Research Article

Anti-inflammatory and Antioxidant effects of Ethanolic extract of Gomphrena celosioides in wistar rats

ADEOTI M Franck[1*], GOGAHY K[1], MONTEOMO G François[1], CAMARA-CISSE M[3], DOSSO Mireille[2], DJAMAN A Joseph[4], SESS Daniel[3]

[1] Laboratory of Medical Biochemistry, University Hospital of Yopougon. Po Box 632 Abidjan 21. [2] Pasteur Institute of Côte d'Ivoire. Po Box 490 Abidjan 01-Côte d'Ivoire. [3] Department of Medical Biochemistry, Faculty of Medicine of Abidjan. Po Box 166 Abidjan 01x- Côte d'Ivoire.[4] Department of Medical Biochemistry and fundamental, Pasteur Institute of Côte d'Ivoire. Po Box 490 Abidjan 01-Côte d'Ivoire.

Correspondence for author: Mansour Adeoti, Department of Medical Biochemistry, Faculty of Medical Sciences of Abidjan,

E-mail: adeotimfranck@gmail.com
Published: 25 December 2016 Received: 28 September 2016
AJBBL December 25, 2016, Volume 05: Issue 01 Page 24-34 Accepted: 23 December 2016

ABSTRACT

This study was aimed to evaluate potential anti-inflammatory and antioxidant activities of Ethanolic extract of *Gomphrena celosioides* (C. Mart). Thirty Wistar rats (180 - 200 g) of either sex, were divided into 5 groups of 6 animals. All animals treated by intraperitoneally with the different solutions. Group 1 to 2 received NacL 0.9%. Group 3 received ethanol extract of *Gomphrena celosioides* (200 mg/kg bw). Group 4 and 5 received respectively Diclofenac (10 mg/kg bw) and vitamin C (100 mg/kg bw). After an hour, Group 2 to 5 received 1mL de carrageenan 1%.The present study showed a significant anti-inflammatory and antioxidant activities of the ethanol extract of *Gomphrena celosioides*, at 200mg/kg. b.w whiches were comparable to the 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 showed a significant anti-inflammatory and antioxidant activities of the ethanol extract of *Gomphrena celosioides*, at 200 mg/kg. b.w.

KEYWORDS: *Gomphrena celosioides*, inflammation, oxidative stress, antioxidant.

1. INTRODUCTION

Medicinal plants are generally used in Côte d'Ivoire for the treatment of many diseases such as malaria, opportunistic infections, and 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, the family Amaranthaceae, is an annual herbaceous plant, a weed of lawns up to 30 cm long. This plant of the Ivorian pharmacopoeia, very little present in West Africa [3], has many uses in traditional medicine. *Gomphrena celosioides* is used in the treatment of jaundice, malaria, dysmenorrhea [4] and skin conditions [5]. It also has analgesic properties, immunostimulant, tonic, carminative and diuretic [6]. This plant is used also in Nigeria for the treatment of various skin diseases [5] and as an abortifacient in South America [6]. The effects induced by plants to facilitate their pharmacological activities are carried out various chemical groups they contain and which forms the scientific basis of traditional therapeutic use of plants studied [7]. Given the various uses of this herb that remains little studied, the objective of this work is not only to identify the active principles of the ethanol extract of *Gomphrena celosioides*, but also to show its effect on inflammation and oxidative status in rats.

2. MATERIAL AND METHODS

2.1. Material

2.1.1. Collection and Extraction

Gomphrena celosioides plants was collected 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 weeks in the laboratory at room temperature. It was powdered and subjected to extraction procedures.

2.1.2. Preparation of ethanolic extract

The powder plant material (100g) was soaked in 1L of 70% ethanol (700 mL of 100% ethanol + 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].

2.1.3. Experimental Animals

Wistar albinos rats (30) 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*.

2.2.1. 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 in Table 1, specifying the primary pharmacological effects.

secondary metabolites	characterization methods	Roles
total phenols	Reaction Folin-Ciocalteu	antioxidant activities [10]
Flavonoids	Cyanidin reaction	anti-inflammatory activities [11]
Alkaloids	Dragendorff and Bouchardat Reagents	anti-inflammatory activities [5]
Tannins	Reagent Stiasny	antioxidant activities [11]
Saponins	Foam production Test	Saponins antioxidant and radical activities [12]
Quinones	Reaction Borntraeger	antioxidant activities [13]
Stérols et triterpenes	Reaction Liebermann	Properties hypotensive and cardiac depressant action [14]
Cardiac glycosides	Reagent Fehling	Activities cardiotonics and vasoconstrictor [15]

Table 1: Qualitative research secondary metabolites in the ethanol extract of *Gomphrena celosioides*.

