

Research Letter

Antimicrobial and anti-inflammatory activities of the leaves of *Clerodendrum splendens* leaves

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ABSTRACT: *Clerodendrum splendens* is a West African climbing shrub used in traditional medicine for wounds and infectious conditions. The petroleum ether, ethyl acetate, and 70% ethanolic extracts of the leaves obtained by successive Soxhlet extraction, inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. The ethyl acetate extract was the most active. Again, all the extracts dose-dependently inhibited carrageenan-induced foot paw oedema in 7-day old chicks. Again, the ethyl acetate extract showed the greatest inhibition. The results of this study provide scientific evidence for the ethnomedicinal use of the leaves of *C. splendens*.

KEY WORDS: *Clerodendrum splendens*, Antimicrobial, Anti-inflammatory activity.

INTRODUCTION

Clerodendrum splendens G. Don (Family: Verbenaceae) also known as the Flaming Glory - bower is a woody or semi-woody evergreen vine which grows in the tropical and subtropical regions of the world. In ethnomedicine, the plant is used to treat wounds and burns,^[1] haemorrhoids, diarrhoea and dysentery.^[2] The leaves have been found to contain reducing sugars, glycosides, unsaturated sterols, triterpenoids and flavonoids.^[3] Recently the plant has been reported to show wound healing, antioxidant and antimicrobial properties.^[4] Various species of *Clerodendrum*, including *C. trichotomum*, *C. indicum* and *C. serratum* which are used traditionally in the management of inflammatory conditions, have been shown to possess potent anti-inflammatory activities.^[5] We have investigated the antimicrobial and anti-inflammatory activities of the leaves of *C. splendens* and in this report provide further support for its ethnomedicinal uses.

MATERIALS AND METHODS

Plant material

The leaves of *C. splendens* were collected from Asokore Mampong in Kumasi in May, 2008. The plant material was authenticated

by Mr. Ntim-Gyakari, the curator of the Herbarium of the Forestry Commission in Kumasi and a voucher specimen (KNUST/HM1/2010/L033) has been deposited at the herbarium of the Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST) Kumasi, Ghana.

Extraction of plant material

The leaves were air dried for four days and ground into a coarse powder. The powder (0.5 kg) was serially extracted using petroleum ether, ethyl acetate and 70% ethanol. The various extracts were evaporated under reduced pressure using a rotary evaporator until a viscous extract of each was produced. The petroleum ether extract gave a yield of 9.19 %^{w/w}, that of ethyl acetate extract was 9.59 %^{w/w} and 13.45 %^{w/w} for the ethanolic extract. Phytochemical screening of the powdered leaves using methods described by Sofowora^[6] and Harborne^[7] showed the presence of flavonoids, tannins and alkaloids.

Test organisms

The microorganisms used in this study were obtained from the stocks of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi. They included; *Staphylococcus aureus* (NCTC 10788), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (NCTC 10662), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 102321). A 24 hour broth culture of the organisms was used. The media used was Nutrient agar (MERCK) for the bacteria and Sabouraud agar (MERCK) for *Candida*.

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Materials used in Anti-inflammatory Studies

Day old post-hatched Cockerels (*Gallus gallus*; strain Shaver 579) were obtained from Akropong Farms, a commercial breeder, in Kumasi. The chicks were housed in standard environmental conditions at the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST. The standard drugs used for the positive control were diclofenac sodium and dexamethasone. Carrageenan sodium (Sigma - Aldrich Inc., St Louis, MO, USA) was used to induce oedema in the chicks.

Preparation of extracts

Extracts of *C. splendens* (10 mg/ml) were prepared in Dimethyl sulphoxide (DMSO) for the antimicrobial assay. Ciprofloxacin and Ketoconazole were used as the positive controls at a concentration of 0.5 mg/ml each.

Agar well diffusion bioassay

The inocula were prepared by inoculating the test organisms in nutrient broth and incubating them for 24 hours at 37°C for the bacteria, while for *Candida albicans* in Sabouraud's dextrose broth was incubated for 48 hours. One milliliter of the diluted cultures was inoculated into a sterile molten nutrient agar at 45°C and poured into a sterile petri dish. Similarly, 1 ml of the diluted fungal suspension was poured into sterile Sabouraud's dextrose agar plates. These were swirled gently and allowed to solidify. Wells were bored into the solidified inoculated nutrient agar plates using cork borer number 6. The wells were filled with equal volume of 0.1 ml of each extract. One hour was allowed for the extract to diffuse into the agar after which the plates were incubated overnight at 37°C and 25°C for fungi and bacteria respectively. At the end of the incubation period, the diameter of inhibition zone(s) were measured with a ruler and recorded. The extracts and standard antibiotics were tested in triplicate and mean zones of inhibition were calculated for each extract and the standard antibiotics.

