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Anti-inflammatory and antioxidant properties of the ethanolic stem bark extract of *Artocarpus Altilis* (Parkinson) Fosberg (Moraceae)

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ABSTRACT

Artocarpus altilis (Parkinson) Fosberg or breadfruit is used in ethnomedicne for the treatment of boils and wounds, hypertension, skin infections, and in pain management. This study therefore evaluated the anti-inflammatory activity, as well as the antioxidant potential of an ethanol stem bark extract of Artocarpus altilis. Foot volume, in chick carrageenan-induced foot edema model, was measured before and after treatment with 30-300 mg/kg of the extract, 0.3-3 mg/kg dexamethasone, and 10-100 mg/kg diclofenac. Total phenol content evaluation, DPPH radical scavenging and phosphomolybdenum antioxidant capacity assays, and phytochemical analysis of the extract were performed. The extract caused a dose – dependent decrease (P<0.05) in foot edema with the highest inhibition of 61.29±1.76% at a dose of 300 mg/kg; comparable to diclofenac and dexamethasone (69.56±1.01% and 68.74±0.98% respectively). The extract, which had a phenolic content of 159.89 mg of tannic acid equivalent (TAE) per gram, showed significant antioxidant activity. Triterpenoids, flavonoids, tannins and coumarins, found in the phytochemical analysis could have been responsible for the activity of the extract. Per the findings, the ethanolic stem bark extract of Artocarpus altilis exhibits anti-inflammatory and antioxidant activities and therefore gives credence to it use in folklore medicine.

Key words: Carrageenan-induced foot edema, DPPH radical scavenging, Phosphomolybdenum antioxidant capacity, *Artocarpus altilis*

INTRODUCTION

Medicinal plants and their products have been successfully used traditionally for a wide range of health problems since prehistoric time [1]. In recent years there has been a resurgence in the consumption of herbal remedies worldwide and this may be attributed to several factors such as patients dissatisfaction with conventional allopathic medicines in terms of effectiveness and/or safety, satisfaction with therapeutic outcome, and the perception that herbal medicines are inherently safe [2].

Inflammation is a complex biological process characterized by swelling, pain and redness which is as a result of exogenous and endogenous aggressions. Inflammatory reactions bring about the production of reactive oxygen species (ROS) [3]. The inflammatory reaction is beneficial to humans but can be harmful when there is excessive production of ROS due to imbalance with biological antioxidants which lead to oxidative stress [3]. Conventional drugs for the treatment of inflammation and pain includes the steroidal and non-steroidal anti-inflammatory drugs (NSAIDS) as well as the narcotic analgesic. The investigation of plant based medicines used in traditional medicine provided lead compounds for the discovery of some of these medicinally important drugs. Thus there is the need to investigate the efficacy of some of the plant based medicines used in ethnomedicine for the treatment of inflammation to serve as alternatives to the NSAIDS and others which have limited use due to undesirable side effects such as gastric irritation, ulceration, bleeding, renal failure, fluid retention and addiction [4-6].

Artocarpus altilis (Parkinson) Fosberg of the family Moraceae is commonly referred to as breadfruit or breadnut. In Ghana it is known as D-ball [7]. Various parts of *Artocarpus altilis* are used in different countries for treatment of various ailments. The juice of its leaves is used as eardrop and the roasted leaves used as a remedy for enlarged spleen [8]. The leaves are used in fever, boils and wounds [9]. Its stems and roots have also been used for treatment of liver cirrhosis and hypertension in Taiwan [10]. Latex from the stem bark is massaged into the skin to heal broken bones and sprains. The stem bark is used to treat fungal and other skin infections [11]. Aqueous extracts of the leaves of *Artocarpus altilis* was reported to have anti-hypertensive effect [12]. A number of flavonoids including artocarpin, hydroxyartocarpin, cyclomorusin, cyclomulberrin, engeletin have been isolated from the plant [10]. However, there is no scientific justification for the use of the stem bark for the treatment of inflammation. Therefore, in this study, the anti-inflammatory and anti-oxidant potential of the ethanolic stem bark of *A. altilis* was evaluated.

