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Pilot studies on the anti-inflammatory activity of leaf extracts of *Buchholzia coriacea* Engl (Capparidaceae)

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ABSTRACT

The anti-inflammatory activity of methanol leaf extract and fractions of *Buccholzia coriaceae* Engl (A. Chev) (Capparidaceae) was studied using xylene-induced topical acute edema of the mouse ear, egg albumin-induced systemic acute edema of the rat paw, cotton pellet-induced granuloma test and formaldehyde arthritis in rats. The results showed that the extract and fractions suppressed acute and chronic inflammation to varying extents. The extract and fractions significantly (P<0.05) inhibited the development of topical edema in the mouse ear and systemic edema of the rat paw. The extract and fractions also significantly (P<0.05) inhibited granuloma tissue growth and the global edematous response to formaldehyde arthritis. The ethyl acetate fraction (EF) caused the greatest inhibitory effect in all the tests. Acute toxicity and lethality tests on the extract established an intraperitoneal and oral LD_{50} >5 g/kg in mice. Phytochemical analysis revealed the presence of saponins, tannins, carbohydrates, reducing sugars, resins, glycosides, flavonoids, alkaloids, terpenoids and steroids in the extract and fractions. The most active fraction, EF, change positive to resins, flavonoids and alkaloids. These findings showed that leaves of *B. coriaceae* possess anti-inflammatory properties in acute and chronic inflammation.

KEYWORDS

Buccholzia coriaceae;
Anti-inflammatory activity;
Chronic inflammation;
Acute inflammation.

INTRODUCTION

Buccholzia coriaceae Engl (A. Chev) (Capparidaceae) is a tree plant widely distributed in West Africa from Guinea to Cameroon and Gabon. The morphology has been described[1]. It is commonly known as the "Musk tree"[2]. In Nigeria, it is variously known as "Wonderful kola" (most common name), "Oji ogwu" (Akokwa, Imo State), "Okekpe" (Abakaliki, Ebonyi State), "Owi" (Bini, Edo State), "Omo" (Ijebu-ode, Ogun State) and "Oponmu" (Akure, Ondo State). In Gabon, in addition to serving medicinal purposes, it is often planted around villages[3].

B. coriaceae enjoys popular use in African traditional medicine practice for treatment of a variety of ailments hence, the name "Wonderful kola". In Ivory Coast, the bark is made into a pulp for inhalation or into a snuff and used to relieve headache, sinusitis and

nasal congestion in colds, otitis, and ophthalmias[4]. A bark decoction is applied externally for chest pains, bronchitis, pleurisy and kidney pains. The Gagou of Ivory Coast administers the bark-sap as an enema for kidney pain[5]. In Ghana, the fresh bark is used for earache while a bark decoction is used to bath persons with small pox. The crushed bark is used to treat skin itch in Gabon, while the leaves are applied to boils and bruised limbs in Sierra Leone[1]. The seeds are used in the management of migraine, severe catarrh, tonsillitis, bronchitis, chest problems and inflammation. The ground seeds or kernels are a component of a traditional and valued aphrodisiac or stimulant sold in local markets in Africa (Cameroon). The seeds or kernels of the plant are edible, have a spicy taste and used as condiment[6]. Extract of the seeds/kernels is an active ingredient of commercially available dermatological preparations licensed in the US for treating diseases and fighting the effects of ageing of human skin[7].

Previous studies have documented the anthelmintic[8] and antimicrobial[2,9,10] activities of this plant as well as the isolation of a number of phytochemical constituents such as the sterols- lupeol, campesterol, and epilupeol[11,12]. Although this plant is used in the treatment of disorders of inflammation, its anti-inflammatory activity is yet to be documented. In continuation of studies to elucidate its pharmacological activities in line with ethnomedicinal uses, we studied the anti-inflammatory activity of the leaves using experimental models of acute and chronic inflammation in rodents.

MATERIALS AND METHODS

Materials

Solvents and Reagents: Methanol (Sigma, Germany), n-hexane (Sigma, Germany), ethylacetate (Sigma, Germany), Tween 85, formaldehyde, xylene

Drugs: Piroxicam (Pfizer, Nigeria).

Animals: Adult mice (15-25 g) and rats (100-250 g) of both sexes obtained from the laboratory animal facility of the Department of Pharmacology and Toxicology, University of Nigeria Nsukka were used. The animals were housed in stainless steel cages (rats) and plastic cages (mice) under standard conditions and fed with standard pellet diet and clean drinking water freely. All animal experiments were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85 – 23, revised 1985).

