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ANTI-INFLAMMATORY ACTIVITY OF EXTRACTS OF ROOT BARK OF *SECURIDACA LONGIPEDUNCULATA* FRES (POLYGALACEAE)

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Abstract

The effect of extract and fractions of the root bark of *Securidaca longipedunculata* Fres (Polygalaceae) on acute inflammation was evaluated. Solvent extraction yielded the crude methanol extract (ME) while solvent-guided extraction yielded a petroleum ether fraction (PF) and methanol fraction (MF). The extract and fractions inhibited topical edema induced by xylene in the mouse ear. In the systemic edema of the rat paw, the methanol extract (ME) and methanol fraction (MF) significantly ($P < 0.05$) suppressed the development of paw edema induced by egg albumin in rats while the petroleum ether fraction (PF) was devoid of such activity. Ulcerogenic assay in rats indicated that the extract and fractions exhibited varying degrees of gastric irritation in rats in the order of magnitude: MF > PF > ME. Phytochemical tests showed that ME and MF tested positive for carbohydrates, reducing sugars, glycosides, flavonoids, terpenoids, sterols and saponins while PF gave positive reaction for resins only. Acute toxicity test for ME in mice established an i.p and p.o LD₅₀ of 11 and 282 mg/kg respectively.

Key words: *Securidaca longipedunculata*, root bark, acute inflammation, systemic edema.

Introduction

Securidaca longipedunculata Fres (Polygalaceae) is a medicinal herb commonly used in parts of Africa. The plant is a savanna shrub with twisted bole or slender erect branches and grows up to 30 ft high (Iwu, 1986). It occurs in various parts of Western, Northern and Eastern Nigeria (Iwu, 1986), and in Malaysia, Guinea, Cuba and several Asian countries (Anonymous, 2000). In Nigeria, *S. longipedunculata* is variously known as “uwar maganigunar” in Hausa and “ezeogwu” in Ibo (Iwu, 1986; Anonymous, 2000).

In herbal medicine practice, the aqueous root extract is used in religious rites due to their psychotropic effects (Hagreaves, 1986). In South Africa, the Chopi, and Kung tribes use the roots as medicine for people “possessed by evil spirits” and in healing sessions (Winkleman and Dobkin de Rios, 1989). Also in South Africa, the roots are inserted into the vagina as a means of committing suicide with death occurring within 12 h (Hagreaves, 1986). The plant root is used in grain storage (Anonymous, 1996). In Nsukka community of Enugu State, Nigeria, a poultice of the root bark of *S. longipedunculata* is popularly used for the treatment of rheumatic conditions and inflammation. Usually, a poultice of the root bark of this plant is applied topically to inflamed joints in herbal treatment of inflammation and rheumatic disorders. The root is also chewed as a stimulant. The cut root possesses a strong aromatic odour which strongly suggests the presence of volatile constituents.

