Investigational
Medicinal Chemistry & Pharmacology

Research Article Open Access

# Translational preclinical evaluation of *Strychnos innocua* leaf extract: Investigating anti-nociceptive and antidepressant activities using in vivo and in silico models

Chinedu Enegide[1]*, Eugene Ehimare[1], Divine Odeworitse[1], Ujunwa Emelda Ezeanochie[1], Israel Ofejiro Efejene[1,2], Michael Eromosele Aisuodionoe[3], Osasengo Precious Osifo[1], Esther Adaeze Ossai[1], Emmanuella Chukwufumnanya Nwegbu[1], Sophia Onyinyechi Agundu[1], Jennifer Orevaoghene Etiku[1]

## Abstract

**Background:** Pain represents a complex physiological response of tissues to injury and often adversely impacts both health and quality of life. Epidemiological data indicate that virtually all individuals will experience some form of pain during their lifetime. *Strychnos innocua* (family Loganiaceae) is employed in ethnomedicine for the treatment of inflammation, pain, central nervous system disorders, and various other ailments. This study investigated the antinociceptive and antidepressant effects of *S. innocua* leaf ethanol extract using established *in vivo* and *in silico* models.

**Methods:** The antinociceptive activity of the extract was evaluated in mice using the acetic acid-induced writhing test, formalin-induced pain, hyperalgesia, and allodynia in carrageenan-induced inflammation. Graded doses of the extract (250 – 1000 mg/kg) were used for these studies. The possible mechanism of action was evaluated by administering the extract in the presence of different antagonists that block antinociception pathways, using the acetic acid-induced writhing model. The tail suspension test was used to evaluate the antidepressant effect of the extract in mice at doses of 250 – 1000 mg/kg in mice. Molecular docking was done using iGemdock.

**Results:** The extract elicited significant ($p < 0.01 – 0.001$) reduction in pain behaviors in acetic acid and formalin-induced pain tests. It also showed potent antihyperalgesic and antiallodynic activities in carrageenan-induced inflammation models. Mechanistic studies indicated involvement of ATP-sensitive potassium channels, alpha-2 adrenergic receptors, and adenosine receptors in the extract's antinociceptive effects. Molecular docking supported these findings, identifying bioactive compounds such as 9,12,15-octadecatrienoic acid and n-hexadecanoic acid as the major culprits. The extract also demonstrated significant antidepressant effects, which were further supported by docking interactions with serotonin and dopamine transporters.

**Conclusion:** The study outcome shows that the extract has potent antinociceptive and antidepressant properties that may be mediated through multiple pathways.

**Keywords:** Antinociceptive; hyperalgesia; allodynia; antidepressant; *in silico*; *in vivo.*

*Correspondence: Tel.: 2347038912400; E-mail address: chinex.snow@gmail.com (Chinedu Enegide)
[1]Department of Pharmacology, College of Medical & Health Sciences, Novena University, Ogume, Delta State, Nigeria; [2]Department of Pharmacology, Faculty of Basic Medical Sciences, Southern Delta University, Ozoro, Delta State, Nigeria; [3]Department of Physiology, Faculty of Basic Medical Sciences, Southern Delta University, Ozoro, Delta State, Nigeria.

*Other authors*
*Email: Ehimareeugene90@gmail.com (Eugene Ehimare), Email: divineodeworitse@gmail.com (Divine Odeworitse), Email: emeldaujunwa706@gmail.com (Ujunwa Emelda Ezeanochie), Email: iefejene@gmail.com (Israel Ofejiro Efejene), Email: eroms2001@yahoo.com (Aisuodionoe Eromosele Michael), Email: osifo2019@gmail.com (Osasengo Precious Osifo), Email: estherossai96@gmail.com (Esther Adaeze Ossai), Email: emmanuellanwegbu@gmail.com (Emmanuella Chukwufumnanya Nwegbu), Email: Sophia.agundu2001@gmail.com (Sophia Onyinyechi Agundu), Email: jenniferetiku@gmail.com (Jennifer Orevaoghene Etiku)*

*Citation on this article: Enegide C, Ehimare E, Odeworitse D, Ezeanochie UE, Efejene IO, Aisuodionoe ME, Osifo OP, Ossai EA, Nwegbu EC, Agundu SO, Etiku JO. Translational preclinical evaluation of Strychnos innocua leaf extract: Investigating anti-nociceptive and antidepressant activities using in vivo and in silico models. Investigational Medicinal Chemistry and Pharmacology (2025) 8(2):118; Doi: https://dx.doi.org/10.31183/imcp.2025.00118*

## Background

Pain represents a complex physiological response of tissues to injury; it often adversely impacts both health and quality of life [1]. Epidemiological data indicate that virtually all individuals will experience some form of pain during their lifetime [2]. Pain serves a crucial protective function by signaling tissue damage and initiating behaviors aimed at minimizing further injury. It is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or resembling such experiences. Beyond its sensory dimensions, pain profoundly influences cognitive, emotional and behavioral responses, reflecting its multifaceted nature. Chronic pain persists as a widespread clinical concern and often contributes to secondary complications such as depression [3]. Pharmacological management of pain commonly relies on non-steroidal anti-inflammatory drugs (NSAIDs). However, the therapeutic use of NSAIDs is frequently limited by adverse effects, notably gastrointestinal ulceration. Similarly, alternative analgesic classes possess significant side effect profiles, underscoring the urgent need for novel therapeutic agents with superior efficacy and safety [4]. In this context, traditional medicine continues to serve as a valuable reservoir for the discovery and development of new pharmacological agents targeting pain and inflammation [5]. Among natural sources, medicinal plants have yielded a wealth of bioactive compounds with promising therapeutic potential [6]. One such plant, *Strychnos innocua* (family Loganiaceae), is employed in ethnomedicine for the treatment of inflammation, pain, central nervous system disorders and various other ailments. Previous phytochemical studies and GC-MS analysis establish that *S. innocua* contains bioactive compounds capable of eliciting analgesic and antidepressant effects [7]. Furthermore, an *in vitro* antioxidant study showed that the leaf ethanol extract exhibits notable antioxidant activity across multiple models, indicative of its ability to elicit anti-inflammatory and antinociceptive effects [8]. Accordingly, the present study investigated the antinociceptive and antidepressant effects of *S. innocua* leaf ethanol extract using established *in vivo* and *in silico* models.

## Methods

*Plant materials*

Fresh leaves of *Strychnos innocua* were harvested from a botanical garden in Plateau state, Nigeria by Mr. Jeffery Azila, a taxonomist at the Federal College of Forestry, Jos. Authentication of the plant was done at the Herbarium Unit, Federal College of Forestry (Voucher number: FHJ875). The harvested plant materials were properly rinsed using clean running water before processing for use in the study.

*Extraction*

The leaves were air-dried openly under shade in the laboratory at room temperature (28-33ºc) until even weight of the dried leaves was gotten. The dried leaves were then pulverized using a motorized milling machine. Following standard procedures outlined by Handa *et al* [9], 2 kg of the pulverized leaves were macerated in 6 L of 70% ethanol at room temperature for 72 h with periodical agitation. The resulting solution was filtered sequentially through a mesh sieve, a cotton wool plug and Whatman filter paper (110 mm). The collected filtrate was concentrated using water bath set at 60ºC.

