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Mechanisms of the anti-inflammatory activity of the leaf extracts of *Culcasia scandens* P. Beauv (Araceae)

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Abstract

The mechanisms of the anti-inflammatory activity of the leaf extracts of *Culcasia scandens* P. Beauv were evaluated. The methanol leaf extract (CE) of *C. scandens* was subjected to bioactivity-guided separation to obtain fraction C as the most active anti-inflammatory fraction. Further activity-guided fractionation of fraction C led to the isolation of an anti-inflammatory principle, CS-1, identified as sitosterol. Phytochemical analysis of CE indicated the presence of carbohydrates, glycosides, alkaloids, saponins, flavonoids, tannins, sterols and terpenoids. Fraction C tested positive for flavonoids, sterols and terpenoids while CS-1 tested positive for sterols. Acute toxicity test on CE in mice established an intraperitoneal (i.p.) and oral (p.o.) LD_{50} of >5 g/kg. The isolated compound, CS-1, inhibited topical edema in the mouse ear while CE and fraction C were devoid of any such activity. The methanol extract (CE), fraction C and CS-1 suppressed paw edema in rats with inhibitory order of magnitude: CS-1>fraction C>CE. The methanol extract (CE), fraction C and CS-1 caused gastrointestinal irritation in rats to varying extents and reduced neutrophil and lymphocyte counts while CE and fraction C reduced total leukocyte count (TLC). The methanol extract (CE) was effective in inhibiting heat-induced hemolysis of human red blood cells (RBCs) but exhibited no inhibitory effect on hypotonicity-induced hemolysis. However, fraction C and CS-1 inhibited both heat- and hypotonicity-induced hemolysis. These results suggest that the leaf extract of *C. scandens* has a good anti-inflammatory profile, indicating a nonspecific mechanism of action.
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Keywords: *Culcasia scandens*; Anti-inflammatory; β-Sitosterol; Membrane stabilization; Leukocyte migration inhibition

1. Introduction

The inflammatory response is a defense mechanism evoked by body tissues in response to injury or microbial invasion. It is a usual accompaniment of most disease processes, and can on its own constitute a source of discomfort thus requiring treatment. Over the centuries, a number of medicinal plants have been in use in the treatment of the disorders associated with the inflammatory response or control of the inflammatory aspects of diseases. These medicinal plants owe their activities to their phytochemical constituents and may exert their anti-inflammatory effect by interfering generally with the inflammatory response pathways or specifically with certain components of the pathway, such as release of proinflammatory mediators, migration of leukocytes under inflammatory stimulus with consequent release of the cytoplasmic contents at inflammation sites, activation of complement sequence, etc. (Okoli et al., 2003). Pharmacological evidence for the anti-inflammatory activity of some of these medicinal plants has been established (Okoli et al., 2003), thus providing a rationale for their medicinal uses and stimulus for further research and development of the plant extracts. One of such plants is *Culcasia scandens* P. Beauv (Araceae), a potent anti-inflammatory herb.

C. scandens P. Beauv is a tall climbing epiphyte with green, simple and entire leaves (Omotoye, 1984). The morphology has been variously described (Morton, 1961; Nielsen, 1965; Omotoye, 1984).

In the southeastern part of Nigeria, *C. scandens* is abundantly present and known as “Oji azu ari nkwu” in

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vernacular. The leaves of *C. scandens* are popularly used in the treatment of tonsillitis, toothache and other inflammatory conditions and claimed to be a very effective remedy. The ethnomedicinal uses in inflammatory conditions and rheumatism have been documented (Kokwaro, 1976; Okoli and Akah, 2000).

In preliminary evaluation, we have reported the anti-inflammatory activity of the methanol leaf extract and its fractions (Okoli and Akah, 2000). These preliminary findings indicated a potent anti-inflammatory activity profile of the leaf extract and fractions of this plant in acute inflammation. The present study was undertaken to identify the possible mechanisms of the anti-inflammatory effect of the leaf extracts and to isolate the anti-inflammatory principle.

2. Experimentals

2.1. Collection of plant material

Fresh leaves of *C. scandens* were collected from plants growing on palm tree trunks, in Nanka, Orumba North L.G.A., Anambra State, Nigeria. The leaves were collected in December 1999 and authenticated by Mr. A.O. Ozioko of the Bioresources Development and Conservation Programme Center (BDCP), Nsukka, Enugu State, Nigeria. A voucher specimen (PC 97028) is preserved in the Pharmacy Herbarium, University of Nigeria, Nsukka (UNN).

2.2. Extraction of plant material

The leaves were cleaned, cut into smaller pieces, dried in the open for 2 days and pulverized to coarse powder using a hand blender. About 5 kg of the powder was extracted by cold maceration in methanol (22.5 L) for 48 h. Concentration of the methanol extract in a rotary evaporator under reduced pressure and subsequent freeze–drying afforded 584.85 g of the crude extract (CE).

2.3. Bioactivity-guided separation of the crude extract

Acute inflammation induced by fresh egg albumin in the rat paw (Akah and Njike, 1990; Okoli and Akah, 2000) was used as biological activity guide in the separation of the crude extract for anti-inflammatory tests.

2.4. Chromatographic separation of the crude extract (CE) and fraction C

About 500 g of CE was separated in a dry-packed silica gel 60, 70–230 Mesh ASTM (EM Science) column successively eluted with ethylacetate (100%) and methanol (100%) to obtain about 500 fractions of 10 ml volume each.

