**Phytochemical screening, anti-inflammatory and antioxidant effects of aqueous extract of *Alternanthera pungens* (Amaranthaceae) in rats**

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**Abstract**

**The protective functions against inflammation of the organism aggression is well established, however, it is now considered a major risk factor for several diseases including cardiovascular disease, cancer and diabetes type II. In this present work, the anti-inflammatory and antioxidant effects of aqueous extract of *Alternanthera pungens* (Amaranthaceae) were evaluated in rats. In addition, phytochemical screening carried out.Thus, a total of 36 Wistar strain rats old to 10 weeks (180 - 200 g) were divided into 6 groups of 6 rats treated with different solutions by intraperitoneal injections. The test group received 200 mg/kg of body weight (b.w.) of aqueous extract from *Alternanthera pungens*. Reference control groups received 10 mg/kg (b.w.) of indomethacin (anti-inflammatory) and 100 mg/kg (b.w.) of vitamin C (antioxidant). One negative control group received only 0.9% NaCl. An hour after these preventive treatments, the animals were stressed with 0.1 mL of carrageenan (1%). The biomarkers of inflammation (CRP) and oxidative stress (TBARS) were determined 5 hrs after. Furthermore, a phytochemical screening was performed. The mean concentrations in serum of CRP and TBARS were respectively of 5.43 ±1.03 mg/mL and 11.56 ±2.36 mmol/L (p < 0.01) in rats treated with the aqueous extract compared to untreated rats. The phytochemical analysis of this extract showed the presence of total phenols, flavonoids, saponins, tannins, terpenoids and steroids that are known for their anti-inflammatory and antioxidant properties. The aqueous extract of *Alternanthera pungens* would possess anti-inflammatory and antioxidant properties related to chemical compounds and confirms the traditional use.**

**Keywords**: *Alternanthera pungens*, Amaranthaceae, anti-inflammatory, antioxidant, screening phytochemical

**INTRODUCTION**

Inflammation is a defense system of organism that is characterized by a series of reactions in response to acute or chronic aggression involved various mechanisms, for eliminate the attack sources. The inflammatory response may be localized or generalized [1]. Nowadays, Chronic inflammation is associated with some a diversity of diseases such as rheumatoid arthritis, type II diabetes, Alzheimer disease or carcinogenesis [2, 3].

Anti-inflammatory drugs currently available, although they provide symptomatic relief to patients, however, It caused various damage such as gastric ulcers for anti-inflammatory drugs (NSAIDs) or toxic effects as the treatment with corticosteroids [4]. Face these side effects resulting of modern drugs, medicinal plants as an alternative to access new bioactive molecules. Indeed, medicinal plants contain active chemical compounds that could be used for therapeutic purposes or as precursors for the synthesis of medicaments [5, 3]. Thus, one of the first recorded applications for the treatment of inflammation and pain was the use of extracts of willow leaves Celsius by the year 30, which led to the discovery of acetylsalicylic acid, the active component of aspirin, anti-inflammatory drug widely used in medical practice and in the composition of many other nonsteroidal anti-inflammatory drugs (NSAIDs) [6, 7].

Today, better knowledge of the properties of commonly used in traditional medicine plants is necessary in terms of emergency because of the growing number of consecutive poisoning to their use and the lack of scientific data related to efficacy, toxicity and safety of most plants used [8]. In this perspective and in order to enhance the Ivorian pharmacopoeia, our interest focused on *Alternanthera pungens*, a herbaceous plant belonging to the Amaranthaceae family, usually known for they antiviral properties [9, 10, 11]. *Alternanthera* is a genus with contains more than 80 species and belongs to the medicinal plants used in the world. In Africa, many species of this genus are used to treat dysentery, diarrhea, gastrointestinal diseases, venereal diseases, cholera, and many parasitic diseases [12, 13]. Other studies have reported that some species of Alternanthera possess traditional use. It is the case of *Alternanthera brasiliana*, species common for analgesic property [14]. *Alternanthera* *philoxeroides*, an antiviral (HIV) [15]. *Alternanthera tenella* known for its immunostimulatory and anti-inflammatory properties [16, 17].

Because of the limited existence of data on biochemical and pharmacological properties of species of *Alternanthera pungens* in Côte d’Ivoire, this plant has been the subject of this study. The present study evaluated a phytochemical screening, anti-inflammatory and antioxidant effects of aqueous extract from leaves of the plant in rats.

