

# The Journal of Phytopharmacology

(Pharmacognosy and phytomedicine Research)

## Research Article

ISSN 2320-480X

JPHYTO 2020; 9(4): 242-245

July- August

Received: 28-05-2020

Accepted: 11-07-2020

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doi: 10.31254/phyto.2020.9405

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## Anti-nociceptive activity of ethanol leaf extract of *Smilax anceps* in swiss albino mice

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### ABSTRACT

*Smilax anceps* is traditionally used in the management of rheumatism. Anti-nociceptive activity of the ethanol extract of the leaf of this plant was investigated. Phytochemical screening and acute toxicity (LD<sub>50</sub>) of the extract were determined. The acetic acid induced writhing and hotplate test were used to evaluate the anti-nociceptive activity in Swiss mice. Fifty animals divided into twenty-five each were used for each assay. Acetylsalicylic acid and tramadol were used as reference drugs while extract was administered at 250, 500 and 1000mg/kg doses via the oral route in writhing and hotplate test respectively. Flavonoids, tannins, saponins and steroids/triterpenoids were found as phytoconstituents. In acetic acid induced writhing test, a statistically significant (P<0.05) difference in anti-nociceptive activity was observed between the test groups and the negative control. However, the extract at 250mg/kg showed a statistically significant (P<0.05) difference in anti-nociceptive activity when compared to the positive control. In the hotplate test, all extract treated groups exhibited a statistically significant (P<0.05) difference when compared to the negative control. Meanwhile, the 250mg/kg extract dose exhibited non-significant increase in latency at the 90,120 and 150minute when compared with the positive control. The findings of this study have shown that ethanol leaf extract of *Smilax anceps* might possess significant peripheral and central anti-nociceptive activities.

**Keywords:** Anti-nociceptive, *Smilax anceps*, Acetic acid, Hot plate, Tramadol, Acetylsalicylic acid.

### INTRODUCTION

Pain could be defined "as an unpleasant emotional experience usually initiated by noxious stimulus and transmitted over a specialized neural network to the central nervous system where it is interpreted as such"<sup>[1]</sup> and in many other medical conditions and can interfere with the general functioning as well as quality of the person's life<sup>[2]</sup>, because while the perception of pain is localized to the area stimulated, the individual's response to life will generally be altered due to the experience. And when there is removal of the noxious stimuli that resulted in the pain and healing of the affected part, most pain resolve immediately though there could be pain in the absence of any observable stimulus, damage or diseases<sup>[3]</sup>. Medicinal plants are believed to have constituents with therapeutic potential that could serve as templates for drug discovery and manufacture<sup>[4]</sup>. The currently available drugs for the management of pain such as non-steroidal anti-inflammatory and opiates are effective for chronic pain, but usually present with abdominal and neurological side-effects.

These would affect the patient's decision on use of such drugs negatively. Hence, there is the need for more research into medicinal plants so as to discover and develop efficacious drugs with lower side-effects<sup>[5]</sup>. *Smilax anceps* is native to South Africa, Nigeria, Cameroon, Zimbabwe, Madagascar. *Smilax anceps* is a shrub or distinctive woody climber with length up to 5m or more. The brown stems and lower leaf stalks are covered with sharp curved thorns about 3mm which is highly effective in scrambling over other plants<sup>[6]</sup>. The leaves of *Smilax anceps* leaves are used traditionally in the treatment of arthritis and rheumatism<sup>[7]</sup>. Osuagwu *et al.*,<sup>[8]</sup> reported that ethanolic leaf extracts of *Smilax anceps* have potential anti-inflammatory and diuretic properties and has been shown to possess antimicrobial activity against *N. gonorrhoea*, *S. flexineri*, *E. coli*, and *S. aureus*<sup>[7]</sup>. Therefore, this study investigated the potential anti-nociceptive activity of the leaves of this plant.