The collected fractions were pooled into four (A, B, C and D) according to the R_f values of the constituents visualized under UV on precoated TLC Uniplate Normal Phase Silica gel, 150 μm (Analtech) plates developed with ethylacetate (100%).

Activity-guided screening of fractions A, B, C and D for anti-inflammatory activity showed that fraction C (10.52 g) caused the greatest inhibition of paw edema. Fraction C was thus subjected to further separation.

Fraction C (6 g) was separated in a column packed as earlier stated and eluted with mixtures of petroluem ether/ethylacetate (1:1, 1:2), ethylacetate/methanol (2:1) and methanol (100%) and collected in 10 ml volumes. The petroleum ether/ethylacetate (1:2) fractions on standing yielded a white coloured amorphous powder (CS-1). The compound was washed with petroleum ether/ethylacetate (1:2) mixture and CS-1 (54.2 mg) precipitated on standing in a refrigerator at 0–4 °C. The purity of the isolate was established by TLC on precoated TLC Uniplate Normal Phase Silica gel, 150 μm (Analtech) plates developed with methylene chloride/acetone (80:20) mixture to reveal a single spot. The purity was further ascertained by TLC eluted with a more polar mixture of methylene chloride/acetone/formic acid (80:20:0.5) to reveal a single spot.

2.5. Phytochemical analysis of extracts

The phytochemical analysis of the CE, fractions C and CS-1 was done using standard methods (Evans, 1989).

2.6. Characterization and identification of CS-1

The structure of the isolated constituent (CS-1) was characterized using H-NMR and ^{13}C-NMR spectroscopy. The identity was established by comparison of the spectral data with those of previously published compounds (Caffaratti et al., 1994; Perez et al., 2003).

2.7. Pharmacological tests

2.7.1. Animals

Adult Swiss albino mice (15–25 g) and rats (150–250 g) of either sex were used. The animals were obtained from the Laboratory Animals' Facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka (UNN). The animals were housed in stainless steel cages (rats) and plastic cages (mice). They were maintained on standard pellets (Bendel Feeds and Flourmills, Benin City, Nigeria) and water ad libitum. All animal experiments were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animal (Pub. No. 85-23, revised 1985).

2.8. Acute toxicity (LD_{50}) test

The LD_{50} of the crude extract (CE) was estimated in mice (15–25 g) by oral (p.o.) and intraperitoneal (i.p.) routes using the method of Lorke (1983).

2.9. Anti-inflammatory tests

2.9.1. Establishment of effect on acute inflammation

2.9.1.1. Topical edema induced by xylene in the mouse ear. The effects of the crude extract (CE), fraction C and CS-1 on acute topical inflammation was evaluated by a modification of the methods of Tubaro et al. (1985) and Atta and Alkohafi (1998).

Adult Swiss albino mice (15–25 g) of either sex were divided into groups of 10 animals. Each group received one of CE, fraction C or CS-1 (5 mg/ear) applied on the anterior surface of the right ear. Topical inflammation was instantly induced on the posterior surface of the same ear by application of xylene (0.05 ml). Control animals received either the vehicle (3% v/v Tween 85) or indomethacin (5 mg/ear). Two hours after induction of inflammation, mice were killed by overdose of ether anaesthesia and both ears removed. Circular sections (7 mm diameter) of both the right (treated) and left (untreated) ears were punched out using a cork borer, and weighed. Edematous response was quantified as the weight difference between the two earplugs. The anti-inflammatory activity was evaluated as percent edema reduction/inhibition in the treated animals relative to control animals (Tubaro et al., 1985; Asuzu et al., 1999) using the relation;

$$\text{Edema Reduction/inhibition } (\%) = \left[100\left(\frac{R_t - L_t}{R_c - L_c}\right)\right]$$

Where R_t=mean weight of right earplug of treated animals, L_t=mean weight of left earplug of treated animals, R_c=mean weight of right earplug of control animals and L_c=mean weight of left earplug of control animals.

2.9.2. Egg-albumin-induced pedal edema in rats

The rat paw edema method of Winter et al. (1962) was used. Increase in the right hind paw volume (Rosa et al., 1994; Bani et al., 2000) induced by the subplantar injection of fresh egg albumin (Akah and Njike, 1990; Okoli and Akah, 2000; Amos et al., 2002) was used as a measure of acute inflammation.

Adult Swiss albino rats (110–210 g) of either sex were divided into five groups of six animals. Each group received intraperitoneal injection of one of CE, fraction C and CS-1, (200 or 400 mg/kg) administered to subgroups of three animals. Control animals received either piroxicam (50 mg/kg) or equivalent volume of 3% v/v Tween 85.Thirty minutes later, acute inflammation was induced by subplantar injection of 0.1 ml of undiluted fresh egg albumin on the right hind paw of the rats. The volume of the paw was measured by water displacement using digital plethysmometer (LETICA, 7500) before and at 0.5, 1, 2, 3 and 4 h after egg albumin injection. Edema formation was assessed in terms of the difference in the zero time paw volume of the injected paw and its volume at the different time intervals after egg albumin injection.

