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*Full Length Research Paper*

## **Anti-Inflammatory and Analgesic Activities of *Securidaca longepedunculata* Fers (Polygalaceae) Leaf and Stem Bark Methanolic Extract**

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### **ABSTRACT**

*Securidaca longepedunculata* Fers (Polygalaceae) is commonly used in many parts of Africa for the treatment of rheumatic conditions, fever, headache and various other inflammatory based diseases. The present study was carried out to evaluate the anti-inflammatory and analgesic activity of *Securidaca longepedunculata* leaf and stem bark methanol extracts using animal model. The anti-inflammatory activity of the methanolic extracts were evaluated using carrageenan induced paw edema in rats while the analgesic activity was determined using acetic acid induced writhing in mice. Both the leaf and stem methanol extracts exhibited anti-inflammatory activity greater than 70% at all doses tested. This activity was dose dependent with the highest being at 800 mg/kg Po and significant at  $P < 0.05$ . The analgesic activity of both extract was however below 50%, though comparable with that of aspirin used as the standard. This study has justified the inclusion of *Securidaca longepedunculata* in remedies used for the management of inflammatory based diseases traditionally.

**Key words:** Analgesic, anti- inflammatory, *Securidaca longepedunculata*, carrageenan, acetic acid

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### **INTRODUCTION**

The use of medicinal plants in pain- relieving drug discovery is well documented in literature. Examples include salicylic acid and morphine which were isolated from plant sources used in traditional medicine as analgesics and to ease pain at child birth (Balandrin, 1985). Opium derived from the latex of unripe capsule of *Papaver somniferum* contains about 25 alkaloids, including morphine, codeine, thebaine, papaverine and pethidine (Trease and Evans, 2006) which are anti-

nociceptive in nature. Cannabinoids from marijuana, and other preparations from *Cannabis sativa* have also been used for centuries to relieve pain. They have been shown to possess anti- nociceptive properties when assessed in several experimental models (Formukong *et al.*, 1988, 1989). It has been reported that plants used to relief pain are chemically confirmed to be rich in secondary metabolites or active principles such as alkaloids, flavonoid, terpenoid and steroids (Cechinel *et al.*; 2000).

*Securidaca longepedunculata* Fers (Polygalaceae) is commonly used as medicine in many parts of Africa

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for the treatment of rheumatic conditions, fever, headache and various other inflammatory conditions. The leaves and stem bark are used in rheumatic pain with Shea butter or *Datura metel*, onions, and groundnut oil as rub. The powdered root with seed is used as snuff for the treatment of headache and feverish pain. (Oliver-Bever, 1986; Assi and Guinko, 1991; Iwu, 1993). In Ghana the root is used as a purgative in small doses as well as for the treatment of syphilis and gonorrhoea. The powdered root is also used in sneezing. The root is used in the treatment of malaria in Zimbabwe and Botswana. It is used in Guinea for urethral discharges of venereal origin. In Senegal, it is used in sleeping sickness as root decoction, in herpes zoster, psoriasis and also as an anti-convulsant. The leaf infusion is used in venereal disease in East Africa. In Sierra Leone is used in the treatment snake bite. (Assi and Guinko, 1991)

Powdered dried roots of the plant is used as pest control agent and have potential as a protectant against insect pests in stored grain (Jayasekara *et. al* 2005). The flavonoids contained in *Securidaca longepedunculata* exhibited anti-microbial activity (Ajali and Chukwurah, 2004). Its xanthenes relaxed the rabbit corpus cavernosum (Rakuambo *et. al*; 2004). *S. longepedunculata* exhibited molluscicidal activity which may probably be due to the active ingredients of saponin – glycosides of oleanolic acid, tannins and vateriannate methylsalicylate (Morais *et. al*; 2005). Anti-inflammatory effect of the root bark on xylene induced topical edema in mouse ear and egg albumin induced rat paw edema was reported by *okoli et. al* 2005. The antimicrobial, analgesic, anti-inflammatory and hypoglycaemic effects of aqueous extract of the root bark have been previously reported (Olaleye *et al*, 1998; Olaleye *et al*, 2002; Ojewole 2008)

This study was carried out to assess the analgesic and anti-inflammatory activities of the leaves and stem bark of the plant with a view to justifying or otherwise, their inclusion in traditional preparations for the management of pain related conditions.

## MATERIALS AND METHODS

### Animals

Adults male Wistar strain albino rats (80-100g) and swiss albino mice (20-25g) were used for this study. The animals were bred in the Pre-clinical animal house, College of Medicine, University of Ibadan. All animals were fasted for 12 hrs with free access to water before starting the experiments. All experimental protocols were in compliance with University of Ibadan Ethics Committee Guidelines as well as internationally accepted principles for laboratory animal use and care as

found in the US guidelines (NIH publication # 85-23, revised in 1985).

### Plant materials

The stem and leaf samples were collected along Eruwa road, Oyo State, Nigeria and authenticated in the herbarium of the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan. Herbarium specimen was deposited at herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan. (FHI NO 109972)

### Preparation of extracts

The plant samples were air dried, finely cut and ground into powder. The powdered samples were soaked separately in methanol for about 72 hours and filtered. The filtrates were concentrated *in vacuo* at 40°C using rotary evaporator.