Pharmacologically, root extracts of the plant increased sodium ion currents and enhanced the contractile response elicited by durable depolarization thus suggesting the possibility of one or more of the constituents acting on the voltage-sensor of excitation-contraction coupling in rat skeletal muscles (Mouzou et al., 1999). Extract of the root bark of *S. longipedunculata* exhibited neuromuscular blocking and negative inotropic and chronotropic cardiac effects (Ojewole et al., 2000) and also demonstrated spasmolytic activity on vascular and extravascular smooth muscles in experimental animals (Ojewole et al., 2001). The root powder exhibited insecticidal activity against a number of insects infesting stored grains (Jayasekara, 2005). Phytochemical studies of the root and root bark has led to the isolation of a number of compounds. Some of these include beta-D-(3,4-disinapoyl)fructofuranosyl-alpha-D-(6-sinapoyl)glucopyranoside and beta-D-(3-sinapoyl)fructofuranosyl-alpha-D-(6-sinapoyl)glucopyranoside which are sucrose derivatives (De Tommasi et al., 1993), the alkaloids elymoclavine, and dehydroelymoclavine, an ergoline compound and cinnamonic acid (Wrobel et al., 1996), methyl 2-hydroxybenzoate (methylsalicylate), methyl 2-hydroxy-6-methoxybenzoate esters and its benzyl analogue (Jayasekara et al., 2002), flavonoids (Ajali and Chukwurah, 2004), and the xanthenes: 1, 7-Dimethoxy-2-hydroxy-xanthone and 1, 4-dihydroxy-7-methoxy-xanthone (Rakuambo et al., 2004). Also, a number of fatty acids and triglycerols such as coriolic (13-hydroxyoctadeca-cis-9, trans-11-dienoic) acid, 11-hydroxyhexadeca-cis-7, trans-9-dienoic acid and 9-hydroxytetradeca-cis-5, trans-7-dienoic acid have been isolated from the seed oil of *S. longipedunculata* (Smith et al., 1979). Some of these compounds like the flavonoids exhibited antimicrobial activity (Ajali and Chukwurah, 2004) while one of the xanthenes, 1, 7-Dimethoxy-2-hydroxy-xanthone relaxed the rabbit corpus cavernosum (Rakuambo et al., 2004).

In our continuing efforts at identifying medicinal plants with anti-inflammatory activity and establishing scientific evidence for activity, the acclaimed potency of the root bark of this plant in inflammatory conditions stimulated our interest to screen the extract for effect on acute inflammation.

Materials and Methods.

Plant material

Fresh roots of *S. longipedunculata* were collected in April 2003 from plants growing in bushes in Edem Ani, Nsukka L.G.A, Enugu State, Nigeria and

authenticated by Mr. A. Ozioko of the Bioresources Development and Conservation Programme (BDGP) center, Nsukka, Enugu State, Nigeria.

The roots were peeled to obtain the bark. The root bark was cut into pieces, dried in the shade to minimize loss of volatile constituents and reduced to size with a pestle in a mortar.

Extraction and fractionation

The plant material (500 g) was extracted by cold maceration in methanol for 48 h to obtain 54.51 g of the methanol extract (ME). A fresh batch of the plant material (500 g) was successively extracted with petroleum ether (40-60%) and methanol by cold maceration for 48 h to obtain 0.87 g of petroleum ether fraction (PF) and 62.97 g of methanol fraction (MF) respectively. The extract and fractions were concentrated in a rotary evaporator at reduced pressure.

Phytochemical analysis

The extract and fractions were subjected to phytochemical analysis for constituent identification using standard protocol (Evans, 1989).

Pharmacological tests

Animals.

Adult Swiss albino mice (20-25 g) and rats (200-250 g) of both sexes were used. They were obtained from the laboratory animal facility of the Department of Pharmacology & Toxicology, University of Nigeria, Nsukka. Animals were housed in steel cages under standard conditions and fed with standard pellets and water *ad libitum*.

Acute toxicity test

The acute toxicity (LD₅₀) of the methanol extract (ME) was determined in mice by the method of Lorke (1983) using the oral and intraperitoneal routes.

Anti-inflammatory tests

Topical edema of the mouse ear

The effect of the extract and fractions on topical acute edema was assessed using xylene-induced ear edema in mice. Swiss albino mice received topical application (5 mg/ear) of one of ME, PF or MF on the anterior surface of the right ear while xylene (0.05 ml) was instantly applied on the posterior surface of the same ear. Control animals received an equivalent volume of the vehicle (3% v/v Tween 85). The left ear was left untreated. Three hours after xylene application, mice were sacrificed and both ears removed. Circular discs were punched out of the ear lobes using a cork borer (6 mm diameter) and weighed. The difference in the weight of discs from the right treated and left untreated ears was calculated and used as a measure of edema (Tubaro et al., 1985; Atta and Alkohafi, 1998). The level of inhibition (%) of edema was calculated using the relation:

Inhibition (%) = $100[1-(Et/Ec)]$ where
 Et= Average edema of the treated group
 Ec= Average edema of the control group

Rat paw edema test.