Collection of plant material and preparation of extract

Fresh leaves of *B. coriacea* were collected in October 2003 from bushes in Edem Ani, Nsukka L.G.A, Enugu State, Nigeria and authenticated by Mr. A .O. Ozioko of the International Center for Ethnomedicine and Drug Development (InterCEDD) Nsukka, Enugu State, Nigeria. The leaves were cleaned, cut into smaller pieces, dried under the sun for 48 h and pulverized to coarse powder using a hand blender. About 1 kg of leaf powder was extracted with 20 litres of methanol by cold maceration for 48 h to obtain the methanol extract (ME: 175 g; 17.5 % w/w). A fresh batch of leaf powder (1 kg) was successively extracted with n-hexane, ethylacetate and methanol to obtain the hexane (HF: 84.6 g; 8.46% w/w), ethylacetate (EF: 70 g; 7% w/w) and methanol (MF: 50 g; 5% w/w) fractions respectively. The extract and fractions were concentrated in a rotary evaporator under reduced pressure and subjected to phytochemical analysis using the methods of Harborne[13].

METHODS

Acute toxicity and lethality (LD_{50}) test

The LD_{50} of the methanol extract (ME) was determined in mice by the oral and intraperitoneal routes using the method described by Lorke[14]. The test was divided into two stages. In stage one; mice were divided into 3 groups (n = 3). Each group received a dose (10, 100 or 1000 mg/kg) of ME suspended in Tween 85 (3% v/v). The doses were administered orally and the treated animals observed for 24 h for deaths. Since no death occurred in this stage, three different higher doses (1600, 2900 and 5000 mg/kg) were administered to a fresh batch of animals (n = 1) in stage two. After 24 h of monitoring, no death was recorded. The procedure was repeated with a fresh batch of mice using the intraperitoneal route and a similar result was obtained. The oral and intraperitoneal LD_{50} of ME in mice was thus estimated to be greater than 5000 mg/kg.

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Effect of extract and fractions on acute inflammation

Topical edema of the mouse ear

The effect of ME, EF, HF and MF on acute topical inflammation was evaluated by a modification of the methods of Tubaro et al.[15] and Atta and Alkohafi[16] as previously described[17]. Briefly, adult Swiss albino mice (15-25 g) of either sex were divided into 5 groups (n = 7). Groups I-IV received topical application of 2 mg/ear of ME, HF, EF and MF in Tween 85 (3% v/v) respectively on the outer (anterior) surface of the right ear. Topical acute inflammation was induced immediately on the inner (posterior) surface of the same ear by application of xylene (0.02 ml). Control animals received either vehicle or piroxicam (2 mg/ear). Two hours after induction of inflammation, mice were sacrificed by overdose of chloroform anaesthesia and both ears removed. Circular discs (6 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 weight difference between the two earplugs. The level of inhibition of edema in the treated animals relative to control animals was calculated using the relation: Inhibition of edema (%) = 100[1- (Rt-Lt/ Rc-Lc)] where Rt = mean weight of right ear plug of treated animals; Lt = mean weight of left ear plug of treated animals; Rc = mean weight of right ear plug of control animals; Lc = mean weight of left ear plug of control animals

Systemic edema of the rat paw

The rat paw edema method of Winter et al.[18] was used. Increase in the right hind paw volume[17,19] induced by the subplantar injection of fresh egg albumin was used as a measure of acute inflammation. Adult Swiss albino rats (100-250 g) of both sexes were divided into 10 groups (n = 6). Groups I-VIII received either 200 or 400 mg/kg of ME, EF, HF or MF suspended in Tween 85 (3% v/v) administered orally. Control animals received either piroxicam (50 mg/kg) or equivalent volume of vehicle. One hour later, inflammation was induced by injection of undiluted fresh egg albumin (0.1 ml) into the subplantar of the right hind paw of rats. The volume of the paw was measured by water displacement 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 times after egg albumin injection. For each dose of extract or fraction, inhibition (%) of edema was calculated using the relation[20]; Inhibition of edema (%) = 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

Effect of extract and fractions on chronic inflammation

Formaldehyde-induced arthritis in rats

The method of Seyle[21] was used. Adult Swiss albino rats (150-270 g) of both sexes were grouped and treated as described above. On day 1 of the experiment, one hour after extract administration, arthritis was induced by injecting 0.1 ml of 2% v/v formaldehyde solution into the subplantar of the left hind paw of rats. Formaldehyde injection was repeated on day 3 while drug (extract or vehicle) administration was continued from day 1 to day 10. Day to day changes in edema was evaluated by measuring the volume of water displaced by the inflamed paw once daily for the 10 days. The global edematous response to formaldehyde arthritis was quantified as the area under the curve (AUC) of the time-course of the arthritic event. The AUC was calculated using the trapezoidal rule. The level of inhibition of arthritis was calculated using the relation: [1 – (AUCt/AUCc)] 100 Where AUCc = AUC of the control group; AUCt = AUC of the treated group

Cotton pellet granuloma test

The effect of the extract and fractions on granulomatous inflammation was evaluated with the cotton pellet granuloma test[22]. Adult Swiss albino rats (150-290 g) of both sexes were grouped and treated as described above. Briefly, sterile autoclaved cotton pellets (50 mg) were implanted subcutaneously one on each side of previously depilated back of ether anaesthetized rats. Extracts were administered orally once daily for 7 consecutive days starting from day 1 post-implantation. On day 8, the animals were sacrificed by overdose of ether anesthesia and the pellets carefully dissected out from

the surrounding tissues, dried overnight in a hot air oven at 60°C to a constant weight and weighed.

