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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS AND THE ANTI-INFLAMMATORY ACTIVITY OF METHANOL ROOT EXTRACT OF *CRYPTOLEPIS SANGUINOLENTA* (PERIPLOCACEAE)

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ABSTRACT

The present study investigates the anti-inflammatory activity of methanolic root extract of *Cryptolepis sanguinolenta* against paw edema induced by egg albumin and carrageenan in rats. Phytochemical analysis and acute toxicity test (LD_{50}) of the ethanol extract was also carried out. The results obtained show that the methanol root extract of *Cryptolepis sanguinolenta* has significant ($P<0.05$) dose-dependent anti-inflammatory activity in the entire model studied. The extract at the doses of 100, 200 and 400 mg/kg showed an inhibition (25.20, 38.21 and 50.41 %) and (43.1, 54.30 and 67.00 %) against acute paw edema-induced by egg albumin and carrageenan respectively at 3 h. The LD_{50} of the extract was 1265.40 mg/kg. Phytochemical analysis of the extract show that it contains carbohydrates, alkaloids, glycosides, saponins, resins, tannins, proteins, steroids and terpenoids. The present study justifies the use of *Cryptolepis sanguinolenta* as an anti-inflammatory agent in traditional medicine.

INTRODUCTION

Plants have formed the basis of herbal medicine system which has been used for thousands of years. Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singly or in combination to treat or to diagnose and prevent illnesses or maintain well being (WHO, 2003). It has been found that the active principles responsible for therapy consists of alkaloids, tannins, glycosides, saponins, gums, resins etc (Evans, 2002). It should also be remembered that the active molecules isolated from traditional medicinal plants might not only provide valuable drugs but are also valuable as 'lead molecules' which might be modified chemically, or serve as templates for the design of synthetic molecules incorporating the pharmacophore responsible for the activity eg Artermisin and its derivatives which are now being used as the drug of choice against malaria parasite is obtained from Chinese herb *Artermisia annua* and its constituent Artemisinin is used as a lead in the synthesis of its derivatives like arthemeter (Evans, 2002). Recently modern day pharmacy employs a systematic screening technique to establish the actual constituents responsible for exerting a given action, isolate them and to be able to determine the quality, purity and if adulterated, the nature of the adulterant. *Cryptolepis* (Periplocaceae) is a shrub found in Africa, grown in the wild but can be cultivated (indigenous to Africa) and commonly known as *nibima* (Ghana) *gangamau* (Hausa) and *Bambara* (Senegal). *Cryptolepis* has been shown to be important in West African traditional medicine, in Zaire and in the Casamance district in Senegal (Ghana Society for Development Dialogue Publication, 2002). The root extract has been found to exhibit anti-rheumatic effect (Ghana Society for Development Dialogue Publication, 2002). The leaf extract induced prolonged vasodilatation causing marked and desirable hypotension and also was shown to have antimicrobial activty due to increase in minimal inhibitory concentration (MIC) (Boakaiji, 1979). The constituent *cryptolopine* was found to be slightly less potent than phentolamine in blocking adrenoreceptor stimulation (Boakaiji, 1979). They are a source of a yellow dye which is used for dyeing leather. *C* is used in Congolese traditional medicine for the treatment of ameobiasis. Hence, this work investigates the phytochemical properties and anti-inflammatory activity of *Cryptolepis sanguinolenta* (Fam: *Periplocaceae*) in order to lend scientific support to its use in traditional medicine as a folk remedy for inflammatory diseases.

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MATERIALS AND METHODS

Plant material

The roots of *Cryptolepis sanguinolenta* were collected during the month of July, 2010 at Nsukka, Enugu State, Nigeria and authenticated by Mr. A. Ozioko of the International Centre for Ethnomedicine and Drug Development (Inter CEDD) Nsukka. The voucher specimen (UNN/PCOG/010/403) was deposited at the herbarium of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka.

Animals

Swiss albino mice (20 -28 g) and Wistar albino rats (160-200 g) of both sexes were obtained from the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were kept in multiple cages at room temperature and maintained on standard animal feed and water *ad libitium* under 12 h light and 12 h dark cycle. All the animals were acclimatized for a week before commencement of the experiment.

Extraction

The dried powdered roots (500 g) were exhaustively extracted with 2.5 litres of 95 % methanol in a soxhlet extractor. The extract was concentrated under reduced pressure using rotary vacuum flash evaporator at 50 ^{0}C. The extract was kept in a desiccator till needed for experimentation.

