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Original Article

Anti-inflammatory and anti-oxidant activity of *Dialium dinklagei* Harms. (Leguminosae)

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ABSTRACT

Dialium dinklagei is a plant used in the management of pain, arthritis, rheumatism, pulmonary diseases and as a laxative in Ghanaian folklore medicine. However, there is no scientific validation of these pharmacological activities. Therefore, in this research, the anti-oedematogenic and antioxidant activity of a hydro-ethanolic extract of *D. dinklagei* was evaluated. *In vivo* anti-inflammatory activity was done by the carrageenan induced oedema in 7 days old chicks. The antioxidant activity was investigated *in vitro* by the DPPH radical scavenging and phosphomolybdenum total antioxidant capacity assays. *D. dinklagei* exhibited a dose-dependent anti-inflammatory activity with ED_{50} of 10.6 mg/kg body weight compared to diclofenac sodium (ED_{50} = 4.03 mg/kg body weight). It showed considerable antiradical activity (EC_{50} = 39.6) and a total antioxidant capacity of 581.58 mg of vitamin C per gram of extract. The phytochemicals tannins, alkaloids and terpenoids present in the plant could be responsible for its anti-inflammatory and antioxidant effects. Thus, the present study has given scientific credence to the use of *D. dinklagei* in ethnomedicine for the management of painful inflammatory conditions.

Keywords: Oedema, DPPH radical, Carrageenan, Total antioxidant capacity, phytochemicals

INTRODUCTION

Free radicals are naturally present in living systems. However, high amounts of free radicals can oxidise biomolecules, leading to tissue damage, cell death or degenerative processes, including aspects of ageing, cancer, cardiovascular diseases, arteriosclerosis, neural disorders, skin irritations and inflammation. Excess free radicals in a living system plays an important role in oxidative stress [1, 2].

Oxidative stress is an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen and nitrogen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism [3]. A number of inflammatory diseases is associated with oxidative stress. Chronic anti-inflammatory diseases such as rheumatoid arthritis are still one of the main health problems of the world's population. At present, although synthetic drugs for treating inflammatory disorders are dominating the market, their toxicity limits their acceptability. Their prolonged use may cause severe adverse effects on chronic administration, the most common being gastrointestinal bleeding and peptic ulcers. Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects. The search for safe and effective anti-inflammatory agents from natural products have been given priority in scientific research [4]

In Africa, plants are used in the treatment of several ailments. Several studies have demonstrated plant extracts to possess antioxidants properties *in vitro* [5, 6]. They are the main source of vitamin E (α-tocopherol), vitamin C (ascorbate) and phenolic

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compounds. These are the most effective free radical scavengers in living organisms. The search for novel natural antioxidants of plant origin has become important because these compounds are safer than synthetic compounds [7]. It is unknown which constituents of plant are associated with reducing the risk of chronic diseases, but antioxidants appear to play a major role in the protective effect of plant medicines in humans [8].

Dialium dinklagei is a deciduous tree with a light spreading crown of pendulous branches which grows usually 7-20 metres tall reaching 30 metres occasionally. The tree is cylindrical and has a thick bark [9, 10]. The plant is locally known in Ghana as 'awenade' (Twi) [11]. *Dialium dinklagei* is predominant in West Africa-Sierra Leone to South Central African Republic, South to Gabon and West Congo. The tree is found around river and lagoon sides, humid gullies, evergreen forests and marshy areas.

The presence of polyterpenes, polyphenols, alkaloids, tannins, steroids, flavonoids, coumarins and saponins were noted in large quantities in the decoction of the leaves [12]. This was confirmed by Akoué *et al*. [9] who noted large quantities of flavonoids, alkaloids, tannins and polyphenols in the ethanolic leaf extract of the plant. In Ghanaian traditional medicine, various parts of the plant are used either alone or in combination therapy for the treatment of various inflammatory conditions [13] including pain, arthritis, rheumatism, pulmonary troubles, and as a laxative[14]. However, there is no scientific justification for its use in the management of these inflammatory conditions. Therefore, the objective of the present study was to investigate its anti-inflammatory and antioxidant activities as purported by folklore medicine.

