Anti-Inflammatory and Antioxidant Effects of Ethanol Extract of *Gomphrena Celosioides* (Amaranthaceae) in Wistar Rats

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**ABSTRACT**

The aim of study was to evaluate potential anti-inflammatory and antioxidant activities of ethanol extract of *Gomphrena celosioides* (C. Mart), related a phytochemical screening analysis. Thirty wistar rats (180-200 g, either sex,) were divided into 5 groups of 6 animals. All animals treated by intraperitoneal with different solutions. The Group 1 and 2 received NaCl 0.9%. Group 3 received ethanol extract of *Gomphrena celosioides* 200 mg/kg of body weight (b.w.) and vitamin C (100 mg/kg b.w.). After one hour, Group 2 to 5 received 1 mL of carrageenan (1%). The biomarkers of inflammation (CRP) and oxidative stress (TBARS) were determined 5 hrs after. This study showed a significant anti-inflammatory and antioxidant activities of the ethanol extract of *Gomphrena celosioides* 200 mg/kg b.w. whichs are comparable to diclofenac and vitamin C inhibition. In anti-inflammatory activity, the extract decreased (p < 0.01) CRP (2.24 mg/mL) similar as diclofenac (2.21 mg/mL). In antioxidant activity, the extract slightly decreased (p<0.01) TBARS (12.66 ±0.66 mmol/L) than vitamin C (10.5±0.54 mmol/L). The present study shows an anti-inflammatory and antioxidant activities of ethanol extract of *Gomphrena celosioides* in rats.

**Keyword:** *Gomphrena celosioides*; inflammation; oxidative stress; antioxidant

**INTRODUCTION**

Medicinal plants are generally used in Côte d’Ivoire for the treatment of many diseases such as malaria, opportunistic infections, degenerative diseases, cardiovascular, HIV /...
AIDS, diabetes and sickle cell anemia [1]. These diseases often cause at patients inflammatory processes development and pathological oxidative stress to remove the consequent aggression [2]. Thus, inflammation, the body’s defense reaction against aggression, is associated with an important reactive oxygen species production.

*Gomphrena celosioides* Mart (*Amaranthaceae*) is an annual herb with popular usage in traditional medecine. In Africa, *Gomphrena celosioides* is called in traditional language “Adukowé” [3]. This plant is very little present in West Africa [4], has many uses in traditional medicine. This plant is used in Nigeria for the treatment of various skin diseases [5] and as an abortifacient in South America [6]. It’s also used for the treatment of jaundice, malaria, dysmenorrhea [7]. It has analgesic, immunostimulant, tonic, carminative and diuretic properties [8].

The present investigation was carried out to evaluate the anti-inflammatory and antioxidant activities of ethanol extract of *Gomphrena celosioides* in rats coupled which a qualitative phytochemical analysis.

**MATERIAL AND METHODS**

**Material**

**Collection and of material plant**

*Gomphrena celosioides* plant was collected from Bingerville, District of Abidjan (Côte d’Ivoire). The plant was identified and authenticated by comparison with herbarium specimens already existing in National Floristic Center (C.N.F.) of University Félix Houphouët-Boigny (Abidjan/Côte d’Ivoire). The identified and authentically plant material (roots, leaves, flowers and stems) was washed and shade air-dried during 2-3 weeks in the laboratory at room temperature. It was powdered and subjected to extraction procedures.

**Preparation of ethanol extract**

We have determined ethanol extract of *Gomphrena celosioides* with the alcohol to respect African traditional uses. The powder plant material (100 g) was soaked in 1 L of 70% ethanol (700 mL of 100% ethanol add 300 mL of distilled water), agitated with an agitator for 24h at 50 °C. The extract was filtered and concentrated to dryness using a rotary flash evaporator and stored at a temperature of -4°C until use [9].

**Experimental animals**

Wistar albinos rats (30) weighing 180-200 g including the two sexes were used. These rats kept for two weeks at the laboratory animal house center of Pharmaceutical Faculty into University Félix Houphouët-Boigny. The animals were maintained under standard housing conditions: temperature (27 ±1 °C), 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. The experimental protocol animals used was in accordance with the guidelines for ethical care of experimental animals of the OECD [10].

**Methods**

**Phytochemical Analysis**

The ethanol extract of *Gomphrena celosioides* was qualitatively tested for the identification of chemical constituents, such as, polyphenols, alkaloids, flavonoids, glycosides, saponins, sterols and terpenes, tannins, quinones and cardiac glycosides. The tests focused on compounds detection methods which are summarized (Table 1), specifying the primary pharmacological effects.
Table 1: Qualitative research of secondary metabolites in ethanol extract of *Gomphrena celosioides*