MATERIALS AND METHODS

Plant material collection and processing

The stem bark of *Artocarpus altilis* (Parkinson) Fosberg (Moraceae) were collected in March, 2013 from the campus of Kwame Nkrumah university of Science and Technology (KNUST), Kumasi, Ghana. It was authenticated at the Department of herbal Medicine where a herbarium specimen has been kept (KNUST/HM 2/2014/S15). It was then air dried for 7 days and milled into coarse powder. The air dried and powdered stem bark of *Artocarpus altilis* (586.65 g) was extracted with 70% ethanol by cold maceration. The extract was concentrated under reduced pressure to a small volume by means of rotavapor (R-114, Buchi, Switzerland) at a temperature of 40ºC, and evaporated to dryness on a water bath to give an extract designated EAA (yield = 7.07%$^{w}/_{w}$).

Drugs and Chemicals Used

All chemicals, with the exception of the drugs, were purchased from Sigma Aldrich Co Ltd. Irvine, UK. Organic solvents were of analytical grade and purchased from BDH Laboratory Supplies (England). Diclofenac and dexamethasone were purchased from Troge, Hamburg, Germany and Pharm-Inter, Brussels, Belgium respectively.

Experimental Animals

Cockerels (*Gallus gallus*; strain shaver 579) were obtained from Akati Farms, Kumasi, Ghana as 1 day post-hatch and were housed in stainless steel cages (34 × 57 × 40 cm3) at a population density of 10-13 chicks per cage. Feed (Chick Mash, GAFCO, Tema, Ghana) and water were available *ad libitum* through 1-quart gravity-fed feeders and water trough. Room temperature was maintained at 29°C, and overhead incandescent illumination was maintained on a 12 hour light-dark cycle. Daily maintenance of the cages was conducted during the first quarter of the light cycle. Chicks were tested at 7 days of age. Group sample sizes of 5 were used throughout the study.

Determination of anti-inflammatory Activity

The carrageenan foot oedema model of inflammation in the chick previously reported by Roach and Sufka [13], was used to evaluate the anti-inflammatory properties of EAA. Seven day-old chicks (weighing between 40-60 g) were put into 10 Groups (n= 5). The initial foot volumes were then taken. Carrageenan (2% $^{w}/_{v}$) was injected intraplantar into the right footpads of the chicks. The foot volumes measured again after one hour. The extract was then dosed orally at 30, 100, and 300 mg/kg to groups one to three. Dexamethasone and diclofenac injected intraperitoneally at 0.3, 1, 3 mg/kg and 10, 30, 100 mg/kg respectively, were administered to chicks in groups 4 to 9. The foot volumes were then measured at hourly intervals for 5 hours by water displacement plethysmography as described by Fereidoni *et al*. [14] using an electronic Von Frey plethysmometer (Model 2888, IITC life science inc. Ca 91367 Canada). The control animals (Group 10) received 2 ml/kg normal saline. The edema component of inflammation

was quantified by measuring the difference in foot volume before carrageenan injection and at the various time intervals.
The foot volumes were individually normalized as percentage of change from their values at time zero then averaged for each treatment group. Total foot volume for each treatment was calculated in arbitrary unit as the area under the curve (AUC). To determine the percentage inhibition for each treatment, the following equation was used.

$$\%\ inhibition\ of\ \ edema = \left(\frac{AUC_{control} - AUC_{treatment}}{AUC_{control}} \right) \times 100$$

The protocol was in compliance with the National Institute of Health guidelines for the care and use of laboratory animals and were approved by the Department of Pharmacology Ethics Committee.