MATERIALS AND METHODS

Plant material collection and processing: The stem bark of *Dialium dinklagei* was collected in June, 2015 from Kwahu Asakraka in the Eastern region of Ghana. The plant material was botanically identified by Dr. George Henry Sam of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical sciences, KNUST, Ghana. It was cleaned of foreign material, air dried and pulverised into fine powder.

Extraction: The powdered plant material (200 g) was extracted with 0.6 L litres of 70% ethanol by cold maceration. The Rotary evaporator (R-114, Buchi, Switzerland) was used to concentrate the extract under a low pressure to a small volume and then evaporated to dryness on a water bath to afford a dark gummy extract (yield of 4.81%$^{w}/_{w}$). The extract was stored in a desiccator.

Drugs, chemicals and instruments used for the experiment: Analytical grade organic solvents purchased from BDH laboratory supplies, England. Diclofenac was purchased from Troge (Germany). All other chemicals used were also of analytical grade and purchased from Sigma Aldrich Co. Ltd. Irvine, UK. UV-visible spectrophotometer (Cecil CE 7200 spectrometer, Cecil instrument limited, Milton Technical Centre, England) and Vernier calliper purchased from Percision India Ltd were employed in the study.

Animals: Cockerels (*Gallus gallus*; Strain shaver 579) were obtained from Akate Farms, Kumasi, Ghana. They were obtained one-day post-hatch and maintained in stainless steel cages (34x57x40 cm3) at 12-13 chicks at room temperature maintained at 29^{o}C. An overhead incandescent illumination was maintained on a 12hr light-dark cycle. The cockerels had free access to feed (Chick Mash, GAFCO, Tema, Ghana) and water *ad libitum.* The experimental procedures were done in accordance with the National Institute of Health guidelines for care and use of laboratory animals (NIH Department of Health Services publication number: 83-23, revised 1985; Committee, 2011) and were approved by the Ethics Committee, Department of Pharmacology, College of Health Sciences of the Kwame Nkrumah University of Science and Technology.

ANTI-INFLAMMATORY ASSAY

The anti-inflammatory effects of the extracts were assessed using the carrageenan induced oedema in 7 days old chicks. Cockerels weighing 40-55 g on day 7 were randomly divided into treatment and control groups of sample size 5. The carrageenan-induced foot oedema model as described by [15] with slight modifications by [16] was used. Diclofenac served as the standard drug. Foot pad oedema was induced by sub-plantar injection of carrageenan (10 µL of a 2%w/v solution in saline) into the right foot pads of the chicks. The extracts were administered orally at 30, 100 and 300 mg/kg and diclofenac at 10, 30, and 100 mg/kg body weight. The control animals received the vehicle saline. Raw values for the foot volumes were measured using Vernier calliper. The paw thickness was measured at an hourly interval for 5 hours using Vernier calliper. The oedema induced by the carrageenan was quantified by measuring the difference in foot thickness before carrageenan injection and at the various time intervals. The values were individually normalized as a percentage change in foot oedema relative to the corresponding values at time zero, and then

averaged for the respective treatment groups. Total foot thickness for each treatment was calculated in arbitrary unit as the area under the curve (AUC). The percentage inhibition for each treatment was determined using the equation:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

ANTIOXIDANT ASSAY

DPPH radical scavenging assay: The free radical scavenging activity of the extract was measured *in vitro*.by 2, 2′- diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described by Govindarajan et al [17]. One ml each of the different concentrations of the extract (500 – 62.5 µg/ml) was added to a methanol solution of DPPH (20 mg/L) in a test tube. The reaction mixture was kept at 25ºC for 30 minutes. Process was repeated for ascorbic acid of concentrations 50-6.25 µg/ml. The absorbance of the residual DPPH was determined at 517 nm using a UV-visible spectrophotometer. The DPPH radical scavenging activity is calculated according to the following equation:
%DPPH radical scavenging = ((A control – A sample) / A control) X 100