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Characterization methods</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenols</td>
<td>Reaction Folin-Ciocalteu</td>
<td>Antioxidant activities [11]</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Cyanidin reaction</td>
<td>Anti-inflammatory activities [12]</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Dragendorff and Bouchardat Reagents</td>
<td>Anti-inflammatory activities [5]</td>
</tr>
<tr>
<td>Tannins</td>
<td>Reagent Stiasny</td>
<td>Antioxidant activities [12]</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam production Test</td>
<td>Saponins antioxidant and radical activities [13]</td>
</tr>
<tr>
<td>Quinones</td>
<td>Reaction Borntraeger</td>
<td>Antioxidant activities [14]</td>
</tr>
<tr>
<td>Stérols et triterpenes</td>
<td>Reaction Liebermann</td>
<td>Properties hypotensive and cardiac depressant action [15]</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Reagent Fehling</td>
<td>Activities cardiotonics and vasoconstrictor [16]</td>
</tr>
</tbody>
</table>

**Induction of Anti-Inflammatory and antioxidant activities**

These activities used carrageenan-induced rat paw oedema test which induced edema in rats after carrageenan administration [17]. Carrageenan, sulfated polysaccharide extracted from seaweed (Chondrus crispus), induces edema at the rat’s paw is considered a characteristic of inflammation and a parameter in the evaluation of more compounds anti-inflammatory activities [18].

Plant extract of *Gomphrena celosioides* (Ethanol extract at 200 mg/kg of body weight (b.w.) administered intra-peritoneally [19]. Control group received vehicle controls normal saline 0.9%. Reference groups received diclofenac (10 mg/kg b.w.) as the reference standard for anti-inflammatory activity [20] and vitamin C (100 mg/kg b.w.) as the reference standard for antioxidant activity [21].

**Evaluation of anti-inflammatory and antioxidant activities**

**Anti-inflammatory activity**

Anti-inflammatory activity at the extract was measured using carrageenan induced rat paw edema essay [17, 22]. Ethanol extract of *Gomphrena* was dissolved in normal saline (0.9%) and administrated intra-peritoneally [19]. C-reactive protein (CRP) is glycoprotein synthesized by the liver cells which is sensitive marker for systemic inflammation.

Thirty (30) rats of either sex were divided into four groups of 6 rats (n = 6). Group 1 (control) received normal saline 0.9% and group 2 received 0.2 mL of carrageenan. Group 3 and 4 received respectively ethanol extract (200 mg/kg b.w.) and diclofenac (10 mg/kg b.w.). After 5hr of carrageenan administration, blood samples in all animals were collected and serum was separated in order to measure levels of C-reactive protein (CRP). The serum level of CRP was determined by enzyme-linked immunosorbent assay (ELISA) [23].

**Antioxidant activity**

The serum obtained previously was used to determine the antioxidant activity of ethanol extract of *Gomphrena celosioides* via lipid peroxidation assay. In this assay as an index of lipid peroxidation, serum MDA concentration was determined by measuring the thiobarbituric acid reactive substances (TBARS) according to spectrophotometric method of SATHO [24]. During the reaction, two molecules of thiobarbituric acid (TBA) react with a
molecule of malondialdehyde (MDA) and lead a pink fluorescent complex after by adding N-butanol. The color of supernatant is measured at 532 nm and corresponds to set of governing substances (TBARS) expressed as MDA. The total antioxidant capacity of serum was determined by measuring its ability to reduce Fe$^{3+}$ to Fe$^{2+}$ by the FRAP test (Ferric Reducing Ability in plasma). The FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe(II)-tripyridyltriazine compound from Fe(III) by action of electron donating antioxidants [25].

**Statistical analysis**

The values expressed as mean ± SEM from 6 animals. The graphical representations of data were carried out with Graph Pad Prism software. The statistical analysis was performed using analysis of variance (ANOVA ONE WAY). The differences between the average values were processed by Dunnett comparison. The observed differences were considered statistically significant at the level of p < 0.05.

**RESULTS**

**Phytochemical Analysis**

The qualitative phytochemical analysis revealed the presence of polyphenols, flavonoids, saponins, sterols and triterpenes, tannins and alkaloids. Quinones and cardiac glycosides have not been found in this extract (Table 2).

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenols</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Catechin tannins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins Gallicas</td>
<td>+</td>
</tr>
<tr>
<td>Sterols et Terpenes</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) : Presence, (-) : Absence

**Anti-inflammatory activity**

After 5$^{th}$ of carrageenan administration serum levels CRP significantly (p < 0.01) increased from 1.95 mg/mL to 6.21 mg/mL. In the same time treatment with ethanolic of *Gomphrena celosioides* (2.25 mg/mL) and diclofenac (2.10 mg/mL) significantly (p < 0.01) decreased CRP level. But there is no significant (p > 0.05) difference between CRP concentration with extract and diclofenac rat groups (Fig. 1).
Antioxidant activity

Serum levels TBARS significantly (p < 0.01) increased in rats treated with carrageenan (25.68 ±0.32 mmol/L), as compared to that before initiation of treatment with carrageenan (8.69 ±0.63 mmol/L). These serum TBARS reduced significantly (p < 0.01) after ethanol extract treatment (12.66 ±0.66 mmol/L) or and vitamin C treatment (10.5 ±0.54 mmol/L). The ethanolic extract statistically showed a similar decrease in TBARS to that of vitamin C (Fig. 2).