ANTIOXIDANT ASSAY

DPPH radical scavenging assay

The free radical scavenging activity was determined as described by Govindarajan [15], with few modifications. A 1 ml quantity of EAA (500, 250, 125 and 62.5 µg/ml) was added to 3 ml methanol solution of DPPH in a test tube and incubated at 25°C for 30 minutes. The absorbance of the mixture was determined at 517 nm in a spectrophotometer (Cecil CE 7200 spectrophotometer, Cecil instrument limited, Milton Technical Centre, England). A solution of 1 ml methanol and 3 ml of DPPH was prepared, incubated at 25°C for 30 minutes and used as control. Ascorbic acid (100, 50, 25, 12.5 and 6.25 µg/ml) was used as a standard free radical scavenger. Results were expressed as percentages of blank. The EC_{50} which is the concentration required to scavenge 50 % of the DPPH molecule was calculated. Each test was carried out using three replicates.

The % DPPH scavenging effect (% of control) of the antioxidant was calculated as follows.

% DPPH scavenging effects = (*Ac* - *At*)/*Ac* x 100

Where: Ac = Absorbance of the control, At = Absorbance of the test drug/ extract.

Concentrations responsible for 50 % of the maximal effect in the DPPH assay (EC_{50}) for each drug/extract were determined using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation [19-20].

$$Y = \frac{a + (b - a)}{(1 + 10^{(LogEC50 - X)})}$$

Where, *X* is the logarithm of dose and *Y* is the response. *Y* starts at *a* (the bottom) and goes to *b* (the top) with a sigmoid shape.

Total antioxidant capacity assay

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 [16]. One millimeter of EAA (500, 250, 125 and 62.5 µg/ml) was added to test tubes containing 3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM disodium phosphate and 4 mM ammonium molybdate) and incubated at 95 °C for 90 minutes. The mixture was allowed to cool to room temperature and the absorbance was determined at 695 nm. Five concentrations of ascorbic acid (100, 50, 25, 12.5, 6.25 µg/ml) were used to construct a calibration curve. A blank solution was prepared by adding every other solution but without extract or standard drug. The antioxidant capacity was expressed as mg of ascorbic acid equivalent (AAE) per g of extract.

Modified Prussian blue assay for total phenol

The Price and Butler method with modification by Graham, [17] to give greater color stability was used in this assay. 1 ml of the extract (62.5-500 mg/ml) was measured into test tubes and 3 ml of distilled water added and shaken thoroughly. 1 ml of potassium ferrocyanate was added followed immediately by 1 ml ferric chloride and immediately vortexed. The mixture was then left to stand for 15 minutes after which 5 ml of stabilizer (30 ml

distilled water, 10 ml 85% H_3PO_4, 10 ml 1% gum Arabic) was added and vortexed. The absorbance of the mixture was measured at 700 nm. Five concentrations of tannic acid (100, 50, 25, 12.5, 6.25 µg/ml) were used to construct a calibration curve. The total phenol was expressed as mg of tannic acid equivalent (TAE) per g of the extract.

Phytochemical analysis

Phtyochemical tests were performed to identify the presence of secondary metabolites such as tannins, phytosterols, alkaloids, saponins, triterpenoids, glycosides, flavonoids and coumarins. These were done according standard methods [18].

Statistical analysis

Graph Pad Prism for Windows version 5.0 (Graph Pad Software, San Diego, CA, USA) was used for all statistical analyses. Differences in AUCs were analyzed by one way analysis of variance (ANOVA) followed by Student-Newman-Keuls' *post hoc* test. $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Inflammation has been shown to be associated with a number of diseases, including rheumatoid arthritis, chronic asthma, multiples sclerosis, inflammatory bowel disease and psoriasis [21]. During inflammation, high levels of reactive oxygen species are produced to exert a defense against pathogens. Scavenging of these ROS is therefore notably helpful in attenuating the inflammatory cascade. Therefore in this research the anti-inflammatory and anti-oxidant properties of *A. altilis* was investigated

Anti-inflammatory assay

Following the induction of edema in the chicks, the foot volume of the control chicks increased gradually and reached a peak after 2 hours (Figure 1a, c & e) and gradually declined throughout the course of the experiment. In chicks given the extract, the foot volume showed significant decrease after 1 hour (Figure 1a) and continued to decrease with time. The decrease in edema was observed to be dose dependent (Figure 1 b) with the highest inhibition of 61.29±1.76 at 300 mg/kg body weight. The NSAID diclofenac and the steroidal anti-inflammatory agent dexamethasone, used as positive controls, also showed significant dose dependent decrease in edema (Figure 1c-f) with percentage inhibitions of 69.56±1.01 and 68.74±0.98 respectively (Table 1). Therefore, the study has shown that the stem bark of *Artocarpus altilis* exhibit anti-inflammatory activity. Thus giving scientific credence to it use for the treatment of inflammation and pain in traditional medicine.

