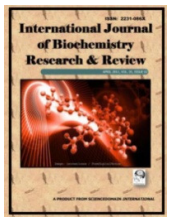

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Evaluation of Anti-Inflammatory Activities of Aqueous and Ethanolic Extracts of *Gomphrena celosioides* Linn.

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Authors' contributions

This work was carried out in collaboration between all authors. Author GK designed the study, reviewed of literature, *performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author FYH supervised and corrected this study. Authors AAE, YD, JDA, FYA and DNJ managed the analyses of this study. 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 *Gomphrena celosioides* (C. Mart) and to determine the most active extract in rat.
Study Design: *Gomphrena celosioides* was collected from Bingerville, District of Abidjan (Cote d'Ivoire). The plant was identified and authenticated by the "Centre National de Floristique", University Felix Houphouet Boigny, Cocody.
Place and Duration of Study: Analysis on the plant samples were done at the ''Laboratoire de Pharmacodynamie-Biochimique, UFR Biosciences, Université de Cocody-Abidjan (Côte d'Ivoire), and Institut Pasteur de Côte d'Ivoire, Département de Biochimie médicale & fondamentale''

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between September 2013 and June 2014.
Methodology: The anti-inflammatory activities were investigated by utilizing carrageenan induced paw edema and CRP concentration in rat. These extracts were administrated intraperitoneally at differents doses (100 and 200mg/kg body weight) to rats.
Result: The present study showed a significant anti-inflammatory activity at 200mg/kg. b.w. to both extracts which were comparable to the Diclofenac (10mg/kg) inhibition.
However, the inflammation inhibition is raised more than 2.6% with the ethanolic extract when this comparison is made with the aqueous extract. This study showed an increased CRP concentration ($p < 0.05$) at rats treated with carrageenan with regard to extracts and Diclofenac rats groups.
But there is no significant difference between CRP concentration with extracts and diclofenac rats groups ($p > 0.05$).
Conclusion: This study showed that aqueous and ethanolic extracts of *Gomphrena celosioides* have a potential anti-inflammatory properties. However, this anti-inflammatory activity is more raised with ethanolic extract and seems to have the most active extract. So, ethanolic and aqueous extracts can be utilized for therapeutic purposes.

Keywords: Gomphrena celosioides; 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]. Some Inflammation standard drugs are Indomethacin, Diclofenac and Voveran. So, there is a need to continue to research newer anti-inflammatory drugs. Medecinal plants are believed to be an important source of new chemical substances. Many herbal preparations are being prescribed widely for the treatment of inflammatory conditions [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].

Gomphrena celosioides Mart (*Amaranthaceae*) is an annual herb with popular usage in traditional medecine. In Africa*, Gomphrena celosioides* is called in traditional language Adukowé [4]. An early pharmaco-chemical study of *Gomphrena celosioides* revealed the presence of saponins, steroids, aminoacids and non-reducing sugars in all the plant parts, phenols and flavonoids in leaves, inflorescences and stem. Betacyanins occurred only in the stem; reducing sugar in the inflorescence, while ketoses were reported found in the root and stem [5,6]. Similar metabolites were found by de Moura et al. [7] in this plant.

Phytochemical study of the Aqueous extract showed that this plant contains Flavonoids, Saponins, sterols and tri-terpens, tanins, coumarins, reducers compounds, sugars and holosises [8]. The ethyl acetate and methanol extracts of *Gomphrena celosioides* was phytochemically examined by Dosumu et al. [9,10] and revealed the presence of secondary metabolites. These metabolites include alkaloids, tannins, saponins, steroids, glycosides and reducing sugars.

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 [11]. In South America, it is used as abortive [12]. Vieira [13] have demonstrated the analgesic, tonic, carminative and diuretic properties of this plant. Recently, Dosumu et al. [14] reported its antimicrobial and anti-helminthic properties. Crude and ethanol extract of the leaf of the plant were found to have antioxidant/antidegradative activity [15]. But, little information was recorded on the anti-inflammatory activity of *Gomphrena celosioides*. In Africa, different plant parts are used in traditional medecine, either as powder soaked in water or ethyl alcohol, or as a toothpick to chew [16].

The present investigation was carried out to evaluate the anti-inflammatory potential of Aqueous and Ethanolic extracts of *Gomphrena celosioides* and to determine the most active extract.