The level of inhibition (%) of edema was calculated using the relation (Perez, 1996);

$$\text{Inhibition of Edema } (\%) = 100\left[1 - \left(\frac{a - x}{b - y}\right)\right]$$

Where a=mean paw volume of treated rats after egg albumin injection, x=mean paw volume of treated rats before egg albumin injection, b=mean paw volume of control rats after egg albumin injection and y=mean paw volume of control rats before egg albumin injection.

2.10. Studies on the mechanisms of anti-inflammatory activity

2.10.1. Ulcerogenic effect in rats

The method of Cashin et al. (1979) was used. Adult Swiss albino rats (150–200 g) were fasted for 24 h. After the fasting period, the crude extract (CE) and fraction C were administered orally to animal subgroups of three at 200 or 400 mg/kg while CS-1 was administered at 400 mg/kg only. Control animals received either indomethacin (100 mg/kg) or equivalent volume of vehicle (3% v/v Tween 85). Three hours after drug administration, animals were killed by ether anaesthesia. The stomachs were removed, cut along the lesser curvature and opened up to expose the mucosal surface. The mucosal surface was washed with normal saline and observed with a convex lens (magnification × 10). Damage to the mucosa was scored 0 to 4 according to an arbitrary scale: 0=no lesions; 0.5=hyperemia; 1=one or two lesions; 2=severe lesions; 3=very severe lesions; 4=mucosa full of lesions (Cashin et al., 1979).

2.10.2. Cell (leukocyte and neutrophil) migration test

The method of Ribeiro et al. (1991) was used. Adult Swiss albino rats (150–210 g) of either sex were used. The methanol extract (CE), fraction C and CS-1 were administered orally at 200 or 400 mg/kg to animal subgroups of three. One hour after drug administration, animals received intraperitoneal injection of 1 ml of 6% w/v dextran in normal saline. Four hours later, the animals were killed and the peritoneal cavities washed with 5 ml of phosphate-buffered saline containing 0.5 ml of 10% EDTA. Total and differential leukocyte counts in the peritoneal wash were taken. The inhibition (%) of neutrophil and lymphocyte migration was calculated.

2.10.3. Membrane stabilization tests

2.10.3.1. Preparation of erythrocyte suspension. Fresh whole human blood (10 ml) was collected and transferred to heparinized centrifuge tubes. The tubes were centrifuged at 3000 rpm for 5 min, and washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as a 40% v/v suspension with

Table 1
Phytochemical constituents of extracts

Extract	Yield (%)	Cho	Alk	Sap	Fla	Tan	Ste	Terp	Gly
CE	12.13	+	+	+	+	+	+	+	+
Fraction C	2.00	−	−	−	+	−	+	+	−
CS-1	0.86	−	−	−	−	−	+	−	−

+=Present; −=absent; Cho=carbohydrate; Alk=alkaloids; Sap=saponins; Fla=flavonoids; Tan=tannins; Ste=sterols; Terp=terpenoids; Gly=glycosides.

isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4). The composition of the buffer solution (g/L) was NaH_2PO_4 (0.2), NaH_2PO_4 (1.15) and NaCl (9.0; Shinde et al., 1999).

2.10.3.2. Heat-induced hemolysis. The isotonic buffer solution (5 ml) containing 200 and 400 μg/ml of CE, fraction C and CS-1 were put in four sets (per concentration) of centrifuge tubes. Control tubes contained 5 ml of the vehicle or 5 ml of 100 μg/ml of hydrocortisone. Erythrocyte suspension (0.05 ml) was added to each tube and gently mixed. A pair of the tubes was incubated at 54 °C for 20 min in a regulated water bath. The other pair was maintained at 0–4 °C in a freezer for 20 min. At the end of the incubation, the reaction mixture was centrifuged at 1300×*g* for 3 min and the absorbance (OD) of the supernatant measured spectrophotometrically at 540 nm using Spectronic 2ID (Milton Roy) spectrophotometer (Shinde et al., 1999). The percent inhibition of hemolysis was calculated using the relation (Shinde et al., 1999):

$$\text{Inhibition of hemolysis } (\%) = 100\left[1 - \frac{OD_2 - OD_1}{OD_3 - OD_1}\right]$$

Where OD_1=absorbance of test sample unheated, OD_2=absorbance of test sample heated and OD_3=absorbance of control sample heated.

2.10.3.3. Hypotonicity-induced hemolysis. The hypotonic solution (distilled water; 5 ml) containing 200 and 400 μg/ml of CE, fraction C and CS-1 were put in two pairs (per dose) of centrifuge tubes. Control tubes contained 5 ml of the vehicle or hydrocortisone (0.5 mg/5 ml). Erythrocyte suspension (0.05 ml) was added to each tube and after gentle mixing, the mixtures were incubated for 1 h at room temperature (31 °C). After incubation, the reaction mixture was centrifuged for 3 min at 1300×*g* and the absorbance (OD) of the supernatant measured spectrophotometrically at 540 nm using Spectronic 21D (Milton Roy) spectrophotometer (Shinde et al., 1999).

The inhibition (%) of hemolysis was calculated using the relation (Shinde et al., 1999):

$$\text{Inhibition of hemolysis } (\%) = 100\left[1 - \frac{OD_2 - OD_1}{OD_3 - OD_1}\right]$$

Where OD_1=absorbance of test sample in isotonic solution, OD_2=absorbance of test sample in hypotonic solution and OD_3=absorbance of control sample hypotonic solution.

