

Original Research Article

**EVALUATION OF ANTI-INFLAMMATORY ACTIVITIES OF
ERYTHROCOCCA ANOMALA AQUEOUS AND ETHANOLIC LEAVES
EXTRACTS IN RAT.**

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 of Felix Houphouet Boigny University.

Place and Duration of Study: Analysis on the plant samples was done in-pharmacodynamic Biochemistry Laboratory of Felix Houphouet Boigny University, the Laboratory of Organic Chemistry and Natural Substances of Felix Houphouet Boigny University and the Laboratory of Pharmacology of Felix Houphouet Boigny University (Côte d'Ivoire) between April and May 2016.

Methodology: The anti-inflammatory activities were investigated by utilizing carrageenan induced paw edema and CRP concentration in rat. These extracts were administrated intraperitoneally at different doses (100 and 200mg/kg body weight) to rats.

Result: Both extracts with 200mg/kg.b.w. showed a good anti-inflammatory activity compared to Diclofenac (25mg/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 (table 2)

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.

30 1. INTRODUCTION

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

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

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

50 The present investigation was carried out to evaluate the anti-inflammatory potential of
51 Aqueous and Ethanolic extracts of *Erythrococca anomala* and to determine the most active
52 extracts.

53

54 2. MATERIALS AND METHODS

55 2.1 Samples Collection and Extraction

56 *Erythrococca anomala* plant was collected in January 2015 from Yakasse-me area in the
57 Department of Adzopé (Côte d'Ivoire). The plant was identified and authenticated by
58 theLaboratory of Botanic of Felix Houphouet Boigny University. The authentically identified

59 plant material (leaves) was washed and dried for 3-4 days in laboratory at room temperature.
60 It was powdered and subjected to different extraction procedures.

61 **2.1.1 Aqueous extract**

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

67 **2.1.2 Ethanolic extract**

68 The powder plant material (100g) was soaked in 1L of 70% ethanol, agitated with an agitator
69 for 24h at 80°C. The extract was filtered and concentrated to dryness using a rotary flash
70 evaporator and stored at a temperature of -4°C until use.

71 **2.2 Experimental Animals**

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

77 **2.3 Test for Anti-inflammatory Activity**

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

81 **2.3.1 Carrageenan induced paw edema model**

82 Thirty six rats either sex were divided into six groups (n=6). Different groups of animals
83 were pretreated with 100 or 200mg/kg.b.w. Diclofenac drugs [12-13]. The control group
84 received normal saline (9%). All the drugs were administrated intraperitoneally to rats. After
85 1h, inflammation was induced by injection of 0.2mL [11-14] carrageenan (1% suspension of
86 carrageenan in normal saline) into the plantar surface of the right hind paw. The paw
87 diameter was measured at hourly intervals for 6h and 24h 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).

$$\% \text{ INH} = (\% \text{ AUG control} - \% \text{ AUG treated}) \times 100 / \% \text{ AUG control}$$

% 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 0.2mL carrageenan, a phlogistic agent. Group III, IV and V received (200mg/kg. b. w.) aqueous and ethanolic extracts and 25mg/kg.b.w. Diclofenac respectively with 0.2mL carrageenan after 1h. After 5h carrageenan administration, all the animals were sacrificed and blood samples 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 $\pm$ 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 200mg/kg.b.w of extracts (aqueous, ethanolic) and Diclofenac treated group, from first hour to 24th hour as compared to 100mg/kg.b.w of the two extracts (table 1). Indeed, there was a significant reduction in the paw diameter observed with Diclofenac treated from 1st h to 6th as compared to control group. Groups treated with the extracts of *Erythrococca anomala* leaves at dose of 100mg/kg.b.w showed significant decrease in paw edema diameter at 2nd h ($p < 0.05$) and sixth hour compared to control group. Groups treated with the aqueous extract of *Erythrococca anomala* leaves at dose of 200mg/kg showed significant decrease in paw edema diameter at 2nd and 6th h ($p < 0.001$) compared to the control group.

However, with aqueous extract (200mg/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 24th h. Diclofenac (25mg/kg) at 1st, 2nd, 3rd, 4th, 5th, 6th, and 24th h had shown 84.07, 71.15, 57.63, 79.65, 88.8, 87.63 and 93.94% inhibition, respectively. At the dose of 200mg/kg the aqueous extract seems have the same inhibition as Diclofenac and had shown 83.46, 61.18, 51.99, 78.50, 88.35, 87.39 and 90.74% inhibition, respectively from the 1st to 24th hour.

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.

Finally, this anti-inflammatory activity is more raised with aqueous extract and seems have an active effect. *Erythroccoca anomalaleaves* seem contained several agents anti-inflammatory-mediator released. In the light of the results on the table 1 and 2, *Erythroccoca anomala* leaves inhibit edema similarly to Diclofenac. So anti-inflammatory activity of *Erythroccoca anomala* leaves seemed to be effective in the two phases of acute inflammation.

Therefore, the anti-inflammatory activity of *Erythroccoca anomalaleaves* 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.

Indeed, 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].

147 **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)	0hr	1hr	2hr	3hr	4hr	5hr	6hr	24hr
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	(100mg/k)	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	(200mg/k)	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	(100mg/k)	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	(200mg/k)	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 mg/kg)	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 *Erythrococa anomala* leaves

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

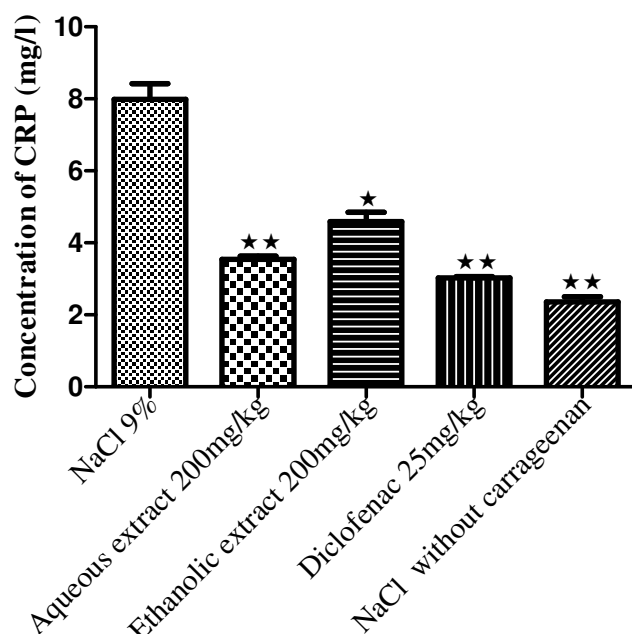


Figure 1 : Changes in the level of serum CRP of rats treated with aqueous, ethanolic extracts and diclofenac at 5th hours during carrageenan induced hind paw edema.

* $p < 0.05$; ** $p < 0.001$

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

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

177 However, CRP synthesized by the liver cells, plays an important role in innate immunity by
 178 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 finally even within atherosclerotic plaques [24].

Our study indentified and characterized the direct interaction between inflammation and CRP. Indeed, treatment with aqueous and ethanolic of *Erythroccoca anomala* leaves and Diclofenac after 5th hour of carrageenan administration a decreased CRP level as compared reflecting 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 obtained showed 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* seemed more active than the ethanolic extract. Also this activity is comparable to those obtained with reference molecule such as diclofenac.

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