Table 1. Percentage inhibition of edema in carrageenan anti-inflammatory model

Drug/Extract	% Inhibition of edema
Extract (EAA)	61.29±1.76
Diclofenac	69.56±1.01
Dexamethasone	68.74±0.98

Antioxidant assay

The DPPH radical scavenging activity of the extract was evaluated using ascorbic acid as the reference antioxidant compound. The extract and ascorbic acid showed a concentration dependent radical scavenging activity (Figure 2) with EC_{50} values of 19.03 and 1.211 µg/ml respectively. In the phosphomolybdate total antioxidant capacity assay (TAC), vitamin C was used as standard antioxidant. The total antioxidant capacity was calculated in mg of ascorbic acid equivalent per gram of extract (AAE). Vitamin C showed a good linearity in the range 50-3.125 µg/ml with a correlation coefficient (r^2) of 0.9994. The extract showed a dose dependent total antioxidant capacity with a high value of 116 mg of ascorbic acid equivalent per gram of extract. Similarly the total phenolic content of extract was determined using tannic acid as standard. It also recorded a high phenolic content of 159.89 mg of tannic acid (TAE) per gram of extract. Thus it has been demonstrated in this study that ethanol extract of the stem bark of *A. altilis* has significant antioxidant activities.

Evidence indicates that inherent antioxidant and other protective defenses mitigate inflammation and can reduce tissue damage. This is because ROS have been implicated in the inflammatory cascade. They have been reported to be released from activated neutrophils and macrophages. Over production of ROS due to oxidative stress leads to tissue injury by damaging macromolecules including proteins, lipoproteins, DNA and lipids [22]. ROS are also known to propagate or augment the state of inflammation by directly activating cytokines such as IL-1, TNF-α and

interferon-γ, which are responsible for the recruitment of additional neutrophils and macrophages. Thus neutralization of free radicals by antioxidants is helpful in reducing the state of inflammation. The antioxidant properties of the stem bark of *A. altilis* may therefore play a role in it anti-inflammatory activities and give credence to it use in folklore medicine.

