

ANTI-INFLAMMATORY, ANTIOXIDANT, AND SELECTIVE ANTIBACTERIAL EFFECTS OF
EUADENIA EMINENS ROOT BARK.Dickson RA^{1*}, Fleischer TC², Ekuadzi E¹, G. Komlaga¹¹Department of Pharmacognosy, ²Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi.

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E-mail: radickson.pharm@knust.edu.gh**Abstract**

Euadenia eminens Hook f. (Capparaceae) has traditional uses in the management of conjunctivitis, iritis, ophthalmia, tuberculosis, otalgia and rectal prolapse. The fruit pulp is also eaten as an aphrodisiac. In this paper, we report on the anti-inflammatory, antioxidant and antibacterial effects of its roots. A 70 % ethanol extract was tested for anti-inflammatory effect using the carrageenan-induced oedema in chicks. Free radical scavenging, total antioxidant and total phenol content were assessed spectrophotometrically. The extract was tested for antibacterial activity using the agar well diffusion method and micro dilution assays. The 70% ethanol extract gave a maximal inhibition of oedema by 74.18 % at 30 mg/kg. The total antioxidant capacity expressed in terms of ascorbic acid was 0.609 mg/g dry weight. The total phenol in terms of tannic acid was 7.25 mg/g dry weight. The extract also demonstrated free radical scavenging activity yielding IC₅₀ value of 1.175 mg/mL. The root extract however, showed selective antibacterial activity, inhibiting growth of two microorganisms; *Bacillus subtilis* and *Bacillus thurigiensis*. The MICs were 500 and 1000 µg/mL respectively. These results may account in part for the ethnopharmacological use of the plant.

Key words: Anti-infective, Anti-inflammatory, Antioxidant, Carrageenan-induced oedema, *Euadenia eminens*.**Introduction**

Medicinal plants represent a largely untapped source of molecules with diverse structures. These bioactive molecules are a unique source of potentially new drug leads necessary to combat the increasing health challenges confronting the world. Antibiotic resistance is one such major threat to public health (Smith et al., 1991). Multi-drug resistance, as well as the emergence of new infectious diseases have prompted the discovery and development of new drug molecules which are not simply based on existing antibiotic templates. Furthermore, the management of chronic inflammatory disorders poses an uncertain outcome. Chronic inflammatory disorders: chronic non-healing ulcers, rheumatoid arthritis, chronic obstructive pulmonary disease, etc., remain one of the major health challenges facing the world (Li et al., 2003). Inflammation is a component of almost every disease condition including arthritis, bronchitis, iritis, etc., and if unchecked would turn chronic.

The relevance of African traditional medicine in the management of various ailments has been well established (Soforowa, 1982). Globally, attention has been turned to herbal medicines; a trend recognized and advocated by the World Health Organization in its Alma Atta declaration for health for all. Medicinal plants are currently being scientifically investigated for their potential in the management of infectious and inflammatory disorders. *Euadenia eminens* Hook f. (Capparidaceae) was selected for this study based on its ethnopharmacological uses in Ghana. A shrub or small tree up to 5 m tall. The juice from cut fruit is used as drops for sore eyes, conjunctivitis, iritis, ophthalmia and trachoma. The fruit pulp is eaten as an aphrodisiac (Abbiw, 1990). Communications with some herbalists in Aburi, Ghana showed that the root decoction is used in tuberculosis, otalgia and rectal prolapse (Agbovie et al., 2002). However, no scientific investigations to the best of our knowledge of these effects have been undertaken. Therefore, the present work aimed to verify the anti-inflammatory, antibacterial and antioxidant effects of the root bark of this plant.

Methods**Plant collection**

The root of *Euadenia eminens* Hook. f. (Capparidaceae) was collected in June 2009, from Kente in the Ashanti Region of Ghana. Botanical identity was confirmed and voucher specimen (KNUST/HM1/2010/R001) was deposited at the Department of Pharmacognosy, College of Health Sciences, Kwame Nkrumah University of Science and Technology herbarium.

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Preparation of extract

The roots of *Euadenia eminens* were chopped, dried and coarsely powdered. The powder (50 g) was soxhlet-extracted using 0.5 L of 70% ethanol for 12 h. The extract was evaporated to a brown mass under reduced pressure in a rotary evaporator, air-dried and kept in a dessicator till required. The yield obtained was 4.9 % w/w.

Chemicals

Organic solvents were of analytical grade and purchased from BDH laboratory supplies, England. Precoated silica gel F₂₅₄ aluminium sheets, purchased from Merck kGaA, Germany, were used for TLC. Dexamethasone and Diclofenac were purchased from Pharm –Intas, Belgium and Troge, Germany respectively. All other chemicals used were also of analytical grade and purchased from Sigma Aldrich Co. Ltd. Irvine, UK.

