

Research Article

## Anti-inflammatory Activity of the Aqueous Macerate of Leaves of *Uvaria chamae* (P. Beauv) (Annonaceae) on Acute Edema of Rat Paw Induced by Carrageenan

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### ABSTRACT

The leaves of *Uvaria chamae* are used in traditional African medicine against hemorrhoids, respiratory disorders, wounds and inflammations. Despite the many virtues of the leaves of *Uvaria chamae*, little information on toxicity and anti-inflammatory activity exists. The aim of the work is to study the phytochemistry, toxicity and anti-inflammatory activity of the aqueous macerate. The model of inflammatory edema of rat paw induced by carrageenin was used for this study. Aqueous macerate of *Uvaria chamae* leaves was used at doses of 100 and 300 mg / kg body weight. After gavage, a macerated dose of 100 and 300 mg / kg significantly prevents ( $P < 0.05$ ) edema of the rat paw induced by carrageenan. The phytochemical screening revealed the presence of sterols, polyterpenes, polyphenols, flavonoids, catechin tannins, and alkaloids. The toxicological study of *U. chamae* macerate determined the LD<sub>50</sub> = 5000 mg / kg bw. The anti-inflammatory activity of the aqueous macerate shows the interest of *U. chamae* leaves in traditional African medicine to prevent or treat inflammation.

**Keywords:** *Uvaria chamae*, acute edema, carrageenan, toxicological, Anti-inflammatory.

### INTRODUCTION

The World Health Organization (WHO) faces enormous challenges in managing public health today, due to the persistence of chronic diseases and the emergence of uncontrolled diseases<sup>1</sup>. Numerous populations of tropical and subtropical regions (Africa and South America), estimated at about 80%, are more and more turned towards traditional medicine using medicinal plants to relieve or cure many pathologies<sup>2</sup>. Metabolic diseases with inflammatory, immunological and hematological components are associated with chronic diseases<sup>3,4</sup>.

Inflammation is a nonspecific defense reaction of the body to various aggressions that may be of hygienic-dietary, physical, chemical, biological (immune response) or infectious origin<sup>5</sup>. Thus, although it is essential for the survival of the aggressed organism, inflammation is no less dangerous<sup>6</sup>. The current treatment for inflammation is based on steroidal (glucocorticoid) and non-steroidal anti-inflammatory drugs such as aspirin. Although these molecules are effective, they most often have undesirable effects that can hinder their long-term use<sup>7</sup>. However,

some plants with anti-inflammatory activity could be an alternative in anti-inflammatory therapy for their better accessibility and less toxicity in general, vis-à-vis conventional anti-inflammatory<sup>8</sup>.

In Africa, among these plants, *Uvaria chamae* Jacq. is used in traditional African medicine in the management of hemorrhoids, respiratory disorders, wounds and sore throats<sup>9,10</sup>. Authors have also highlighted the antibacterial and antioxidant activities<sup>11</sup>, antifungal and anti-inflammatory<sup>12</sup> of *Uvaria chamae*. Despite some studies on *Uvaria chamae* leaves, data on phytochemistry, toxicity and anti-inflammatory activity may define the potential medical interest of this plant.

The objective of this study was to perform a phytochemical study and to evaluate the acute toxicity and anti-inflammatory activity of the aqueous macerate of *Uvaria chamae* leaves on an animal model of carrageenan-induced edema.

### MATERIALS AND METHODS

#### Equipment

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Table 1: Effect of aqueous macerate of leaves of *Uvaria chamae* on carrageenan induced rat paw edema 1% in the rat. % increase in paw volume

Treatments	Doses (mg/kg bw)	1h	2h	3h	4h	5h	6h
NaCl 0.9%		12.17±1.10	20.39±1.17	27.93±0.95	41.38±1.00	40.34±1.50	39.11±0.82
Diclofenac	50	4.89±0.43*	5.55±0.81*	6.68±0.98*	16.01±0.89*	14.36±0.62*	13.77±0.87*
<i>U.chamae</i>	100	8.97±0.38*	12.98±0.66*	20.48±0.65*	21.6±1.03*	18.12±0.54*	16.91±1.03*
<i>U.chamae</i>	300	5.89±0.61*	9.72±0.81*	17.56±1.00*	16.67±1.93*	15.33±1.03*	14.40±0.91*

The data are expressed as mean ± standard error at the mean. Significance at \*P < 0.5 versus NaCl 0.9% control.