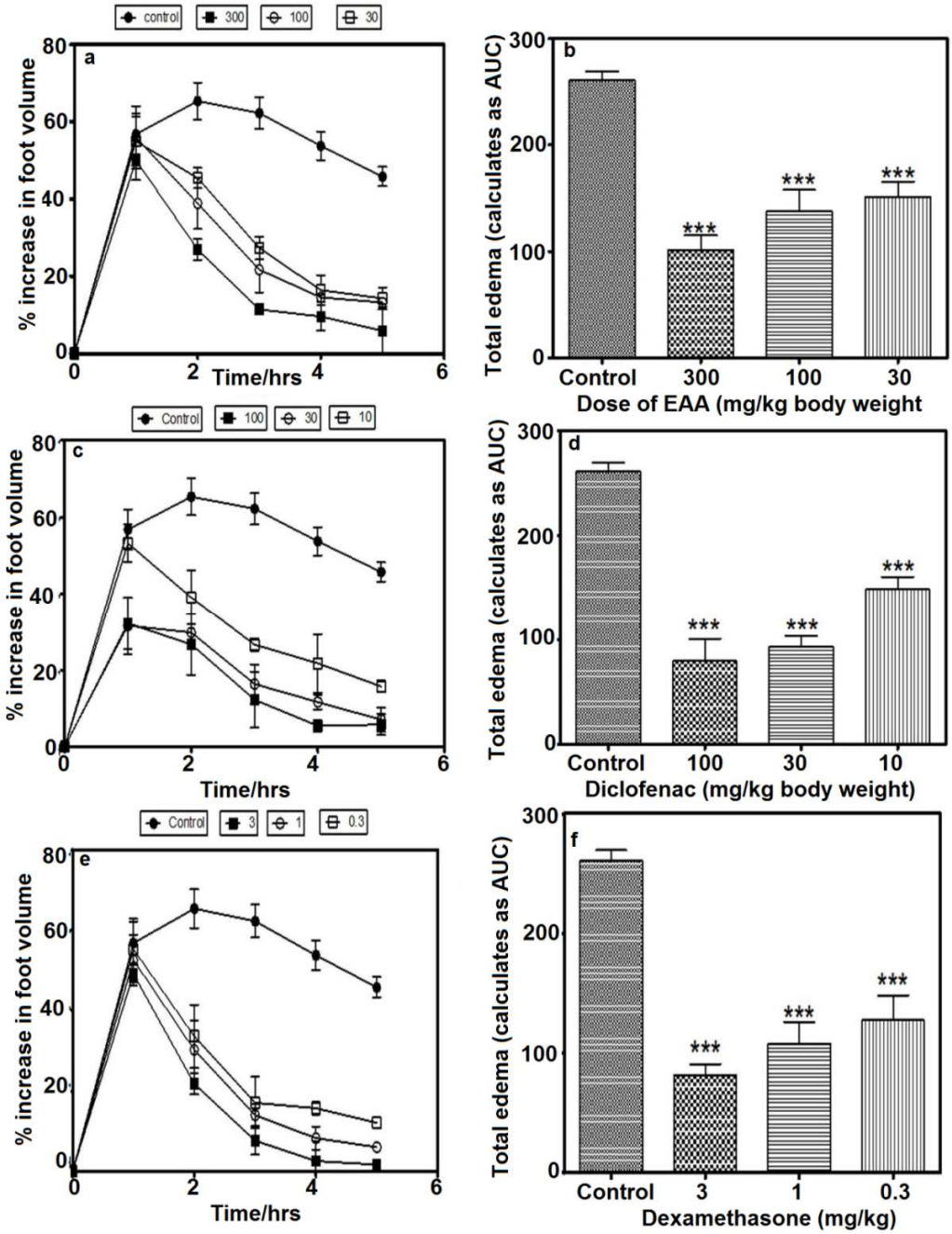

Figure 1 Effect of *A. altilis* [(30-300 mgkg^{-1}oral)(a-b)] , diclofenac [(10-100 mg/kg; *i.p*)(c-d)] and dexamethasone [(0.1-3 mg/kg; *i.p*)(e-f)] on time course curve and the total oedema response, calculated as AUC's, for 5 hours, in carrageenan induced paw oedema in chicks. Values are means ± S.E.M (n=5) *** p < 0.001, ** p < 0.01. *P<0.05 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keul's post hoc test)

The antioxidant activity demonstrated by the extract may also support the traditional use of the plant in Trinidad and Bahamas for the treatment of wounds. This is because in acute wounds, there is increase in the level of reactive oxygen species. Antioxidants maybe useful in scavenging the reactive oxygen species leading to gradual return of cells to the state of redox homeostasis thereby preventing complications and promoting healing [23].