Statistical analysis

Data obtained was analyzed using ANOVA and subjected to Fischer LSD post hoc test. Differences between means were accepted significant at $P<0.05$. Results are presented as Mean ± SEM.

RESULTS AND DISCUSSION

B. coriaceae is a popularly acclaimed effective remedy for several disorders of inflammation. Experimental evaluation of the anti-inflammatory activity of the leaves in rodents showed that the extract and fractions suppressed the development of acute and chronic inflammation to varying extents. Studies on acute inflammation showed that the extract and fractions inhibited acute topical edema of the mouse ear induced by xylene with the EF causing a significant ($P<0.05$) effect (TABLE 1). The extract and fractions also significantly ($P<0.05$) suppressed the development of systemic acute edema induced by egg albumin in the rat paw in a dose-related manner (TABLE 2). The effect on systemic acute edema indicates that constituents of the leaves possess true anti-inflammatory activity and did not inhibit topical edema through a counter-irritant effect. Counter irritants are known to exert

TABLE 1: Effect of extract and fractions on acute topical edema of the mouse ear

Treatment	Dose (mg/ear)	Edema (mg)	Inhibition (%)
Control	-	9.29 ± 2.02	-
ME	2	5.73 ± 1.30	38.32
HF	2	7.84 ± 1.48	15.61
EF	2	4.30 ± 2.00*	53.71
MF	2	7.17 ± 1.84	22.82
Piroxicam	2	4.29 ± 1.30*	53.82

n = 7; *$P<0.05$ compared to control (*One Way ANOVA; LSD post hoc*); Inhibition (%) of edema was calculated relative to the Control; Values of edema shown are Mean ± SEM; ME = methanol extract; HF = Hexane fraction; EF = Ethyl acetate fraction; MF = Methanol fraction.

TABLE 2 : Effects of extract and fractions on systemic acute edema in rats

Treatment	Dose (mg/kg)	Edema (ml)				
		0.5 h	1 h	2 h	3 h	4 h
ME	200	0.67 ± 0.03* (19.28)	0.53 ± 0.01* (32.05)	0.48 ± 0.02* (38.46)	0.47 ± 0.06* (39.74)	0.40 ± 0.05* (44.44)
	400	0.58 ± 0.06* (30.12)	0.42 ± 0.04* (46.15)	0.43 ± 0.05* (44.87)	0.33 ± 0.03* (57.69)	0.30 ± 0.02* (58.33)
HF	200	0.68 ± 0.01* (18.07)	0.55 ± 0.00* (29.45)	0.58 ± 0.01* (25.64)	0.48 ± 0.01* (38.46)	0.48 ± 0.01* (33.33)
	400	0.52 ± 0.01* (37.35)	0.43 ± 0.01* (44.87)	0.40 ± 0.03* (48.72)	0.37 ± 0.02* (52.56)	0.35 ± 0.00* (51.39)
EF	200	0.48 ± 0.05* (42.17)	0.32 ± 0.03* (58.97)	0.27 ± 0.05* (63.38)	0.20 ± 0.02* (74.36)	0.20 ± 0.02* (72.22)
	400	0.40 ± 0.03* (51.81)	0.30 ± 0.04* (61.54)	0.25 ± 0.03* (67.95)	0.20 ± 0.02* (74.36)	0.13 ± 0.04* (81.94)
MF	200	0.70 ± 0.06* (15.66)	0.58 ± 0.07* (25.64)	0.53 ± 0.07* (29.49)	0.53 ± 0.06* (32.05)	0.50 ± 0.07* (30.55)
	400	0.47 ± 0.05* (43.37)	0.43 ± 0.06* (44.87)	0.38 ± 0.06* (51.28)	0.37 ± 0.06* (52.56)	0.37 ± 0.06* (48.61)
Piroxicam	50	0.45 ± 0.05* (45.78)	0.30 ± 0.02* (61.54)	0.20 ± 0.02* (74.36)	0.15 ± 0.02* (80.77)	0.13 ± 0.01* (81.94)
Control	-	0.83 ± 0.01	0.78 ± 0.02	0.78 ± 0.01	0.78 ± 0.01	0.72 ± 0.01

n = 6. *$P<0.05$ compared to control (*One Way ANOVA; LSD post hoc*); Values of edema shown are Mean ± SEM; Values in parenthesis represent Inhibition (%) of edema calculated relative to the Control; ME = methanol extract; HF = Hexane fraction; EF = Ethyl acetate fraction; MF = Methanol fraction

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anti-inflammatory activity by diverting hyperemia away from sites of inflammation[23]. The topical anti-inflammatory effect may also contribute to the relief obtained when the leaf poultice is used in treatment of boils. The mechanism of anti-inflammatory effect in acute inflammation is not known but may not be unrelated to inhibition of mediator release and cellular migration which play well-established roles in its pathogenesis.