### MATERIALS AND METHODS

#### Plant material collection

The leaves of the plant *Smilax anceps Willd* were collected from Ibaa town in Emohua Local Government Area of Rivers State, Nigeria in their natural habitat. Plant identification was done by Dr. Oladele (Department of Forestry and Wildlife), while authentication was carried out by Dr. C. Ekeke of the

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### Plant Extraction

The leaves were shredded into smaller pieces and air-dried under room temperature for two weeks. The air-dried plant materials were pulverized by grinding with the aid of an electrical grinder and stored in an airtight container. A 2.475kg of the pulverized plant was macerated with 18.29 L of absolute ethanol for 72 hours in a macerating jar with intermittent shaking of the macerating jars. The extract was filtered using whatmann filter paper, and the extract filtrate concentrated using rotary evaporator. The concentrated extract filtrate was carefully dried. The dried extract was weighed, weight recorded and the extract stored properly in airtight container, appropriately labeled and kept in the refrigerator.

### Animals used

Sixty-eight healthy Swiss albino mice of weight ranging from (13-27g) were housed in cages of five animals each at normal room temperature in the animal house. They were allowed to acclimatize before the commencement of the experiment. They had unhindered access to water and feed.

### Drugs and chemicals used

Acetyl salicylic acid 300 mg tablet (Emprin®) Emzor. Nigeria.

Tramadol 100mg capsule (Trammed®) Zin Pharmaceuticals. India

Absolute ethanol (JHD Guangdong Guanghua Sci-Tech.Co. Ltd. Shantou, Guangdong, China)

### Determination of phytochemical constituents

Standard procedures were used to evaluate the phytoconstituents of *Smilax anceps* leaf extract [9].

### Experimental protocol

#### Acute toxicological evaluation

Eighteen albino mice (average weight: 22g) of both sexes were used in the study. The animals were deprived of food from the night prior to the study. The study was divided into two phases each of 9 animals. The 9 animals in the first phase were grouped into three, each of three animals. The route of administration used for the study was oral and the doses for groups 1, 2, and 3 were 10, 100 and 1000 mg/kg of the *Smilax anceps* leaf extract respectively. No sign of toxicity or death occurred within twenty-four hours in either of the groups. The 9 animals in the second phase were grouped as in phase one but differing doses used were 1600, 2900 and 5000 mg/kg of the extract [10].

#### Anti-nociceptive activity

##### Acetic acid induced writhing

Twenty-five animals were divided into five groups of five animals each. Oral route was used for the study. Group one was given normal saline, group two (100 mg/kg of acetyl salicylic acid), group three, four and five were administered 250, 500 and 1000 mg/kg of extract respectively. One hour after the administration of each of these agents; 10ml/kg of acetic acid in distilled water (0.6% v/v) was given

intraperitoneally to each of the animal and number of writhes in thirty minutes was counted with intermittent recording of the value obtained every five minutes. A writhe is said to have occurred if there is a twisting movement of the abdomen or stretching of the hind-limb [11].

#### Hot-plate analgesic activity test

Twenty-five albino mice of both sexes were allowed to adjust to the laboratory environment one hour prior to the study. The animals underwent pre-testing on hot-plate set at  $55 \pm 0.1^\circ\text{C}$  while those with latency time above 15seconds on hot-plate during the pre-testing were removed. Hot-plate analgesic test was performed as reported by Brochet *et al.*, [12] with little alterations. Mice were arranged into 5units each consisting of 5. Group one was administered with normal saline (10ml/kg), group two (tramadol 30mg/kg i.p), groups III, IV, and V were jointly exposed to 250, 500 and 1000mg/kg i.p. Thirty minutes afterward, each mouse was positioned on hot plate and the time during which the mouse remains quiet on the hot plate without flicking or licking the hind limb and not trying to escape was measured in seconds and recorded as the latency period. The mice were subjected to a let-off or resting time of 30 seconds so as to protect the tissue from injury. Readings were determined at 30 minutes' interval for 3hours (180minutes). The number of constrictions was compared with the normal saline group and the percent decline of writhes count was computed as follows:

$$\% = (N_{\text{control}} - N_{\text{test}}) / N_{\text{control}} \times 100$$

$N$  = the mean number of writhes for each group.

#### Statistical analysis

The data were expressed as Mean  $\pm$  SEM. Analysis was carried out using one-way analysis of variance (ANOVA) followed by Turkey's t-test using GraphPad Prism, version 7.03 to determine differences between treatments. P-values lower than 0.05 were taken as significant.

## RESULTS

### Qualitative phytochemical screening

Preliminary phytochemical qualitative evaluation of *S. anceps* leaves the indicated steroidal/triterpenoid, tannins, saponins, flavonoids and carbohydrate as it's phytoconstituents while alkaloids and anthraquinone were absent.