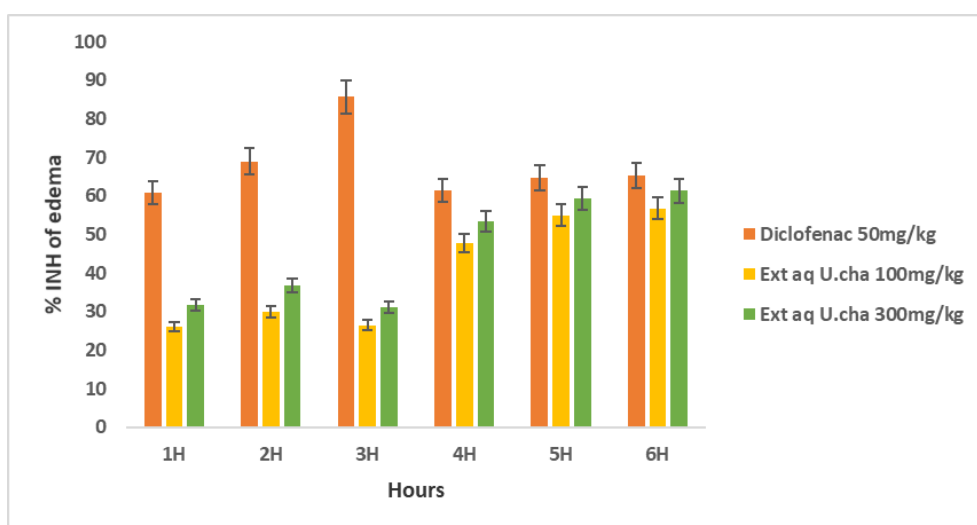


Figure 1 : Percent inhibition of acute inflammation with Diclofenac and aqueous macerate (100 and 300 mg/kg) of *Uvaria chamae* leaves.

#### Plant material

It consists of *Uvaria chamae* leaves harvested in October 2016 in the Poro (Korhogo) region of northern Côte d'Ivoire.

#### Animal material

Adult rats of Wistar strains weighing between 115 and 210 grams were used for this study. These rats were raised in the vivarium of the Ecole Normale Supérieure (ENS) of Côte d'Ivoire with free access to food and water.

#### Methods

##### Aqueous macerated leaves of *Uvaria chamae*

##### Identification and leaf powder of *Uvaria chamae*

The botanical identification was carried out at the National Floristic Center of the University Felix Houphouët Boigny (Ivory Coast). The leaves were cut into small pieces and then dried out of the sun, at room temperature (25 to 30 °C) to better preserve the molecules sensitive to heat and light for 14 days. The dried leaves were ground to a fine powder using a traditional mortar. The powder obtained was stored in an airtight bottle and was used for the preparation of the aqueous extract.

##### Preparation of the aqueous macerate

The aqueous macerate of the leaves of *Uvaria chamae* was prepared according to the method of Olakunle<sup>13</sup> with slight modifications. One hundred grams (100 g) of the plant powder was macerated in 1000 ml of distilled water with

blender stirring (Philips, Boroglass®), then wrung out in a square of clean tissue, successively filtered twice on cotton hydrophilic and once on filter paper (Whatman®3mm paper). This filtrate was then slowly dried in an oven at 50 °C to constant weight. The resulting powder (total aqueous macerate) is stored in a sealed jar and stored in the refrigerator at + 4 °C<sup>14</sup>. The yield obtained after extraction was evaluated.

##### Anti-inflammatory activity

A preventive experimental approach that tracks the evolution of tubal edema induced by the administration of carrageenin in rats has been used to evaluate the anti-inflammatory effect according to Winter<sup>15</sup>. This approach consists of pre-treating the rats with the extract before creating an inflammatory focus and finally monitoring how the treatment prevents inflammation. The rats, divided into 4 lots of 3, were weighed and then fasted 18 hours before the experiment.

For each rat, the initial diameter (Do) of the right hind paw was measured using a digital micrometer prior to treatment administration. The different treatments were administered by gavage:

Control batch (n = 3): a solution of NaCl (0.9%) due to 10 ml / kg

Reference lot (n = 3): the anti-inflammatory drug diclofenac (50 mg / kg) dissolved in 0.9% NaCl.

Table 2 : Chemical composition of the aqueous macerate of *Uvaria chamae*.

	Stérols and Polyterpenes	Polyphenols	Flavonoids	Tanins		Quinones	Alkaloids	Saponoside
				Gallic	Catechic			
Aqueous macerated	+	++	++	-	++	-	++	-

- : Absence of the compound ; + : Presence of the compound ; ++ : Strong presence of compound

Lot U.ch (n = 3): aqueous macerate of *Uvaria chamae* at a dose of 100 mg / kg of PC dissolved in 0.9% NaCl.