*Drugs and chemicals*

The following drugs and chemicals were used: acetic acid (BDH, poole, UK), aspirin (Prestige brands, United States); formalin (Balaji formalin Pvt. Ltd, India); dihydrocodeine (Accord pharmaceutical, Nepals); diclofenac (Embassy pharmaceutical and chemicals limited, Nigeria); glibenclamide (Sygen Pharmaceuticals Ltd, Nigeria); nifedipine (Unicure Pharmaceutical Co. Ltd, Nigeria); atropine (Ancalima Life Sciences Ltd, India); naloxone (Jackson laboratories Pvt Ltd, India); theophylline (Eminent drugs and pharmaceutical Ltd, Nigeria). All drugs used were of high standard grade and solutions from these were freshly prepared daily before use.

*In vivo studies*

*Animals*

Both male and female Swiss albino mice (weighing between 20 – 28 g) were utilized in this study. The animals were maintained at the animal facility of the Department of Pharmacology, Novena University, housed in standard cages under controlled laboratory conditions, including regulated room temperature and humidity. They had unrestricted access to water and standard rodent feed. All animal experiments were conducted in strict compliance with the NIH revised guidelines for the care and use of laboratory animals [10], as well as the ethical codes and regulations established by Novena University for laboratory animal research. Ethical clearance was obtained from Novena University's Department of Pharmacology ethical committee on the use of animals (NUO/ECUA/0122).

*Acetic acid-induced writhing test*

Acetic acid-induced writhing test was carried out using the method described previously by Woode *et al* [11]. Twenty-five Swiss albino mice of either sex were used for the study. They were randomly assigned to five groups of five animals each. The animals were pre-treated orally with the vehicle, extract or standard drug. Group 1 (control) received normal saline (vehicle) 10 mL/kg; groups 2 – 4 received kg extract doses of 250, 500 and 1000 mg/ respectively, while group 5 received aspirin 150 mg/kg. One hour after administration, the animals were administered acetic acid intraperitoneally (0.6%, 0.1 ml/10 g body weight). The mice were placed in individual cages, and the number of writhes was counted for each mouse for 15 minutes following a 5-minute latency period. The percentage inhibition of writhing was calculated using the following formula:

% inhibition = [(Mean number of writhes control – Mean number of writhes treated)/Mean number of writhes control] × 100

*Formalin-induced pain*

Formalin test was carried out according to the method of Hunskaar and Hole [12]. Twenty-five Swiss albino mice of both sexes randomly assigned to six groups of five animals each. The animals were pre-treated orally with the vehicle (normal saline), extract or standard drugs. Group 1 (control) received normal saline 10 mL/kg; groups 2 – 4 received extract doses 250 – 1000 mg/kg; while group 5 received dihydrocodiene 30 mg/kg. One hour after administration, each mouse was injected with 0.02 ml of 2.7 % formalin into the right hind paw. The animals were placed

individually in transparent observation chambers and observed for 30 min post-injection. The duration (in seconds) spent licking and biting the injected hind paw was recorded as an indicator of nociceptive behavior. Nociceptive scores were taken at 0 – 5 min (early phase/ neurogenic pain) and 15 – 30 min (late phase/ peripheral pain) following formalin injection.

*Hyperalgesia*

Thermal hyperalgesia test was conducted following the method described by Amoateng *et al*. [13] with modifications. Twenty-five Swiss albino mice of both sexes were used. Baseline reaction time (latency to withdraw paw) for each animal was recorded using a digital hotplate set at 50 ± 1 $^0$C. Animals were randomly assigned to five groups of five animals each. Animals in the respective groups were pre-treated as follows: group 1 (negative control) received normal saline 10 mL/kg, groups 2 – 4 received graded extract doses (250 - 1000 mg/kg), and group 5 received diclofenac 10 mg/kg. One hour after treatment, inflammation was induced by injecting 20 µL of 2% w/v carrageenan into the dorsal surface of the right hind paw. Reaction times were recorded hourly for three hours starting at 1 hour post-carrageenan administration. Latency to paw withdrawal (in seconds) was recorded with a stopwatch from the moment the animal was placed on the hot plate to the first sign of discomfort, such as licking, shaking of the ipsilateral hind paw or jumping off from the surface of the hot plate apparatus. A significant increase in latency was considered indicative of antihyperalgesic activity. A cut-off time of 20 seconds was applied to prevent tissue damage.

*Cold allodynia (acetone test)*

The test was conducted following the method described by Brener *et al*. [14] with minor modifications. Twenty-five Swiss albino mice of both sexes were used. Each animal was placed in the test chamber with a mesh floor, and the baseline reaction time (withdrawal) of the right hind paw was recorded following irrigation with 10 µL of acetone. The animals were randomly assigned to five groups of five animals each. The groups were pre-treated orally with vehicle, extract or standard drug as follows: group 1 (negative control) received normal saline 10 mL/kg; groups 2 – 4 received graded extract doses (250 - 1000 mg/kg); and group 5 received diclofenac 10 mg/kg. One hour after administration, the right hind paw of each mouse was inflamed by injecting 20 µL of 2 % w/v carrageenan into the dorsal surface. The reaction time to acetone irrigation of the right hind paw was recorded hourly for three consecutive hours, starting one hour post-carrageenan administration.

*Mechanism of antinociception*

Acetic acid-induced writhing test was employed for determining the possible involvement of specific pathways through which the extract elicits its antinociceptive effect, as described by Enegide *et al*. [15]. Swiss albino mice of either sex were randomly assigned to nine groups of five mice each. The animals in groups 3 – 8 were pre-treated with six different inhibitors/antagonists: atropine (a nonselective muscarinic receptor antagonist), naloxone (a nonselective opioid receptor antagonist), ondasetron (serotonin receptor antagonist), glibenclamide (an ATP sensitive potassium channel inhibitor), yohimbine (alpha-2 receptor antagonist) and theophylline (a non-selective adenosine receptor antagonist). These anatgonists were administered 15 minutes intraperitoneally and 30 minutes orally before administration of the extract (1000 mg/kg *p.o*). Group 1 served as negative control and received normal saline (10 mL/kg *p.o*), group 2 received the extract alone (1000 mg/kg *p.o*), and group nine served as the positive control and received aspirin (150 mg/kg *p.o*). one hour after administration of normal saline, extract or aspirin, the animals were injected intraperitoneally with acetic acid (0.6 % 0.1 ml/10 g body weight *i.p*). Five minutes after acetic acid administration, the mice were placed individually in cages, and the number of writhes was counted for 15 minutes.

*Involvement of the muscarinic cholinergic system*

Animals in group 3 were pre-treated with atropine (5 mg/kg, *i.p.*) 15 minutes before the administration of the extract (1000 mg/kg, *p.o*.) and their nociceptive responses were observed.

*Involvement of the opioid System*

Animals in group 4 were pre-treated with naloxone (2 mg/kg, *i.p.*) 15 minutes before the administration of the extract (1000 mg/kg, *p.o*.) and their nociceptive responses were observed.

*Involvement of serotonergic system*

Animals in group 5 were pre-treated with ondasetron (0.5 mg/kg, *i.p*) 15 minutes before the administration of the extract (1000 mg/kg, *p.o*.) and their nociceptive responses were observed.