2.11. Statistical analysis

Results were analyzed by SPSS version 11.0 using one-way ANOVA and subjected to Fischer LSD post hoc test and expressed as mean±S.E.M. Differences between means were accepted to be significant at $P<0.05$.

3. Results

3.1. Extraction, fractionation and chromatographic separation

The yield of the crude methanol extract (CE) was 12.13% (w/w). Chromatographic separation of CE gave 2% (w/w) of fraction C while chromatographic separation of fraction C yielded 0.86% (w/w) of CS-1 (Table 1).

3.2. Phytochemical analysis of extracts

Phytochemical tests on CE gave positive reactions to carbohydrates, glycosides, alkaloids, saponins, flavonoids, tannins, sterols and terpenoids (Table 1). Fraction C tested positive to flavonoids, and sterols and terpenoids while CS-1 tested positive to sterols (Table 1).

3.3. Identification of CS-1

Comparison of the spectral data with those of known compounds established the identity of CS-1 to be sitosterol (Fig. 1).

3.4. Acute toxicity test

The oral and i.p. LD_{50} of the methanol extract (CE) estimated in mice, were >5 g/kg.

3.5. Effect of C. scandens leaf extracts on topical (acute) inflammation

On the xylene-induced ear edema in mice, CS-1 (5 mg/ear) markedly inhibited edema formation. The effect of CS-1 was greater than that of indomethacin (5 mg/ear). Fraction C and CE were devoid of effect on topical edema (Table 2).

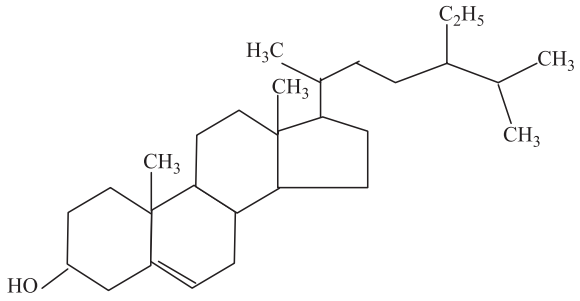

Fig. 1. Structure of sitosterol (Anderson et al., 1926).

Table 2
Effect of extract and fractions on xylene-induced acute topical edema in the mouse ear

Extract	Dose (mg/ear)	Edema (mg)	Inhibition (%)
CE	5.0	0.96±0.49	–
Fraction C	5.0	2.89±1.28	–
CS-1	5.0	0.15±0.05	81.01
Indomethacin	5.0	0.72±0.19	8.86
Control	–	0.79±0.12	–

n=10.
–=No inhibition.

3.6. Effect of C. scandens leaf extracts on rat paw edema

The tested extracts and fractions all suppressed paw edema induced by egg albumin to varying degrees (Table 3). The methanol extract (CE) produced a nondose-related inhibition of paw edema and at 200 mg/kg, the effect was significant (P<0.01) when compared to the control and also greater than that of piroxicam (50 mg/kg; Table 3). Fraction C produced a significant (P<0.01) dose-related inhibition of paw edema. Both doses of fraction C caused greater suppression of edema than piroxicam (50 mg/kg). The active principle (CS-1) caused a significant (P<0.01) dose-related inhibition of paw edema (Table 3) with the 400-mg/kg dose being markedly better than piroxicam. The magnitude of activity is in the order—CS-1>fraction C>CE.

3.7. Effect of C. scandens leaf extracts on gastrointestinal irritation

The methanol extract (CE), fraction C and CS-1 all produced gastrointestinal irritation in rats. The methanol extract (CE) exhibited a dose-related irritation of the stomach mucosa. Fraction C exhibited a nondose-related effect with the 200-mg/kg dose producing a greater irritation (Table 4).

3.8. Effect of C. scandens leaf extracts on in vivo leukocyte migration

The methanol extract (CE) reduced total leukocyte count (TLC) in a dose-related manner. Only 400 mg/kg was effective in inhibiting total leukocyte migration. In the differential count, CE (200 mg/kg) inhibited neutrophil migration while exhibiting no inhibitory effect on lymphocyte counts. Conversely, CE (400 mg/kg) exhibited no inhibitory effect on neutrophil migration but was the least effective in inhibiting lymphocyte migration (Table 5).

Fraction C caused a nondose-related reduction in TLC and at 200 mg/kg, it caused the highest inhibition of total leukocyte migration, while at 400 mg/kg, it was not effective in inhibiting TLC. In the differential count, Fraction C exhibited a dose-related inhibition of neutrophil migration with little or no activity against lymphocyte migration (Table 5).

The active principle (CS-1) did not reduce TLC. In the differential cell count, CS-1 exhibited a nondose-related inhibition of neutrophil migration and a dose-related inhibition of lymphocyte migration (Table 5).

3.9. Effect of C. scandens leaf extracts on heat-induced hemolysis of human red blood cells (RBCs)

The methanol extract (CE), fraction C and CS-1 all inhibited heat-induced hemolysis of RBCs to varying degrees. Both the methanol extract (CE) and fraction C

Table 3
Effect of extracts on egg-albumin-induced acute inflammation of the rat paw

Extract	Dose (mg/kg)	Inhibition of edema (%)				
		0.5	1	2	3	4
CE	200	43.66*	50.00*	50.82	50.00	42.55
	400	29.58	45.45*	39.34	31.48	36.17
Fraction C	200	40.85*	46.97*	42.62	53.70	51.06
	400	53.52*	54.55*	55.74*	55.56	63.83
CS-1	400	70.42*	62.12*	75.41*	72.22*	76.60
Piroxicam	50	4.23	12.12	21.31	31.48	46.81

* Significant at P<0.05 (one-way ANOVA; Fischer LSD post hoc test).