Lot U.ch (n = 3): aqueous macerate of *Uvaria chamae* at a dose of 300 mg / kg of PC dissolved in 0.9% NaCl.

One hour after the administration of the different treatments, each rat received by subplant injection in the right hind paw, 0.1 ml of a 0.5% carrageenan suspension dissolved in 0.9% NaCl. The diameters of the injected paws were measured using a digital micrometer after induction of edema at one-hour intervals for six hours. The importance of edema was assessed by determining the average percent increase (% AUG) in the diameter of the rat paw according to the formula: % AUG = (Dn-Do) × 100 / Do; Dn: diameter of the paw the 1st hour after the injection of carrageenan. Do: diameter of the paw before the injection of carrageenan. The anti-inflammatory activity was evaluated by calculating the percent inhibition (% INH) of the edema according to the formula: % INH = (% AUG control-% AUG treated) × 100 / % AUG control.

#### Phytochemical Screening

Phytochemical screening was performed according to the methods used by Békro<sup>16</sup>, Bedié<sup>17</sup>. It makes it possible to identify groups of chemical constituents of pharmacological interest such as sterols, polyterpenes, polyphenols, flavonoids, tannins, quinone substances, alkaloids and saponosides.

Two (2) g of lyophilizate was dissolved in 50 mL of distilled water to form the aqueous macerate of *Uvaria chamae*. The search for sterols and polyterpenes was done thanks to the reaction of Liebermann.

The reaction with ferric chloride and with cyanidine enable the compounds of the polyphenol and flavonoid group to be characterized respectively.

Then, those belonging to the group of free or combined tannins and quinonics were revealed by the reaction of Stiasny and that of Borntraeger respectively.

Finally, the search for alkaloids and saponosides has been made using general alkaloid characterization reagents and the properties of aqueous solutions containing saponosides. This study was conducted to determine the chemical constituents that could explain the effects of *Uvaria chamae*.

#### Acute toxicity

The acute oral toxicity of aqueous macerate of *U. chamae* leaves was evaluated in rats according to the recommendations of the OCDE Test Guideline 423 for the testing of chemicals<sup>18</sup>. The method used makes it possible to formulate a classification judgment of the test substance in a toxicity class delimited by previously fixed LD50 values. A mass of 2 g of the extract was dissolved in 15 ml of distilled water and homogenized for 2 min using a magnetic stirrer. The volume of solution administered to the animals was calculated according to the expression:

Let V = Volume of the solution to be administered (mL); D = Dose (mg / kg body weight);

P = weight of the animal (kg); C = Concentration of the solution to be administered (mg / mL).

The maximum volume of fluid administered as a single dose was  $V_{max} = 2\text{mL} / 100\text{g body weight}^{18}$ . The rats are placed in the laboratory at about 22 ° C for at least five days before the experiment. At each dose, three randomly selected animals for the experiment were labeled (T, C, Q) to allow individual identification.

According to guidelines 423 of the OCDE<sup>18</sup>, rats are deprived of food for 16 hours, but no water. After the fasting period, the dose was administered in a single dose by gavage using an oesophageal tube.

The animals were again deprived of food for 4 hours. The initial dose of 300 mg macerated / kg body weight was administered to the animals followed by an intermediate dose of 2000 mg macerated / kg body weight, after starvation for 4 h. The administration of the 5000 mg dose of the macerate / kg body weight is carried out when the previously treated animals have survived. The animals are observed individually and permanently for the first 30 minutes. Then they are observed every hour during the first 24 hours. Special attention is paid daily to observing the various manifestations of tremor, convulsion, salivation, diarrhea, lethargy, sleep and coma for 14 days after administration of the substance.

#### Statistical analyzes

These results are also analyzed by the ANOVA one way test followed by the Tukey test for multiple comparisons and determination of significance rates. Values of  $P \leq 0.05$  are considered statistically significant.

## RESULTS

### Anti-inflammatory activities of the aqueous macerate of *Uvaria chamae*

Table 1 shows the effects of aqueous macerate of leaves of *Uvaria chamae*, Diclofenac (50 mg / kg) and NaCl 0.9% on the evolution of carrageenin-induced edema in the left hind paw of rats (No. = 3). Injection of the carrageenin under the footpad causes a gradual increase in the volume of edema in rats treated with 0.9% NaCl solution during the 6 hours of the experiment.