### In vivo studies

#### *Carrageenan-induced paw edema in rats*

Pedal inflammation in rats was induced essentially as described by Winter *et al*; (1962). Fifty (50) Wistar rats were divided into 6 groups with five animals in each group. An injection was made of 0.1ml of 1% carrageenan suspension into the right hind foot of each rat. The test groups of rats were treated with 100, 200, 400 and 800 mg/kg (po) of *Securidaca longepedunculata* extract 1hr before carrageenan injection. The negative and positive control groups received 0.2 ml normal saline and 150 mg/kg Aspirin po respectively. The leaves and stem extracts were administered to separate groups of animals.

Paw diameter measurement was measured by wrapping a piece of cotton thread round the paw of each rat and measuring the circumference with a metre rule (Adeyemi *et. al* 2002, Ratheesh and Helen 2007). This procedure was done prior to irritant injection and 1, 2, 3, 4, 5 hours subsequently. The percentage edema inhibition in drug treated rats versus control was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Ct} - \text{Co}) \text{ control} - (\text{Ct} - \text{Co}) \text{ treated}}{\text{Ct} - \text{Co}} \times 100$$

$$(\text{Ct} - \text{Co}) \text{ control}$$

Where Ct=paw size 1 h, 2 h, 3 h, 4 h or 5 h after carrageenan injection

Co=paw size before carrageenan injection

#### *Acetic acid-induced writhing in mice*

Fifty Swiss mice were divided into ten treatment and control groups of five mice per group. The test groups were administered 100, 200, 400 and 800 mg/kg of *Securidaca longepedunculata* extract, while the

negative and positive control group received 0.2 ml normal saline 100 mg/kg aspirin po respectively. The animals were fasted for 16 h prior to treatments. Writhing was induced by the method of Koster *et. al.*, (1959). One hour after treatment, the mice were injected i.p with 0.2 ml of 3% acetic acid solution to induce the writhing. The number of abdominal constrictions (writhing) and stretching with a jerk of the hind limb was counted between 5 and 15 minutes after acetic acid injection. The response of the extract and aspirin treated groups were compared with those of the animals in the control group (0.2 ml saline). Percentage protection against writhing movement (% inhibition of writhing) was taken as an index of analgesia and it was calculated as follows:

$$\% \text{ inhibition} = \frac{Wr(\text{control}) - Wr(\text{test group})}{wr(\text{control})}$$

Where *Wr* = mean number of writhing

### Statistical analysis

The results are expressed as mean ± standard deviation. Statistical analysis was done using analysis of variance (ANOVA) followed by Post Hoc Test.

### RESULTS

The inhibitory effects of *S. longepedunculata* methanolic leaf extract, methanolic stem extract and aspirin on carrageenan induced paw edema are as reported in table 1 and 2. In the experimental conditions used for this study, the stem and leaf methanol extracts of *S. longepedunculata* shows significant inhibition of carrageenan paw edema in rats as reflected in the table below. The reported values represent circumference of the rat paws in centimeter as well as the level of inhibition in percentages.

**Table 1:**

Anti-inflammatory effect of methanol extract of *S. longepedunculata* leaf on carrageenan induced paw edema in rats.

GROUP/DOSE(mg/kg)	0 hr	1 hr	2 hrs	3 hrs	4 hrs	5 hrs
Control (Normal saline)	1.07±0.5	2.31±0.3	2.63±0.3	4.05±0.2	3.91±0.2	3.19±0.3
ASPIRIN 150	1.65±0.1	1.92±0.3	2.12±0.2*	2.11±0.3**	2.27±0.2**	1.86±0.2*
		78.2%	69.8%	70.5%	78.2%	90.1%
EXTRACT 800	1.43±0.4	1.78±0.4**	1.89±0.6**	2.21±0.1*	1.88±0.3	1.72±0.2*
		71.7%	70.5%	73.8%	84.2%	86.3%
EXTRACT 400	1.87±0.2	2.27±0.1*	2.37±0.3*	2.66±0.4	2.45±0.1	2.23±0.2*
		67.7%	67.9%	73.5%	79.6%	83.0%
EXTRACT 200	1.38±0.1	1.87±0.3	1.93±0.2*	1.86±0.5**	1.73±0.1**	1.62±0.1*
		60.5%	55.6%	61.3%	71.7%	80.6%
EXTRACT 100	1.72±0.3	2.25±0.1*	2.50±0.0*	2.86±0.2*	2.67±0.1**	2.31±0.1*
		57.3%	50.0%	61.7%	66.5%	72.2%

\* Significant difference between groups at 0.01 (P <0.01); \*\* Significant difference between groups at 0.05 (P <0.05)

**Table 2:**

Anti-inflammatory effect of methanol extract of *S. longepedunculata* stem bark on carrageenan induced paw edema in rats.