Anti inflammatory Assay

The anti-inflammatory properties of the extracts were evaluated using the carrageenan-induced foot oedema in 7-day old chicks as described by Roach and Sufka^[8] with some modifications. The experiment was performed to evaluate the prophylactic effects of the petroleum ether, ethyl acetate and 70% ethanolic extracts on the oedema component of inflammation. Dexamethasone, a steroidal anti-inflammatory drug and diclofenac, a non-steroidal anti-inflammatory drug (NSAID) were used as positive controls. In this method, chicks were randomly selected, grouped (5 per group) and fasted for 24 hours before the experiment. Water was available *ad libitum*. The test samples were prepared by dissolving the fluid extracts in 2% tragacanth in distilled water. Doses of 30, 100 and 300 mg/kg were prepared and given orally (*p.o*) 1h before the carrageenan challenge and for the diclofenac (10, 30 and 100 mg/kg) and dexamethasone (0.1, 1.0 and 3 mg/kg) were given intraperitoneally (*i.p*) 30 minutes before the carrageenan challenge. The foot thickness of each chick was measured before carrageenan injection (baseline measurement) and then at hourly

intervals over the next 6 hours post carrageenan injection. The right footpads of the chicks were injected *intraplantar* with carrageenan (10 µl of a 1% solution in saline). The change in foot thickness for the various groups was recorded hourly for six hours by means of a digital caliper. The oedema component of inflammation was quantified by measuring the foot thickness before carrageenan injection and at the various time points.

Statistical analysis of data

The extracts were tested against test organisms in triplicates and the results were presented as the mean ± the standard error of means (SEM). Raw scores for the right foot thickness were individually normalized as percentage of change from their values at time 0 and then averaged for each treatment group. The time-course curves for foot thickness were subjected to two-way (*treatment × time*) repeated measures analysis of variance with Bonferroni's *post hoc t* test. Total foot thickness for each treatment was calculated in arbitrary units as the area under the curve (AUC) and to determine the percentage inhibition for each treatment, the following equation was used.

$$\% \text{ Inhibition of oedema} = \frac{\text{AUC control} - \text{AUC treatment}}{\text{AUC control}} \times 100$$

RESULTS AND DISCUSSION

Undeniably, plants have played very important roles in the lives of humans for centuries. *C. splendens* enjoys traditional use as anti-inflammatory and antimicrobial agents. This study was conducted on the leaves of *C. splendens* to validate these folkloric uses.

All the extracts showed some level of antimicrobial activity against *Staph. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli* and *C. albicans in vitro*, with the ethyl acetate extract exhibiting the highest activity (Table 1). The zones of inhibition ranged from 4.00 ± 0.54 mm to 9.0 ± 0.16 mm. The activity of the petroleum ether and ethanolic extracts ranged between 3.50 ± 0.50 mm to 4.67 ± 0.33 mm and 3.33 ± 0.47 mm to 4.3 ± 0.47 mm respectively. The least susceptible organism to the extracts was *P. aeruginosa*. *Staph. aureus* which generally causes infections that are very difficult to combat due to their multi drug resistance^[9,10] was found to be susceptible to all extracts. Generally, the activities of the extracts were weak compared to the activities of the standard antibiotics used in the study.

