

ANTI - INFLAMMATORY AND ANALGESIC ACTIVITIES OF *NOTHOSPONDIAS STAUDTII*

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Summary: The aqueous (AENS), methanolic (MENS) and chloroform (CENS) extracts of the leaves of *Nothospondias staudtii* Engl (Anacardiaceae) were screened for analgesic and anti-inflammatory activities in mice and rats. Pain responses were studied in mice using the tail immersion and acetic acid induced writhing while carrageenan induced paw oedema was used to access anti-inflammatory activity. The three extracts exhibited significant analgesic compared with the control (saline, 10ml/kg) as evidenced by (i) increased escape latency in the tail immersion assay (ii) reduction in abdominal writhing induced by acetic acid. The analgesic activity were higher in MENS & CENS compared to aspirin (150mg/kg). The extracts progressively reduced rat paw oedema induced by subplantar injection of carrageenan, the methanolic extract showing more pronounced effect than the aqueous and chloroform extracts. Preliminary phyto-chemical screening shows the presence of alkaloids, sugars, proteins and anti-oxidants in the extracts.

Key Words: *Nothospondias staudtii*, inflammation,, analgesic activity, alkaloid

Introduction

Nothospondias Staudtii Engl. (family simarubaceae) is an understory forest tree with large and conspicuous leaves. The leaves are large and long. It is confined to the forest regions Nigeria, Gabon, Zaire and Congo where the fresh juice from the leaves are employed in the dressing of fresh wounds. Extractive from the leaves are also employed traditionally for relieve of headache and inflammations (Keay 1989).. There is a dearth of information on the biological activity of *Nothospondias staudtii*. We have therefore carried out a preliminary screening for its anti-inflammatory and analgesic activities of the leaf extracts of this plant.

Materials and Method

Plant Material

N. Staudtii leaves were harvested from their natural habitat at Gambari forest reserve, Oyo State, Nigeria in January, 2000 and authenticated by Mr. T.K. Odewo, a Taxonomist of the forestry research Institute of Nigeria (FRIN) Ibadan. A voucher specimen (FHI 105679) was deposited in the Herbarium of the same forestry research institute.

Extract Preparation

Air-dried and powdered leaves of *N. Staudtii* Engl. were extracted with water, methanol and chloroform at 80°, 40° C and room temperature respectively. The dried extract was stored at 4°C until used. The yield of AENS MENS and CENS were 1.5g, 5g and 1.5g/150ml water, 30.0g/300ml methanol and 20.0g/250ml chloroform

respectively. AENS was dissolved in 0.9% saline while MENS and CENS were each dissolved in 2.5% Tween 80 and subsequently in normal saline. Animals

Adult male and female Swiss mice (20 - 28g) and albino rats (120 - 150g) obtained from the animal house, College of Medicine, University of Ibadan, Nigeria were used. They were housed in cages at room temperature and fed with mouse cubes (Ladokun feeds; Ibadan) water was provided *ad libitum*.

Phytochemical Analyses

The crude extract of the leaves of *Nothospondias staudtii* were tested for the presence of secondary metabolites using standard methods (Persino and Quimby, 1967; Odebiyi and Sofowora, 1979). The dichloromethane extract gave positive tests for alkaloids while the methanolic extract indicates the presence of Tannins, Proteins and amino acids, fixed oils and fats (Persino and Quimby, 1967; Odebiyi and Sofowora, 1979). Reacting with Benedicts solution indicates the presence of reducing sugars.

About 0.01gm of the methanolic extract was dissolved in methanol and spotted on the TLC (Aluminum foil TLC 254 - Merck) and developed in the system (ethylacetale/methanol, 90:10). The TLC plate was allowed to dry and then 254nm and sprayed with DPPH assay (Poteract, 1997) yellow spots against pink background confirm the presence of flavonoidal compounds.

Anti-Inflammatory Activity

The effect of oral administration of 100mg/kg of the extract of *Nothospondias staudtii* (AENS, MENS & CENS), 150mg/kg Aspirin (Dyspirin[®] by Reckitt & Coleman) or vehicle (Saline, 10ml/kg) on the hind-paw oedema induced by subplantar injection of 0.1ml carrageenan (1% w/v) was evaluated according to the method described by Winter *et al* (1962). Paw oedema was measured by wrapping a piece of cotton thread round the paw and measuring the circumference with a metre rule (Hess and Miloning, 1972, Bamgbose and Noamesi, 1981). Measurement was carried out immediately before and 3hrs following carrageenan injection. Percent inhibition of test drugs was calculated in comparison with vehicle control (100%).

Analgesic Activity

The *Nothospondias staudtii* leaf extracts (AENS, MENS and CENS) were evaluated for analgesic activity in mice using Tail Immersion (Jansen and Jagenau 1959) and acetic acid induced writhing (Koster *et al*, 1959) tests described below.

Tail Immersion:- Mice more treated orally with 100mg/kg of the leaf extract (AENS, MENS and CENS), reference drug (150mg/kg, Aspirin) and vehicle (Saline, 10ml/kg) 1hr before the measurement of extract effect. Water was heated to

50.0 ± 1.0°C in a water bath. The time taken for the animal to remove its tails out of the water was recorded. Percentage protection was calculated in comparison to control (100%).