The rat paw edema method of Winter et al (1962) was used. The methanol extract (ME) and fractions (PF) and (MF) were administered (5 or 10 mg/kg) intraperitoneally to animal groups of 3 per dose. Control animals received equivalent volume of vehicle (3% v/v Tween 85) or 50 mg/kg piroxicam. Thirty minutes after extract administration, inflammation was induced by subplantar injection of 0.1 ml of fresh undiluted egg albumin (Okoli and Akah, 2000). Edema was assessed in terms of volume of distilled water displaced by the paw before and at 0.5, 1, 2, 3, and 4 hours after induction of inflammation. The level of inhibition of edema was calculated for each extract using the relation (Perez, 1986)

Inhibition (%) = $100(1 - (a-x/b-y))$

Where a = mean paw volume of treated animals after egg albumin injection

x = mean paw volume of treated animals before egg albumin injection

b = mean paw volume of control animals after egg albumin injection

y = mean paw volume of control animals before egg albumin injection

Ulcerogenic assay

The method of Cashin et al., (1979) was employed. Food was withheld from the animals for 18 h prior to the experiment. The fasted animals (n = 4) received the methanol extract (ME), the petroleum ether fraction (PF) or methanol fraction (MF) administered orally at 500 mg/kg. Control animals received equivalent volume of vehicle (3% v/v Tween 85) or indomethacin (30 mg/kg). Three hours later, animals were killed and the stomachs removed and cut open along the lesser curvature. The opened stomach was washed with normal saline and observed with a magnifying lens (x10). Lesions on the mucosal surface were scored according to an arbitrary scale: 0 = no lesion; 0.5 = hyperemia; 1 = one or two lesions; 2 = severe lesions; 3 = very severe lesions; 4 = mucosa full of lesions (Bani et al., 2000).

Statistical analysis

Results were analyzed using One way analysis of variance (ANOVA) and expressed as Mean \pm SEM. Data was further subjected to Fischer LSD post hoc test and differences between means were regarded significant at $P < 0.05$.

Results

The extraction process yielded 10.9% of the methanol extract (ME), 0.174% of petroleum ether fraction (PF) and 12.6% of methanol fraction (MF) (Table 1). Phytochemical tests showed that ME and MF tested positive for carbohydrates, reducing sugars, glycosides, flavonoids, terpenoids sterols and saponins. The petroleum fraction (PF) gave positive reaction for resins only (Table 1). The acute toxicity test for ME in mice established an i.p and p.o LD₅₀ of 11 and 282 mg/kg respectively.

Topical acute edema of the mouse ear

The extract and fractions inhibited topical edema induced by xylene in the mouse ear. The fractions (PF and MF) caused greater inhibition than the methanol extract (ME) in the order PF>MF>ME (Table 2).

Paw edema induced by egg albumin in the rat.

The methanol extract (ME) and methanol fraction (MF) suppressed the development of paw edema induced by egg albumin in rats. The methanol extract (ME) evoked a non-dose related inhibition while the methanol fraction (MF) caused the reverse from 2 h. The petroleum ether fraction (PF) was did not exhibit anti-inflammatory activity (Table 3).

Table 1: Phytochemical constituents of extracts and fractions

Constituent	Extract and fractions		
	ME (10.9 %)	PF (0.17 %)	MF (12.6 %)
Carbohydrates	+	-	+
Reducing sugars	+	-	+
Glycosides	+	-	+
Flavonoids	+	-	+
Saponins	+	-	+
Tannins	-	-	-
Terpenoids	+	-	+
Alkaloids	-	-	-
Sterols	+	-	+
Resins	-	+	-

ME = methanol extract; PF = petroleum ether fraction; MF = methanol fraction
+ = present; - = absent; percent values in parenthesis represent extractive yield.