**MATERIAL AND METHODS**

**Plant material**

The leaves of *Alternanthera pungens* were collected in forest areas of Abidjan, 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) [13]. These leaves were washed with distilled water and were shade-dried at room temperature during ten days (10 days), and subsequently reduced to coarse powder using a grinder and stored at room temperature.

**Preparation of aqueous extract**

One hundred grams (100 g) of powder were macerated in 1000 mL of distilled water during 24 hours. The homogenate were filtered three times in succession absorbent cotton and then once on Wattman paper. Then, the filtrate was lyophilized using freeze-dryer model Telstar-LyoQuest-55 to obtain the dried aqueous extract. The sample was kept in tightly bottles and stored under refrigeration at 8 °C until use for the biological testing and phytochemical analysis.

**Animal material**

All animals provided of animal house center of University Félix Houphouët-Boigny. A total of 36 Wistar strain rats (18 males and 18 females), all old to 10 weeks and whose weights range from 180 to 200 grams were used to study the anti-inflammatory and antioxidant effects. The experimental protocol animals used was in accordance with the guidelines for ethical care of experimental animals of the OECD [18]. All rats were screening then maintained in animal house center of the University during two weeks before to start the experiments. The following conditions were met: Temperature at room of 25 °C, level humidity of 40 to 50% with 12 hours of light and 12 hours of darkness. The animals were fed with the granules of FACI® (fabricant), and had free access to water and food without discontinuity.

**Screening phytochemical**

A phytochemical screening was performed on aqueous extract according to the conventional protocol [19]. The presence of different bioactive compounds such as alkaloids, polyphenols, flavonoids, tannins, saponins, terpenes, quinones and cardiac glycosides has been carried out after revelation by using specific reagents solutions. The saponins have been characterized thanks to their foaming properties in aqueous solution shaked [20].

**Inflammatory reaction induction in rats**

The adopted methodology is a preventive experimental approach [21, 22], using 6 groups of 6 rats. We have administered initially a preventive dose of aqueous extract of *A. pungens* at two groups of rats.

One hour after this treatment, the stress was induced in animals by intraperitoneal injection (i.p.) with 0.1 mL of carrageenan (1%), a pro-inflammatory agent. Then, anti-inflammatory and antioxidant effects were assessed by determination of certain markers of inflammation and stress. The preventive dose of *A. pungens* were performed with of 200 mg/kg body weight (b.w.) of aqueous extract in each group tested.

The control of anti-inflammatory activity were performed with a dose of 10 mg/kg (b.w.) of methylated indole derivative (indomethacin), a reference non-steroidal anti-inflammatory molecule [22]. The control of antioxidant activity were carried out with the vitamin C (ascorbic acid), a reference molecule used to 100 mg/kg (b.w.) [23]. The positive control of carrageenan effect was measured in one group. Negative control received only saline solution (0.9% NaCl).

**Determination of biomarkers**

Protein C-Reactive (CRP) and Thiobarbiturates Acid Reactive Substances (TBARS) are biomarkers selected for monitor respectively inflammation and oxidative stress. For this, 500 ml of whole blood were collected 5 hours after the different treatments in each rat on dry tube and centrifuged at 4000 rpm/min for 5 minutes; the serum obtained were collected for the determination of these biomarkers.

**Determination of Protein C-Reactive**

CRP concentrations were determined using enzyme-linked immunosorbent ELISA [24], with the unit Roche Diagnostics Germany origin.

**Determination of Thiobarbiturates Acid Reactive Substances**

The dosage of Thiobarbiturates Acid Reactive Substances (TBARS) was performed by the determination of certain markers of lipid peroxidation. The principle is based on the determination in medium acid (TCA and sulfuric acid), final products of lipid peroxidation (MDA alkenals and alkanals). During the reaction, two molecules of thiobarbituric acid (TBA) react with one molecule of malondialdehyde (MDA) and leads to the formation of a pink complex, made fluorescent in ajouant N-butanol. The coloration obtained the supernatant is measured at 532 nm and corresponds to the set of the governing substances (TBARS) expressed in MDA [25].

**Statistical analysis**

The obtained results were expressed as mean ±standard error of mean (mean ±SEM). 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**

**Screening phytochemical**

Phytochemical analysis of aqueous extract from *Alternanthera pungens* revealed the presence of total phenols, flavonoids, tannins, saponins, cardiac glycosides and sterol-triterpenes (Table I).