Test Animals

Cockerels (*Gallus gallus*; Strain shaver 579) were obtained from Akropong Farms, Kumasi, Ghana. They were obtained one-day post-hatch and maintained in stainless steel cages (34x57x40 cm³) at 12-13 chicks at a controlled temperature (29±1^oC). An overhead incandescent illumination was maintained on a 12 hr light-dark cycle. The cockerels had free access to feed (Chick Mash, GAFCO, Tema, Ghana) and water *ad libitum*.

Microorganisms used

The antibacterial activities of the *Euadenia eminens* root extract were assessed against eight (8) bacterial species which included both Gram positive (*Enterococcus faecalis* ATCC 29212, *Bacillus thurigiensis* ATCC 13838, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* NCTC 10073) and Gram negative (*Salmonella typhi* NCTC 6017, *Escherichia coli* NCTC 9002, *Proteus vulgaris* NCTC 4175 and *Pseudomonas aeruginosa* ATCC 27853) bacteria. The strains were obtained from the University of Ghana Medical School.

Anti-inflammatory assay

Carrageenan-induced oedema in chicks

Cockerels weighing 40-55 g on day 7 were randomly divided into treatment and control groups, and were used in the anti-inflammatory activity evaluation (Woode et al., 2007; Roach and Sufka, 2003). Group sample sizes of 5 were used throughout the study. Foot pad oedema was induced by subplantar injection of carrageenan (10 µL of a 2% w/v solution in saline) into the right foot pads of the chicks. Raw values for the foot volumes were measured by water displacement plethysmography at 0, 1, 2, 3, 4, and 5 hour (Fereidoni et al., 2000). The test groups received the *Euadenia eminens* extract (30, 100 and 300 mg/kg *p.o.*), standard groups received diclofenac (5, 15 and 50 mg/kg *i.p.*) and dexamethasone (1, 3 and 10 mg/kg *i.p.*) and the control animals received the vehicle only. All treatments were administered pre-emptively 30 minutes for *i.p.* route and 1 hour for oral route. All experimental protocols were 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.

Statistical analysis

Raw scores for right foot volumes were individually normalized as percentage of change from their values at time 0 then averaged for each treatment group. The time-course curves for foot volume was subjected to two-way (treatment × time) repeated measures analysis of variance with Bonferroni's *post hoc t* test. Total foot volume for each treatment was calculated in arbitrary unit as the area under the curve (AUC). The percentage inhibitions of carrageenan-induced oedema were calculated for each animal group in comparison with the vehicle-treated group. Differences in AUCs were analyzed by ANOVA followed by Newman-Keul's *post hoc t* test. ED₅₀ (dose responsible for 50% of the maximal effect) for each drug was determined using the inhibition percentage of oedema. GraphPad Prism for Windows version 5.00 (GraphPad Software, San Diego, CA, USA) was used for all statistical analysis and ED₅₀ determinations. P < 0.05 was considered statistically significant.

Antimicrobial assays

Agar well diffusion method

The extract of *Euadenia eminens* root was tested for antimicrobial activity using agar well diffusion as described by Vanden Berghe and Vlietnick (Vanden Berghe and Vlietnick, 1991). Wells of 9 mm diameter were made in 20 mL nutrient agar with 2 loopfuls of a suspension of organisms (10⁵ CFU/mL) aseptically. The extracts were tested at 100 µL of 5 mg/mL solution in 10 % aqueous DMSO. The plates were incubated at 37^oC for 24 hours after which zones of inhibition were measured. The test results are the averages of three replicates.

Micro dilution assay

Minimum inhibitory concentration (MIC) was determined using the 96-well micro titre-plate-based serial dilution method (Eloff, 1998). 20 μ L of a bacterial suspension (10^5 CFU/mL) was dispensed into each well containing 100 μ L of double strength nutrient broth. 70% ethanol extract was reconstituted in 10 % aqueous DMSO to obtain a stock solution of 1mg/mL. To each well was added 100 μ L of the test extract at various concentrations. Amoxicillin was included as a positive control. The microplates were incubated for 24 hours at 37 $^{\circ}$ C. 20 μ L of a 5% solution of p-iodonitrotetrazolium was incorporated as an indicator of cell growth. The test results are the means of 3 replicates.

Antioxidant assays**Rapid screening for antioxidants**

The dried extract was reconstituted in methanol and spotted on pre-coated silica gel F₂₅₄ aluminium sheets. The plates were developed in chloroform-methanol (9:1v/v) and sprayed with 20 mg/L solution of stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. Zones where the colour changed within 30 minutes of spraying were taken as positive results (Cuendet et al., 1997).

DPPH assay

Assay was performed by the DPPH method described by Blois (Blois, 1958). One mL of various concentrations (0.375-3 mg/mL) of the extract in methanol was added to 3 mL of DPPH solution (20 mg/L in methanol). After 30 minutes incubation at room temperature, absorbance was measured at 517 nm.