Acetic acid induced writhing: Mice were injected intraperitoneally with 0.6% aqueous acetic acid (10ml/kg) 1hr after oral administration of 100mg/kg of AENS MENS, and CENS or vehicle (Saline, 10ml/kg). The reference group was given 150mg/kg of Aspirin. The number of writhing movement of each mouse was counted for 10min, starting from 5 min after the injection of acetic acid.

Statistical Analysis

All values were expressed as Mean ± S.E.M. statistical significant was determined using the student's t-test. Values with P<0.05 were considered significant.

Results**Anti-Inflammatory Activity**

The results obtained with 100mg/kg of AENS, MENS and CENS on carrageenan-induced rat hind-paw oedema are shown in Table 1. The extracts significantly (P<0.05) inhibited the inflammatory oedema; however the inhibition was highest in CENS.

Table 1. Effect of the various extract of *Nothospondias staudtii* leaves on Carrageenan - induced paw oedema in rats

Treatment	Dose	Paw size (mean± S.E.M)	Inhibition %
Control (saline)	-	3.14 ± 0.07	-
AENS	100	2.90 ± 0.03*	13.64
MENS	100	2.84 ± 0.05*	25.0
CENS	100	2.78 ± 0.07*	38.4
Aspirin	150	2.62 ± 0.04*	61.4

*p<0.05 (C.f; vehicle), n = 5, student's t-test

Analgesic Activity

Table 2 shows the responses of mice to tail immersion. The animals were significantly protected from the thermal stimuli by 100mg/kg of AENS, MENS and CENS. The latency and percentage protection were comparable to that of 150mg/kg of Aspirin. The responses of Mice are

shown in Table 3. Treatment with 100mg/kg of AENS., MENS and CENS significantly (P<0.05) inhibited the number of writhing furthermore at the dose of 100mg/kg MENS (87.46%) and CENS (85.37%) show greater percentage inhibition of writhing than 150mg/kg of Aspirin (79.4%).

Table 2: Effect of the various extracts of *Nothospondias staudtii* leaves on Tail immersion in $50 \pm 1^\circ\text{C}$ hot water (Mice)

Treatment	Tolerance ^a	Pre-treatment 0 min	Post-treatment	
			60 min	90 min
Control (Saline)	Latency (Sec) % Protection	8.25 \pm 1.05	11.25 \pm 1.05	11.88 \pm 0.44
AENS (100mg/kg)	Latency (Sec) % Protection	10.38 \pm 0.42	17.75 \pm 1.01*	18.5 \pm 0.65
MENS (100mg/kg)	Latency (Sec) % Protection	7.88 \pm 0.30	23.63 \pm 1.32*	23.38 \pm 0.71*
CENS (100mg/kg)	Latency (Sec) % Protection	7.38 \pm 0.53	20.37 \pm 0.46*	21.50 \pm 0.38*
Aspirin (150mg/kg)	Latency (Sec) % Protection	11.00 \pm 0.32	17.38 \pm 0.87*	20.75 \pm 1.36*

$$^a\text{Percentage protection} = \frac{\text{Latency (test)} - \text{Latency (control)}}{\text{Latency (control)}} \times 100$$

* $p < 0.05$ (C.f; vehicle), $n = 8$

Table 3: Effect of the various extracts of *Nothospondias staudtii* leaves on acetic acid - induced writhing test in Mice

Treatment	Dose	No of writhing (mean \pm S.E.M)	Inhibition (%)
Control (Saline)	-	35.88 \pm 1.86	-
AENS	100	12.88 \pm 0.64*	64.1
MENS	100	4.50 \pm 0.57*	87.5
CENS	100	5.25 \pm 0.62*	85.4
Aspirin	150	7.38 \pm 0.32*	79.4

* $p < 0.001$, $n = 8$

Discussion

In the present study we have demonstrated that the leaf of *Nothospondias staudtii* Engl. has analgesic and anti-inflammatory effect. The tail immersion and acetic acid tests reveal that this plant has high analgesic activity. However we take the acetic acid writhing as a more accurate means of quantifying the extracts effect on nociception. [This is because some form of error may be introduced with the animal handling while the test is being elicited. Both tests show highest degree of analgesia in MENS compared to other extracts and Aspirin.]

All the extracts significantly inhibited the rat paw oedema at 3hr. However anti-inflammatory activity was higher in MENS and lowest in AENS. The activities were not as high as the aspirin treated. It is not unusual to find certain extract of plants showing stronger anti-inflammatory activity than others.

Phytochemical screening of the methanolic extract of the plant under investigation shows that it contains flavones amongst other secondary metabolites. Flavonoids are a class of phenolic compounds widely distributed in plants. These compounds have medical function such as diuretic laxative, antispasmodic, anti-hypertensive and anti-inflammatory actions (Mellors and Tappel A.C. 1966). But flavonoids have also been reported to possess anti-oxidant and anti-radical properties (Poteract, 1997). Thus the bioactivity elicited by this extract may be due partly to its flavonoidal contents, since flavonoids have been shown to have anti-inflammatory activity.

However, the anti-oxidative activity of the plant under study is of great importance, hence further study on the anti-oxidative activity of the plant is still going on in our laboratories.

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