**Anti-inflammatory activity**

Five hours (5 hrs) after induction of inflammation, the mean of CRP concentration into serum in animals that received the aqueous extract of *Alternanthera pungens* as preventive measure was 5.43 ±1.02 mg/L. That of animals that have not received prevention was 8.70 ±0.33 mg/L (Figure 1). These results indicate a significant difference (p < 0.001) of CRP concentration levels in animals treated with the aqueous extract of *Alternanthera Pungens*. Those that were injected by indimetacin as anti-inflammatory reference also have their decreased CRP (4.38 ±0.83 mg/L). *A. pungen*s could have anti-inflammatory properties.

**Antioxidant Activity**

In animals that received the aqueous extract before being treated with carrageenan, there was a mean concentration of TBARS in serum was of 11.56 ±2.36 mmol/L. by against that of animals that have not received prevention was of 18.14 ±2.38 mmol/L (Figure 2). These results show very reduced levels of TBARS concentration (p < 0.01) in animals receiving the aqueous extract of *A. pungens* as preventive product, compared to those untreated animals with the aqueous extract of *A. pungens* and vitamin C. The extract of *A. pungens* could possess antioxidant properties.

**DISCUSSION**

**Anti-inflammatory activity**

Inflammation caused in rats by carrageenan believed to be biphasic [26]. The first phase of a period of one hour (1 hr) is mainly due to the release of histamine serotonin and kinin whose rates increase. A second phase, of duration of more than one to five hours (1-5 hrs duration) would be characterized by the increased levels of prostaglandin, proteases and lysosomes [27]. Significant elevation of serum concentration of Protein C-reactive (CRP), which passes from 3.95 mg/mL to 8.75 mg/mL shows the installation of inflammatory stress in rats and confirms the pro-inflammatory properties of carrageenan. Our results are in agreement with the work of Gabay et al. [28], which showed a significant elevation of CRP in the acute phase of inflammation.

The significant decrease of serum concentrations of CRP in inflamed rats having received the aqueous extract of *A. pungens* may be related to inhibition of the inflammation process caused by carrageenan. The presence of secondary metabolites in the aqueous extract from *A pungens*, especially the flavonoids revelated by this study would be indicated the anti-inflammatory effect. In fact, flavonoids are known for their anti-inflammatory properties [5]. Like the anti-inflammatory drugs, substances with anti-inflammatory activity such as flavonoids contained in the aqueous extract of *Alternanthera pungens*, inhibit some phases of inflammation [29]. These results could justify the use of this plant in ethno-medicine for the treatment of wounds, burns and ulcers [30].

**Antioxidant activity**

We have noted significant increase of serum concentrations of TBARS only in inflamed rats without receiving prevention. This result reveals a link between inflammation and the occurrence of oxidative stress, and it is characterized by increased production of oxygen free radicals [31].Preventive treatment of animals by the extract of *A. pungens* or vitamin C caused a significant reduction of serum concentration levels of TBARS. This result translates the presence of antioxidant activity of the aqueous extract of *A. pungens* similar to that of vitamin C. The probable antioxidant property of the aqueous extract of *Alternanthera pungens* would be related to the presence of tannins, flavonoids, saponins, terpenoids. These compounds have demonstrated their antioxidant, anti-inflammatory and antimicrobial properties [32, 33, 34]. In Côte d’Ivoire, the antioxidant and immunostimulant properties of *Alternanthera pungens* have already been shown in a clinical trial of the extract used as an adjunct in the medical care of people living with HIV-1 [9, 10]. The stimuli activities of humoral immune system of *Alternanthera* extracts which flavonoids were isolated has also been reported by other authors [17].

**CONCLUSION**

The aqueous extract of *Alternanthera pungens* presente anti-inflammatory and antioxidant properties that could be used in the treatment of disorders involving inflammatory processes. This study provides a scientific basis for the traditional use of *A. pungens* during treatment of chronic infections such as HIV/AIDS. These results need to carry out additional work that could lead to the isolation and identification of bioactive molecules to study their mechanism of action.