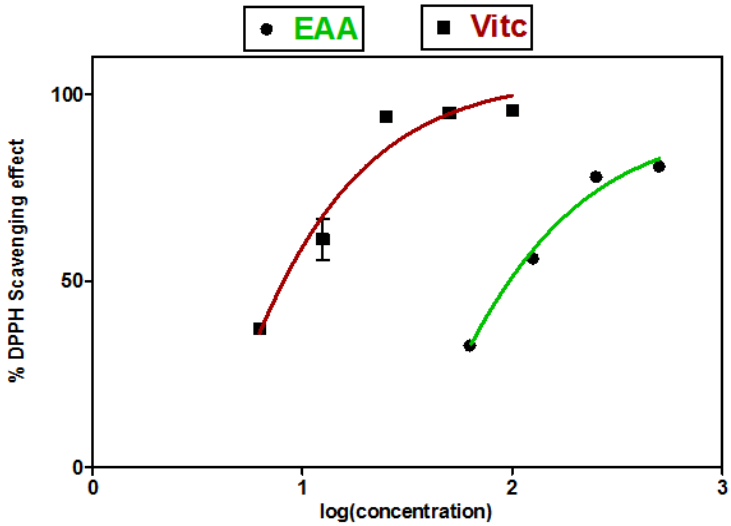

Figure 2. DPPH radical scavenging activity of extract and vitamin C
EAA; A. altilis ethanolic extract, Vitc; Vitamin C

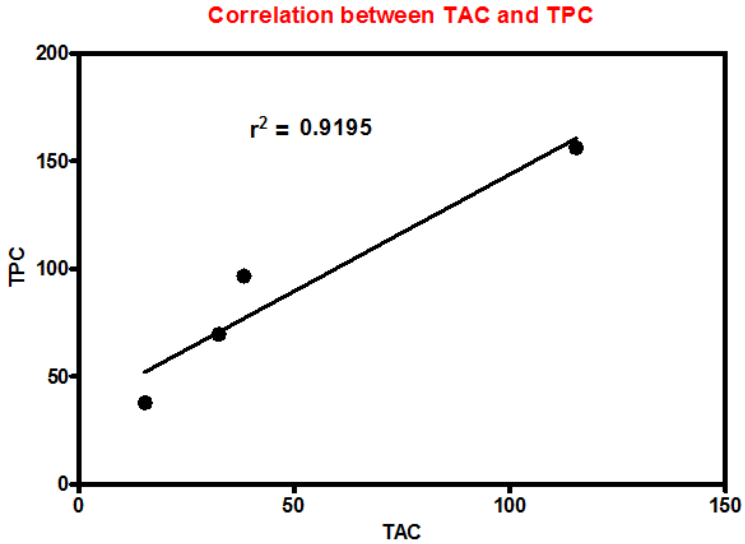

Figure 3. Correlation between TAC and TPC
TAC: total antioxidant capacity, TPC; total phenolic content

There was a high positive correlation between the total antioxidant capacity and the phenolic content (r^2=0.9195)(Figure 3). This means that about 92% of the antioxidant activity of *A. altilis* may be due to its phenolic content, with the remaining secondary metabolites contributing 8% of the antioxidant activity. Phytochemical analysis of the stem bark revealed the presence of secondary metabolites such coumarins, flavonoids, triterpenoids, glycosides and tannins (Table 2). Artocarpus species are rich in phenolics such as flavonoids [9]. Flavonoids have been reported to have antiviral, antiallergic, antiplatelet, anti-inflammatory, antitumor, and antioxidant activities [24-25]. Ant-inflammatory activity of flavonoids have been linked to their antioxidative and radical scavenging activities, regulation of cellular activities of inflammation-related cells, modulation of the activities of arachidonic acid metabolism enzymes (phospholipase A2, cyclooxygenase, lipoxygenase) and nitric oxide synthase, modulation

of the production of other pro-inflammatory molecules and modulation of pro-inflammatory gene expression. Therefore their presence in *Artocarpus altilis*, may be responsible for its antioxidant and anti-inflammatory activities. This is the first report of its antioxidant and anti-inflammatory activities.

Table 2. Phytochemical screening on the stem bark of *Artocarpus altilis*

Phytochemical constituents	Powdered bark
Alkaloids	-
Coumarins	+
Flavonoids	+
Glycosides	+
Phytosterols	-
Saponins	-
Tannins	+
Triterpenoids	+

Key : - Absent, + Present

CONCLUSION

The study has shown that ethanol extract of the stem bark of *Artocarpus altilis* exhibit ant-inflammatory and antioxidant activities. This gives scientific justification of it use for the management of inflammation pain and wound healing.

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