Total antioxidant capacity

It was observed significantly (p < 0.05) an increased serum levels total antioxidant capacity in rats receiving ethanol extract of *Gomphrena celosioides* (10.44 ±0.36 μmol Fe^{2+}/L) and vitamin C (10.54 ± 0.30 μmol Fe^{2+}/L) as compared to value obtained in rats treated with carrageenan (4.26 ± 0.67μmol Fe^{2+}/L). Vitamin C and the ethanol extract showed statistically similar activities (Fig. 3)
Fig. 3: Serum change of total antioxidant capacity level in rats treated with ethanol extract and diclofenac at 5th h during carrageenan induced hind paw edema. EE_{200}: ethanol extract at 200 mg/kg of body weight (b.w.) of Gomphrena celosioides. Vit C_{100}: vitamin C at 100 mg/kg b.w. Carra: carragenan.

DISCUSSION

Phytochemical analysis
Qualitative phytochemical analysis of ethanol extract of Gomphrena celosioides revealed the presence of polyphenols, flavonoids, saponins, sterols and triterpenes, tannins and alkaloids. These results agree with those of Maxime et al [26] who showed that the aqueous extract of this plant contains flavonoids, saponins, tannins, sterols and Tri-terpenes. However, Onocha et al [5] noted an absence of alkaloids, tannins and saponins in the extract ethyl acetate from Gomphrena celosioides.

Anti-inflammatory activity
Anti-inflammatory activity of ethanol extract of Gomphrena celosioides was evaluated by carrageenan induced rat paw edema method [22] and determination serum level of C-reactive protein using commercial kit, according to manufacturer instructions. C-reactive protein (CRP) is the classic acute phase reactant and a sensitive marker for systemic inflammation. 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 [2]. During an inflammatory response, its output increases [27]. This study showed an increased serum levels CRP significantly with carrageenan indicating an inflammatory process. Carrageenan induced paw edema is widely used for determining the acute phase of inflammation [28]. Edema formation due to carrageenan in the rat paw is a biphasic [29]. 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 [30, 31]. Anti-inflammatory drugs inhibit different stages of inflammation [32]. The flavonoids, saponins and tannins might be responsible in part for the observed anti-inflammatory effect [33]. This inhibition may be related to inhibition of pro-inflammatory cytokines such as IL 6, IL 1TNF, responsible for the synthesis of CRP. Treatment with ethanol of Gomphrena celosioides and diclofenac significantly
decreased CRP level. Therefore, *Gomphrena celosioides* and diclofenac may exert an anti-inflammatory effect. This anti-inflammatory activity may be due their several anti-inflammatory agents which inhibit mediators of the inflammation. The ability of the extract to inhibit carrageenan induced paw edema suggested that it possessed a significant effect against acute inflammation. The extract (200 mg/kg of body weight) also caused marked inhibition of carrageenan induced hind paw edema in rats as compared with diclofenac sodium (10mg/kg), the standard anti-inflammatory agent used.

**Antioxidant activity**

The inflammatory process induced by carrageenan increased serum levels reactive oxygen species [34], such as thiobarbituric acid reactive substances (TBARS) which are markers of lipid peroxidation produced during stress in rats treated with carrageenan. These oxygen species are involved in the genesis of the inflammation and oxidative stress. Ethanol extract reduced TBARS in serum, suggesting an antioxidant activity of *Gomphrena celosioides*. This antioxidant property could be attributed to antioxidant compounds contained in extract such as tannins, saponins, polyphenols and flavonoids.

Indeed, Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson’s diseases, mongolism, ageing process and perhaps dementias [35]. These polyphenols include flavonoids are powerful antioxidants that may inhibit the formation of free radicals and resist oxidation of macromolecules [12].

**Total antioxidant capacity**

Ethanol extract of *Gomphrena celosioides* significantly attenuate the increase in TBARS (MDA) a marker of increased oxidative stress. Furthermore, it was observed significantly an increased total antioxidant capacity in rats receiving ethanol extract of *Gomphrena celosioides*. The ability of this extract suggests that it possesses a significant reduction in activity ferric ion Fe³⁺ to ferrous ion Fe²⁺. This activity is probably due to presence of hydroxyl groups in phenol compounds that may be used as the electron donor [36]. This test of Ferric reducing ability of plasma confirmed the antioxidant properties of ethanol extract studied in rats.

**CONCLUSION**

The results obtained from the present study demonstrated that ethanol extract of *Gomphrena celosioides* exhibited anti-inflammatory and antioxidant activities which support the traditional utilization in Africa, particularly in Côte d’Ivoire. Secondary metabolites contained in ethanol extract were responsible for these effects. In our previous studies, Ethanol extract seemed more active than the aqueous extract and this activity is comparable to those obtained with diclofenac as reference molecule. This extract may be used as part of the search for new therapeutic molecules for prevention of various diseases whose frequency increases with aging.

**CONFLICT OF INTEREST STATEMENT**

The authors declare that they have no conflict of interests.

**ACKNOWLEDGEMENTS**

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