Phytochemical studies

The preliminary phytochemical screening of the extract was performed following standard quanlitative chemical tests (Harbourne, 1973; Trease and Evans, 1994). The classes of phytoconstituents tested for include; alkaloids, tannins, flavonoids, resins, saponins, glycosides, proteins, fats and oils, steroids, terpenoids and carbohydrates.

PHARMACOLOGICAL STUDY

Acute toxicity study

The method of Lorke (1983) was adopted and a total of twenty one mice were used for this study. The animals were fasted before the study, but were allowed water *ad libitum*. In the initial phase, three groups (n=3) were given 10,100 and 1000 mg /kg of the extract intraperitoneally (i.p) respectively. They were then observed for 24 h for signs of toxicity or deaths. In the second phase, another four groups (n=3) were given 2000, 3000, 4000 and 5000 mg /kg of the extract i.p and were observed for 24 h for signs of toxicity or deaths. The lethal dose (LD_{50}) was then calculated.

Anti-inflammatory evaluation

Egg albumin-induced inflammation

Twenty-five animals divided into five groups of five animals each were used for the experiment. They were fasted for about 8 h before the experiment and deprived of water only during the experiment. The deprivation of water was to ensure uniform hydration and to minimize variability in edematous response. The animals were given intra peritoneal injection of methanol extract of *Cryptolepis sanguinolenta* at doses of 50, 100, and 200 mg/kg. The negative control animals were treated with normal saline. The positive control group received 25 mg/kg diclofenac sodium. All substances except diclofenac sodium were administered 30 min before the administration of the inflammatory agent (0.1 ml of fresh diluted egg albumin) in the sub plantar region of the right hind paw after intra peritoneal administration of the extract. The positive control was administered 1 h before induction of inflammation. The paw volume measurements were taken at 0, 1, 2 and 3 h after egg albumin induction. Edema was assayed in terms of volume of water displaced by the treated paw using a phethysmometer.

Carrageenan-induced rat paw edema

This was performed according to the method of Winter *et al*. (1962). Animals were divided into five groups of five each. Edema in the right hind paws of wistar rats was induced by subcutaneously injecting 0.1 ml of 10 % (w/v) carrageenan in 1 % sodium carboxymethylcellulose. The paw volume of each rat was measured before carrageenan injection and then at 30 min intervals up to five times using a pleythysometer 7150 (UGO BASIL, ITALY). Three groups of the rats were treated with different (100, 200 and 300 mg / kg, i.p) 30 min prior to carrageenan injection. The control animals were given vehicle (normal saline). Another group of rats was administered diclofenac sodium (25 mg /kg, i.p). The difference between the initial and subsequent reading gave the actual edema volume. Percentage inhibition of inflammation was calculated using the formula:

$$\% \text{ Inhibition} = \frac{VC - VT}{VC} \times \frac{100}{1}$$

Where VT represents edema volume in the treated rats and VC represents edema volume in the untreated.

Statistical analysis

Values were expressed as mean ± S.E.M. Statistical significance was determined by student's t-test (Snedecor, 1967). Values within $P < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The percentage yield of the methanol extract was found to be 12.7 % w/w. The LD_{50} was estimated to be 1265.40 mg/kg i.p in mice. *Cryptolepis sanguinolenta* methanol leaf extract (100, 200 and 300 mg/kg) produced a significant ($P<0.05$) dose-dependent inhibition against acute paw edema-induced by egg albumin and carrageenan as compared to diclofenac sodium, a standard anti-inflammatory drug (Tables 2 and 3). The methanolic extract of *Cryptolepis sanquinolenta* (400 mg/kg) prevented the formation of edema induced by egg albumin and carrageenan and thus showed significant anti-inflammatory activity ($p<0.05$). The methanolic extract of *Cryptolepis sanquinolenta* (400 mg/kg) reduced the edema induced by egg albumin and carrageenan by 50.41 and 67.00 % after 3h injection of noxious agent as compared to the control vehicle treat group. Diclofenac sodium at 25 mg/kg inhibited the

Table 1. Phytochemical analysis of the root of *Cryptolepis sanguinolenta*

Constituent	Inference
Carbohydrates	++
Reducing sugars	+
Alkaloids	+++
Glycosides	+
Saponins	+
Tannins	+
Flavonoids	+
Resins	+
Proteins	+
Fats and Oils	-
Steroids	+
Terpenoids	++
Acidic compounds	-