2.2.2. Induction of anti-inflammatory and antioxidant activities

These activities used carrageenan-induced rat paw oedema test which induced edema in rats after carrageenan administration [16]. 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 [17]. Plant extract of *Gomphrena celosioides* (Ethanol extract, 200 mg/kg bw) administered intra-peritoneally [18]. Control group received vehicle controls normal saline 0,9 %. Reference groups received Diclofenac (10 mg/kg bw) as the reference standard for anti-inflammatory activity [19] and Vitamin C (100 mg/kg bw) as the reference standard for antioxidant activity [20].

2.2.3. Evaluation of anti-inflammatory and antioxidant activities

2.2.3.1. Anti-inflammatory activity method for the evaluation of anti-inflammatory effect

Anti-inflammatory activity at the extract was measured using carrageenan induced rat paw edema essay [16,21]. Ethanol extract of Gomphrena was dissolved in normal saline (0.9%) and administrated intra-peritoneally [18].

2.2.3.2. Quantitative measurement of rat C Reactive Protein (CRP) in serum

Twenty four rats of either sex were divided into four groups (n=6). Group I received normal saline 0.9% (control). Group II received 0.2 mL carrageenan. Group III and IV received ethanolic extract (200 mg/kg bw) and Diclofenac (10 mg/kg bw) respectively. After 5hr of carrageenan administration, all the animals were sacrificed and blood samples were collected. Serum was separated and used to measure levels of C-reactive protein (CRP). C-reactive protein (CRP), glycoprotein synthesized by the liver cells is a sensitive marker for systemic inflammation. Its serum levels was determined by enzyme-linked immunosorbent assay (ELISA) according ABCAM® essay procedure [22].

2.2.3.3. Antioxidant activity

The serum obtained previously was used to determine the antioxidant activity of the ethanol extract of *Gomphrena celosioides*.

2.2.3.3.1. Tests for antioxidant activity

a. Lipid peroxidation assay

In this assay as an index of lipid peroxydation, serum MDA concentration was determined by measuring the thiobarbituric acid reactive substances (TBARS) according to the spectrophotometric method of SATHO [23]. 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 a wavelength of 532 nm and corresponds to the set of the governing substances (TBARS) expressed as MDA.

b. Total antioxidant capacity

The total antioxidant capacity of serum was determined by measuring its ability to reduce Fe^{3+} to Fe^{2+} by the FRAP (Ferric Reducing Ability of Plasma) test. 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 the action of electron donating antioxidants [24].

2.2.4. 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

3.1. Phytochemical analysis

Phytochemical qualitative 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).

Secondary metabolites	Ethanolic extract
Polyphenols	+
Flavonoids	+
Catechin tannins	+
Tannins Gallicas	+
Sterols et Terpenes	+
Saponins	+
Quinones	-
Cardiac glycosides	-
Alkaloids	+

Table 2: Phytochemical composition of ethanol extract of *Gomphrena celosioides*

+ = Presence **-** = Absence

3.2**.** Evaluation anti-inflammatory and antioxidant activities of ethanolic extract of *Gomphrena celosioides*

3.2**.**1. Effect of ethanolic extract on serum levels CRP at 5th h during carrageenan induced hind paw edema in rats

After 5th 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 difference between CRP concentration with extract and diclofenac rats groups ($p > 0.01$) (Figure 1).