On chronic inflammation, chronic administration of the extract and fractions significantly ($P<0.05$) inhibited granuloma tissue growth induced by cotton pellets (TABLE 3) as well as the global edematous response to formaldehyde arthritis (TABLE 4). Granuloma tissue formed on an inert foreign body in a dead space comprises an accumulation of modified macrophages[24] and other cells and tissues[24,25]. Although the precise mechanisms of anti-inflammatory effect of the extract and fractions are yet to

TABLE 3: Effect of extract and fractions on granuloma tissue growth

Extract	Dose (mg/kg)	Granuloma tissue weight (mg)	Inhibition (%)
Control	-	109.2 ± 6.64	-
ME	200	61.7 ± 3.80*	43.49
	400	68.3 ± 4.77*	37.45
HF	200	98.5 ± 0.9	9.8
	400	90.8 ± 4.55*	16.84
EF	200	53.3 ± 2.47*	51.19
	400	57.5 ± 3.82*	47.34
MF	200	88.3 ± 7.03*	19.14
	400	100.8 ± 10.8	7.69
Piroxicam	50	49.2 ± 1.54*	54.95

n = 6. *$P<0.05$ compared to control (*One Way ANOVA; LSD post hoc*); Values of granuloma tissue weight shown are Mean ± SEM; Inhibition (%) of granuloma tissue growth was calculated relative to the Control; ME = methanol extract; HF = Hexane fraction; EF = Ethyl acetate fraction; MF = Methanol fraction.

be elucidated, inhibition of granuloma tissue growth suggests they may interfere with cellular migration. Inhibition of cellular migration reduces the severity or magnitude of the inflammatory response.

Solvent-guided extraction of the leaves which afforded the fractions, was performed to relate biological activity to constituents. A comparison of the magnitude of anti-inflammatory activity of the extract and fractions

TABLE 4: Effect of extract and fractions on global edematous response to formaldehyde arthritis

Extract	Dose (mg/kg)	AUC (ml/day)	Inhibition (%)
Control	-	3.23 ± 0.43	-
ME	200	2.13 ± 0.23*	34.06
	400	1.87 ± 0.34*	42.11
HF	200	1.82 ± 0.40*	43.65
	400	2.27 ± 0.06*	29.72
EF	200	0.68 ± 0.28*	78.95
	400	1.00 ± 0.01*	69.04
MF	200	2.30 ± 0.34*	28.79
	400	1.22 ± 0.12*	62.23
Piroxicam	50	0.66 ± 0.04*	79.57

n = 6. *$P<0.05$ compared to control (*One Way ANOVA; LSD post hoc*); Values of AUC shown are Mean ± SEM; Inhibition (%) of AUC was calculated relative to the Control; ME = methanol extract; HF = Hexane fraction; EF = Ethyl acetate fraction; MF = Methanol fraction; AUC = Area under the curve of edema time curve.

showed that the ethyl acetate fraction (EF) caused the greatest and most consistent inhibition of acute and chronic inflammation. While the extract and other fractions tested positive to alkaloids, carbohydrates, flavonoids, glycosides, saponins, tannins, terpenoids and steroids, phytochemical analysis showed that alkaloids, flavonoids and resins were detected in the ethyl acetate fraction. It is thus reasonable to suggest that these constituents may account for the anti-inflammatory activity of leaves of this plant, in addition to the sterols[11,12] earlier isolated. Sterols like lupeol, have also been isolated from other plants and shown to possess anti-inflammatory activity[26]. In the acute toxicity and lethality studies, oral and intraperitoneal administration of up to 5 g/kg of the extract caused no death in mice. Thus, the oral and intraperintoeal LD_{50} of the extract in mice was estimated to be >5 g/kg. This high LD_{50} value suggests that the leaves may be generally regarded as safe and possesses a remote risk of acute intoxication.

In conclusion, this study showed that leaves of *B. coriaceae* possess anti-inflammatory properties in acute and chronic inflammation which provides a rationale for its use in traditional medicine practice for disorders of inflammation. The constituents such as alkaloids, flavonoids and resins detected in the most active solvent fraction may account for the anti-inflammatory activity

in addition to the sterols earlier isolated.

SUPPLEMENTARY INFORMATION

Phytochemical constituents of extract and fractions: The results of the phytochemical analysis of extract and fractions are shown.

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