Total antioxidant capacity assay: The total antioxidant capacity of the fractions was determined by phosphomolybdate method using ascorbic acid as a standard. The assay is based on the reduction of molybdenum, Mo^{+6} to Mo^{+5}, by the extracts and subsequent formation of a green phosphate-molybdate (Mo^{+5}) complex at acidic pH [18]. The reagent solution was prepared by adding Ammonium molybdate (4 mM), disodium hydrogen phosphate (28 mM) and sulphuric acid (0.6 M). A blank solution was prepared by adding every other solution but without the extract. 1ml each of the different concentrations of the extract (500 µg/ml - 62.5 µg/m) and ascorbic acid (50 µg/ml - 6.25 µg/ml) were added to test tubes containing 3 ml of the reagent solution and incubated at 95ºC for 90 minutes. After the mixture, had cooled to room temperature, the absorbances of the solutions were measured at 695 nm using the UV-visible spectrophotometer. Ascorbic acid (50 µg/ml - 6.25 µg/ml) was used as standard to construct a calibration curve from which the TAC of extract was extrapolated. The antioxidant capacity was thus estimated as ascorbic acid equivalent (AAE) in milligram per gram (mg/g) of dried extract.

Phytochemical Analysis

The presence of saponins, alkaloids, triterpenes, flavonoids, glycoside, reducing sugars and tannins were tested by simple qualitative and quantitative methods of Trease and Evans [19] and Sofowora [20].

Statistical evaluations: The statistical analysis was done by using Graph pad prism for windows version 5.0 (Graph pad software, San Diego, CA, USA). The differences in AUCs were analysed by one-way analysis of variance (ANOVA) followed by Students-Newman-Keul's *post hoc* test.

RESULTS AND DISCUSSION

Carrageenan-induced oedema test [21], a classical model of acute inflammation, has been used widely to screen new anti-inflammatory drugs [22]. The inflammatory response induced by carrageenan is characterized by a biphasic response [23] with marked oedema formation resulting from the rapid production of several inflammatory mediators such as histamine, serotonin and bradykinin (first phase), which is subsequently sustained by the release of prostaglandins and nitric oxide (second-phase) with peak at 3 h, produced by inducible isoforms of COX (COX-2) and nitric oxide synthase (iNOS), respectively [24, 25]. The second (late) phase is sensitive to most clinically effective anti-inflammatory drugs [22, 23]. In this study, chicks rather than rodents were used. Chicks are easier to handle and more economical than rodent models.
Administration of carrageenan (10 µl, 2 % suspension) induced moderate inflammation resulting in foot oedema in the 7-day old chicks which began 1 h after intraplantar injection of carrageenan and peaking at 2-3 h as described by Roach and Sufka (2003)(Figure 1). The time course effects of *Dialium dinklagei* and diclofenac on carrageenan-induced oedema are shown in Figure 1. Oral administration of the hydro-ethanolic stem bark extract (30-300 mg/kg), dose dependently and significantly (P<0.001) reduced the increase in foot volume induced by injection of carrageenan (Figure 1a) with ED_{50} of 10.60 mg/kg compared with 4.03 mg/kg of diclofenac (Figure 2). The extract inhibited the increase in foot volume significantly (P<0.001) from the second hour (Figure 1a) and thus possibly, inhibited the synthesis and release of prostaglandins. Similarly, the NSAID diclofenac significantly (P<0.001) and dose-dependently reduced the foot oedema (Figure 1b and 1d) over the period of the experiment. The study has shown that extracts of the stem bark of *Dialium dinklagei* possesses significant anti-oedematogenic effect on foot pad oedema induced by carrageenan. This justifies the use of the plant traditionally in the management of inflammatory conditions. *D. dinklagei* inhibited both early and late phases of oedema suggesting that it could be acting through the inhibition of the release and/or action of those inflammatory mediators involved in

carrageenan-induced oedema which include cytoplasmic enzymes, histamine, serotonin, bradykinin, prostaglandins and other cyclooxygenase products. However, the actual mechanism of action in inflammation is unknown, and needs to be established.

