



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 brasiliiana*, 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 shaken [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 \pm standard error of mean (mean \pm 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).

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*

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

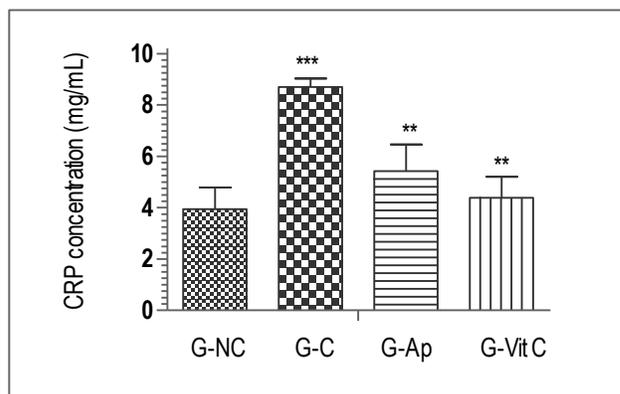


Figure 1: Mean concentrations of CRP in serum 5 hrs after induction of inflammation with carrageenan. G-NC: Untreated group with carrageenan (Negative control). G-C: group treated with carrageenan (control carrageenan effect). G-Ap: group treated with carrageenan and aqueous extract of *A. pungens* at 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.

Anti-inflammatory reference also have their decreased CRP (4.38 ± 0.83 mg/L). *A. pungens* 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 (Figure2). These results show very reduced levels of TBARS concentration ($p < 0.01$) in



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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.

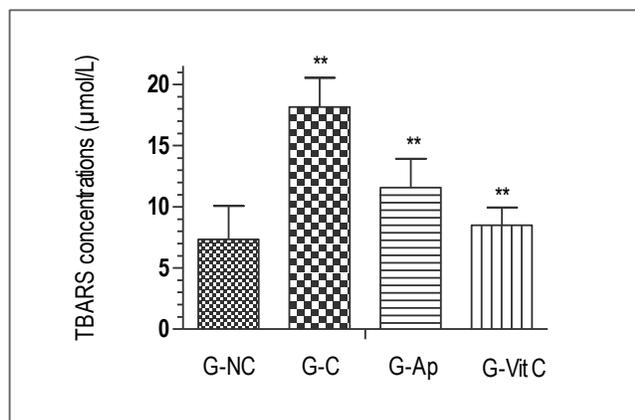


Figure 2: Mean concentrations of TBARS in serum 5 hrs after induction of inflammation with carrageenan. G-NC: Untreated group with carrageenan (Negative control). G-C: group treated with carrageenan (control carrageenan effect). G-Ap: group treated with carrageenan and aqueous extract of *A. pungens* 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.

DISCUSSION

Phytochemical analysis and anti-inflammatory activity

Phytochemical analysis of aqueous extract of *Alternanthera pungens* revealed the presence of secondary metabolites including polyphenols, flavonoids, tannins, saponins, cardiac glycosides, terpenes and sterols. These secondary metabolites

synthesized in plants in response to external stimuli, have often demonstrated beneficial effects for humans [3, 5, 13]. These beneficial effects were reported in this study, through the anti-inflammatory and antioxidant properties of aqueous extract of *Alternanthera pungens*.

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 revealed by this study would be indicated the anti-inflammatory effect. In fact, flavonoids are known for their anti-



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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* presents 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

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