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# Evaluation of Anti-inflammatory Activities of *Erythrococca anomala* Aqueous and Ethanolic Extracts from Leaves in Rat

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author MBAP designed this study, reviewed of literature, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author YHF supervised and corrected this study. Authors OT and KSG managed the analyses of this study. Author KYKF contributed to the manuscript correction. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** This study was therefore aimed to evaluate potential anti-inflammatory activity of Aqueous and Ethanolic extracts of *Erythrococca anomala* leaves (Euphorbiaceae) and to determine the most active extract in rat.
**Study Design:** *Erythrococca anomala* leaves were collected from Yakasse-Me area in the Department of Adzopé (Côte d'Ivoire). The plant was identified and authenticated by the Botanic Laboratory, University Felix Houphouet Boigny, Cocody (Côte d'Ivoire).

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**Place and Duration of Study:** Analysis on the plant samples was done in pharmacodynamic Biochemistry Laboratory, University Felix Houphouet Boigny, the Laboratory of Organic Chemistry and Natural Substances, University Felix Houphouet Boigny and the Laboratory of Pharmacology, University Felix Houphouet Boigny between April and May 2016.
**Methodology:** The anti-inflammatory activities were investigated by utilizing carrageenan induced paw edema and CRP (C-reactive protein) concentration in rat. These extracts were administrated intraperitonealy at different doses (100 and 200 mg/kg body weight) to rats.
**Results:** Both extracts with 200 mg/kg.b.w. showed a good anti-inflammatory activity compared to Diclofenac (25 mg/kg). This study also showed an increased CRP concentration ($p$<0.05). However, the inflammation inhibition is raised more than 26.25% with the aqueous extract compared to ethanolic extract.
**Conclusion:** This study showed that aqueous and ethanolic extracts of *Erythrococca anomala* leaves possess a potential anti-inflammatory properties. However, this anti-inflammatory activity is more raised with aqueous extract and seems to have the most active extract. So aqueous and ethanolic extracts can be utilized for therapeutic purposes.

*Keywords: Erythrococca anomala; anti-inflammatory; carrageenan; diclofenac; CRP.*

## 1. INTRODUCTION

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents as well as to remove the consequent necrosed cells and tissue [1]. Medical plants are believed to be an important source of new chemical substances. Many herbal preparations are being prescribed widely for the treatment of inflammatory condition [2]. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs [3].

*Erythrococca anomala* (Euphorbiaceae) is an annual plant with popular usage in traditional medicine. Phytochemical study for *Erythrococca anomala* revealed the presence of saponins, sterols, phenols and flavonoids in the leaves [4]. These different types of chemical interground blended highlighted in extracts of this plant have therapeutic effects. It can be used for the treatment of human and animal diseases [5].

In Ivory Coast, leaf powder, alone or mixed with that of *Psychotria peduncularis* and clay works by friction against malaria and meningitis in children [6]. In Cameroon, the decoction or maceration of laxative and purgative leaves is taken to expel tapeworms or treat dental pain while in Nigeria the bark is used against arthritis and rheumatism [7]. But, little information was recorded on the anti-inflammatory activity of *Erythrococca anomala.*

The present investigation was carried out to evaluate the anti-inflammatory potential of aqueous and ethanolic extracts of *Erythrococca anomala* and to determine the most active extracts.

## 2. MATERIALS AND METHODS

### 2.1 Samples Collection and Extraction

*Erythrococca anomala* plant was collected in January 2015 from Yakasse-me area in the Department of Adzopé (Côte d'Ivoire). The plant was identified and authenticated by the Laboratory of Botanic, University Felix Houphouet Boigny. The authentically identified plant material (leaves) was washed and dried for 3-4 days in laboratory at room temperature. It was powdered and subjected to different extraction procedures.

#### 2.1.1 Aqueous extract

The aqueous extract was prepared by decoction method [8]. The powdered material was suspended in distilled water (100 g/1000 mL) agitated with an agitator for 24 h at 80°C. The extract was filtered through absorbent cotton then with Whatman N°1 filter paper to get the filtrate. The filtrate was dried under reduced pressure using a rotary flash evaporator and stored at a temperature of -4°C until use.