*Involvement of ATP-sensitive $K^+$ channels*

Animals in group 6 were pre-treated with glibenclamide (8 mg/kg, *p.o.*) 30 minutes before the administration of the extract (1000 mg/kg, *p.o*.) and their nociceptive responses were observed.

*Involvement of adrenergic system*

Animals in group 7 were pre-treated with yohimbine (3 mg/kg, *p.o.*) 30 minutes before the administration of the extract (1000 mg/kg, *p.o*.) and their nociceptive responses were observed.

*Involvement of the adenosinergic system*

Group 8 animals were pre-treated with theophylline (10 mg/kg, *p.o.*) 30 minutes before the administration of the extract (1000 mg/kg, *p.o*.) and their nociceptive responses were recorded.

*Tail suspension test*

The tail suspension test was conducted according to the method described by Can *et al*. [16]. Twenty-five Swiss albino mice of both sexes were randomly divided into five groups of five animals each. They were treated with vehicle, extract or standard drug. Group 1 (negative control) received normal saline at 10 mL/kg, groups 2–4 received graded doses of the extract (250–1000 mg/kg, p.o.), and group 5 was administered fluoxetine at 10 mg/kg p.o. One hour post-treatment, each mouse was suspended by the tail, 70 cm above the floor, on the edge of a wooden platform using adhesive tape placed approximately 1 cm from the tip of the tail. The suspension lasted for 360 seconds, during which immobility time was recorded using a stopwatch. Immobility was defined as the absence of any limb or body movement except for movements caused by respiration, with the mouse hanging passively and completely motionless.

*In silico studies*
*PASS prediction*

The potential biological activities of the compounds identified in the extract via GC-MS analysis in our prior study [7] were predicted using the Prediction of Activity Spectra for Substances (PASS) software, an online computational tool. PASS facilitates the estimation of diverse biological activities for a broad range of chemical entities, including organic compounds with molecular weights between 50 and 1250 Da, as well as phytochemicals. This software evaluates candidate molecules by analyzing their structure-activity relationships (SAR) through comparison with a comprehensive training dataset comprising approximately 205,000 chemical structures, which collectively exhibit nearly 3,750 distinct biological activities. Compounds selected for subsequent molecular docking studies were required to demonstrate a PASS-predicted activity (Pa) score greater than 0.7, ensuring a high probability of relevant bioactivity.

*Ligand retrieval and preparation*

Ligands employed in this study were sourced from the PubChem database, a comprehensive repository of chemical information. The downloaded compounds, initially obtained in SD file format, were subsequently imported into Biovia Discovery Studio 2021 Client (version 21.1) for structural conversion to PDB format. This conversion was necessary because the docking software, iGemdock, exclusively accepts ligand input in the PDB file format, ensuring compatibility and accurate processing.

*Molecular docking*

Molecular docking simulations were performed using iGemdock (version 2.1) based on the outcome of the *in vivo* studies. Prepared ligands were docked into the respective active sites of their target proteins employing the standard precision (SP) protocol. Post-docking, the resultant ligand–protein complexes were analyzed and visualized *via* Biovia Discovery Studio 2021 Client to elucidate binding interactions and assess docking conformations.

*Statistical analysis*

The data obtained from the *in vivo* studies was expressed as mean standard error of mean (Mean ± SEM). One way analysis of variance (ANOVA) followed by Dunnet's post hoc test was used to test for significance $p < 0.05$ was considered significant. Graph pad prism (version 8.0) was used for the analysis.

## Results

*Acetic acid-induced writhing test*

Results from the study showed that pre-treatment of the experimental animals with graded doses of the extract (250 – 1000 mg/kg) one hour before the administration of acetic acid (0.6 %) significantly ($p < 0.001$) reduced abdominal writhing compared to the control (untreated) animals (Figure 1). The effect was dose-dependent, with the highest dose producing the highest activity (78.8%) during the study. Aspirin also demonstrated significant activity ($p < 0.001$), reducing writhing by 75.8% (Table 1).

*Formalin-induced pain*

In the formalin-induced pain test, all doses of the extract (250 – 1000 mg/kg) significantly ($p < 0.01 – 0.001$) reduced nociceptive behaviors (i.e biting and licking) that characterize administration of formalin into mice hind paw (Figure 2). The effect of the extract was significant against both the early phase (neurogenic) and late phase (inflammatory) pain. Dihydrocodeine also demonstrated significant ($p < 0.001$) activity against both pain forms during the study.

*Hyperalgesia*

In the result for hyperalgesia due to carrageenan-induced inflammation, hyperalgesia was observed in the control animals after 1 hour of carrageenan administration, shown by significant decrease ($p < 0.01 – 0.001$) in reaction time compared to baseline which progressed throughout the study (Figure 3). All doses of the extract (250 – 1000 mg/kg) prevented this trend, higher doses (500 and 1000 mg/kg) even elicited significant ($p < 0.05$) increase in reaction time at 2 and 3 hours after carrageenan administration when compared with baseline (Figure 3). Diclofenac also prevented the trend observed in the control group, as there was no significant ($p > 0.05$) change in reaction time throughout the study (Figure 3).

*Allodynia*

In the study for allodynia due to carrageenan-induced inflammation, a significant decrease ($p < 0.01 – 0.001$) in reaction (withdrawal) time was observed in the control animals compared to baseline after 1 hour of carrageenan administration, persisting throughout the study which was indicative of allodynia. However, extract doses of 500 and 1000 mg/kg completely prevented this trend, significantly ($p < 0.05$) increasing the reaction time at 2 and 3 hours after carrageenan administration when compared to baseline (Figure 4). Diclofenac also prevented the significant decrease in reaction time observed in the control animals (Figure 4).

*Mechanism of antinociception*

Results from the study revealed that pre-treatment of the experimental animals with atropine, naloxone and ondasetrone did not prevent the antinociceptive effect of the extract. But glibenclamide, yohimbine and theophylline blocked the antinociceptive activity of the extract in acetic acid-induced writhing test (Figure 5).

*Tail suspension test*

Results from the study showed that all doses of the extract (250 – 1000 mg/kg) significantly ($p < 0.05$) decreased immobility time in the experimental animals compared to control group. Fluoxetine also demonstrated significant ($p < 0.001$) activity during the study (Figure 6).

*Molecular docking*

Result from the Prediction of Activity Spectra for Substances (PASS) virtual screening showed that among the 40 compounds identified in the extract by GC-MS analysis reported in an earlier

publication by the authors [7], three compounds may be among the major constituents responsible for the effects observed during the study. These are 9,12,15-octadecatrienoic acid (Z,Z,Z), n-hexadecanoic acid and dl-threitol. Further molecular docking studies result showed that 9,12,15-octadecatrienoic acid (Z,Z,Z) had good binding affinity (-6.13 kcal/mol) with ATP-sensitive potassium channel forming akyl bonds at ILE182, ARG201 and LEU205 (Figure 7, Table 2). It also had good binding affinity with alpha2 adrenergic receptor, adenosine receptor $A_1$ and serotonin transporter (Figure 8 – 10, Table 2). Also, n-hexadecanoic acid had good binding interaction with adenosine receptor $A_1$, serotonin transporter and dopamine transporter having binding affinity of -6.27, -5.00 and -6.75 kcal/mol, respectively, while dl-threitol had modest binding affinity (-4.20 kcal/mol) with adenosine receptor $A_1$ (Figure 8 – 11, Table 2).