Total Phenol Content

Total phenol was evaluated using Folin Ciocalteu reagent (McDonald et al., 2001). Various concentrations of the extract (1 mL of 0.125-2.5 mg/mL) or tannic acid (1 mL of 0.03-0.1 mg/mL) was mixed with 1 mL of Folin Ciocalteu's and 1 mL aqueous Na₂CO₃ (2%) solution. The mixtures were allowed to stand for 2 hours, centrifuged, and absorbance was recorded at 760 nm. The standard curve was prepared using tannic acid solutions (0.03-0.1 mg/mL) in methanol. Total phenol content was expressed in terms of tannic acid equivalent (mg/g of dry mass).

Total Antioxidant Capacity

The total antioxidant capacity was determined as described by Prieto (Prieto et al., 1999), using ascorbic acid as the standard control. One mL of the extract (0.125-2.5 mg/mL) was delivered into a test tube containing 3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM disodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95 $^{\circ}$ C for 90 minutes, cooled to room temperature, and absorbance measured at 695 nm against a blank. Antioxidant capacity was expressed as ascorbic acid equivalent (mg/g of dry mass).

Phytochemical screening

The presence of plant secondary metabolites in the powdered roots of *Euadenia eminens* were determined by simple qualitative phytochemical methods (Evans, 2009).

Results and Discussion**Phytochemical screening**

Phytochemical screening of the powdered roots of *Euadenia eminens* indicated the presence of phenolics, glycosides, triterpenoids and flavonoids. The anti-inflammatory, antibacterial and antioxidant effects observed may be related to the presence of one or more of these secondary metabolites in the plant extract.

Anti-inflammatory assay

The 70% ethanol extract of *Euadenia eminens* root, and the standard drugs significantly decreased the foot pad oedema. This is shown as time-course curves and AUCs (Figure 1). From the ED₅₀s, the extract was 7-9 times less potent compared to the standard treatments (Table 1). The synthesis and/or release of histamine, serotonin, kinins, prostaglandins and cyclooxygenase-2 have been suggested to contribute to the mechanism of inflammation induced by carrageenan (Asongalem et al., 2004). These inflammatory mediators increase vascular permeability and subsequently cause the observed oedema (Vasudevan et al., 2007). The anti-oedematous effect observed suggest an inhibitory effect of the extract on the synthesis and/or release of any one of the inflammatory mediators. Although direct evidence of the phytoconstituents responsible for the anti-inflammatory effect observed

has not been determined, in vivo and in vitro anti-inflammatory effects have been reported for flavonoids (Clavin et al., 2007). Thus, the presence of flavonoids in the extract may be responsible for the observed activity.