#### 2.1.2 Ethanolic extract

The powder plant material (100 g) was soaked in 1 L of 70% ethanol, agitated with an agitator for 24 h at 80°C. The extract was filtered and

concentrated to dryness using a rotary flash evaporator and stored at a temperature of -4℃ until use.

## 2.2 Experimental Animals

Wistar albinos rats weighing 190-200 g of each sex kept for three weeks at the laboratory animal home of UFR Pharmaceutical and Biologic Sciences, University of Felix Houphouet Boigny, Côte d'Ivoire were used. The animals were maintained under standard housing conditions: temperature (27℃±1C), humidity (55-60%), light/dark cycle (12:12 h) and had free access to standard rodent pellet diet and water ad libitum.

## 2.3 Test for Anti-inflammatory Activity

Anti-inflammatory activity on the extracts was measured using carrageenan induced rat paw edema essay [9-10]. Extracts of *Erythrococca anomala* leaves were dissolved in normal saline (9%) and administrated intraperitonealy [11].

### 2.3.1 Carrageenan induced paw edema model

Thirty six rats either sex were divided into six groups (n=6). Different groups of animals were pretreated with 100 or 200 mg/kg.b.w. Diclofenac drugs [12-13]. The control group received normal saline (9%). All the drugs were administrated intraperitonealy to rats. After 1 h, inflammation was induced by injection of 0.2 mL [11-14] carrageenan (1% suspension of carrageenan in normal saline) into the plantar surface of the right hind paw. The paw diameter was measured at hourly intervals for 6 and 24 hours using digital paw edema meter. The importance of the edema was assessed by measuring the diameter of the paw edema over time and determining the percentage inhibition of edema (% INH).

> % INH = (% AUG control-% AUG treated) x100 / % AUG control
>
> % AUG control and % AUG treated are percentage of augmentation edema diameter of control and treated group respectively.

### 2.3.2 Quantitative measurement of rat C-reactive-protein (CRP) in serum

Thirty rats were divided into 6 groups, each containing six animals. Group I received normal saline 9% (control). Group II received 0.2 mL carrageenan, a phlogistic agent. Group III, IV and V received (200 mg/kg. b. w.) aqueous and ethanolic extracts and 25 mg/kg.b.w. Diclofenac respectively with 0.2 mL carrageenan after 1 h. After 5 h carrageenan administration, all the animals were sacrified and blood samoles were collected and serum was separated. The dosage of the CRP was performed according to the method of Immunoturbidimetry improved particles in the COBAS C311 (Roche Diagnostic GmbH, Mannheim; Germany) [15]. It is based on the principle of agglutination of latex particles coated with specific antibodies.

## 2.4 Statistical Analysis

The values expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using one way ANOVA followed by Dunnett's test to significant, *p* values less than 0.05 were considered significant.

## 3. RESULTS AND DISCUSSION

### 3.1 Carrageenan-induced Paw Edema Model

The results showed a significant reduction in the paw diameter with 200 mg/kg.b.w of extracts (aqueous, ethanolic) and Diclofenac treated group, from first hour to 24$^{th}$ hour as compared to 100 mg/kg.b.w of the two extracts (Table 1). Indeed, there was a significant reduction in the paw diameter observed with Diclofenac treated from 1$^{st}$ h to 6$^{th}$ as compared to control group. Groups treated with the extracts of *Erythrococca anomala* leaves at dose of 100 mg/kg.b.w showed significant decrease in paw edema diameter at 2$^{nd}$ h ($p$< 0.05) and sixth hour compared to control group. Groups treated with the aqueous extract of *Erythrococca anomala* leaves at dose of 200 mg/kg showed significant decrease in paw edema diameter at 2$^{nd}$ and 6$^{th}$ h ($p$<0.001) compared to the control group.

However, with aqueous extract (200 mg/kg.b.w) the inflammation inhibition is raised more than 26.25% compared to ethanolic extract and 3.09% compared to Diclofenac (Table 2).