## Discussion

*Strychnos innocua* is an herbal plant that has been utilized in ethnomedicine for different therapeutic purposes. Reports from earlier studies on the leaf ethanol extract by the authors revealed the presence of several phytochemicals including alkaloids, flavonoids, saponins, tannins and phenols [8]. The authors also reported presence of 40 volatile compounds from GC-MS analysis of the extract, including 9,12,15-octadecatrienoic acid (Z,Z,Z), n-hexadecanoic acid and dl-threitol [7]. Furthermore, the authors chronicled that the acute oral toxicity dose of the extract was greater than 5000 mg/kg in mice [8]. This study evaluated the antinociceptive and antidepressant activities of the extract utilizing *in vivo* and *in vitro* models.

Among the *in vivo* models used to evaluate the antinociceptive activity of the extract is the acetic acid-induced writhing test, which is known to be very sensitive in screening candidate analgesic agents. The test is characterized by nociceptive behaviors such as pelvic rotation, abdominal constrictions and hind limb stretching which result from sensitization of C fibres by pro-inflammatory mediators such as serotonin, histamine, bradykinin and prostaglandins [17]. Analgesics may act via different pathways influencing these mediators. During the study, the extract demonstrated significant antinociceptive activity in a dose-dependent manner. Furthermore, the formalin-induced pain model was used to evaluate its anti-nociceptive effect. It is a highly predictive biphasic pain model known to mimic acute clinical pain resulting from tissue injury [18]. During the study, the extract demonstrated effective antinociceptive effects against both neurogenic and inflammatory pain at all test doses. The early phase, neurogenic pain, occurs almost immediately after formalin injection and is elicited by the direct chemical activation of central nociceptive afferent terminals of the Aδ nerve fibres. The late phase, inflammatory pain, is due to central sensitization in the dorsal horn and the direct stimulation of chemical nociceptors, resulting in increased input from C fibres [18, 19]. This finding corroborates the outcomes from previous studies reporting the extract's potent antioxidant activity and presence of phytochemicals known to elicit potent antinociceptive effects including alkaloids, phenols and flavonoids [8]. However, the extract may exert its antinociceptive effect via multiple pathways.

The effect of the extract on special pain manifestations (i.e hyperalgesia and allodynia) was evaluated using heat hyperalgesia and cold allodynia (acetone test) in carrageenan-induced inflamed mice. Carrageenan-induced inflammation in mice triggers hyperalgesia and allodynia following complex inflammatory cascade involving the release of pro-inflammatory mediators including bradykinin, histamine and cytokines, leading to sensitization of both peripheral and central nociceptive pathways. This culminates in decreased pain thresholds (hyperalgesia) and pain perception from naturally non-painful stimuli (allodynia) [20 – 22]. Most commonly used analgesic agents are usually not effective against these special pain manifestations. The study outcome showed that the extract demonstrated potent antihyperalgesic and antiallodynic effects. Since these manifestations are common features with neuropathic pain, the extract may therefore be useful in its management.

The *in vivo* study to unravel the mechanism of action was carried out by administering the extract in the presence of different antagonists that block different analgesic pathways. It revealed that glibenclamide, yohimbine and theophylline blocked the antinociceptive activity of the extract in acetic acid-induced writhing test, suggesting that the mechanism of antinociception may involve interaction with the ATP-sensitive potassium channel, alpha-2 adrenergic receptor and adenosine receptor. ATP-sensitive potassium channels play a role in pain modulation by regulating neuronal excitability. Activation of these channels at local, systemic and supraspinal levels reduces pain, making them important targets for pain management. Notably, ATP-sensitive potassium channel activators such as diazoxide are known for their strong analgesic effects [23]. Alpha-2 adrenergic receptor agonists, such as dexmedetomidine, clonidine and tizanidine, elicit potent analgesic effect by stimulating alpha-2 adrenergic receptors in the central nervous system, particularly in the dorsal horn of the spinal cord and brainstem nuclei like the locus ceruleus, leading to blockade of potassium and calcium channels [24]. Presynaptic alpha-2 adrenergic receptors inhibit the release of substance P and other neuropeptides that stimulate nociceptive transmission, while post-synaptic alpha-2 adrenergic receptors hyperpolarize neurons membranes and decrease the activity of nociceptive neurons [25]. At peripheral nerve terminals in rodents, adenosine $A_1$ receptor activation produces antinociception by decreasing cyclic AMP levels in the sensory nerve terminal [26]. Horiuchi *et al*., reported that adenosine inhibits hyperalgesia via stimulation of A1 receptors, whereby agonist acting at this receptor elicit analgesic effects [27]. Hence, the extract may have exerted its antinociceptive activity through these pathways.

Molecular docking studies revealed that 9,12,15-octadecatrienoic acid (Z,Z,Z) identified in the extract demonstrated good binding interactions (-6.13 and -6.82 kcal/mol) with ATP-sensitive potassium channel and α-2 adrenergic receptor, respectively. These interactions may have contributed to the antinociceptive activity observed, aligning with *in vivo* findings. Additionally, 9,12,15-octadecatrienoic acid (Z,Z,Z) and n-hexadecanoic acid showed good binding interaction with the $A_1$ adenosine receptor, with binding affinities of -6.27 and -6.23 kcal/mol, respectively, supporting the involvement of the adenosinergic pathway in the extracts antinociception mechanism observed *in vivo*.

The antidepressant potential of the extract was assessed using the tail suspension model in mice. This test relies on immobility induced by an inescapable stressful situation as a measure of despair-like behavior. Immobility time is used to assess depression in this model [28]. The extract demonstrated potent antidepressant activity, significantly decreasing immobility time in treated animals compared to control. Inhibition of neurotransmitter transporters including serotonin and dopamine transporters is a known antidepressant mechanism [29]. *In silico* studies showed that compounds such as 9,12,15-octadecatrienoic acid (Z,Z,Z) and n-hexadecanoic acid exhibited good binding affinities with

serotonin transporter (-5.63 and -5.00 kcal/mol, respectively), while n-hexadecanoic acid and dl-threitol had good affinities with dopamine transporter (-6.75 and -4.90 kcal/mol respectively). These binding interactions may have inhibited serotonin and dopamine transporters, contributing to the antidepressant activity observed.

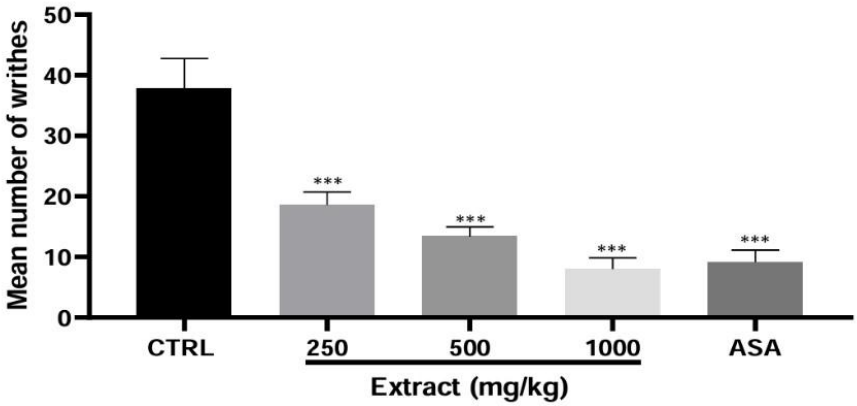


**Figure 1.** Antinociceptive activity of the extract in acetic acid induced writhing test.
Values expressed as Mean ± SEM, n = 5, ***$p$ < 0.001 using one-way ANOVA. CTRL = Control, ASA = Aspirin

