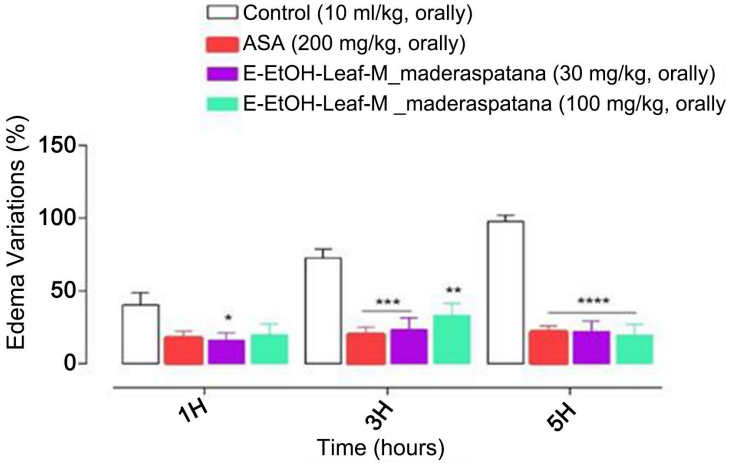

Figure 2. Mean percentage increase in edema after administration of crude ethanolic extract of *Melothria maderaspatana* stems at doses of 30 and 100 mg/kg and aspirin at a dose of 200 mg/kg to Wistar rats.

4. Discussion

This study highlighted the anti-inflammatory properties of the ethanolic extract of *Melothria maderaspatana* leaves and stems and characterized the chemical groups contained in these extracts. The induction of edema by carrageenan in the rat paw is a well-established animal model for evaluating the anti-inflammatory effect of natural products as well as synthetic chemical compounds [11]. Phytochemical characterization of the leaf and stem extracts revealed the presence of reducing compounds, tannins, sterols and polyterpenes, polyphenols, and flavonoids. These results are similar to those found by Yougbaré-Ziébrou *et al.* (2016), who attributed numerous biological properties, including anti-inflammatory activity, to terpenic compounds such as monoterpenes and sesquiterpenes [12]. Furthermore, the presence of steroid compounds, and in particular corticosteroids, is the reason for their widespread therapeutic application, notably as anti-inflammatory agents [13].

The anti-inflammatory activity, tested using the acute inflammation model (induced by carrageenan), proved effective in preventing carrageenan-induced edema. However, this anti-inflammatory effect is weak in the initial phase of the edema but significant in the later phase (5 hours). Carrageenan injection into the paw triggers a biphasic inflammatory response, the initial phase of which, lasting approximately 2.5 hours after injection, is attributed to tissue damage that induces histamine synthesis [14]. The ethanolic extract of *Melothria maderaspatana* leaves significantly reduces carrageenan-induced edema. However, the anti-edema effect of the extract is greater at the 5th hour with the dose of 100 mg/kg of the stem extract, with a percentage reduction of 19.61% ± 3.01%. The extract acted progressively on the edema and showed better prevention of induced rat leg edema. These results suggest that the ethanolic extract of the plant has an effect that counteracts the action of endogenous pro-inflammatory mediators. However, to confirm the dose-dependent activity, the number of treated samples will need to be increased, which is estimated here at four, statistically insufficient for a general conclusion. This action appears to be exerted primarily on cyclooxygenase, the enzyme responsible for prostaglandin synthesis, and is attributed to the presence of bioactive compounds such as tannins, sterols, polyterpenes, and polyphenols, which give both extracts an anti-inflammatory mechanism of action comparable to that of non-steroidal anti-inflammatory drugs [15].

Similarly, the results of the phytochemical characterization of the ethanolic extract of *Melothria maderaspatana* leaves and stems confirm the results obtained by Mame *et al.* (2023), who indeed showed the presence of tannins, flavonoids, and polyterpenes. These compounds, particularly tannins and flavonoids, inhibit oxidative stress by effectively scavenging free radicals [16]. This anti-oxidant property is essential for protecting cells from oxidative damage associated with aging and various inflammation-related diseases. Furthermore, recent work by Peña *et al.* (2024) has demonstrated colorectal anti-cancer properties, showing a potent antiproliferative effect on several colorectal cancer cell lines, as well as synergy

with oxaliplatin (OXA). Several mechanisms, including cytokinesis inhibition with G2/M phase arrest, an extrinsic apoptotic pathway, and autophagy, could be involved. The extracts could also reduce tumor invasiveness and malignancy by decreasing the clonogenic and migratory capacity of tumors and by downregulating the expression of cancer stem cell (CSC)-specific genes in tumor cells, as well as by inhibiting angiogenesis. Furthermore, the anti-oxidant and chemopreventive activities demonstrated by the extracts could be used as a strategy for the prevention of colorectal cancer [17].

However, the more pronounced anti-inflammatory activity of the ethanolic stem extract could be explained by the presence of flavonoids. Numerous *in vivo* studies have shown that flavonoids, such as quercetin and rutin, possess potent anti-inflammatory activity in models of acute and chronic inflammation by inhibiting lipid peroxidation, platelet aggregation and capillary permeability [18]-[20]. Indeed, flavanones, particularly rutin, are known for their capillary-protective properties and their ability to inhibit phospholipase A2 (PLA2) and modulate neutrophil recruitment [21]. Furthermore, quercetin, as a flavanol, exhibits a potential anti-inflammatory effect by reducing the production of various inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1), and by reducing the expression of adhesion molecules like VCAM-1 and CD80 on vascular endothelial cells, thereby reducing leukocyte extravasation [20]. Studies by Nakadate *et al.* (2025) explore the molecular mechanisms by which these compounds attenuate chronic inflammation, highlighting their potential role in disease prevention, show that these secondary metabolites modulate inflammatory pathways, such as nuclear factor (NF-κB) and mitogen-activated protein kinase, reduce oxidative stress, and inhibit pro-inflammatory cytokines [22].

5. Conclusions

Today, phytotherapy represents a true asset in the field of public health, where the diversity of biological properties is certainly linked to the therapeutic virtues attributed to an extraordinary range of bioactive molecules synthesized by the plant. The objective of this work was to conduct a phytochemical study and evaluate the *in vivo* anti-inflammatory activity of the ethanolic extract of the leaves and stems of *Melothria maderaspatana*. Phytochemical screening of the two extracts (leaves and stems) demonstrated the plant's richness in secondary metabolites. The evaluation of *in vivo* anti-inflammatory activity revealed that both parts of the plant exhibit good anti-inflammatory activity in the rat model of carrageenan-induced edema.

Based on the results obtained, it can be concluded that the ethanolic extract of the stems at a dose of 100 mg/kg showed anti-inflammatory potential after 5 hours of observation, with a significant reduction in edema corresponding to a small percentage increase. This explains and confirms the use of these plants in traditional Senegalese medicine. However, this research should be extended to other biological activities, such as cytotoxicity, in order to better understand and enhance the

therapeutic properties of this plant.

Conflicts of Interest

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

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