In rats treated with aqueous macerate of *Uvaria chamae* leaves (100 and 300 mg / kg) as well as with Diclofenac, there was a significant decrease in the volume of edema from the fourth hour to the sixth hour (Table 1). Diclofenac (50 mg / kg) administered significantly inhibited paw edema in the first hour and reached its maximum ( $85.72 \pm 1.69$ ) at the third hour. On the other hand, the aqueous macerate of the leaves of *Uvaria chamae* (100 and 300 mg / kg) has a dose-inhibiting action depending on the edema and progresses to the sixth hour (Figure 1).

### Phytochemical Screening

The different chemical groups contained in the aqueous macerate of *Uvaria chamae* were presented in Table 2. The results of the phytochemical characterization revealed that the aqueous macerate used for the anti-inflammatory and acute toxicity tests contained sterols, polyterpenes, polyphenols, flavonoids, catechin tannins, and alkaloids (Table 2).

### Acute toxicity

With respect to the results of the acute toxicity study, there were no signs of toxicity following doses of 300, 2000 and 5000 mg / kg body weight (bw) of aqueous macerate of *Uvaria chamae*. All animals survived after 14 days of observation, implying that the LD50 is greater than 5000 mg / kg bw.

### DISCUSSION

Oral administration of aqueous macerate of *Uvaria chamae* leaves has been shown to be dose-dependent (100 and 300 mg / kg) effective in the prevention of carrageenan inflammatory edema. This same observation was made by Leme<sup>19</sup>. An increase in rat paw volume of all batches was noted during the six hours after carrageenin injection in control and treated rats. However, the increase in paw volume in the control group was greater than the treated groups. This increase indicates that carrageenin induced an inflammatory reaction causing edema. (Table 1).

Indeed, carrageenan is a sulfated mucopolysaccharide from a rhodophyceae, it causes inflammation typically related to the activation of cyclooxygenase<sup>20</sup>. It is known that in living animals, carrageenin in a first phase causes the synthesis of chemical mediators such as histamine and serotonin which maintain inflammation<sup>20</sup>. In a second phase, this reference molecule induces the synthesis of mainly prostaglandins, proteases and lysosomes. This last step is sensitive to synthesis antagonists of prostaglandins and natural or synthetic anti-inflammatories such as glucocorticoids<sup>21,22,23</sup>.

In addition, the administration of Diclofenac at a dose of 50 mg / kg orally significantly prevents the increase in paw volume of the rats. It is  $4.89 \pm 0.43$ ,  $5.55 \pm 0.81$ ,  $6.68 \pm 0.98$ ,  $16.01 \pm 0.89$ ,  $14.36 \pm 0.62$  and  $13.77 \pm 0.87$  at the 1st, 2nd, 3rd, 4th, 5th and 6th hour respectively after administration. carrageenan. These results are significantly different from those of physiological control *Uvaria chamae* aqueous macerate was found to be more active (61.29%) precisely at the sixth hour with the 300 mg / kg bw dose compared to the same macerated at the same time at the dose. 100 mg / kg bw with edema inhibition of the order of 56.75%. These results are consistent with several studies showing that the macerated anti-inflammatory activity can be explained in part by the presence in the leaves of polyphenolic compounds such as tannins and flavonoids<sup>24,25,26</sup>.

In addition, the phytochemical screening revealed the presence of sterols and polyterpenes, polyphenols, flavonoids, tannins and alkaloids in the aqueous macerate. Flavonoids, natural antioxidants, have been reported to play a very important role in the treatment of inflammations, tumors and bacterial diseases<sup>27,28</sup>.

Single administration of aqueous macerate of *U. chamae* by the oral route revealed a lethal dose of 50% (LD50) greater than 5000 mg / kg bw. According to the OCDE Globally Harmonized Classification System<sup>18</sup>, which means that the aqueous macerate in this study can be categorized as Category 5 and considered a non-toxic substance by the oral route.

### CONCLUSION

The significant results obtained during this study showed an anti-inflammatory effect of the aqueous macerate of leaves of *Uvaria chamae* on the acute model of carrageenan inflammatory edema in the rat. At high dose, this activity would be more important and could be related to the inhibition of cyclooxygenases and lipoxygenases in the late phase of inflammatory edema to carrageenan. These results constitute a scientific basis that justifies the traditional use of *Uvaria chamae* in the management of pathologies with inflammatory components.

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### DECLARATION OF INTEREST

The authors declare that there is no conflict of interest.

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