

The antiinflammatory activity of extracts from the root of *Combretum dolichopetalum*

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Summary

The methanol and chloroform extracts of the root of *Combretum dolichopetalum* were obtained and gave yields of 6.48% w/w and 0.38% w/w respectively. The methanol extract significantly inhibited carrageenin-induced mouse paw edema in a dose-dependent manner after an oral treatment. The maximum effect was achieved at a dose of 600 mg/kg and the result was comparable to that of indomethacin (10 mg/kg). The antiinflammatory activity of the chloroform extract against croton-oil induced mouse ear edema was significant ($p < 0.001$) and increased with the dose. The maximum effect (93%) was achieved with 1.0 mg of the extract per ear.

Key words: *Combretum dolichopetalum*, root extract, carrageenin edema, Croton oil edema, antiinflammation.

Introduction

The extract of *Combretum dolichopetalum* GILS ex Engl. (Combretaceae) in alcohol is used in folklore medicine to relieve stomach aches, blood in the stool, diarrhea, cramps and related gastrointestinal disorders (Asuzu and Njoku, 1992).

The ethanol extract of the root's bark has been shown to significantly protect rats against gastric and duodenal ulcers induced by cold stress and indomethacin administration (Asuzu and Onu, 1990). In another study, the ethanol extract protected rats in a dose-dependent manner from gastric ulcers induced by pyloric ligation with histamine administration. The antiulcer activity was associated with the flavonoid fractions that showed antihistamine and anticholinergic activities (Asuzu and Njoku, 1992). The extract also delayed gastric emptying in rats a result that was consistent with the antidiarrheal activity for which the plant extract is used locally.

A number of flavonoids have been shown to exhibit antiinflammatory activities (Akaraz and Jimenez, 1988). The aim of the present study is to investigate the antiinflammatory activity of the methanol and chloro-

form extracts of *C. dolichopetalum*, *in vivo* and *in vitro*, respectively.

Materials and Methods

Animals

White albino mice (both sexes) used in the experiments were bred in the Laboratory Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They weighed between 25 and 28 g and were kept in clean stainless steel cages with an adequate supply of feed and water.

Plant collection and extraction

The plant material was collected in January 1996 from the Nsukka Local Government Area and identified as *Combretum dolichopetalum*, Gils ex Engl. (Combretaceae) by Mr. A. Ozioko of the Department of Botany, University of Nigeria, Nsukka. The freshly detached roots were dried under mild sunshine and subsequently

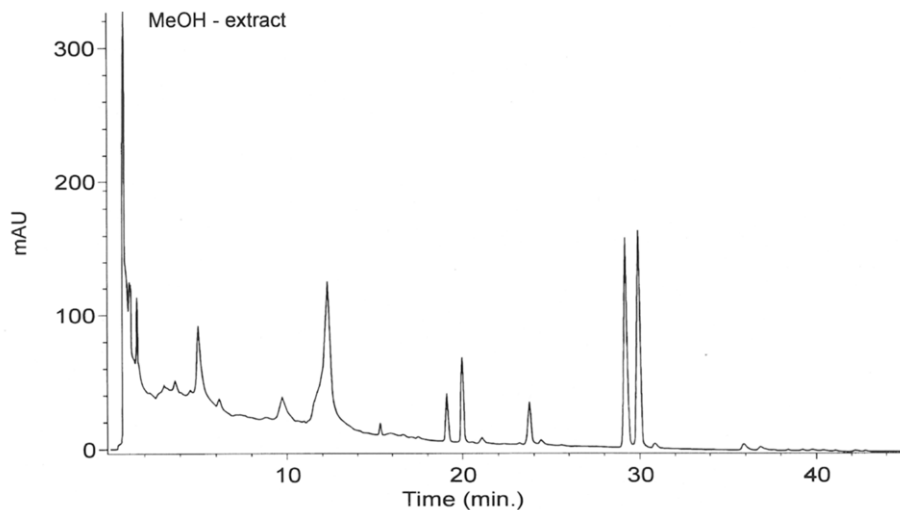


Fig. 1. The peaks between Rt 12–31 min show the characteristic UV-spectrum of flavonoids.

reduced to coarse powder by pounding in a mortar with pestle.

About 100 g of the coarse root powder was macerated in 50% methanol in a large bottle with tight cork and shaken intermittently for 24 h. The resulting extract was obtained by filtering through a filter paper. The methanol extract was concentrated to dryness under reduced pressure using a rotary evaporator (Buchi) at 40 °C and gave a yield of 6.48% w/w.