Table 4
Effect of extract and fractions on gastrointestinal irritation in rats

Extract	Dose (mg/kg)	Ulcer index (mean±S.E.M.)
CE	200	0.50±0.50*
	400	0.75±0.25*,#
Fraction C	200	1.50±0.50*,#
	400	0.00±0.00#
CS-1	400	0.25±0.25#
Indomethacin	100	3.00±0.00*
Control	–	0.00±0.00

n=3. Damage to the mucosa was scored 0 to 4 according to an arbitrary scale: 0=no lesions; 0.5=hyperemia; 1=one or two lesions; 2=severe lesions; 3=very severe lesions; 4=mucosa full of lesions (Cashin et al., 1979).
* Significant at P<0.05 (ANOVA; Fischer LSD post hoc test) compared to the negative control.
# Significant at P<0.05 (ANOVA; Fischer LSD post hoc test) compared to indomethacin.

Table 5
Effect of extracts on in vivo leukocyte migration in rats.

Extract	Dose (mg/kg)	% Inhibition of leukocyte migration		
		TLC	DLC	
			Neutrophils	Lymphocytes
CE	200	–	11.11	–
	400	15.54	–	3.16
Fraction C	200	33.90	3.70	–
	400	–	18.52	–
CS-1	200	–	25.93	–
	400	–	–	5.41

n=3. TLC=Total leukocyte count; DLC=differential leukocyte count; –=no inhibition.

Table 6
Effect of the extract and fractions on human red blood cell hemolysis induced by heat and hypotonicity

Extract	Concentration (μg/ml)	Inhibition of hemolysis (%)	
		Heat	Hypotonicity
CE	200	3.54±2.83	–
	400	–	–
Fraction C	200	6.95±4.20	
	400	5.79±1.47	7.87±0.46
CS-1	200	–	34.89±21.38#
	400	12.91±5.59*	202.57±3.23#
Hydrocortisone	100	9.91±4.20*	7.05±0.88

–=No inhibition.
* Significant at $P<0.05$ (one-way ANOVA; Fischer LSD post hoc test) compared to crude extract (CE).
# $P<0.05$ compared to CE, fraction C and hydrocortisone.

evoked a nondose-related inhibition of hemolysis. The active principle (CS-1) exhibited the most potent and dose-dependent inhibition of hemolytic activity. The inhibitory effect produced by the 400-μg/ml concentration was greater than that caused by hydrocortisone (100 μg/ml; Table 6).

3.10. Effect of C. scandens leaf extracts on hypotonicity-induced hemolysis of human red blood cells (RBCs)

The methanol extract (CE) did not exhibit inhibitory activity against hypotonicity-induced hemolysis of RBCs. However, fraction C and CS-1 inhibited RBC hemolysis induced by hypotonicity. Fraction C caused a dose-related inhibitory effect greater than that produced by hydrocortisone (100 μg/ml). The isolated compound (CS-1) exhibited a dose-related inhibitory activity that was greater than that of both fraction C and hydrocortisone (Table 6).

4. Discussion and conclusion

The extraction and bioactivity-guided fractionation of the methanol leaf extract of *C. scandens* have led to the isolation of CS-1 identified as sitosterol and which exhibited potent anti-inflammatory activity.

Acute toxicity study of the methanol extract (CE) established an intraperitoneal and oral LD_{50}>5 g/kg in mice. Thus, the methanol leaf extract (CE) of *C. scandens* could be regarded as safe (Lorke, 1983), suggesting a remote risk of acute toxicity as well as good tolerability.

The phytochemical constituents of the methanol extract (CE) and fraction C were identified as carbohydrates, glycosides, alkaloids, saponins, tannins, flavonoids, sterols and terpenoids. The composition of fraction C is less complex being made up of only flavonoids, sterols and terpenoids. The isolated compound, CS-1, contained only sterol. These typical plant principles are known to be biologically active, eliciting a variety of pharmacological actions, including anti-inflammatory effects (Gabor, 1972; Barik et al., 1992; Recio et al., 1995; Aydin et al., 1996; Shinde et al., 1999). These plant principles have been shown to inhibit arachidonic acid metabolism (Bauer et al., 1996; McGaw et al., 1997), inhibit increase in vascular permeability (Pereira da Silva and Parente, 2001) and exhibit anticomplementary activity, (Srivastava and Kulshreshta, 1989) which are all mechanisms of inflammation.