The anti-inflammatory activity of the leaves of *C. splendens* was established using the carrageenan-induced oedema in chicks, a common experimental animal model used to evaluate NSAIDs.^[11] It is believed to act in a biphasic manner. The initial phase of inflammation (0-2 h) has been attributed to the release of histamine and kinins, followed by a late phase (2.5-6 h) mainly sustained by release of prostaglandins.^[12] The second phase is sensitive to most clinically effective anti-inflammatory drugs.^[13] In this study, the time course curves revealed a dose-dependent effect of the extracts on oedema (Figure 1). Furthermore, when

Table 1: Antimicrobial Activities of *C. splendens* extracts

Extracts	Mean Zones of Inhibition (mm)				
	<i>E.coli</i>	<i>B. subtilis</i>	<i>Staph aureus</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
Pet ether	4.2 ± 0.17	4.3 ± 0.67	4.2 ± 0.17	3.5 ± 0.50	4.7 ± 0.33
Ethyl acetate	9.0 ± 0.17	7.0 ± 0.17	5.2 ± 0.44	4.0 ± 0.50	6.3 ± 1.17
70% ethanol	3.7 ± 0.33	4.0 ± 0.56	4.3 ± 0.33	3.3 ± 0.33	3.7 ± 0.33
Ciprofloxacin ^a	20 ± 0.67	24 ± 0.50	17.5 ± 0.71	23.5 ± 0.43	—
Ketoconazole ^b	—	—	—	—	18 ± 0.30
2% DMSO	0	0	0	0	0

—; no assay performed, the data are shown as mean ± Standard Error of the Mean (SEM),
^{a,b}; positive controls