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**COMPETING INTERESTS**

Authors have declared no competing interests exist

**BIBLIOGRAPHIE**

1. Lacerda R, De Lima C, da Silva L, et al. Discovery of novel analgesic and anti-inflammatory 3\_arylamine\_imidazo [1, 2-a] pyridine symbiotic prototypes. *Biorg. Med. Chem. 2009; p79-84, Vol 17.*
2. Kiecotl-Glaser JK, Gouin J and Hantsoo L. Close relationship, inflammation and health. *J. Neu. Bio. Rev. 2010; p33-38, Vol 35*.
3. Adebayo SA, Dezoyem JP, Shai LJ, et al. The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in South African *Bio. Med. Central. 2015; p159, Vol 15*.
4. Jaiswal SR and Santakke SD. Experimental evaluation of analgesic and anti-inflammatory activity of simvastatin and atorvastation. *Indian. J. Pharmacol.* 2012; *p475-479, Vol 44*.
5. Borokini TI and Omotayo OF. Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. *J. Med. Plants Res. 2012; p1106-1118, Vol 6*.
6. Vane JR and Botting RM. Anti-inflammatory drugs and their mechanism of action. *Infamm. Res.* *1998; p78–87, Vol 47*.
7. Yuan G, Wahlqvist ML, He G, et al. Natural products and anti-inflammatory activity. *Asia. Pac. J. Clin. Nutr. 2006; p143-52, Vol 15.*
8. **Pousset J**. Plantes médicinales d'Afrique : Comment les connaître et les utiliser ? *13090 Aix-en-Provence, Secum/Edisud* 2004; 288p.
9. Djinhi J, Adéoti MF, Lohoues EE, et al. Effet du traitement à base d’*Alternathera pungens* sur le profil anti-oxydant, nutritionnel et immunitaire chez les personnes vivant avec le VIH 1 à Abidjan. *Rev. Iv. Odonto-Stomatol. 2012; p59-66, Vol 14*.
10. Djinhi J, Lohoues EE, Zirihi G, et al. Extrait acqueux de *Alternanthera pungens* : effet sur l’activité des enzymes antioxydantes chez les personnes vivant avec le VIH (PVVIH). *J. sci. pharm. biol.* *2008; 6-12, Vol 9*.
11. Zongo C, Aly S, Somda M, et al. In vitro evaluation of the antimicrobial and antioxidant properties of extracts from whole plant of *Alternanthera pungens* H.B. & K. and leaves of *Combretum sericeum* G. Don. *Int. J. Phytomed. 2011; p182-191, Vol 3*.
12. Gronhaug T, Glaeserud S, Skogsrud M, et al. Ethnopharmacological survey of six medicinal plants from Mali, West-Africa. *J. Ethnobiol. Ethnomed.* *2008; p26, Vol 4.*
13. Zirihi G, N’guessan K, Dibie E, et al. Ethnopharmacological study of plants used to treat malaria in traditional medicine, by Bete Populations of Issia (Côte d’Ivoire). *J. Pharm. Sci. Res. 2010; p216-227, Vol 2*.
14. Macedo AF, Barbosa NC, Esquibel MA, et al. Pharmacological and phytochemical studies of callus culture extracts from *Alternanthera brasiliana. Pharmazie. 1999; p776-777, Vol 54*.
15. Zhang SM, He YS, Tabba JD, et al. Inhibitor against the human immunodeficiency virus in aqueous extract of *Alternanthera philoxeroides. Chin. Med. J. 1988; p861-866, Vol 101*.
16. Guerra RNM, Pereira H-AW, Silveira LMS, et al. Immunomodulatory properties of *Alternanthera tenella* Colla aqueous extracts in mice. *Braz. J. Med. Biol. Res. 2003; p1215-1219, Vol 36*.
17. Biella CA and Salvador MJ*.* Evaluation of immunomodulatory and anti-inflammatory effects and phytochemical screening of *Alternanthera tenella* Colla (Amaranthaceae) aqueous extracts. *Mem. Inst. Oswaldo. Cruz. Rio de Janeiro. 2008; p569-577, Vol 103*.
18. Organisation for Economic Cooperation and Development (OECD), “Guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation”. *OECD, Paris 2000;*

*[www.olis.oecd.org/olis/2000doc.nsf/LinkTo/env-jmmono(2000)7]*.