Key: + = slightly present, ++ = moderately present, +++ = highly present, - = Absent

Table 2. Effect of alcoholic root extract of *Cryptolepis sanguinolenta* on egg albumin-induced paw edema in rats at 3h

Treatment	Doses (mg/kg)	Paw volume (ml) after 3h	Percentage inhibition (%)
Vehicle (1 ml/kg)	-	1.23 ±0.01	00.00
Extract	100	0.92 ±0.03*	25.20
	200	0.76 ±0.007*	38.21
	300	0.61 ± 0.02*	50.41
Diclofenac sodium	25	0.49 ± 0.004*	61.63

*Indicate significant anti-inflammatory activity at P< 0.05 compared to control. Values are presented as mean ± S. E. M, n = 5.

Table 3. Effect of the methanol extract of *Cryptolepis sanguinolenta s* leaf on carrageenan- induced paw edema in rats

Treatment	Doses (mg/kg)	Paw volume (ml) after 3 h	Percentage of Inhibition (%)
Vehicle (1 ml/kg)	-	1.00± 0.02	00.00
Diclofenac sodium	25	0.21± 0. 006*	79.00
Extract	100	0.57± 0.01*	43.01
	200	0.46 ± 0.009*	54.30
	300	0.33±0.005*	67.00

*Indicate significant anti-inflammatory activity at P< 0.05 compared to control. Values are presented as Mean ± SEM, n=5.

edema volume by 61.63 and 79.00 % for egg albumin and carrageenan respectively. On carrageenan induced inflammation model the methanolic extract (400 mg/kg) produced better inhibition of paw edema

Albumin and Carrageenan respectively

The significant (P<0.05) anti-inflammatory activity exhibited by the extract at the doses studied (100, 200 and 300 mg/kg) against edema induced by egg albumin and carrageenan in rats compared to the control group is an indication that, the plant might serve as a useful source of anti-inflammatory agent. The tendency of a drug to have an anti-inflammatory activity depends on the physiological change accompanying inflammatory diseases. The anti-inflammatory property can easily be seen when the drug prevents swelling due to inflammation. Deprivation of water was to ensure uniform hydration and to minimize variability in edematous response during the experiment. Carrageenan induced paw edema and egg white induced rat paw methods are suitable for screening agents for anti-inflammatory activity, which are frequently used to assess the anti-edematous effect of natural products (Akah *et al.*, 1993; Amos *et al.*, 2002)). Several inflammatory mediators like histamine, kinins, protagladins and pro-inflammatory cytokines have been suggested to play a role in the mechanism of inflammation (Rosa et al., 1968; Hirschelman and Berkemeier, 1993).

It is assumed that at least some of these; mediators are subjects of inhibition by the extract. Edema which develops after carrageenan in inflammation is a biphasic event. The initial phase is attributed to the release of histamine and serotonin (Castro *et al.*, 1968). The edema maintained between the first and second phase is due to kinin like substances (Crunkhan, 1971). The first phase begins immediately after injection and dimishes in 1 h. The second phase begins at 1 h and remains for 3 h. On the other hand, the delayed phase of carrageenan induced edema result mainly from the potentiating effect of prostaglandins on mediator releases, especially of bradykinni and neutrophil-derived free radicals. The phytochemical analysis of the extract revealed the presence of active constituents (Table 1). These phytochemical constituents of the extract might be responsible for the observed pharmacological activities of the extract. It has not been established which of the compounds is responsible but alkaloids are suspected for the plant anti-infllammatory activity because cryptolepine an alkaloid obtained from the leaf of *Cryptolepis sanguinolenta* has been shown to possess anti-inflammatory activity (Olumayokun, 2002). Analgesic and anti-inflammatory effects have been observed in flavonoids as well as tannin (Alhmadiani *et al.*, 2000). Certain flavonoids posses potent inhibitory activity against a wide range array of enzymes such as protein kinase C, protein tyrosine kinases, phospholipase A2, phosphodiesterases and others (Middleton, 1998). Inhibition of these key enzymes provides the mechanism by which flavonoids inhibit inflammatory processes (Manthey *et al*., 2001). In conclusion, this study has shown that the extract does possess significant anti-inflammatory effect in experimental animals at the doses investigated. The results support the traditional use of *Cryptolepis sanguinolenta* in some painful and inflammatory condition and also suggest the presence of biologically active principle, which may worth further investigation and elucidation.

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