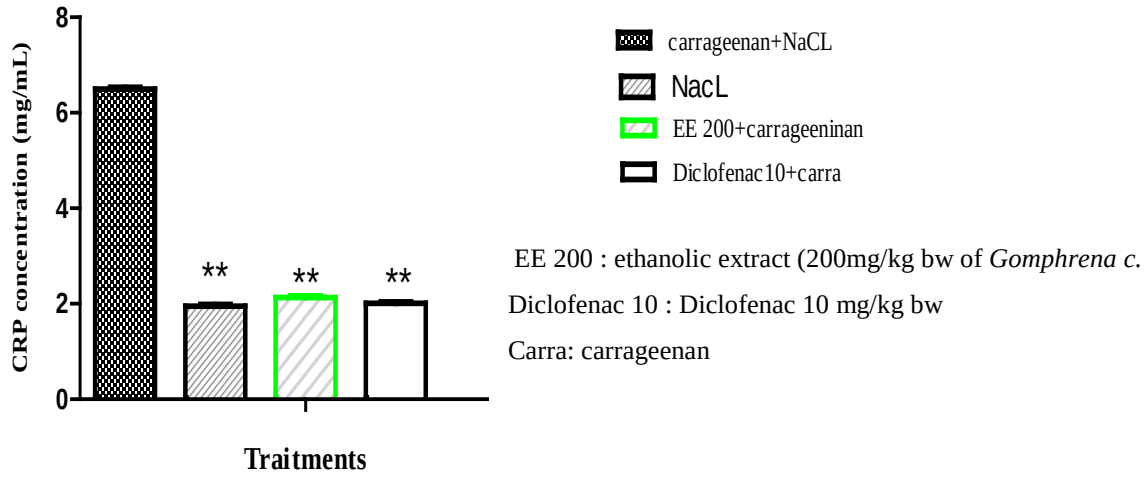

Figure 1: Changes in the level of serum CRP of rats treated with ethanolic extract and diclofenac at 5th h during carrageenan induced hind paw edema.

3.2.2. Lipid peroxidation assay (TBARS assay)

Serum levels TBARS significantly ($p < 0.01$) increased in rats treated wich 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 (Figure 2).

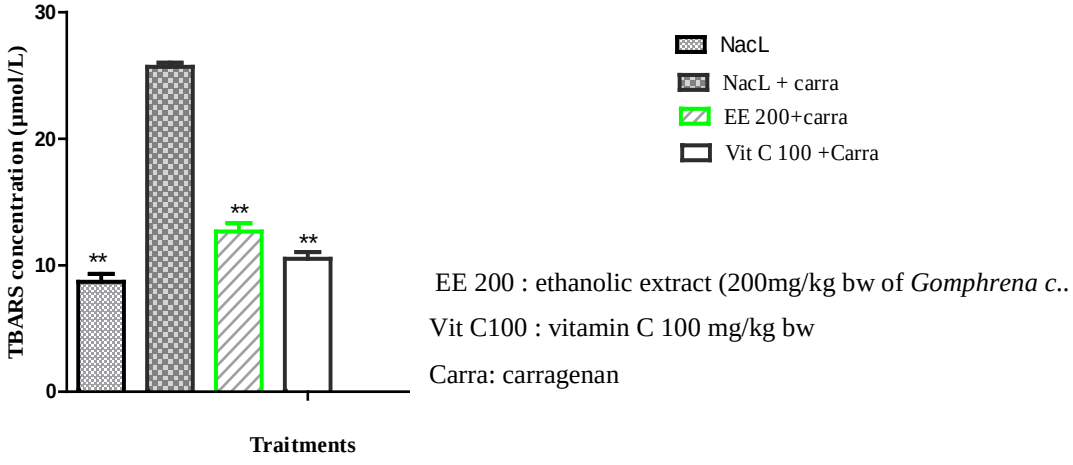

Figure 2**:** Changes in the level of serum TBARS of rats treated with ethanolic extract and diclofenac at 5th h during carrageenan induced hind paw edema.

3.2.3. 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 (Figure 3).

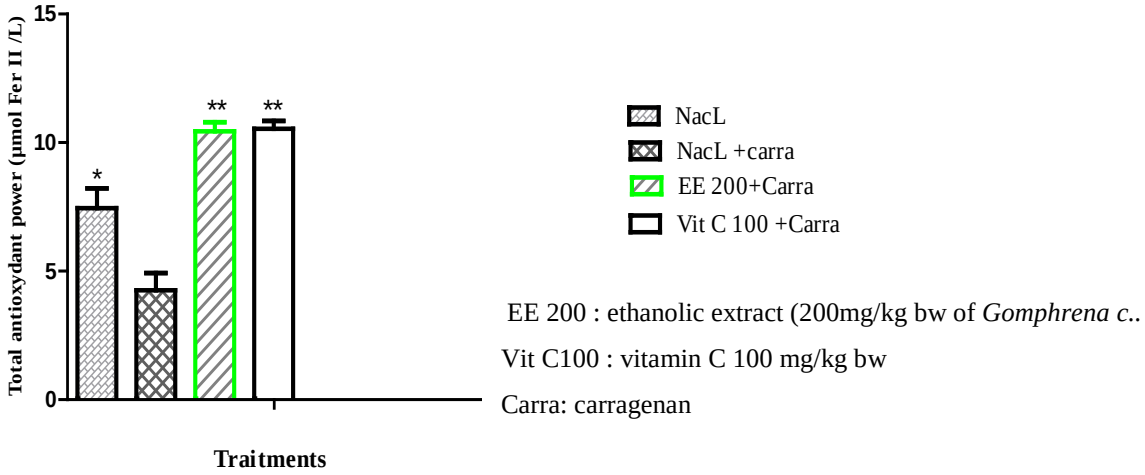

Figure 3: Changes in the level of serum total antioxidant capacity of rats treated with ethanolic extract and diclofenac at 5th h during carrageenan induced hind paw edema.