Characterization of CS-1 revealed that it is a phytosterol–sitosterol. Sitosterol is an unsaturated secondary alcohol containing one double bond (Anderson et al., 1926) and one of the principal phytosterols (Finar, 1986). Sterols are steroids, which occur in plant, and animal oils and fats (Finar, 1986) and sitosterol, which may occur with stigmasterol, has been reported in some plants (Evans, 1989). It occurs as a mixture of three isomers—α-, β-, and γ-sitosterol. Although the actual isomer isolated is yet to be identified, it may likely be the β-isomer, which has been earlier identified in the leaf extract of *C. scandens* (Udeh, 1986). β-sitosterol was isolated from wheat germ oil (Anderson et al., 1926). Topical application of CS-1 potently suppressed the development of mouse ear edema, provoking an inhibition greater than that of indomethacin. Ear edema induced by different phlogistic substances is mediated by a variety of agents, such as leukocytes and prostanoids, which have been shown to mediate croton oil ear edema (Tubaro et al., 1985). It is possible that these or similar mediators are involved in the xylene-induced ear edema and the topical anti-inflammatory activity of CS-1 in the xylene-induced ear edema may likely indicate antiphlogistic effect (Atta and Alkohafi, 1998).

The topical anti-inflammatory effect of CS-1 may suggest that it has a lipophilic character, which enabled it to cross the skin barrier and exert antiphlogistic action (Asuzu et al., 1999). This is consistent with the chemical nature of β-sitosterol and may thus explain the inability of CE and fraction C to inhibit xylene-induced mouse ear edema. In traditional medicine, a leaf poultice of *C. scandens* is usually applied on inflamed parts mixed with *Capsicum frutescens* (Kokwaro, 1976), which may serve to enhance skin permeation. The topical anti-inflammatory activities of CS-1 may represent true anti-inflammatory activity and not counterirritant effect. Counterirritants or rubefacients exert anti-inflammatory effect by diverting hyperemia away from sites of inflammation (Oliver-Bever, 1986). In addition, CS-1 exhibited anti-inflammatory effect following systemic administration further suggesting that it is not a counterirritant (Garbacki et al., 1999).

In the egg-albumin-induced paw edema, systemic administration of CE, fraction C and CS-1 suppressed edema formation in the rat paw. These agents did not exhibit a phase-dependent anti-inflammatory activity, because suppression of edema was progressive and consistent from 0.5 through 4 h postinjection of irritant. They may have inhibited the effects of proinflammatory mediators, such as histamine, 5-HT, bradykinin and prostaglandins (Di Rosa

and Sorrentino, 1969; Vinegar et al., 1969; Flower et al., 1972; Ferriera et al., 1974).

Gastrointestinal irritation test provides information on the possible involvement of prostaglandin synthesis inhibition as a mechanism of anti-inflammatory activity and extent of gastric tolerability of the extract and fractions. CE, fraction C and CS-1 caused varying degrees of gastric irritation. It is possible that the gastric irritation was caused by cyclo-oxygenase (COX) inhibition, which mediates the synthesis of prostaglandin. Extracts of plants, such as *Trichilia dregeana* (Jager et al., 1996), *Cryptocarya latifolia*, *Euclea natalensis*, *Felicia muricata* and *Mohria caffrorum* (McGaw et al., 1997), have been reported to possess COX inhibitory activity. Thus, the inhibition of the COX enzyme may partly be responsible for the anti-inflammatory activity of the leaf extract and fractions. β-sitosterol is poorly absorbed form the stomach (Bowman and Rand, 1988). As such, the fact that the it caused only hyperemia, even at the very high dose tested, may imply a remote possibility of severe direct mucosal damage as well as good gastric tolerability.

The effect of the extract and fractions on dextran-induced in vivo leukocyte migration was studied because leukocytes play a pivotal role in the development of inflammation. The methanol extract (CE), fraction C and CS-1 all inhibited dextran-induced leukocyte migration. Dextran has been shown to cause the accumulation of edema fluid containing proteins and neutrophils (Lo et al., 1982). Leukocyte migration in response to inflammatory stimulus involves interaction of leukocytes with chemotactic/chemoattractant and adhesion molecules. Various phlogistic agents induce leukocyte migration by different mechanisms. Carrageenan-induced neutrophil migration is dependent on the release of chemotactic mediators by resident cells (Souza et al., 1988) while *N*-formyl-methionyl-L-leucyl-L-phenylalanine (fMLP)-induced neutrophil recruitment occurs by a direct chemoattractant effect that is independent of protein synthesis and resident cells (Cybulsky et al., 1989). Dextran-induced accumulation of neutrophils has been shown to occur through mast cell degranulation (Lo et al., 1982). Although the precise mechanism of inhibition of leukocyte migration by these agents is not known, it is possible that these agents inhibited any or all of these processes. Plant principles, like some flavonoids, have been shown to inhibit the expression of adhesion molecules in endothelial cells (Gerristen et al., 1995), which correlated with their anti-inflammatory activity (Gerristen et al., 1995). Inhibition of leukocyte activation can also occur by inhibition of complement activation. Inhibition of complement activation by aqueous stem bark extract of Neem plant has been shown to correlate with the anti-inflammatory and anti-rheumatic effects of Neem oil (Labadie et al., 1989). The precise mechanism of inhibition of leukocyte migration by these agents remains to be elucidated. However, the possibility of inhibition of mast cell degranulation may have played a role in inhibiting the acute inflammatory response induced by egg albumin, by inhibiting histamine release.

Inhibition of leukocyte migration by these agents may certainly have modulated the inflammation because leukocyte migration into sites of inflammation aggravates the inflammatory response. Thus, inhibition of leukocyte migration may confer the additional advantage of offering protection from the destructive effects of superoxide radicals released by activated neutrophils during phagocytosis (Weissmann et al., 1980; Perez and Weissmann, 1981) which cause further tissue inflammation and injury (Perez and Weissmann, 1981). Thus, these agents may reduce the incidence of superoxide radicals' release by inhibiting leukocyte migration.