Indeed, inhibition of carrageenan induced hind paw edema in rats by Diclofenac started at 1st h and which was maintained up to 24$^{th}$ h. Diclofenac (25 mg/kg ) at 1$^{st}$, 2$^{nd}$ , 3$^{rd}$, 4$^{th}$, 5$^{th}$, 6$^{th}$, and 24$^{th}$ h had shown 84.07, 71.15, 57.63, 79.65, 88.8, 87.63 and 93.94% inhibition, respectively. At the dose of 200 mg/kg the aqueous extract seems have the same inhibition such as Dilofenac and had shown 83.46, 61.18, 51.99, 78.50, 88.35, 87.39 and 90.74% inhibition, respectively from the 1$^{st}$ to 24$^{th}$ hour.

**Table 1. Effect of aqueous and ethanolic extracts of *Erythroccoca anomala* leaves and diclofenac on carrageenan induced paw in rats**

| Treatment groups | Edema diameter (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dose (mg/kg) | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 24 hr |
| Normal saline (control) | 9% | 5.75±7.5 | 9.30±4.5* | 9.82±4.8* | 11.13±0.21** | 11.37±7.1** | 11.39±1.2** | 10.78±7 * | 10.11±0.2** |
| Aqueous extract | 100 | 5.78±2.5 | 6.33±4.7 | 7.05± 7.7 | 7.34± 0.19 | 6.25±2.55 | 6.08±5.5 | 6.03±1 | 5.99±0.27 |
| Aqueous extract | 200 | 5.45±4.55 | 5.78±0.21 | 6.69±0.20 | 7.71±0.19 | 6.23±0.16 | 5.85±1.53 | 5.89±2 | 5.70±0.77 |
| Ethanolic extract | 100 | 5.45±4.55 | 7.54±0.18 | 7.78±0.24 | 8.28±1.36 | 8.96±1.33* | 9.43±4.12* | 9.12±2* | 8.34±3.25* |
| Ethanolic extract | 200 | 5.07±1.75 | 6.78±2.14 | 7.09±0.43 | 7.36±0.77 | 7.86±4.67* | 8.03±0.23* | 7.43±4* | 7.10±5.33* |
| Diclofenac (standard) | 25 | 5.45±1.25 | 5.76±0.75 | 6.32±3.76 | 6.93±1.12 | 6.18±1.77 | 5.82±2.67 | 5.88±3 | 5.54±3.79 |

*Each value is mean ± SEM, N= 6 rats, the data was analyzed by using One Way ANOVA followed by Dunnett's test **p<0.001, *p<0.05, where Extracts and control were compared with Diclofenac*

**Table 2. Percentage inhibition of paw edema exhibited by aqueous and ethanolic extracts of *Erythroccoca anomala* leaves**

| Treatment groups | Inhibition percentage (%) at various times intervals | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dose (mg/kg) | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr | 24 hr | Means of inhibition |
| Normal saline (control) | 9 % | - | - | - | - | - | - | - | - |
| Aqueous extract | 100 | 66.74 | 51.49 | 47.53 | 71.91 | 71.91 | 84.76 | 84.83 | 70.14±0.18 |
| Aqueous extract | 200 | 83.46 | 61.18 | 51.99 | 78.50 | 88.35 | 87.39 | 90.74 | 77.32±0.28 |
| Ethanolic extract | 100 | 49.54 | 50.12 | 41.89 | 36.13 | 33.50 | 35.01 | 59.45 | 43.66±0.30 |
| Ethanolic extract | 200 | 53.57 | 53.70 | 43.92 | 42.32 | 43.57 | 58.17 | 62.25 | 51.07±0.20 |
| Diclofenac (standard) | 25 | 84.07 | 71.15 | 57.63 | 79.65 | 88.8 | 87.63 | 93.94 | 80.41±0.19 |

### 3.2 Effect of the Two Extracts and Diclofenac on Serum Levels CRP at 5th Hour during Carrageenan Induced Hind Paw Edema in Rats

This study showed an increased CRP concentration ($p< 0.001$) at rats treated with carrageenan with regard to extracts and Diclofenac rats groups (Fig. 1). But there is no significant difference between CRP concentration with extracts and Diclofenac rats groups.