The DPPH assay determines the ability of an agent to scavenge free radicals. The DPPH radical scavenging assay of the extract and control (Ascorbic acid) showed a concentration dependent radical scavenging activity (Figure 3) with EC_{50} values of 39.6 and 22.59 µg/ml respectively. In the total antioxidant assay, ascorbic acid was used as the reference antioxidant. The total antioxidant capacity was expressed as ascorbic acid equivalent in mg/g weight of extract. Ascorbic acid showed a dose dependent total antioxidant activity of 581.58 mg of ascorbic acid per gram of extract. The anti-oxidant activity may partly be responsible for the anti-inflammatory activity of *D. dinklagei* established earlier. This is because inflammatory tissue injuries are mediated by reactive oxygen metabolites from phagocytic leukocytes (e.g. neutrophils, monocytes, macrophages and eosinophils) that invade the tissues. These radicals may injure cells and tissues directly via oxidative degradation of essential cellular components [26].

Preliminary phytochemical screening of the plant revealed the presence of tannins, alkaloids and triterpenoids (Table 1). The anti-inflammatory and antioxidant actions shown by the stem bark extract of *Dialium dinklagei* may be attributed to these metabolites. Alkaloids have diverse pharmacologic activity and are proven to have remarkable anti-inflammatory activity [27]. Many triterpenes have shown activity in different *in vivo* experimental models such as hind paw oedema induced by carrageenan, serotonin and phospholipase A2; ear oedema induced by phorbol esters, ethylphenylpropiolate, arachidonic acid and capsaicin. Other effects have been demonstrated in a number of *in vitro* experiments [28]. Tannins have been suggested to produce effects in a non-specific manner by their astringent properties on cell membranes thus affecting cell functions [29] Tannins, together with flavonoids, are a more reliable source of antioxidants compared with vitamins C, E and beta carotenes[30]. Triterpenes have the ability to scavenge free radicals indicating its potential use as an antioxidant [31].

CONCLUSION

This study has demonstrated that the stem bark extract of *Dialium dinklagei* possesses considerable anti-inflammatory and anti-oxidant activity. This provides justification for the use of the stem bark of the plant for treating inflammatory conditions.

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Table 1: Secondary metabolites present in the stem bark of *Dialium dinklagei*

Phytochemical	**Inference**
Tannins	+
Terpenoids	+
Flavonoids	-
Alkaloids	+
Phytosterols	-
Reducing sugars	+