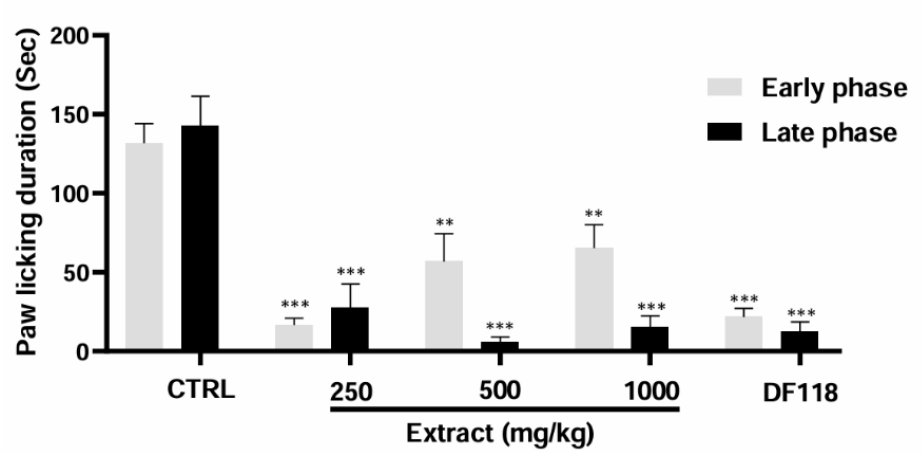


**Figure 2.** Antinociceptive activity of the extract in formalin-induced pain.
Values expressed as Mean ± SEM, n = 5, **$p$ < 0.01, ***$p$ < 0.001 using one-way ANOVA. CTRL = Control, DF118 = Dihydrocodeine

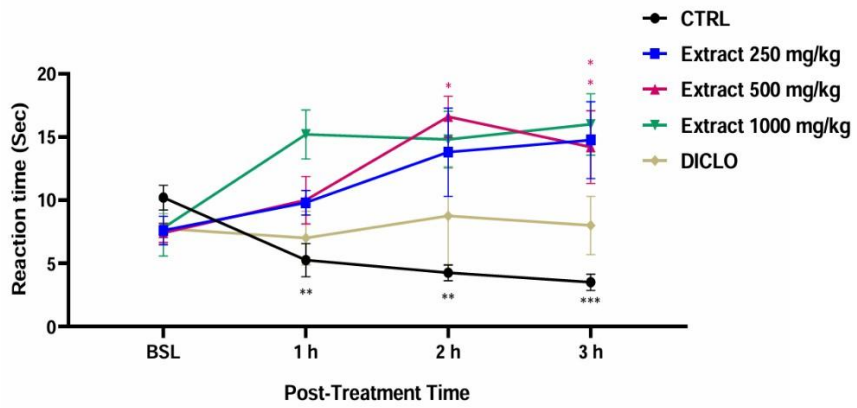


**Figure 3.** Antinociceptive activity of the extract in hyperalgesia associated with carrageenan-induced inflammation.
Values expressed as Mean ± SEM, n = 5, *$p$ < 0.05, **$p$ < 0.01, ***$p$ < 0.001 using one-way ANOVA. DICLO = Diclofenac

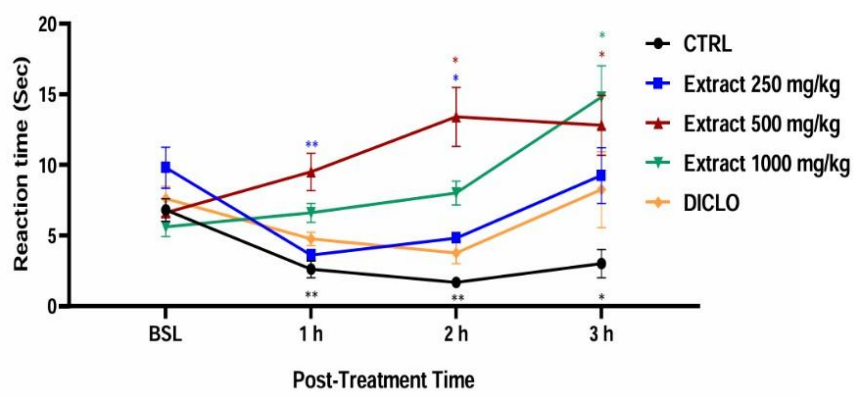


**Figure 4.** Antinociceptive activity of the extract in allodynia associated with carrageenan-induced inflammation.
Values expressed as Mean ± SEM, n = 5, *$p$ < 0.05, **$p$ < 0.01, ***$p$ < 0.001 using one-way ANOVA. DICLO = Diclofenac

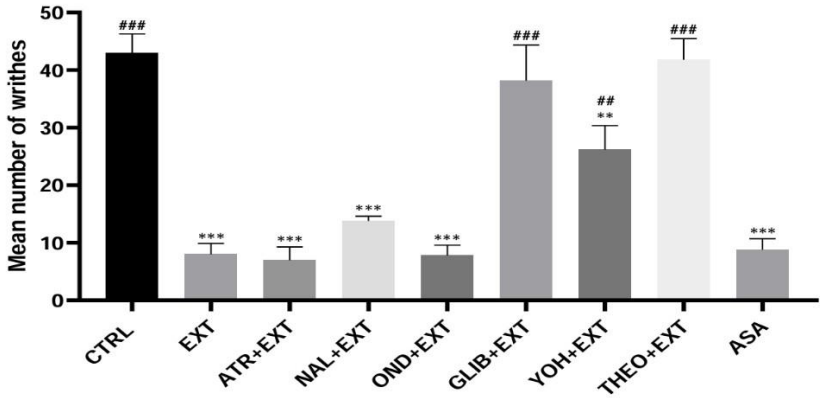


**Figure 5.** Antinociceptive effect of the extract in presence of antagonists in acetic acid induced writhing test for possible mechanism of action. Values expressed as Mean ± SEM, where n=5. Using one-way ANOVA, **$p$ < 0.01 compared with control, ***$p$ < 0.001 compared with control, $^{\#\#\#}p$ < 0.001 compared with Extract alone, $^{\#\#}p$ < 0.01 compared with Extract alone. ATR = Atropine, NALOX = Naloxone, OND = Ondasetron, GLIB = Glibenclamide, YOH = Yohimbine, NIF = Nifedipine, THEO = Theophyline, ASA = Aspirin, EXT = Extract, CTRL = Control.