Table 2: Effect of extract and fractions on topical edema of the mouse ear

Extract	Dose (mg/ear)	Edema (g)	Inhibition %
ME	5.0	0.019 ± 0.002	40.63
PF	5.0	0.011 ± 0.0004	65.63
MF	5.0	0.015 ± 0.001	53.13
Control	-	0.032 ± 0.02	-

n = 10; ME = Methanol extract; PF = Petroleum ether fraction; MF = Methanol fraction.

Ulcerogenic assay in rats

The extract and fractions exhibited varying degrees of gastric irritation in rats (Table 4). The magnitude of ulcerogenic capability is of the order: MF > PF > ME.

Discussion

Folkloric treatment of inflammation of various etiologies, using medicinal plants, is well known to masters of the art of traditional medicine practice. The vernacular names of *S. longipedunculata* on translation mean “king of medicines” which is suggestive of high therapeutic potency in disease conditions especially in inflammatory disorders where it enjoys popular use.

Pharmacological screening of root bark extracts of *S. longipedunculata* has revealed that the root bark possesses potent anti-inflammatory effect in the topical and systemic models of acute inflammation. These extracts may have inhibited the release

Table 3: Effect of extracts and fractions on egg albumin-induced paw edema in rats.

Extract	Dose (mg/kg)	Edema (ml) (Mean \pm SEM)				
		0.5	1	2	3	4
ME	5	0.30 \pm 0.04* (57.14)	0.33 \pm 0.06* (57.14)	0.30 \pm 0.07* (50.00)	0.23 \pm 0.05 (37.83)	0.17 \pm 0.06* (48.48)
	10	0.57 \pm 0.06 (18.57)	0.60 \pm 0.08 (22.00)	0.47 \pm 0.08 (21.67)	0.23 \pm 0.06 (37.83)	0.20 \pm 0.04* (39.39)
PF	5	1.00 \pm 0.08 (NI)	0.87 \pm 0.09 (NI)	0.63 \pm 0.10 (NI)	0.53 \pm 0.08 (NI)	0.43 \pm 0.05 (NI)
	10	1.10 \pm 0.04 (NI)	0.67 \pm 0.05 (12.98)	0.57 \pm 0.08 (5.00)	0.40 \pm 0.00 (NI)	0.28 \pm 0.01 (15.15)
MF	5	0.57 \pm 0.02 (18.57)	0.53 \pm 0.08* (31.17)	0.47 \pm 0.06 (21.67)	0.27 \pm 0.05 (27.03)	0.13 \pm 0.06* (60.61)
	10	0.63 \pm 0.05 (10.00)	0.57 \pm 0.08 (25.97)	0.43 \pm 0.06 (28.33)	0.17 \pm 0.02* (54.05)	0.10 \pm 0.04* (69.69)
Piroxicam	50	0.53 \pm 0.05* (24.29)	0.50 \pm 0.04* (35.06)	0.50 \pm 0.04 (16.67)	0.27 \pm 0.05 (27.02)	0.13 \pm 0.02* (60.61)
Control	-	0.70 \pm 0.04	0.77 \pm 0.02	0.60 \pm 0.00	0.37 \pm 0.02	0.33 \pm 0.05

n = 4; * P<0.05 compared to Control (One way ANOVA, Fischer LSD Post Hoc test).

Values in parenthesis represent percent inhibition of edema.