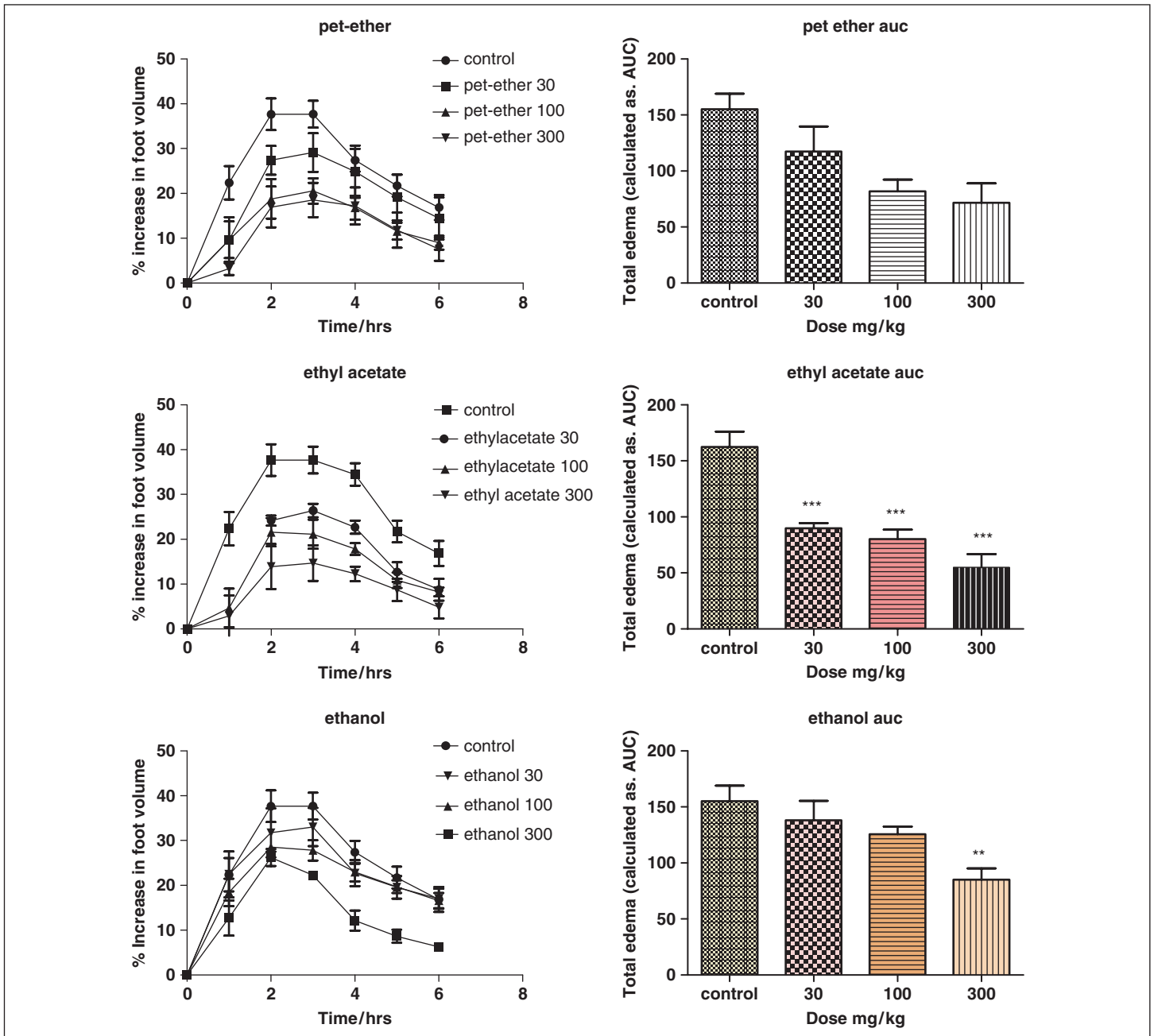


Figure1: Time course effects of Petroleum Ether, Ethyl acetate and Ethanol Extracts (10-300 mg kg⁻¹ p.o.) in the prophylactic protocol on carrageenan induced foot oedema in the chick and their respective total oedema responses for 6 h [defined as the area under the time course curve (AUC)]. Each point on the column represents the Mean ± S.E.M. (n = 5). ***P < 0.001, **P < 0.01, *P < 0.05.

total oedema over the period of the experiment was represented arbitrarily as AUC of the time course curves, all the extracts significantly reduced total oedema with a maximal inhibitory effect of $47.29 \pm 8.65\%$, $66.09 \pm 13.13\%$ and $45.19 \pm 5.09\%$ respectively at 300 mg/kg (Table 2). Diclofenac (10-100 mg/kg, *i.p*) also showed significant effect on the time course curve and total oedema with maximal inhibitory effect of $79.56 \pm 18.24\%$ at 100 mg/kg as seen in Figure 2. Similarly, treatment with dexamethasone, a steroidal anti-inflammatory agent, (0.3-3 mg/kg, *i.p*) exhibited a significant effect on the time course curve of carrageenan-induced oedema (Figure. 2) with a maximal inhibitory effect of $78.69 \pm 3.91\%$ at 3 mg/kg. Thus, all the extracts inhibited oedema from the second hour (Figure. 1). The extracts may therefore be acting in the late phase of the inflammation by

inhibiting chemical mediators such as prostaglandins. The ethyl acetate extract exhibited the highest inhibitory effect in a dose-dependent manner at all doses with a maximal effect of $66.1 \pm 3.67\%$ at 300 mg/kg body weight. The extent of inhibition of the foot oedema by the extracts was less than the standard anti-inflammatory drugs, diclofenac and dexamethasone. Phytochemical screening revealed the presence of tannins, alkaloids, flavonoids, glycosides and sterols in the leaves. Some of these metabolites have been reported to possess antimicrobial activity.^[10] Our results agree with that observed by Gbedema *et al.*^[4] and lend further support to the use of the leaves of *C. splendens* for the treatment of wounds and microbial infections in traditional medicine. The results again provide support for the ethnomedicinal use of *C. splendens* in the treatment of inflammatory diseases.

Table 2: Inhibitory effects of Petroleum ether, ethyl acetate and 70% ethanolic extract on carrageenan-induced oedema on 7-day old chicks.

Extract	300 mg/kg	100 mg/kg	30 mg/kg
Pet ether	$47.29 \pm 8.65\%$	46.43 ± 2.98	$24.41 \pm 3.97\%$
Ethyl acetate	$66.1 \pm 3.67\%$	$50.57 \pm 0.67\%$	$44.65 \pm 4.77\%$
70% Ethanol	$45.19 \pm 5.09\%$	$19 \pm 5.34\%$	$11.11 \pm 9.77\%$
Diclofenac (100 mg/kg)	$79.56 \pm 18.24\%$		
Dexamethasone (3 mg/kg)	$78.69 \pm 3.91\%$		