GROUP/DOSE(mg/kg)	0hr	1 hr	2 hrs	3 hrs	4 hrs	5 hrs
Control (Normal saline)	1.95±0.3	2.12±0.1	2.49±0.5	2.98±0.3	2.57±0.1	2.22±0.1
ASPIRIN 150	1.42±0.1	1.46±0.2*	1.56±0.1*	1.73±0.0*	1.56±0.1*	1.45±0.2**
		76.4%	74.1%	69.9%	77.4%	88.8%
EXTRACT 800	1.73±0.1	2.11±0.4*	1.92±0.3**	2.05±0.2	1.86±0.2	1.78±0.1
		67.5%	64.8%	68.9%	79.0%	81.4%
EXTRACT 400	1.89±0.4	2.35±0.1*	2.17±0.3	2.32±0.1*	2.09±0.0*	1.05±0.0*
		60.6%	48.2%	58.2%	67.7%	77.7%
EXTRACT 200	1.67±0.2	2.20±0.1*	1.97±0.4**	2.14±0.1	1.89±0.3**	1.74±0.1*
		54.7%	44.4%	54.3%	64.5%	74.1%
EXTRACT 100	1.48±0.1	2.07±0.3*	1.80±0.1*	1.97±0.2*	1.72±0.2	1.56±0.0
		49.5%	40.7%	52.4%	61.3%	70.4%

\* Significant difference between groups at 0.01 (P <0.01) \*\* Significant difference between groups at 0.05 (P <0.05)

**Table 3:**  
Number of writhing and percentage inhibition by stem and leave extracts

Group/dose (mg/kg)	Number of writhing		Percentage inhibition	
	Stem extract	Leaves extract	Stem extract	Leave extract
800	17	14.6	38.2	46.9
400	18.4	15.8	33.0	42.5
200	21.8	19.3	20.7	30.0
100	23.7	23.6	13.9	14.2
Aspirin 100	14.0	14.0	49.1	49.1

## DISCUSSION

Plants are a viable source of drugs and they have contributed especially to the discovery of analgesic and anti-inflammatory drugs. *Securidaca longepedunculata* is well documented in ethnomedicine as pain reliever (Oliver-Bever, 1986; Assi and Guinko, 1991). Phytochemical screening of the root bark extract (Auwal *et al.*, 2013 and Okoli *et al.*, 2005) and the leaves (Ndamitso *et al.*, 2013) revealed the presence of saponins, tannins, flavonoids, cardiac glycosides, alkaloids, reducing sugar and anthraquinones.

In this study, the leaves and the stem bark of this plant were screened for their anti-inflammatory and analgesic activity in experimental animals using carrageenan induces paw edema and acetic acid induced writhing respectively. Carrageenan induced paw edema is believed to be in two phases. Phase one (1 hr) involves the release of serotonin and histamine while phase two (after 1 hr) is mediated by prostaglandins cyclooxygenase product and the continuity between the two phases is due to kinins (Perianayagam *et al.*, 2006, Loganayaki *et al.*, 2012).

The anti-inflammatory results showed an increase in rat paw circumference from the first to third hour in animals given the extract, after which a decrease was observed. The percentage inhibition of edema decreased at the second hour for both the leaf and stem extract, with an increase in the third, fourth and fifth hour. These results are significant at different P values of either 0.01 or 0.05 as indicated on the table. The percentage inhibition was also dose dependent with the leaf extract having a greater percentage inhibition than the stem extract at the same dose. The percentage inhibition increased as the doses administered increased.

The results obtained confirmed that edema formation was inhibited in all treated animals except the control animals which were given normal saline. The percentage inhibition was shown to be the highest at the fifth hour with aspirin showing the highest percentage inhibition of 90.27%. It is however to be expected that purified extract of *Securidaca longepedunculata* may exhibit a higher anti-inflammatory activity than reported

in the crude extract. Carrageenan-induced rat paw edema is a valuable test used in predicting the value of anti-inflammatory agents acting by inhibiting the mediators of acute inflammation (Mossa *et al.*, 1995).

The methanol extract of *Securidaca longepedunculata* leaf and stem also exhibited analgesic activity in mice by inhibiting the acetic acid –induced writhing. The analgesic activity exhibited was observed to be dose dependent, with an increase in percentage inhibition of writhing as the dose increased from 100mg/kg to 800mg/kg. The leaf extract exhibited more analgesic activity than the stem extract at the same dose. The highest percentage inhibition was observed for aspirin (49.09%).

The result of this research is in agreement with previous work on the plant. Ojewole, 2008 and Okoli *et al.*, 2005 have reported the anti-inflammatory and analgesic activities of the root extract. This is however a first report of the analgesic and anti-inflammatory activities of the leaves and stem bark.

From the research findings, it can be concluded that *Securidaca longepedunculata* leaf and stem methanol extract contain substances with anti-inflammatory and analgesic properties. An extension of this study could involve fractionation and isolation of the active constituents through various techniques with the aim of identifying specific agent(s) responsible for the anti-inflammatory and analgesic activities of this plant. This should be followed with structure elucidation of compounds and other biological and pharmacological evaluation with a new understanding of the mode of actions.

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