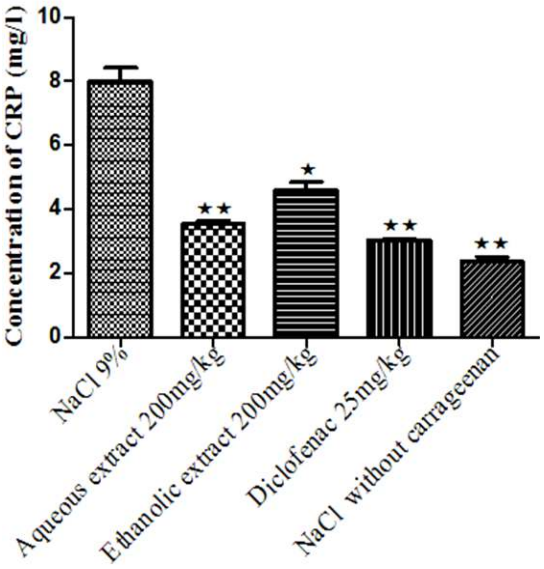


**Fig. 1. Changes in the level of seum crp of rats treated with aqueous, ethanolic extracts and diclofen AC at 5th hours during carrageenan induced hind paw edema**

*★p< 0.05; ★★ p< 0.001*

Finaly, this anti-inflammatory activity is more raised with aqueous extract and seems to be very active effect. *Erythroccoca anomala* leaves seem to led anti-inflammatory-mediator released. In the light of the results on the Tables 1 and 2, *Erythroccoca anomala* leaves inhibit edema similarly to Diclofenac. Therefore the anti-inflammatory activity of *Erythroccoca anomala* leaves seemed to be effective in the two phases of acute inflammation.

The only anti-inflammatory activity of *Erythroccoca anomala* leaves may be due to inhibition of inflammation mediators such as histamine, serotonin released during the first phase of inflammation. Prostaglandins, bradykinins and leucotrienes are released during the second phase of this inflammation.

Actually, Carrageenan induced paw edema is widely used for determining the acute phase of inflammation [14]. Carrageenan as a phlogistic agent non antigenic and is devoid of apparent systemic effect [15].

The model is based on the principle of release of various inflammatory mediators by carrageenan [16]. Edema formation due to carrageenan in the rat paw is biphasic [17]. The first phase mainly due the release of histamine and serotonin in the first hour. The late phase is sustained by prostaglandin release and mediated by bradykin, polynuclear and prostaglandins produced by tissue macrophages [18]. Anti-inflammatory drugs inhibit different stages of inflammation [19]. C-reactive protein (CRP) is the classic and sensitive marker of systemic inflammation.

The body produces C-reactive protein during the general process of inflammation. CRP is a marker for inflammation, meaning its presence indicates an increased state of inflammation in the body. Measurement of CRP may provide a useful method of assessing the prognosis of disease with systemic inflammation [20].

However, CRP synthesized by the liver cells, plays an important role in innate immunity by its properties opsonization, activation of complement and receptor binding immunoglobulins [21]. The site secretion main, but not exclusive hepatocytes is responsible for basal levels of plasma CRP. During an inflammatory response, its output increases. Secretion exists in neurons where production is increased in dementia of the Alzheimer type [22] in some cells [23] and finaly even within atherosclerotic plaques [24].

Our study indentified and characterized the direct interaction between inflammation and CRP. Indeed, treatment with aqueous and ethanolic extracts of *Erythroccoca anomala* leaves and Diclofenac after 5th hour of carrageenan administration a decreased CRP level as compared to reflect the inhibition of inflammation.

In view of these results of inhibition, we note that the aqueous extract reduced more than ethanolic extract and is almost identical to the reference molecule. So, the average value of reduction is more than 26.25% with regard to the reference molecule.

## 4. CONCLUSION

The anti-inflammatory effect of *Erythroccoca anomala* leaves extracts was evaluated in this

work. The results showed that the extracts have anti-inflammatory activity. Though, this activity is different depending on the degree solubility of secondary compounds from *Erythroccoca anomala* leaves. Aqueous extract from the leaves of *Erythroccoca anomala* seem to be more active than the ethanolic extract. Also this activity is comparable to those obtained with reference molecule such as diclofenac.

## ETHICAL APPROVAL

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University Félix Houphouet-Boigny. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals. All efforts were made to minimize animal suffering and reduce the number of animals used.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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