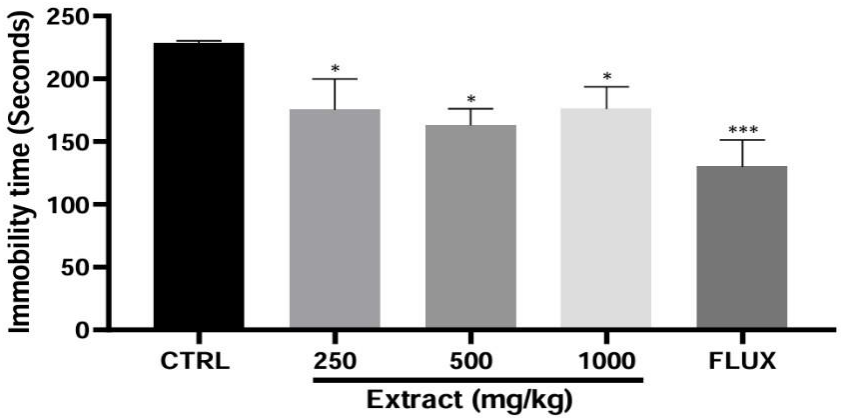


**Figure 6.** Antidepressant activity of the extract in tail suspension test.
Values expressed as Mean ± SEM, n = 5, *$p$ < 0.05, ***$p$ < 0.001 using one-way ANOVA. FLUX = Fluoxetine

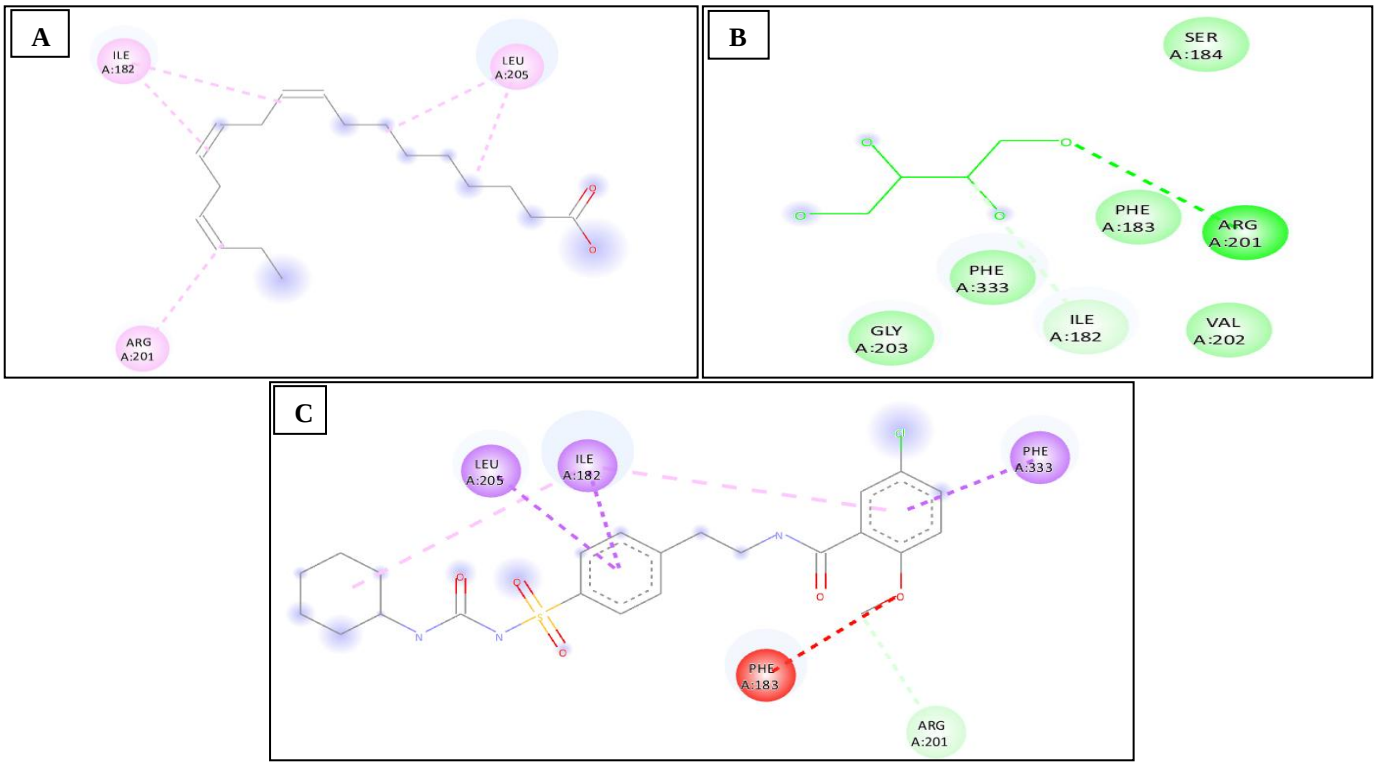


**Figure 7.** Docking orientation and interaction of the identified compounds and reference compound at the active binding site of the ATP-sensitive potassium channel.
[A] 9,12,15-Octadecatrienoic acid (Z,Z,Z) [B] dl-Threitol [C] Glibenclamide

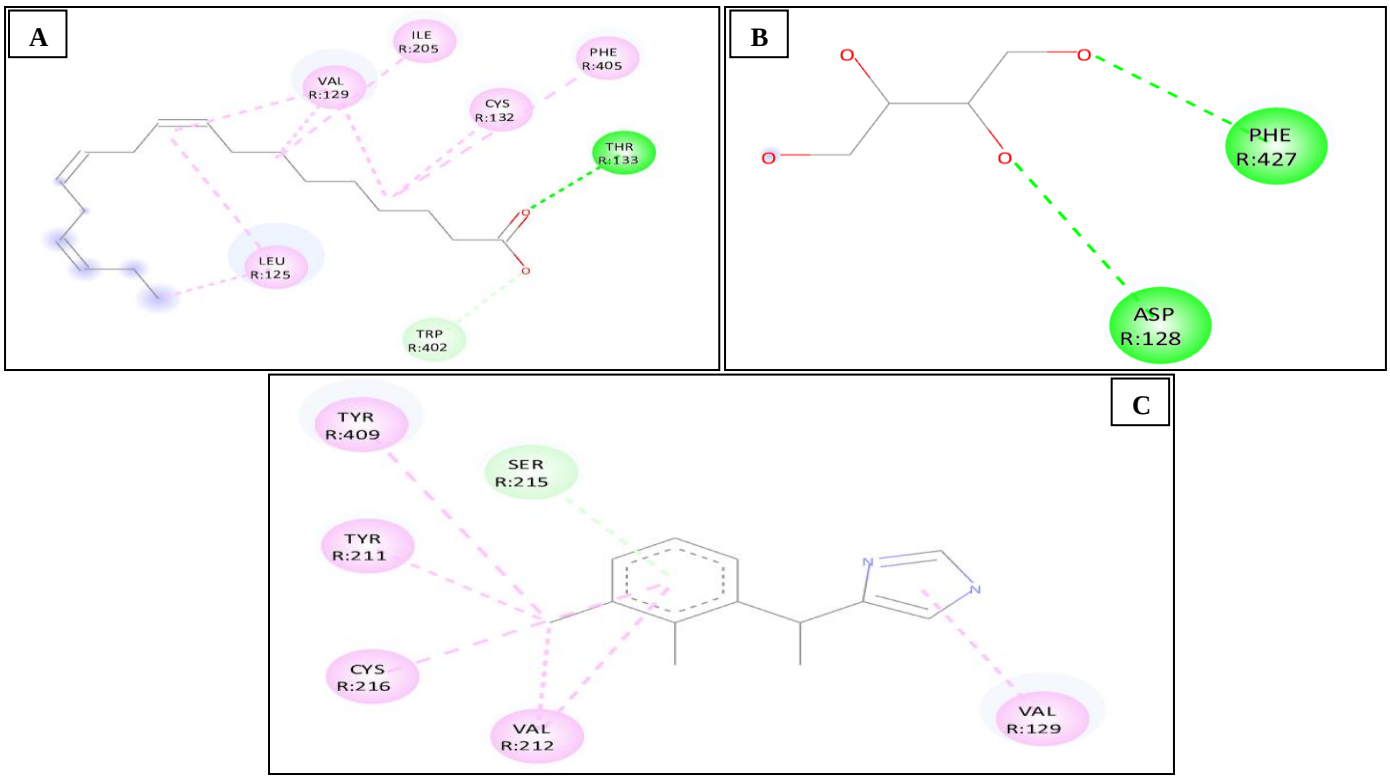


**Figure 8.** Docking orientation and interaction of the identified compounds and reference compound at the active binding site of the alpha-2 adrenergic receptor.
[A] 9,12,15-Octadecatrienoic acid (Z,Z,Z) [B] dl-Threitol [C] Dexmedetomidine