2. MATERIALS AND METHODS

2.1 Samples Collection and Extraction

Gomphrena celosioides plants was collected in September 2013 from Bingerville, District of Abidjan (Cote d'Ivoire). The plant was identified

and authenticated by botanist of "Centre National de Floristique", University Felix Houphouet Boigny, Cocody The authentically identified plant material (roots, leaves, flowers and stems) was washed and shade air- dried for 2-3 days in the 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 [17]. The powdered material was suspended in distilled water (100g/1000mL) agitated with an agitator for 24h 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 (100g) was soaked in 1L of 70% ethanol, agitated with an agitator for 24h at 80°C. The extract was filtered and concentrated to dryness using a rotary flash evaporator and stored at a temperature of -4°C until use.

2.2 Experimental Animals

Wistar albinos rats weighing 180- 200g of each sex kept for two weeks at the laboratory Animal home of the Faculty of Pharmaceutical (Biochemistry), University of Felix Houphouet Boigny, Cote d'Ivoire were used. The animals were maintained under standard housing conditions: temperature (27°±1C), humidity (55-60%), light/dark cycle (12:12h) and had free access to standard rodent pellet diet (products of FACI®, Côte d'Ivoire) and water *ad libitum*.

2.3 Tests for Anti-inflammatory Activity

Anti-inflammatory activity at the extracts was measured using carrageenan induced rat paw edema essay [18,19]. Extracts of Gomphrena were dissolved in normal saline (0.9%) and administrated intra-peritoneally [20].

2.3.1 Carrageenan–induced paw edema model

Thirty six rats of either sex were divided into six groups (n=6). Different groups of animals were pretreated with 100 or 200mg/kg.b.w. of each extract or 10mg/kg.b.w. Diclofenac as reference drug [21,22]. The control group received normal saline (0.9%). All the drugs were administrated intra-peritoneally to rats. After 1h, inflammation was induced by injection of 0.2mL [20,23] 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 h and at 24h hours using digital paw edema meter**.** Percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{(Vc - Vt) \times 100}{Vc}$$

Where Vc and Vt are average 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 0.9% (control). Group II received 0.2ml carrageenan, a phlogistic agent. Groups III, IV and V received (200mg/kg.b.w) aqueous and ethanolic extracts and 10mg/kg.b.w. Diclofenac respectively. + 0.2ml carrageenan after 1h. After 5hr of carrageenan administration, all the animals were sacrificed and blood samples were collected and serum was separated. Serum levels of CRP (C Reactive Protein, Rat ELISA Kit, ab108827) were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit supplied by abcam ®, UK, as per the manufacturer's instructions.

Essay procedure [24]

All materials and prepared reagents were equilibrated prior to use. Standards, controls and samples were prepared in duplicate. Reagents, working standards and samples were prepared and equilibrated as instructed. The assay was performed at room temperature (18-25°C). Excess microplate strips from the plate frame was removed and returned immediately to the foil pouch with desiccant inside. The pouch was resealed securely to minimize exposure to water vapor and stored in a vacuum desiccators. 50µL of C Reactive Protein Standard or sample were added to the wells and covered with a sealing tape and incubated for two hours. The timer started after the last sample addition. The plates were washed five times with 200µl of the wash Buffer manually and then inverted each time and the contents were decanted on absorbent paper towel to remove the liquid. 50µL of 1X Biotinylated C Reactive Protein Antibody were

added to each well and the plates were incubated for one hour. The microplates were washed as described above. 50µL of 1X SP Conjugate were added to each well and incubated for 30 minutes. The microplates reader were turned on and the program was set up in advance. The microplates were washed as described above.50µL of Chromogen Substrate were added per well and incubated for about 20 minutes or till the optimal blue colour density developped. 50µL of Stop Solution were added to each well. The color should be changed from blue to yellow. The absorbance was read on a microplate reader at a wavelength of 450 nm immediately.

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 verify the 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 estracts (aqueous, ethanolic) and Diclofenac treated group, from 1sth to 24thh as compared to 100mg/kg.b.w of the two extracts (Table1). Indeed, there was a significant reduction in the paw volume observed with Diclofenac treated group from 1st h to 6th h as compared to control group. Groups treated with the extracts of Gomphrena celosioides at dose of 100mg/kg showed significant decrease in paw edema volume from at 2nd h (p<0.05) and 6th h compared to control group. Groups treated with the ethanolic extract of Gomphrena celosioides at dose of 200mg/kg showed significant decrease in paw edema volume from at 2nd and, 6th h (p<0.01) compared to control group.