+ = detected - = not detected

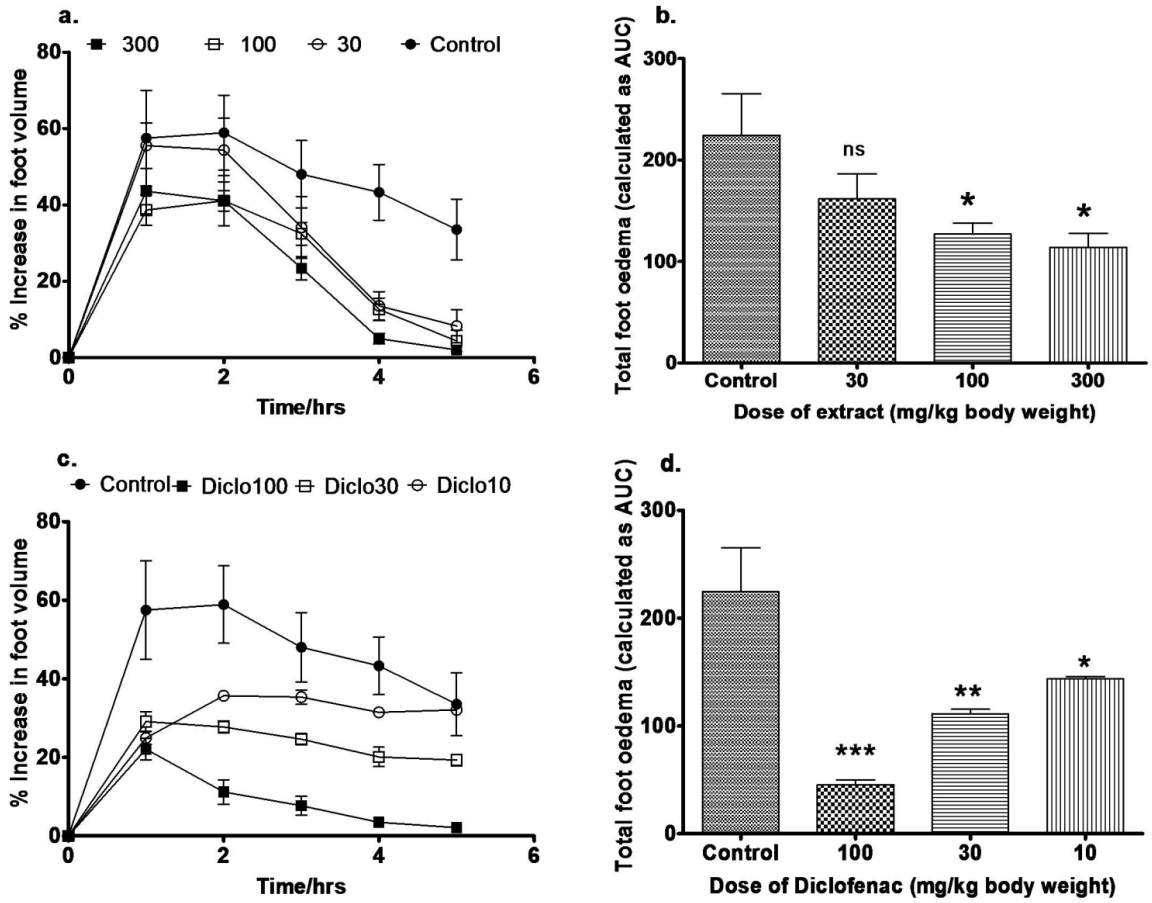

Figure 1: Effect of *Dialium dinklagei* extract (a, b) (30 – 300 mg/kg; *p.o*) and diclofenac (c, d) (1 – 10 mg/kg; *p.o*) on the time course curve (a&c) and total oedema response (expressed as the AUC b&d) in carrageenan induced oedema in chicks. Values are means ± SEM. (n =5). $^{***}P < 0.0001$;$^{**}P < 0.001$; $^{*}P < 0.05$ compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's post hoc test). ***P < 0.0001; **P < 0.001;*P < 0.05 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keul's *post hoc* test).