Stabilization of the membrane was studied to further establish the mechanism of anti-inflammatory action of these compounds. Fraction C and CS-1 inhibited both heat- and hypotonicity-induced lysis while CE only inhibited heat-induced lysis. These provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. These extract and fractions may possibly inhibit the release of lysosomal contents of neutrophils at sites of inflammation. These neutrophil lysosomal constituents include bactericidal enzymes and proteases, which upon extracellular release cause further tissue inflammation and damage (Chou, 1997). Although the precise mechanism of this membrane stabilization is yet to be elucidated, it is possible that the extract and fractions produced this effect by increasing the surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or shrinkage of the cell and an interaction with membrane proteins (Shinde et al., 1999). In addition, interaction of these extract and fractions with membrane proteins may have induced cytoprotection of the red cell membrane against heat-induced lysis. Hypotonicity induces lyses probably by causing cell shrinkage due to osmotic loss of intracellular electrolyte and fluid components. Inhibition of hypotonicity-induced lysis clearly involves processes that prevent the migration of these intracellular components outside the cell. It has been shown that cell deformability and cell volumes of erythrocytes are closely related to their intracellular content of calcium (Shinde et al., 1999). Thus, the membrane stabilization effect by these agents may be due to alteration of the influx of calcium into the erythrocytes (Shinde et al., 1999). The precise mechanism for these effects remains to be elucidated.

In conclusion, the results of this study suggest that the anti-inflammatory activity of leaf extracts of *C. scandens* may be mediated through inhibition of prostaglandin synthesis, inhibition of leukocyte migration and membrane stabilization. The isolated compound, CS-1, identified as sitosterol, is the anti-inflammatory principle of the leaves of *C. scandens*.

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References

Akah PA, Njike HA. Some pharmacological effects of rhizome aqueous extract of *Auchomanes diformins*. Fitoterapia 1990;61:368–70.

Amos S, Chindo B, Edmond I, Akah P, Wambebe C, Gamaniel K. Anti-inflammatory and antinociceptive effects of *Ficus platyphylla* extract in mice and rats. J Herbs Spices Med Plants 2002;9(1):47–53.

Anderson RJ, Shriner RL, Burr GO. The phytosterols of wheat germ oil. J Am Chem Soc 1926;48:2987–96.

Asuzu IU, Sosa S, Della LR. The anti-inflammatory activity of *Icacina trichantha* tuber. Phytomedecine 1999;6(4):267–72.

Atta AH, Alkohafi A. Antinociceptive and anti-inflammatory effects of some Jondanian medicinal plants extracts. J Ethnopharmacol 1998;60:117–24.

Aydin S, Ozturk Y, Beiss R, Baser KHC. Investigation of *Origanum onites*, *Sideritis congesta*, and *Satureja cuneifolia* essential oils for analgesic activity. Phytother Res 1996;10:342–4.

Bani S, Kaul A, Jaggi BS, Suri KA, Suri OP, Sharma OP. Anti-inflammatory activity of the hydrosoluble fraction of *Euphorbia royleana* latex. Fitoterapia 2000;71:655–62.

Barik BR, Bhowmik T, Dey AK, Patra A, Chatterjee A, Joy S, et al. Premnazole, an isoxazole alkaloid of *Premna integrifolia* and *Gmelina arborea* with anti-inflammatory activity. Fitoterapia 1992; 53:295–9.

Bauer R, Probstle A, Lotter H, Wagner-redecker W, Matthiesen U. Cyclooxygenase inhibitory constituents from *Houttuynia cordata*. Phyto Med 1996;2(4):305–8.

Bowman WC, Rand MJ. Textbook of pharmacology. 2nd edition. Oxford: Blackwell Scientific Publications; 1988.

Caffaratti M, Ortega MG, Scarafia ME, Ariza Espinar L, Juliani HR. Prenylated flavones from *Dalea elegans*. Phytochemical 1994; 36:1083–4.

Cashin CH, Dawson W, Kitchen EA. The pharmacology of benoxaprofen (2-4-chlorophenyl-methyl-5-benzoxazole acetic acid) LRCL3694, a new compound with anti-inflammatory activity apparently unrelated to inhibition of prostaglandin synthetase. J Pharm Pharmacol 1979; 29:330–6.

Chou CT. The anti-inflammatory effect of *Tripterygium wilfordii* Hook F on adjuvant-induced paw edema in rats and inflammatory mediators release. Phytother Res 1997;11:152–4.

Cybulsky MI, McComb DJ, Mova HZ. Protein synthesis dependent and independent mechanisms of neutrophil emigration Different mechanisms of inflammation in rabbits induced by interleukin-1, tumor necrosis factor alpha or endotoxins versus leukocyte chemoattractants. Am J Pathol 1989;135:227–37.

Di Rosa M, Sorrentino L. The mechanism of the inflammatory effect of carrageenin. Eur J Pharmacol 1969;4:340–2.

Evans WC. Trease and Evans textbook of pharmacognosy. 13th edition. London: Bailliere Tindall; 1989.

Ferriera SH, Moncado S, Parsons M, Vane JR. The concomittant release of bradykinin and prostaglandins in the inflammatory response to carrageenin. Br J Pharmacol 1974;52:108–9.