However, with ethanolic extract (200mg/kg.bw) the inflammation inhibition is raised more than 2.6% when this comparison is made with the aqueous extract and 0.39% with diclofenac (Table 2).

Indeed , inhibition of carrageenan induced hind paw edema in rats by Diclofenac started at 1st hr and which was maintained up to 24th hr. Diclofenac at the dose of 10mg/kg at 1st, 2nd, 3rd, 4th, 5th 6th and 24th h has shown 10.65, 20.17, 24.68, 29.65, 32.18, 32.56, and 13,46 % inhibition, respectively. At the dose of 200mg/kg of the ethanolic extract seem have the better inhibition than diclofenac and has shown 12.85, 21.61, 25.79, 30.45,31.91,31.46 and 13.83% inhibition, respectively from the 1st to 24th h.

3.2 Effect of the two Extracts and Diclofenac on Serum Levels CRP at 5th h During Carrageenan Induced Hind Paw Edema in Rats

This study showed an increased CRP concentration (p<0.05) 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 (p>0.05).

Finally, this anti-inflammatory activity is more raised with ethanolic extract and seems have an active effect.

We have to determine these two extracts with the water and the alcohol to respect the working conditions of the African traditional uses. Thus, the anti-inflammatory activity of aqueous and ethanolic extracts of *Gomphrena celosioides* was evaluated by carrageenan induced rat paw edema method and determination serum level of C-reactive protein using commercial kit, according to manufacturer instructions.

In our previous studies, *Gomphrena celosioides* seems contained several agents anti-inflammatory and result in the partial inhibition of inflammatory-mediator released. In the light of the results on the Table 1 and 2*, Gomphrena celosioides* inhibits edema similarly to Diclofenac. So, anti-inflammatory activity of *Gomphrena celosioides* seemed to be effective in the two phases of acute inflammation. Therefore, the anti-inflammatory activity of *Gomphrena celosioides* may be due to inhibition of mediators of the inflammation such as histamine, serotonin released during the first phase of inflammation and prostaglandins, bradykinin, leukotrienes which released during the second phase of inflammation.

Indeed, Carrageenan induced paw edema is widely used for determining the acute phase of inflammation [25]. Carrageenan as a phlogistic agent is non antigenic and is devoid of apparent systemic effect [26].

Table 1. Effect of Aqueous and ethanolic extracts of *Gomphrena celosioides* and Diclofenac on carrageenan induced paw edema in rats

Treatment groups	Dose (mg/kg)	Edema diameter (mm)							
		0hr	1hr	2hr	3hr	4hr	5hr	6hr	24hr
Normal saline (control)	-	4.79±0. 11	6.38±0.41	6.94±0.55*	7.21±0.47**	7.52±0.38**	7.52±0.46**	7.31 ± 0.51**	5.42 ± 0.16
Aqueous extract	100	4.6 1±0.09	5.57±0.24	5.50±0.23	5.43±0.24	5.56±0.35	6.20± 0.48	6.86 ± 0.45*	5.08 ± 0.26
Aqueous extract	200	4.78±0.15	5.75±0.13	5.44±0.08	5.38±0.06	5.33±0.06	5.26±0.05	5.23 ± 0.06	5.18 ± 0.04
Ethanolic extract	100	4.88±0.05	5.88±0 .28	6.08±0.19	6.21±0.13	6.43±0.25	6.78±0.25*	6.54 ± 0.16 *	5.05 ± 0.18
Ethanolic extract	200	4.59±0.10	5.56±0.07	5.44±0.09	5.35±0.10	5.23±0.11	5.12±0.07	5.01 ± 0.08	4.67 ± 0.05
Diclofenac (Standard)	10	4.41±0.02	5.70±0.03	5.54±0.01	5.43±0.01	5.29±0.02	5.10±0.02	4.93 ± 0.06	4.69 ± 0.06

*Each value is mean ± SEM, N= 6 rats, the data was analyzed by using One Way ANOVA followed by Dunnett's test, **p<0.01, *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 *Gomphrena celosioides* and diclofenac