### Acute toxicity result

The result obtained from the acute toxicity test of the ethanol extract of *Smilax anceps* leaf showed that the median lethal dose (LD<sub>50</sub>) exceeds 5000 mg/kg since no death was recorded.

### Anti-nociceptive activity

#### Acetic acid induced writhing test

The result showed that on comparison of the treatment groups with the normal saline, a statistically significant ( $P < 0.05$ ) anti-nociceptive activity was observed after 30 minutes at the three extract doses. However, comparing the acetylsalicylic acid and the treated groups, it was observed that the plant extract at 250mg/kg dose exhibited a statistically significant ( $P < 0.05$ ) anti-nociceptive activity while the 500mg/kg and 1000mg/kg doses had no statistically significant ( $P > 0.05$ ) difference anti-nociceptive activity (Table 1).

**Table 1:** Evaluation of ethanol leaf extract of *Smilax anceps* on acetic acid induced writhing test in mice (Mean ± S.E.M)

Groups	Dose (mg/kg)	Number of writhes in 30 minutes (Mean±SEM)	Reduction in writhes count (%)
Normal saline	10ml	239.00±4.53	0
Acetylsalicylic acid	100	143±16.9	40.17
Extract	250	81.80±11.80*	65.77
Extract	500	99.80±15.10*	58
Extract	1000	123.00±8.82*	48.53

n=5, \* P<0.05 in comparison to the negative control

**Hot plate test**

The findings of the hot plate test revealed a statistically significant (P<0.05) anti-nociceptive activity in all the extract treated groups with an increase in latency time at the various 30 minutes' interval within

the 3 hours of observation when compared with normal saline. However, only the 250mg/kg dose of extract showed a non-statistically significant (P<0.05) difference in anti-nociceptive activity at the 90,120 and 150minute in comparison with the tramadol group (Table 2).

**Table 2:** Evaluation of anti-nociceptive activity of ethanol leaf extract of *Smilax anceps* in hot plate test (Mean ± S.E.M)

Treatment	Dose (mg/kg)	Latency time (seconds)±S.E.M						
		0mins	30mins	60mins	90mins	120mins	150mins	180mins
Normal saline	10ml	4.19±0.46	4.34±0.52	4.50±0.64	4.15±0.34	4.34±0.55	4.68±0.15	4.25±0.12
Tramadol	100	7.50±0.31	7.92±0.35	9.49±0.65	8.92±0.27	9.31±0.51	8.09±0.12	7.77±0.13
Extract	250	6.67±0.12*	7.40±0.23*	7.70±0.23*	10.12±0.80*	10.46±0.34*	9.04±0.33*	7.62±0.29*
Extract	500	7.30±0.05*	8.00±0.2*	8.59±0.18*	9.92±0.47*	8.54±0.46*	7.84±0.32*	7.41±0.21*
Extract	1000	8.14±0.19*	9.02±0.65*	9.80±0.53*	8.62±0.57*	8.26±0.59*	7.59±0.19*	7.34±0.05*

n=5.\* P<0.05 in comparison with normal saline and tramadol

**DISCUSSION**

Medicinal plants have been known for a long time as templates for drug discovery with many pharmaceuticals derived from natural products.

Toxicity which is the inherent property of a substance to cause an adverse biological effect can be determined through acute toxicity evaluation. Acute toxicity is the biological effect or effects which occur within a short period of time after a short-term exposure of a single oral administration or a dermal or inhalational exposure not exceeding 24 hours or multiple exposures in a short period of time. *Smilax anceps* ethanol leaves extract LD<sub>50</sub> was found to be greater than 5000mg/kg and can be said to be relatively safe [10]. Steroid/triterpenoid carbohydrate, saponins, tannins and flavonoids were observed as the phytoconstituents of this plant.

Substances with analgesic properties modulates the nervous system either peripherally or centrally to ease pain without altering alertness and cognition [13]. Acetic acid induced writhing assay is used to evaluate analgesics for peripheral inhibition of pain mechanism [14]. It is characterized by a reduction in the number of writhes produced when an effective analgesic drug is given to an animal [11]. Acetic acid produces pain through the enhancement of pain mediators release arachidonic acid metabolism pathway by virtue of prostaglandin biosynthesis (PGE2 and PGF2α) from tissue phospholipids, which may be accompanied by a corresponding increase in lipoxigenase end products in peritoneal fluids [15, 16]. Prostaglandin and lipoxigenase end products are responsible for inflammation and pain by promoting increase in capillary membrane permeability. Any substance that is able to inhibit the writhing achieves this through the inhibition of peripheral mechanism of pain induction Gupta and [17, 16].