The chloroform extract was obtained by first defatting 100 g of the coarsely powdered root with petroleum ether (40°–60°). The dried marc obtained was extracted with chloroform by the same method as described for the methanol extract. It gave a yield of 0.38% w/w. The extracts were stored in the refrigerator at 4 °C before the experiments.

HPLC-fingerprint analysis

Instrumentation: HP 1090 liquid chromatograph with HP 1050 photodiode array detection system and HP DOS Chemstation (Hewlett Packard, Waldbronn)

Column: LiChroCART 125-4 with LiChrospher 100 RP 18,5 µm (Merck)

Precolumn: LiChroCART 4-4 with LiChrospher 100 RP 18,5 µm (Merck)

Solvent: A = water,
B = acetonitril

● Methode for methanolic extract of *C. dolichopetalum* root (A)

100 mg of the dried extract were dissolved in 5,0 ml of a mixture of methanol and water using subsonic bath followed by filtration of the solution over a Millipore® filtration unit type HV 0,45 µm.

Gradient: 5–50% B linear in 40 min, 50% B 5 min isocratic

Flow: 1,0 ml/min

Detection: UV 254 nm

Injection: 3 µl

● Methode for chloroform extract of *C. dolichopetalum* root (B)

100 mg of the dried extract were dissolved in 5,0 ml of ethanol abs. using subsonic bath followed by filtration of the solution over a Millipore® filtration unit type HV 0,45 µm.

Gradient: 30–70% B linear in 30 min, 70–95% B linear in 10 min, 95% B 5 min isocratic

Flow: 1,0 ml/min

Detection: UV 210 nm

Injection: 5 µl

Effect of the methanol extract on carrageenin paw edema

Fifty mice were weighed and divided into 5 groups (A–E) of 10 mice each. Each mouse was injected with 0.05 ml of 1% carrageenin (Viscarine Marine Colloids, Rockland, ME, USA) in saline at the subplanter region of the right limb following a standard-method (Winter et al., 1962). The groups were treated with varying doses (200, 400 and 600 mg/kg) of the methanol extract or indomethacin (10 mg/kg) by gastric intubation 1 h before carrageenin as follows:

Group A: 200 mg/kg p.o. extract. Group B: 400 mg/kg p.o. extract. Group C: 600 mg/kg p.o. extract. Group D: 10 mg/kg p.o. indomethacin. Group E: saline solution p.o.

The paw volumes of the mice were determined with a water plethysmometer (Ugo Basile) 1 h, 3 h, 4 h and 5 h after the injection of carrageenin. The percentage of

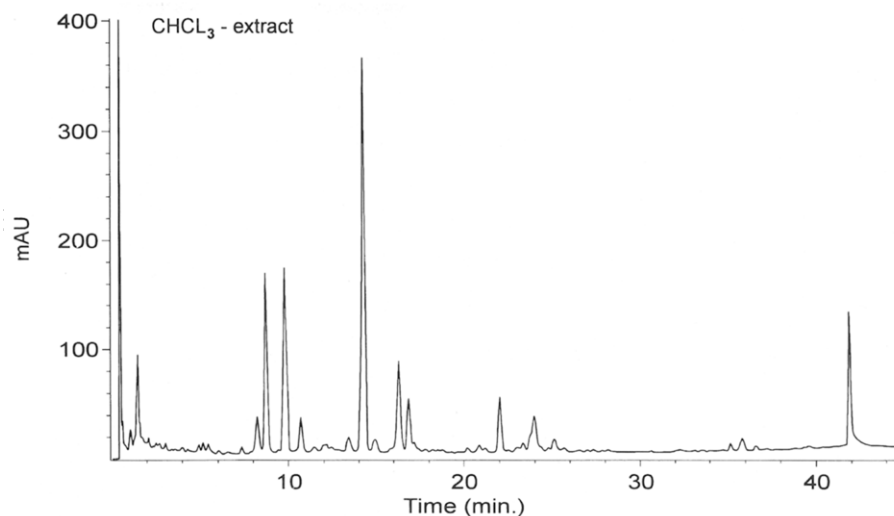


Fig. 2. The peaks at Rt 8.25, 8.72 and 13.40 show the characteristic UV-spectra of flavonoids, the peaks between Rt 14.00–41.2 endabsorption only.

Table 1. The effect of the methanol extract of *Combretum dolichopetalum* on carrageenin-induced edema.