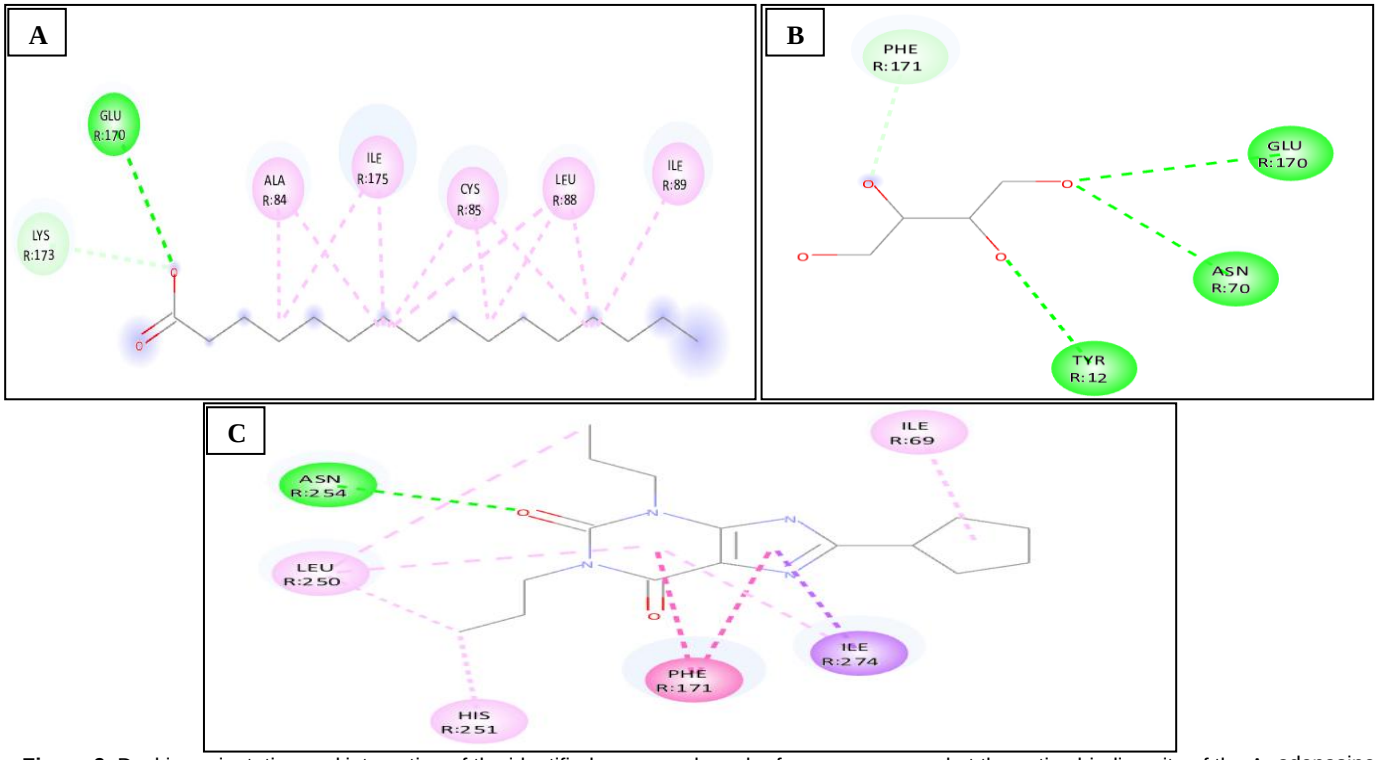


**Figure 9.** Docking orientation and interaction of the identified compounds and reference compound at the active binding site of the $A_1$ adenosine receptor.
[A] n-Hexadecanoic acid [B] dl-Threitol [C] 1,3-dipropyl-8-cyclopentylxanthine