Acetylsalicylic acid is a non-steroidal anti-inflammatory drugs (NSAIDs) which exerts its analgesic, antipyretic and anti-inflammatory properties through its non- selective inhibition of cyclooxygenase enzyme, thus preventing the formation and release of prostaglandins that cause inflammation, pain and fever [18].

There was a statistically significant (P<0.05) decline in abdominal contractions brought about by acetic acid in all extract treated groups when compared with the negative control. The extract at 250mg/kg also showed a statistically significant (P<0.05) difference in anti-nociceptive activity when compared to acetylsalicylic acid (100mg/kg) group while the 500 and 1000mg/kg did not exhibit any statistically significant (P>0.05) difference. The reports of this study corresponds to that of Khan *et al*, [15] that the leaf extract of *Terminalia coriacea* possesses anti-nociceptive activity.

Hot-plate test is one of the thermal induced model assays that is used to evaluate central anti-nociceptive activity. The substance will be considered to possess central anti-nociceptive activity if it is able to increase the pain threshold which is measured as an increase in the latency time [19].

Tramadol (4-phenyl-piperidine, synthetic derivative of codeine) is an analgesic with central effects but with minimal affinity for opioid receptors but shows selectivity for μ receptors. Naloxone is the only opioid antagonist with partial inhibitory effects on tramadol. Tramadol analgesic effect might be produced through stimulation of the monoamine neurotransmitters and also likely through ancillary stimulation of post-synaptic alpha adrenergic receptors jointly in the spinal cord and brain [20]. This study revealed that, there was a statistically significant (P<0.05) anti-nociceptive activity of the *Smilax anceps* leaf extract treated groups when compared with the negative control with an increase in latency time at 0 minute to 180-minute interval after intra-peritoneal administration. However, on comparison with the positive control, only the extract dose of 250mg/kg at the 90,120 and150 minute interval produced a non- statistically significant (P<0.05) anti-nociceptive activity, while the rest showed no statistically significant (P>0.05) anti-nociceptive activity. The results obtained corroborates that of Khan *et al*, [15] which stated that the methanolic leaf extract of *Terminalia coriacea* possesses anti-nociceptive activity and also the report of Nwafor *et al*, [19] that the ethanolic extract of *Smilax kraussiana* leaf possesses anti-nociceptive activities through acetic acid induced and hot plate assay methods. The outcome of this experiment has revealed that the ethanol leaf extract of *Smilax anceps*

possesses both peripheral which may be through the inhibition of prostaglandins synthesis and central anti-nociceptive activities which may likely be attributed to the steroids, tannins, saponins and flavonoids contained in the crude extract.

Saponins, tannins including flavonoids have been reported to possess the ability to reduce sensitivity to pain by preventing the synthesis of prostaglandins through the inhibition of enzymes involved in arachidonic acid metabolic pathway<sup>[21, 22]</sup>.

## CONCLUSION

The findings of this investigation has revealed that the ethanol leaf extract of *Smilax anceps* have remarkable anti-nociceptive activity on both the peripheral and central nervous system. The presence of saponins, triterpenoids/steroids, flavonoids as well as tannins as phytochemical constituents in this plant may be responsible for this observed activity.

## Ethical Compliance

The University of Port Harcourt research ethics committee gave the consent for the protocol adopted in this experiment in line with International guidelines with number UPH/CEREMAD/REC/MM/59/035.

## Conflict of Interest Statement

There is none.

## Statement on Authors Participation

Shorinwa Olusayo Aderonke planned, superintended the experimental study and drafted the article. Uchendu Precious Nwabueze executed the laboratory work.

## Source of Funding

The study was financed by the authors.

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## HOW TO CITE THIS ARTICLE

Shorinwa OA, Uchendu PN. Anti-nociceptive activity of ethanol leaf extract of *Smilax anceps* in swiss albino mice. *J Phytopharmacol* 2020; 9(4):242-245.