Finar IL, 1986. 5th ed. Textbook of Organic Chemisry, vol 2. England: Longman Group; 1986.

Flower R, Gryglewski R, Herbaczynska-cedro K, Vane JR. Effects of antiinflammatory drugs on prostaglandins biosynthesis. Nat New Biol 1972;238:104–6.

Gabor M. The anti-inflammatory activity of flavonoids. Hungary: Akademia Kiado-Budapest; 1972.

Garbacki N, Gloaguen V, Damas J, Bodarf P, Tits M, Angenot L. Anti-inflammatory and immunological effects of *Centaurea cyanus* flower heads. J Ethnopharmacol 1999;68:235–41.

Gerristen ME, Carley WW, Rangers GE, Shen CP, Phan SA, Ligon GF, et al. Flavonoids inhibit cytokine-induced endothelial cell adhesion protein gene expression. Am J Pathol 1995;147:278–92.

Jager AK, Hutchings A, van Staden J. Screening of Zulu medicinal plants for prostaglandin synthesis inhibition. J Ethnopharmacol 1996;52:95–100.

Kokwaro JO. Medicinal plants of East Africa. 2nd edition. Kenya: General Printers; 1976.

Labadie RP, Van der Nat JM, Simons JM, Kroes S, Kosasi S, Van der Berg AJJ, et al. Advances in immuno-modulatory agents from medicinal plants and traditional preparations. Proc 11th Asian Symp Med Plants and Species 24–28th Jan, Bandung, Indonesia;1989. p. 407–28.

Lo TN, Almeida AP, Beavan MA. Dextran and carrageenan evoke different inflammatory response in rat with respect to composition of infiltrates and effect of indomethacin. J Pharmacol Exp Ther 1982; 221:261–7.

Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983;53:275–89.

McGaw LJ, Anna J, van Staden J. Prostaglandins synthesis inhibitory activity in Zulu, Xhosa and Sotho medicinal plants. Phytother Res 1997;11:113–7.

Morton JK. West African lilies and orchids. London: William Cloves and sons; 1961.

Nielsen MS. Introduction to the flowering plants of West Africa. London: University of London Press; 1965.

Okoli CO, Akah PA. A pilot evaluation of the anti-inflammatory activity of *Culcasia scandens*, a traditional antirheumatic agent. J Altern Complement Med 2000;6(5):423–7.

Okoli CO, Akah PA, Nwafor SV. Anti-inflammatory activity of plants. J Nat Rem 2003;3/1:1–30.

Oliver-Bever B. Medicinal plants in tropical West Africa. London: Cambridge University Press; 1986.

Omotoye O. Taxonomy of West African flowering plants. U.S.A.: Longman Group; 1984.

Pereira da Silva B, Parente JP. Anti-inflammatory and immunomodulatory polysaccharide from *Orbignya phalerata*. Fitoterapia 2001;72: 887–93.

Perez HD, Weissmann G. Lysozymes as mediators of inflammation. In: Keller W, et al,, editor. Textbook of rheumatology, 1st edition. Philadelphia: W.B Saunders; 1981. p. 179–94.

Perez GRM. Anti-inflammatory activity of *Ambrosia artemisaefolia* and *Rheo spathacea*. Phytomedecine 1996;3(2):163–7.

Perez C, Tiraboschi IN, Ortega MG, Aagnese AM, Cabrera JL. Further antimicrobial studies of 2′ 4′ -dihhidroxy-5′ -(1‴-dimethylallyl)-6-prenylpinocembrin from *Dalea elegans*. Pharm Biol 2003;41:171–4.

Recio MC, Giner RM, Manes S, Gubells L, Gueh J, Julien HR, et al. Anti-inflammatory activity of flavonoid glycosides from *Erythrospermum moticolum* depending on single or repeated local TPA administration. Planta Med 1995;61:502–4.

Ribeiro RA, Flores CA, Cunha FQ, Ferreira SH. IL-8 causes in vivo neutrophil migration by a cell dependent mechanism. Immunology 1991;73:472–7.

Rosa M, Perez G, Salud PG, Guauhtemoc PG, Miguel A, Zavala S, et al. Anti-inflammatory activity of *Cissus trifoliata*. Fitoterapia 1994;64: 103–7.

Shinde UA, Phadke AS, Nari AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Membrane stabilization activity—a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodora* wood oil. Fitoterapia 1999;70:251–7.

Souza GEP, Cunha FQ, Melo R, Ferreira SH. Neutrophil migration induced by inflammatory stimuli is reduced by macrophage depletion. Agents Actions 1988;24:377–80.

Srivastava R, Kulshreshta DK. Bioactive polysaccharide from plants. Phytochemical 1989;28:2877–83.

Tubaro A, Dri P, Delbello G, Zilli C, Della LR. The Croton oil ear test revisited. Agents Actions 1985;17:347–9.

Udeh, O. Phytochemical and antimicrobial investigations of *Culcasia scandens* P. Beauv (fam: Araceae). A B. Pharm. thesis presented to the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka;1986.

Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin edema in rats. J Pharmacol Exp Ther 1969;166:96–103.

Weissmann G, Smolen JE, Korchak HM. Release of inflammatory mediators from stimulated neutrophils. New Engl J Med 1980;303: 24–7.

Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962;111:544–7.