1. Wagner H and Bladt S Plant drug analysis-a thin layer chromatography atlas. *Springer. 2nd eds. 1996; p384*.
2. Mangambou MJD, Kasali MF and Kadima NJ. Contribution à l’étude phytochimique de quelques plantes médicinales antidiabétiques de la ville de Bukavu et ses environs (SudKivu, R.D. Congo). *J. Appl. Biosci. 2014; p6211-6220, Vol 75.*
3. Winter C, Risley E and Nuse G. Carrageenan-induced oedema in hind paw of rat as an assay for anti-inflammatory drugs. *Exp. Biol. And. Med. 1962; p544-547, Vol 111.*
4. Bezerra TO, Carvalho D, et al. Anti-inflammatory and Antinociceptive effects of the aqueous extract of the bark of *Chrysobalanus icaco Linnaeus. British Journal of Pharmaceutical Research. 2014; p1253-1268, Vol 4.*
5. Gogahy K. Effets des extraits aqueux et ethanolique de *Gomphrena celosioides (amaranthaceae)* sur le processus inflammatoire, le stress oxydant et l’immunité cellulaire. Thèse de Doctorat Univ FHB Abidjan. 2015: p136.
6. Benmammar R. Intérêt du dosage de la CRP dans le dépistage des infections nosocomiales à l’unité de néonatologie de l’EHS mère- enfants de Tlemcen du 14 mai au 22 juin 2012. Master en biologie moléculaire et cellulaire,Université Abou Bekrbelkaid (Algérie), 2012; p46.
7. Satho K. Serum lipid peroxidation in cerebrovascular disorders determined by a new colorimetric method. *Clin. Chim. Acta. 1978; p37-43, Vol 90*.
8. Vinegar R, Schreiber W and Hugo R. Biphasic development of carrageenan edema in rats. *J Pharmacol. Exp. Ther. 1969; p96-103, Vol 166.*
9. Crunkhon P and Meacock S. Mediators of the inflammation induced in the rat paw by carrageenan. *Br. J. Pharmacol. 1971; p392-402, Vol 42.*
10. Gabay C and Kushner I. Acute - phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* 1999*; p448-454, Vol 340*.
11. Jyothi M, Jayanthi M and Suresha R. Evaluation of anti-inflammatory activity of Aegle marmelos (Bilwa) root. *I. J. P. 2011; p393-397, Vol 43*.
12. OkwuD. Phytochemicals and vitamin content of indigenous spices *o\~*Southeastern Nigeria. *J. Sustain. Agric. Environ. 2004; p30-37, Vol 6*.
13. Kim D, Chun O, Kim Y, et al. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *J. Agric. Food. Chem.* *2003; p6509-6515, Vol 51*.
14. Metodiewa D and Koska C. Reactive oxygen species and reactive nitrogen species: Relevance to cyto (neuro) toxic events and neurologic disorders: An overview. *Neurotox. Res.* 2000; *p197-233 Vol 1*.
15. Iwalewa EO, McGaw LJ, Naidoo V, et al. Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *Afr. J. Biotech. 2007; p2868–2885, Vol 6*.
16. Hodzic Z, Pasalic H, Memisevic A, et al. The influence of total phenols content on antioxidant capacity in the whole grain extract. *Eur. J. Sci. Res.* *2009; p471-7, Vol 28.*

**Tables and Figures**

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| --- | --- |
| Secondary metabolites | Aqueous extract |
| Total phenols | + |
| Alkaloids | - |
| Flavonoids | + |
| Tanins | + |
| Saponnins | + |
| Sterols et triterpenes | + |
| Cardiac glycosides | + |
| *(+) : Presence ; (-) : Absence* |

**Table I: Secondary metabolites of aqueous extract of *A. pungens***



**Figure 1: Mean concentrations of CRP in serum 5 hrs after induction of inflammation with carrageenan**

*G-NC: Untreated group with carrageenan (Negative control). G-C: group treated with carrageenan (control carrageenan effect). G-Ap: group treated with carrageenan and aqueous extract of A. pungens at 200 mg/kg body weight (test group),). G-Ind: group treated with carrageenan and indomethacin at 10 mg/kg b.w. (control group reference). (\*\*): p < 0.01 vs G-NC. (\*\*\*): p < 0.001 vs G-NC.*



**Figure 2: Mean concentrations of TBARS in serum 5 hrs after induction of inflammation with carrageenan**

*G-NC: Untreated group with carrageenan (Negative control). G-C: group treated with carrageenan (control carrageenan effect). G-Ap: group treated with carrageenan and aqueous extract of A. pungens at 200 mg/kg body weight (tested group). G-Vit.C: group treated with carrageenan and vitamin C at 100 mg/kg b.w. (control group reference). (\*\*): p < 0.01 vs G-NC.*