Values are mean \pm S.E.M ($n = 5$), $P < 0.001$

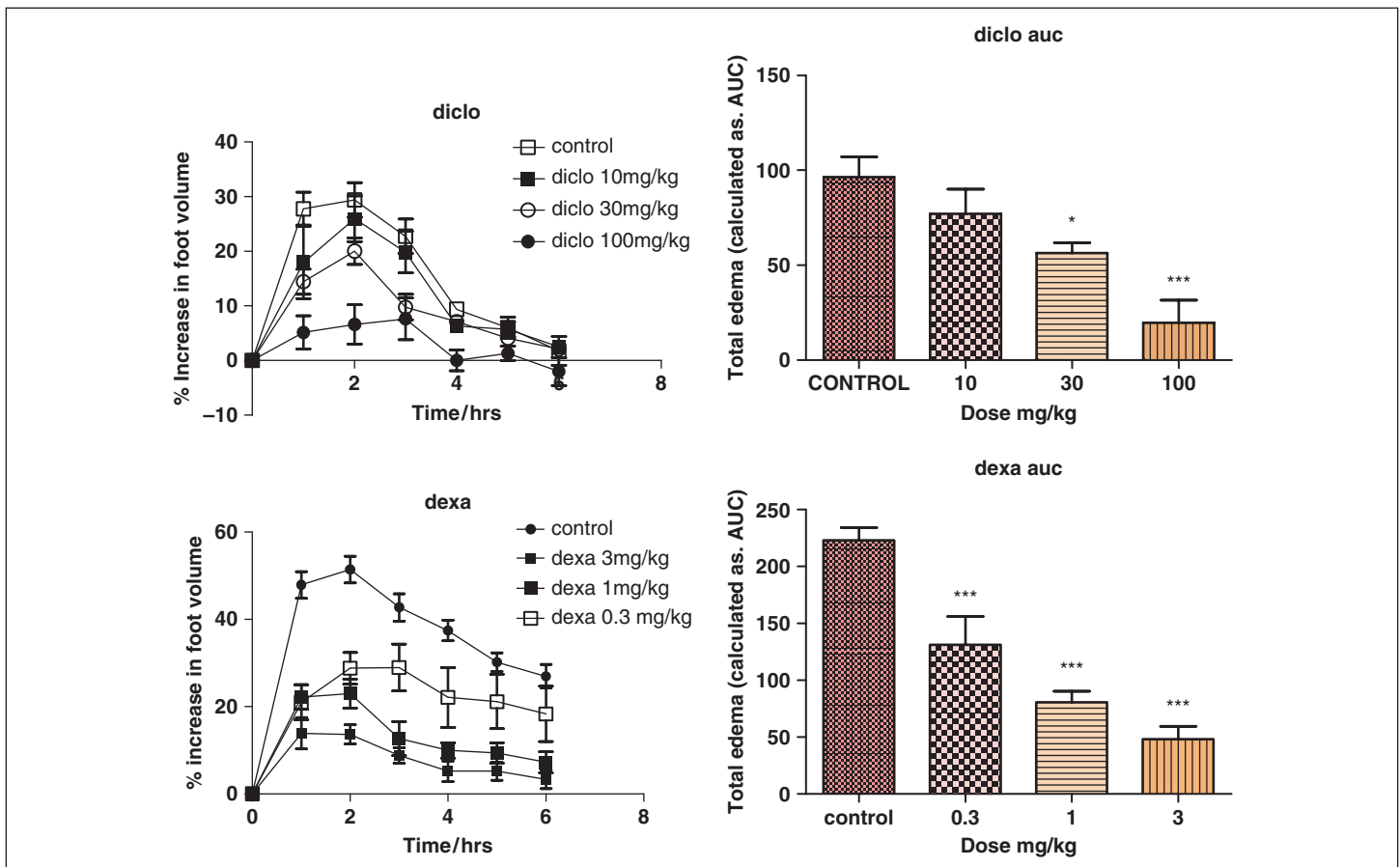


Figure 2: Time course effects of Diclofenac (10-100 mg kg⁻¹, *i.p*) and Dexamethasone (0.3-3.0 mg kg⁻¹ *i.p*) in the prophylactic protocol on carrageenan induced foot oedema in the chick and the total oedema response for 6 h. Each point and column represents the mean \pm S.E.M. ($n = 5$) *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

CONCLUSION

The present study demonstrates a weak antimicrobial activity compared to standard antibiotics and a good anti-inflammatory activity in chicks. Of the various extracts tested, the medium polar EtOAc extract showed the highest activity. The results support the wound healing activities of earlier reports, and provide the rationale for the ethnomedicinal use of the leaves of *C. splendens* in the management of inflammatory disorders. Flavonoids, tannins and alkaloids which were found present in the leaves of the plant may be responsible for these antimicrobial and anti-inflammatory activities.

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