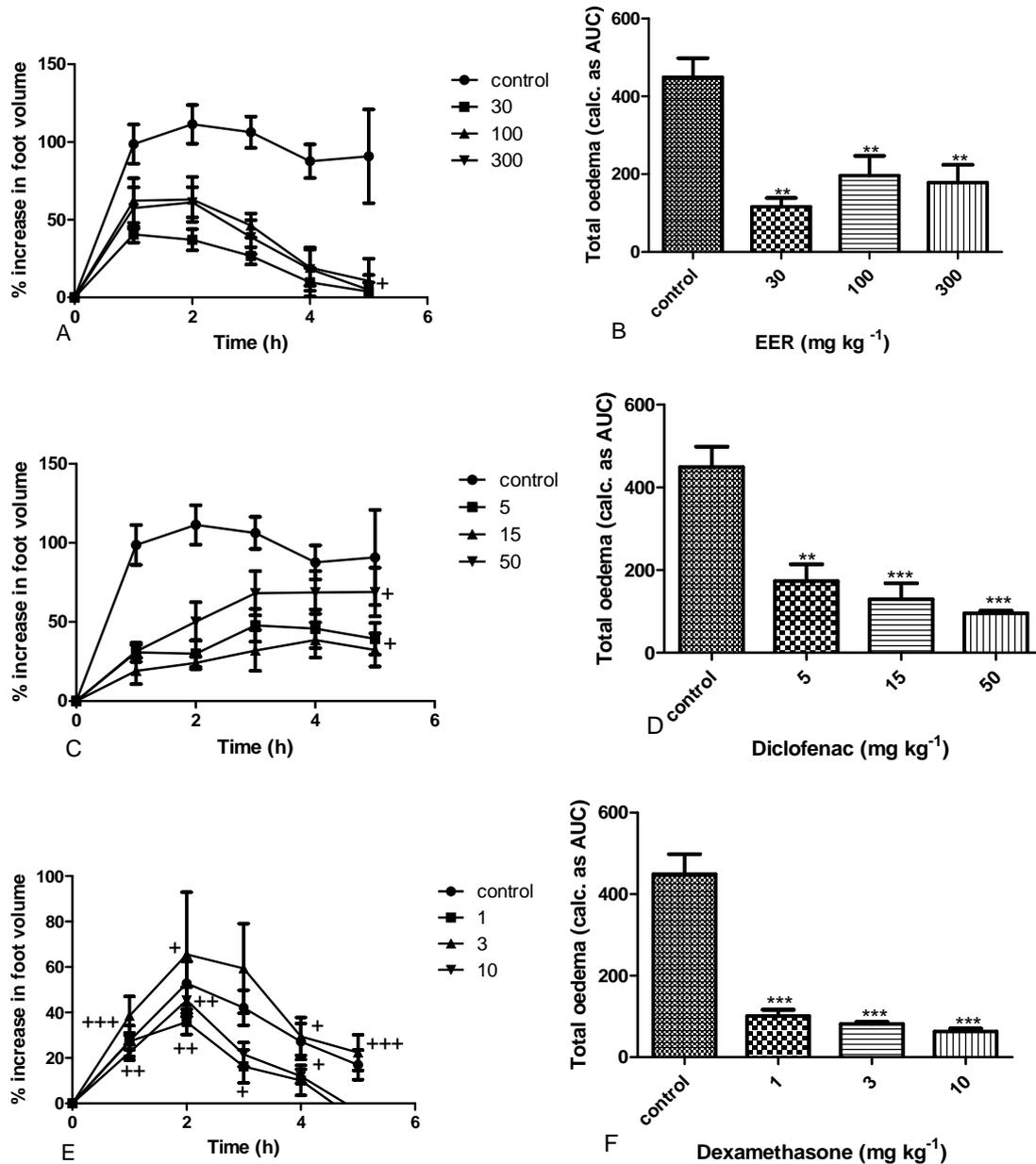


Figure 1: Effect of diclofenac (10 – 100 mg/kg; i.p.), dexamethasone (1 – 10 mg/kg; i.p.) and *Euedenia eminens* root extract (30 – 300 mg/kg; p.o) on time course curve (A, C, and E) and the total oedema response (B, D and F respectively) in carrageenan-induced oedema in chicks. Values are means ± SEM. (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).

Table 1: ED₅₀ values for the anti-inflammatory effect of *Euedenia eminens* root, diclofenac and dexamethasone.

Drug	ED ₅₀ (mg/kg)
<i>Euedenia eminens</i> root	30.71
Diclofenac	4.42
Dexamethasone	3.43

Antimicrobial assays

In the preliminary assessment, the extract showed moderate antibacterial activity against two Gram positive bacteria: *Bacillus subtilis* NCTC 10073 (12.67±0.33 mm) and *Bacillus thuringiensis* ATCC 13838 (12.33±0.88 mm). However, the extract failed to inhibit the growth of the other microorganisms used in this study. Minimum inhibitory concentrations were observed for the susceptible microorganisms, 500 and 1000 µg/mL for *Bacillus subtilis* and *Bacillus thuringiensis* respectively. Terpenoids, polyphenols and flavonoids present in the extract have been found to be effective antimicrobial substances (Cowan, 1999). Even though, the extract may not have exhibited broad-spectrum activity, the results remain interesting since they lay the grounds for further research to determine the compound(s) responsible for the observed activity.

Antioxidant assays

The active compounds were detected as yellow spots on a violet background. These results indicated the potential of the extract as a proton or electron donor. This potential was assessed quantitatively using spectrophotometry. The extract reduced DPPH to the yellow coloured product, diphenylpicrylhydrazine, with an IC₅₀ of 1.175 mg/mL as against 0.004 mg/mL by n-propyl gallate (positive control). Phenolic compounds have been recognized as antioxidant compounds which act as free radical terminators (Shahidi and Wanasundara, 1992). The phenol content of the extract in terms of tannic acid was 7.25 mg/g dry weight. The total antioxidant capacity of the extract expressed in terms of ascorbic acid was 0.609 mg/g dry weight. Free radicals may be generated as by products of biological reactions or from exogenous factors. The involvement of free radicals in the development and progression of many disease processes is well documented (Chen et al., 2002). Paradoxically, they are produced by many cells as a protective mechanism (Lunec et al., 2002). The root of *Euadenia eminens* exhibited potent scavenging properties and may be useful as a preventive intervention for the ailments it finds use in. The ailments may be infectious and/or inflammatory in nature, but are usually accompanied by oxidative stress. Thus, making an antioxidant intervention relevant.

In conclusion, the 70% ethanol extract of *Euadenia eminens* roots possess anti-inflammatory, selective antibacterial activity and antioxidant effects. These results suggest that the traditional uses *Euadenia eminens* may be quite useful and reasonable. Further isolation of the various constituents responsible for these activities is in progress in our laboratories.

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