Treatment	Dose mg/kg	Percentage inhibition (%) at various times intervals							Mean of % Inhibition
		1hr	2hr	3hr	4hr	5hr	6hr	24hr	
Normal saline (control)	-	-	-	-	-	-	-	-	-
Aqueous Extract	100	12.69	20.74	24.68	26.06	17.55	6.15	6.27	16.91±3.59
Aqueous Extract	200	9.87	21.61	25.38	29.12	30.05	28.45	4.42	23.17±3.95
Ethanolic Extract	100	7.83	12.39	13.86	14.49	9.84	10.53	6.82	11.32±1.16*
Ethanolic Extract	200	12.85	21.61	25.79	30.45	31.91	31.46	13.83	25.84±2.89
Diclofenac	10	10.65	20.17	24.68	29.65	32.18	32.56	13.46	25.45±3.08

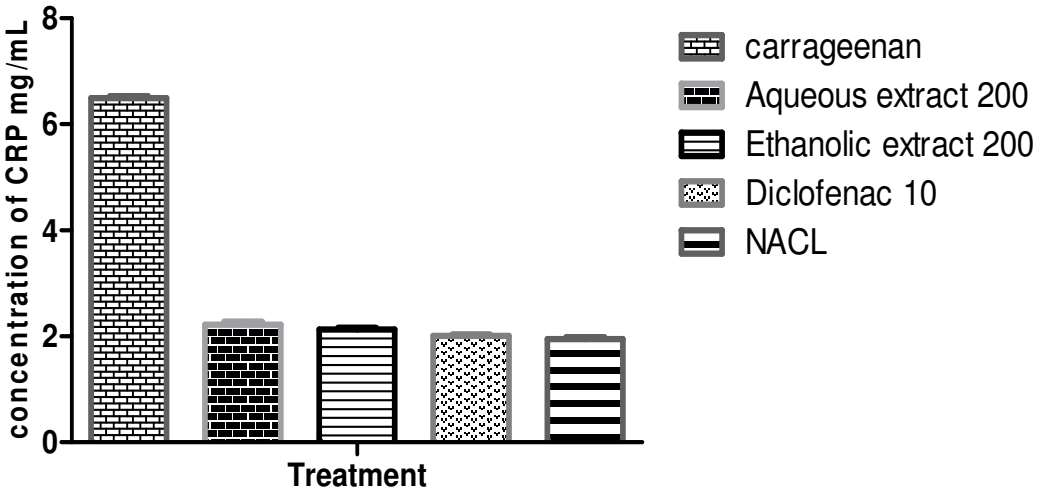

Fig. 1. Changes in the level of serum CRP of rats treated with aqueous extracts and diclofenac at 5th h during carrageenan induced hind paw edema

This model is based on the principle of release of various inflammatory mediators by carrageenan [27]. Edema formation due to carrageenan in the rat paw is a biphasic [28]. The first phase is mainly due the release of histamine and serotonin in the first hour. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polynuclear (neutrophils and monocytes) and prostaglandins produced by tissue macrophages [29,1]. Anti-inflammatory drugs inhibit different stages of inflammation [30].

C-reactive protein (CRP) is the classic acute-phase reactant and a sensitive marker for systemic inflammation. The body produces C-reactive protein or CRP 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 [31].

However, The CRP synthesized by the liver cells, plays an important role in innate immunity by its properties opsonization, activation of complement and receptor binding immunoglobulins [32]. The site secretion main, but not exclusive hepatocytes is responsible for basal levels of plasma CRP. During an inflammatory response, its output increases. Secretion (extrahepatic) exists in neurons where production is increased in dementia of the Alzheimer type [33] in some cells [34] and finally even within atherosclerotic plaques [35].

Our study identifies and characterizes the direct interaction between inflammation and CRP. Indeed, treatment with aqueous and ethanolic of *Gomphrena celosioides* and diclofenac after 5th 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 ethanolic extract reduced more than the aqueous extract and is almost identical to the reference molecule. So, the average value of reduction is more than 2,6% With regard to the reference molecule.

4. CONCLUSION

The results obtained from the present study have clearly demonstrated that the extracts of *Gomphrena celosioides* possessed anti-inflammatory activities which support the traditional utilization in Africa, particularly in Côte d’Ivoire. Ethanol extract seemed more active than the aqueous extract and this activity is comparable to those obtained with reference molecules such as diclofenac.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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