4. DISCUSSION

4.1. Phytochemical analysis

Phytochemical qualitative analysis of the 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* [25] 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*.

We have to determine ethanol extract of *Gomphrena celosioides* with the alcohol to respect African traditional uses.

4.2. Evaluation anti-inflammatory and antioxidant activities of ethanolic extract of *Gomphrena celosioides*

4.2.1. Anti-inflammatory activity

Anti-inflammatory activity of ethanolic extract of *Gomphrena celosioides* was evaluated by carrageenan induced rat paw edema method [21] 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 [26].

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 [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, 30]. Anti-inflammatory drugs inhibit different stages of inflammation [31]. The flavonoids, saponins and tannins might be responsible in part for the observed anti-inflammatory effect. [32]. 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 ethanolic 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 witches 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 bw) 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.

4.2.2. Antioxidant activities

4.2.2.1. TBARS assay

The inflammatory process induced by carrageenan increased serum levels reactive oxygen species [33], such as 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 serum TBARS suggesting an antioxidant activity of *Gomphrena celosioides*. This antioxidant property could be attributed to the antioxidant compounds contained in extract such as tannins, saponins, polyphenols, 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, neurodegenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias [34]. These polyphenols include flavonoids are powerful antioxidants that may inhibit the

formation of free radicals and resist oxidation of macromolecules [11].

4.2.2.2. 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 the extract suggests that it possesses a significant reduction in activity ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). This activity is probably due to the presence of hydroxyl groups in the phenolic compounds that can be used as the electron donor [35]. This test of Ferric reducing ability of plasma confirmed the antioxidant properties of ethanol extract studied in rats.

5. 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.

Competing interests
Authors have declared that no competing interests exist.

REFERENCES:

1. Ake-Assi L, Guinko S. Plantes utilisées dans la médecine traditionnelle en Afrique de l'ouest. *Edition Roche-Basel, Switzerland,*1991,151p.

2. Dupuy M, Terrier N et Sénécal L. La CRP est-elle plus qu'un marqueur de l'inflammation ? Labo. Bioch. CHU Montpellier. Rev. Nephro., 2003 (7) : 337 – 341.

3. Adonou AC, Yedomonhan H, Djossa B, Legba SI, Oumorou M et al. Etude Ethnobotanique des plantes médicinales vendues dans le marché d'Abomey-Calavi au Benin. Int. J. Biol. Chem. Sci, 2012; 6(2):745-772

4. Vieira CCJ, Mercier H, Chu EP, Figueiredo-Ribeiro RCL. Gomphrena species (globe amaranth) in vitro culture and production of secondary metabolites. In: Bajaj, YPS., ed. Biotechnol. Agr. Forest. Medicinal and Aromatic Plants VII,1994 (28):257-270.

5. Onocha PA, Ajaiyeoban EO, Dosumu OO, Ekundayo O. Phytochemical screening and biological activities of *Gomphrena celosioides* (C. Mart) extracts. *Nigerian Soc Exp Biol J,* 2005 (5): 59 - 67.

6. Burkill HM. The useful plants of West Tropical Africa. Families A-D. *Royal Botanical Garden kew*. 1984 (1). 441-415.

7. Adjanohoun E, Adjakidjè V, Ahyi M, Ake-Assi L, Akoegninou A et *al.* Médecine traditionnelle et pharmacopée. Contribution aux études ethnobotaniques et floristiques en République Populaire du Bénin. *Agence de Coopération Culturelle et Technique*, 1989, 895p.

8. Gessler MC, Nkunya MH, Mwasumbi LB, Heinrich M, Tanner M. Screening Tanzanian medicinal plants for antimalarial activity. *Acta Trop*, 1994(56) : 65-77.

9. Guédé - Guina F, Manda M et Harouna D. Proteins of MISCA a plant source concentrate against fungi. *J Ethnopharmacol.,* 1993 (105): 30 - 45.

10. Hayase F et Kato H. Antioxidative components of sweet potatoes. *J Nutr Sci Vitaminol*,1984, 30 (1): 37 - 46

11. Timbo B. Etude phytochimique et des activités biologiques de *Trichilia emetica VAHL* (*Meliaceae).*Thèse de pharmacie, 112 p. Université de Bamako (Mali). 2003.