NI = No inhibition

of pro-inflammatory mediators of acute inflammation such as histamine and prostaglandin. Interestingly, the extracts caused gastrointestinal irritation in rats typical of anti-inflammatory prostaglandin inhibitors such as the non-steroidal anti-inflammatory drugs NSAIDs (Rang and Dale, 1988). Thus, these extracts may exert anti-inflammatory effect by inhibiting the synthesis of prostaglandin. The presence of methylsalicylate, an anti-inflammatory constituent with prostaglandin inhibitory activity has been isolated from the roots of *S. longipedunculata* (Costa et al 1992; Jayasekara et al., 2002). The methylsalicylate content accounts for about 90% of the volatile materials in the root bark (Jayasekara et al., 2002). These volatile constituents including methylsalicylate may be chiefly present in the petroleum ether fraction which was shown to contain resins. Resins are often associated with a variety of compounds such as volatile oils, acids, alcohols, phenols etc (Evans, 1989). Methylsalicylate is also a common ingredient of most topical anti-inflammatory/analgesic preparations. In addition to cyclooxygenase enzyme inhibition, methylsalicylate exerts counter-irritant effect on topical application (Bowman and Rand, 1988), which reduces inflammation by diverting hyperemia away from inflamed sites (Oliver-Bever, 1986) and may be responsible for the topical anti-inflammatory effect of the extract and fractions and that of the root poultice when applied topically in herbal therapy of inflammatory disorders.

Table 4: Effect of extract and fractions on gastric irritation in rats.

Extract	Dose (mg/kg)	Ulcer incidence	Ulcer score
ME	500	3/4	0.50 ± 0.20
PF	500	4/4	0.75 ± 0.14
MF	500	4/4	1.75 ± 0.25
Indomethacin	30	4/4	3.00 ± 0.58
Control	-	0/4	0.00 ± 0.00

n = 3. Lesions on the mucosal surface were scored according to an arbitrary scale: 0 = no lesion; 0.5 = hyperemia; 1 = one or two lesions; 2 = severe lesions; 3 = very severe lesions; 4 = mucosa full of lesions.

In the systemic edema test, however, the petroleum ether fraction failed to inhibit acute inflammation. This may still be attributed to the predominant presence of methylsalicylate in the fraction. Methylsalicylate undergoes delayed gastric absorption (Roberts and Morrow, 2001) and its systemic absorption has not been found to correlate with its systemic anti-inflammatory effect (Bowman and Rand, 1988). Thus, impaired absorption of the methylsalicylate content may have affected the activity of the fraction on paw edema at the doses used. Consequently, it is likely that the methanol fraction which exhibited activity in both topical and systemic models, and demonstrated greatest

ulcerogenic potential, may owe its activity in large part to other constituents than methylsalicylate. It is thus likely that plant principles other than the volatile constituents may contribute to the anti-inflammatory activity of the root bark of this plant.

Besides methylsalicylate, other phytochemical constituents isolated from the root bark of *S. longipedunculata* such as flavonoids are known to possess anti-inflammatory activity. Anti-inflammatory constituents such as oleanic acid, beta-sitosterol (Yang et al., 2002), salicylic acid and benzoic acid (Yang et al., 2001) have also been reported in *S. inappendiculata*, a specie related to *S. longipedunculata*. These constituents may also occur in *S. longipedunculata* to varying extents and contribute to the anti-inflammatory activity.

The low LD₅₀ values suggest a possible risk of acute toxicity. The toxicity may not be unrelated to the molluscicidal (Kela et al., 1989a, b), and insecticidal or insect repellent properties of *S. longipedunculata* which may underlie the use of the roots in grains storage (Anonymous, 1996). The insecticidal property has been attributed to the fumigant effect of the methylsalicylate constituent (Jayasekara et al., 2005) which may also be responsible for the acute toxicity and the use of the plant root for suicide (Hagreaves, 1986). However, the results of the acute toxicity also showed that the risk of acute intoxication might be route-dependent as the value of LD₅₀ using the oral route was higher. Thus oral administration may reduce the risk of acute toxicity probably due to the impaired absorption of methylsalicylate.

The magnitude of activity obtained at the two dose levels used indicates high potency of anti-inflammatory effect and together with the array of compounds already isolated from the plant provide impetus to continue the search for novel anti-inflammatory constituents from this plant.

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