Dose of extract or drug(mg/kg)	Edema increase %				Edema inhibition %			
	1 h	3 h	4 h	5 h	1 h	3 h	4 h	5 h
Carrageenin only	21.85	33.11	30.46	31.78	–	–	–	–
Extract (200 mg/kg)	20.86	30.68	9.65	10.22	4.53	7.34	68.32	67.68
Extract (400 mg/kg)	10.88	0.00	8.81	13.47	50.20	100.0	71.10	57.84
Extract (600 mg/kg)	0.00	0.00	0.00	8.00	100.0	100.0	100.0	74.82
Indomet (10 mg/kg)	0.00	0.00	0.00	3.76	100.0	100.0	100.0	88.10

edema inhibition by the extract and indomethacin were calculated following a standard method (Winter et al., 1962).

Effect of the chloroform extract on mouse ear edema

Mouse ear edema was induced by applying 35 μ g of Croton oil dissolved in 15 μ l acetone per ear (Tubaro et al., 1985; Tragni et al., 1985). A total of 40 mice were divided into 5 groups (A–E) of 6 mice each. They were weighed, anaesthetized with ketamine HCl (150 mg/kg, i.p.) and treated as follows: Group A the control, had the inflammation-inducing solution applied on the inner surface of the right ear. Group B had 0.25 mg of the chloroform extract dissolved in 15 μ l of the inflammation-inducing solution applied on the inner surface of the right ear. Groups C and D were given the same treatment as in group B but with 0.5 and 1.0 mg/ear, respectively, of the extract dissolved in the inflammatory solution. Group E had indomethacin (0.25 mg/ear) dissolved in 15 μ l of the inflammatory solution, applied to the inner surface of the right ear.

After 6 h, the mice were killed by cervical dislocation and 6 mm diameter plugs were obtained from both ears and weighed with a Metler analytical balance. The difference between the right (treated) ear and the left (control) ear weights (EPW) was determined as the mean

change of EPW, and the edema inhibition was calculated as the percent reduction with respect to the edema of the control (untreated) group. The statistical significance of the difference between the mean edema of the treated groups and that of the control was evaluated by means of analysis of variance (ANOVA).

Results

Effect of the methanol extract on carrageenin paw edema

The methanol extract of *C. dolichopetalum* inhibited carrageenin-induced edema in the mouse paw in a dose-dependent manner. The maximum inhibition (100%) was achieved with 600 mg/kg of the extract within 4 hours of the induction of the inflammation (Table 1).

Effect of the chloroform extract on mouse ear edema

The chloroform extract significantly ($p < .001$) inhibited mouse ear edema induced by Croton oil (Table 2). The inhibition of the edema increased with the dose of extract and reached the maximum (93%) at 1.0 mg/ear.

Table 2. Inhibitory effect of the chloroform extract of *Combretum dolichopetalum* on mouse ear Croton oil induced inflammation.

Dose of extract or Drug	Δ Edema (g) mean \pm s.e.	Edema inhibition (%)
Control	1.778 \pm .44	–
Extr. (0.25 mg/ear)	0.375 \pm .18*	78.9
Extr. (0.5 mg/ear)	0.222 \pm .14*	89.5
Extr. (1.0 mg/ear)	0.125 \pm .09*	93.0
Indomet (0.25 mg/ear)	0.625 \pm .57	35.1

* significant at $P < .001$

Discussion

The methanol extract of *Combretum dolichopetalum* root significantly inhibited carrageenin paw edema in mice after an oral administration. The inhibitory effect of the extract was dose-dependent and comparable to indomethacin (10 mg/kg, p.o.) at 600 mg/kg. It was also observed to act maximally between three to four hours after administration. The methanol extract could not inhibit mouse ear edema induced by Croton oil in a preliminary experiment. The reason could be related to the inability of the extract (which is polar) to penetrate the skin of the ear. The chloroform extract was used for this reason to test for antiinflammatory activity with the Croton oil ear edema model and was found to have significant inhibitory effect. The result suggests that the antiinflammatory principle could be non-polar or lipophilic, since it was present in the chloroform extract. The antiinflammatory activities of the extracts could be attributed to their flavonoid content proposed in an earlier study (Asuzu and Njoku, 1992) and the inhibition of inflammatory response may contribute to the overall effectiveness of the extracts in curing gastrointestinal problems in traditional medical practice.

In conclusion, the methanol and chloroform extracts of *Combretum dolichopetalum* induced significant inhibitory effects against edema induced with carrageenin *in vivo* and Croton oil *in vitro*, respectively.

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