12. Huong N, Matsumoto K, Kasai R *et al*. In vitro antioxidant activity of Vietnamese ginseng saponin and its components. Biol Pharm Bull,1998 (21): 978-981

13. Villamil F, Dubin M, Galeffi C. Effects of mansonones on lipid peroxidation, P450 monooxygenase activity, and superoxide anion generation by rat liver microsomes. Bioch Pharmacol, 1990 (40): 2343–2351.

14. Temitope I et Omotayo O. Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. *J Med Plants Res*, 2012. 6(7):1106 - 1118.

15. Allain P. Les médicaments. *CdM Editions*, 2000. Paris, 500p

16. Gepdiremen A, Mshvidadze V et Elias R. Acute anti-inflammatoryactivity of four saponins isolated from ivy: alphahederin, hederasaponin-c, hederacolchiside-E and hederacolchiside-F in Carrageenan induced rat paw edema. *Phytomedecine*, 2005 (12): 440-444.

17. Morris C. Carrageenan-Induced Paw Edema in the Rat and Mouse. Inflammation Protocols. Totowa, NJ, Humana Press Inc, 2003, 115 – 121.

18. Ratheesh M, Helen A. Anti-inflammatory activity of *Ruta graveolens* Linn on carrageenan induced paw edema in wistar male rats. AJB, 2007;5(10);1209-1211

19. Rubina M, Zargar MA, Latief Ahmad. Evaluation of anti-inflammatory potential of Atropa acuminate in carrageenan induced inflammation in rats. J. Med. Plants Res. 2012, 6 (43):5586-5592.

20. Aka AE. Activités anti-inflammatoire, antioxydante et action stimulatrice des lymphocytes totaux sur les extraits aqueux et hydoaéthanolique de *Cola nitida* (Sterculiaceae). Thèse de Doctorat. Univ FHB Abidjan. 131p. 2014.

21. Winter CA, Risely EA, Nuss GW. Carragenan-induced oedema in the hind Paw of the rats as an assay for anti-inflammatory drugs. EBM,1962;111:554-547

22. Anomyme; 2014. Available: www.abcam.com/www.abcam.cn/www.abcam.co.jp.

23. Satho K. Serum lipid peroxidation in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta,1978; 90: 37-43.

24. Benzie I and Strain J. Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Ann Biochem,1996 ;239: 70 -76.

25. Maxime MS, Bale B, Mama AB, Jean MA, Karim LD. Composition chimique de l'extrait aqueux de *Gomphrena celosioides Mart* et étude de ses effets toxicologiques chez le foie du rat Wistar. Rev ScLib Ed Mers, 2012. 4: 2106-2111.

26. Jyothi MB, Jayanthi MK, Suresha RN. Evaluation of anti-inflammatory activity of *Aegle marmelos* (Bilwa) root. IJP, 2011;43(4):393-397

27. Patil P, Prasd K, Nitim M, Vijay KM, Sreenivasa RK. Evaluation of anti-inflammatory effect of *Catotropis procero* root extract against different mediators of inflammation in albino rats. IRJP, 2011.2(3): 279-284.

28.Vinegar R, Truax JF, Selph JH, Johnson PR, Venable AL, Mckenzie KK. Pathway to carrageenan-induced inflammation in the hind limb of the rat. Fed Proc, 1987;46: 118-126

29. Brito ARMS, Antonio MA. Oral anti-inflammatory and antiulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (*Turneraceae*), J. Ethnopharmacol,1998; 61: 215-228

30. Anupama AS, Kishor NR, Rahul DK, Kanchan SM. Evaluation of anti-inflammatory and analgesic activities of *Tamarindus indica* Seeds. IJPSDR, 2012; 4(3):213-217

31. Jyothi MB, Jayanthi MK, Suresha RN. Evaluation of anti-inflammatory activity of *Aegle marmelos* (Bilwa) root. IJP, 2011;43(4):393-397

32. Calixto JB, Beirith A, Ferreira J, Santos AR, Cechinel FV and al. Naturally occurring antinociceptive substances from plant. Phytotherapy Research, 2000;14:401-418.

33. Kim D, Chun O, Kim Y. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *J Agric Food Chem*., 2003 (51): 6509-6515

34. Polterait O. Antioxidants and free-radical Scavengers of Natural origin. Current Org. Chem., 1997, 1:415-440

35. Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* L. Walp.) seed extracts. Food Chem., 2007; 101(1): 10-19