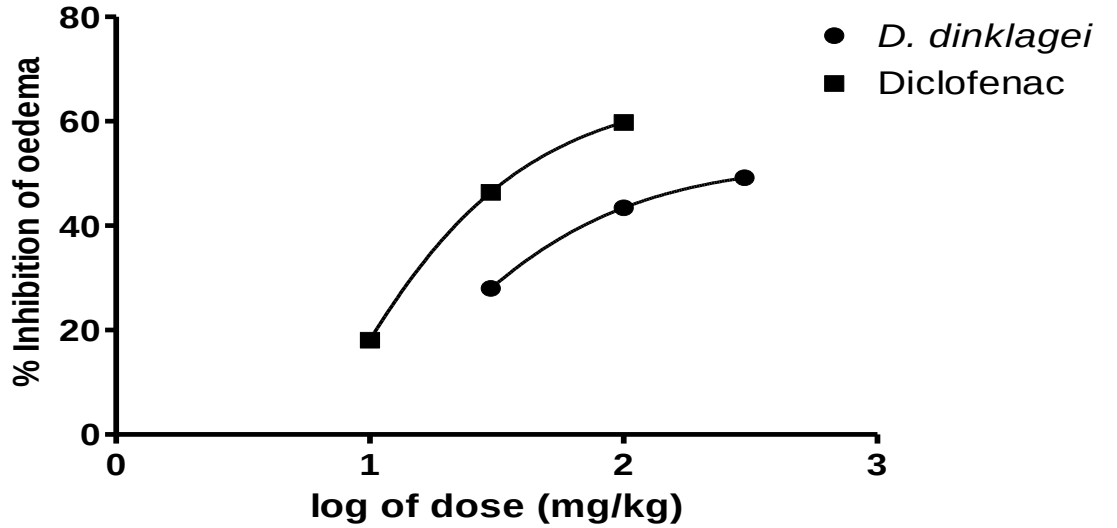

Figure 2: Dose response graph to evaluate the ED_{50} of *D. dinklagei* and diclofenac in the chick carrageenan anti-inflammatory model

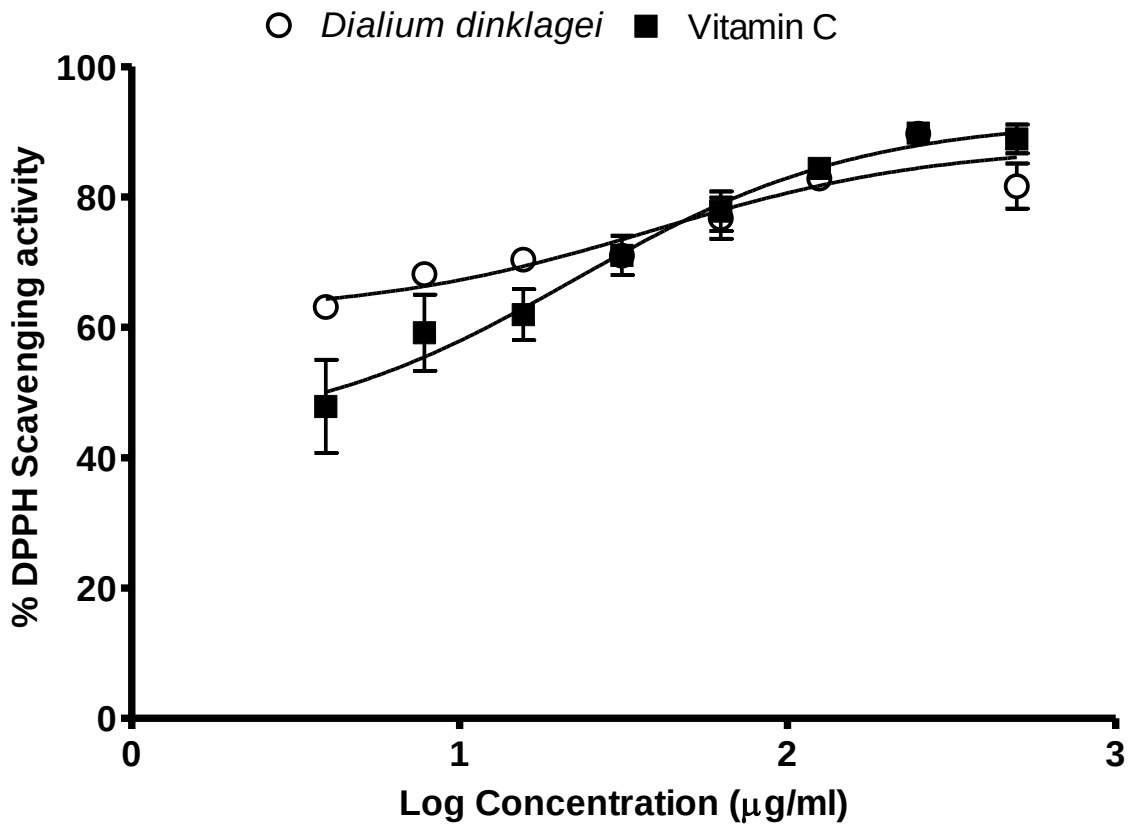

Figure 3: DPPH radical scavenging effect of *D. dinklagei* and the reference antioxidant vitamin C

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