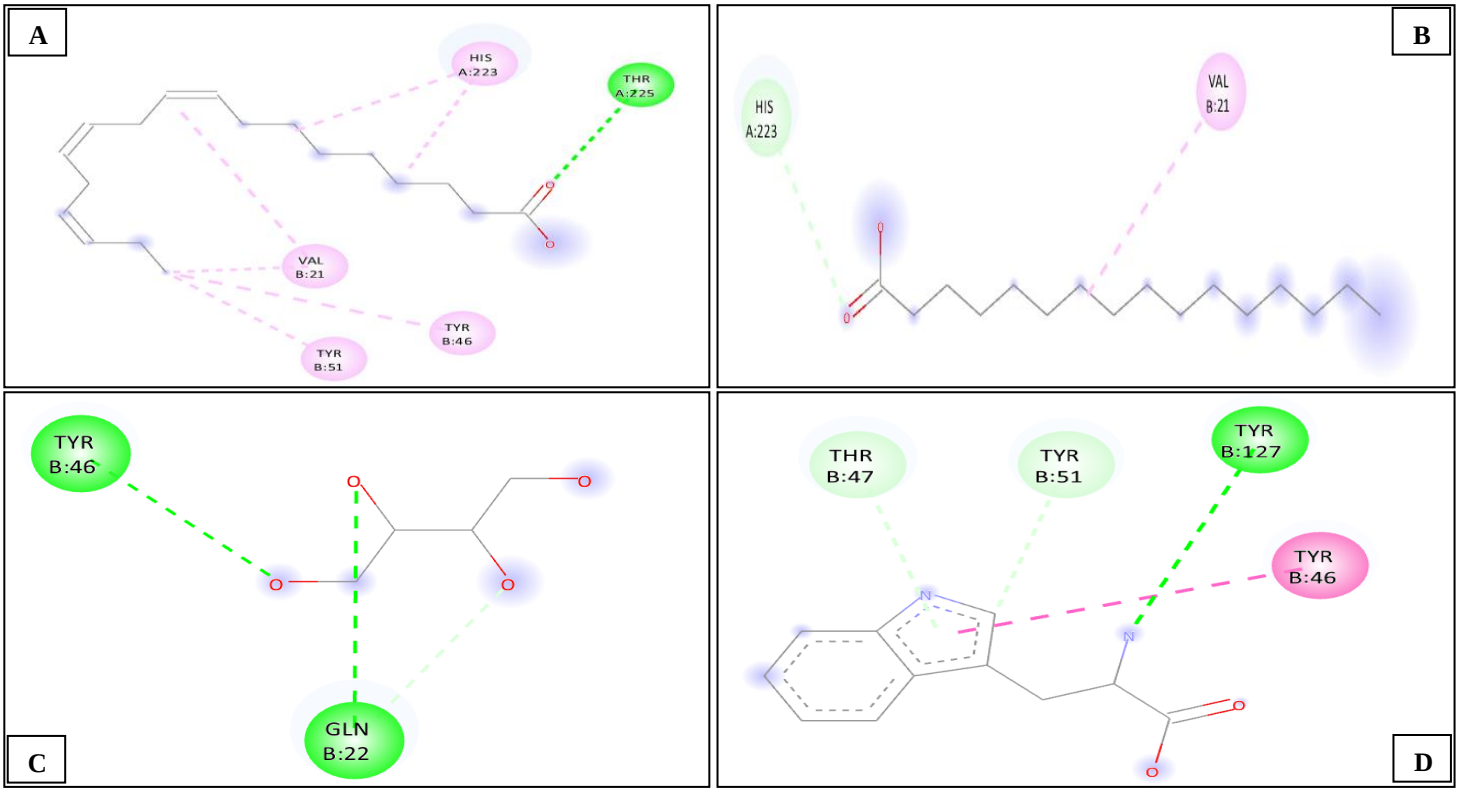


**Figure 10.** Docking orientation and interaction of the identified compounds and reference compound at the active binding site of the Serotonin transporter.
[A] 9,12,15-Octadecatrienoic acid (Z,Z,Z) [B] n-Hexadecanoic acid [C] dl-Threitol [D] Tryptophan

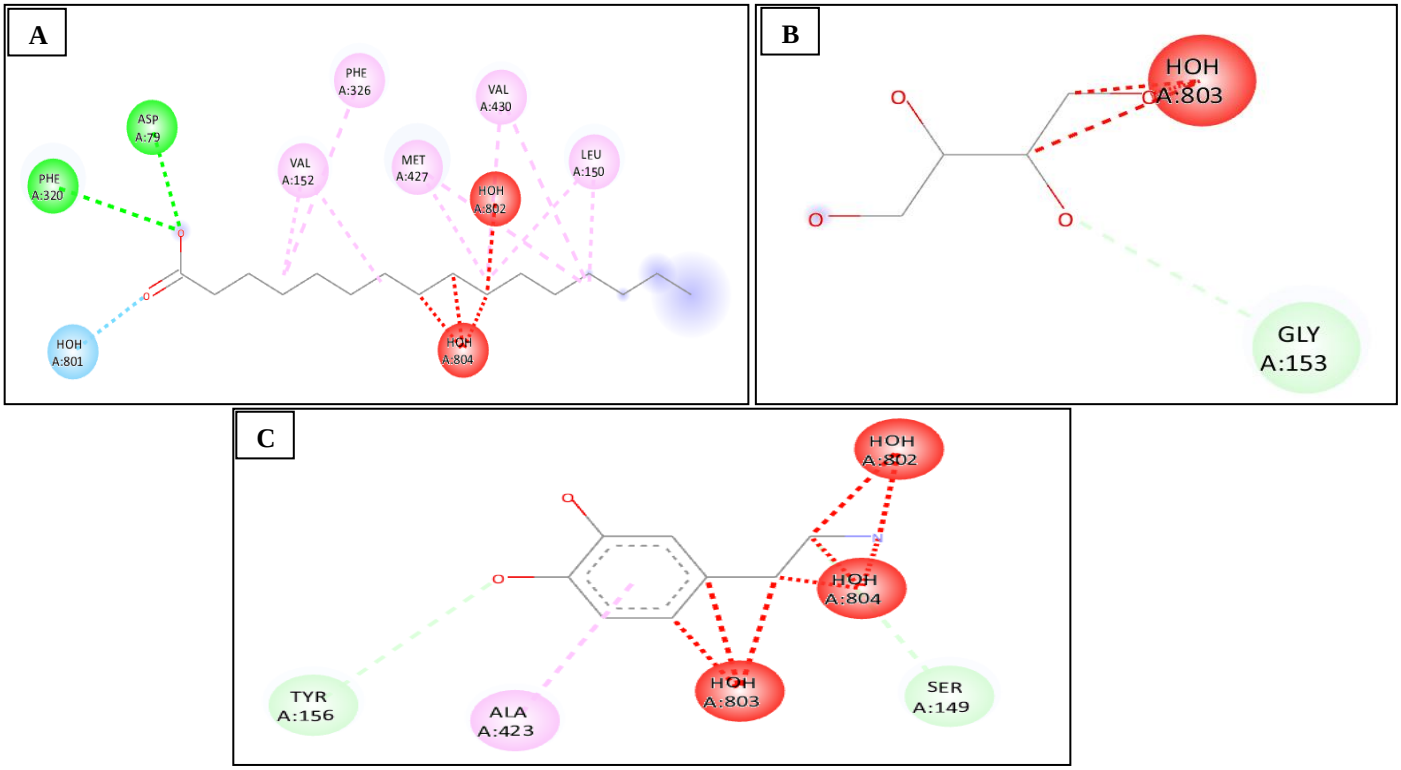


**Figure 11.** Docking orientation and interaction of the identified compounds and reference compound at the active binding site of the Dopamine transporter.
[A] n-Hexadecanoic acid [B] dl-Threitol [C] Dopamine

**Table 1.** Percent antinociceptive activity of the extract in acetic acid-induced writhing test.

| Treatment | Dose (mg/kg) | Percent activity (%) |
|---|---|---|
| Control (Normal Saline) | 10 mL/kg | – |
| Extract | 250 | 50.8 |
| | 500 | 64.6 |
| | 1000 | 78.8 |
| ASA | 150 | 75.7 |

ASA: Aspirin

**Table 2.** Identified compounds binding affinities and interaction details.

| Protein | Compounds | Binding affinity (KCal/Mol) | Interactions |
|---|---|---|---|
| ATP sensitive potassium channel | 9,12,15-Octadecatrienoic acid (Z,Z,Z) | -6.13 | Akyl bond: ILE182, ARG201, LEU205 |
| | dl-Threitol | -3.72 | Carbon Hydrogen Bond: ILE182<br>Conventional Hydrogen Bond: ARG201 |
| | *Glibenclamide | -8.01 | Pi-Sigma Bond: ILE182, LEU205, PHE333<br>Pi-Akyl Bond: ILE182<br>Carbon Hydrogen bond: ARG201 |
| Alpha-2 adrenergic receptor | 9,12,15-Octadecatrienoic acid (Z,Z,Z) | -6.82 | Carbon Hydrogen Bond: THR133<br>Pi-Akyl Bond: VAL129, LEU125<br>Akyl Bond: VAL129, CYS132, ILE205, PHE405<br>Pi-Donor Hydrogen Bond: TRP402 |
| | dl-Threitol | -4.30 | Conventional Hydrogen Bond: ASP 129, PHE427<br>Pi-Donor Hydrogen Bond: PHE427 |
| | *Dexmedetomidine | -7.11 | Pi-Donor Hydrogen Bond: SER215<br>Pi-Akyl Bond: VAL212<br>Akyl Bond: VAL129, TYR211, CYS216, TYR409 |
| Adenosine $A_1$ receptor | 9,12,15-Octadecatrienoic acid (Z,Z,Z) | -6.27 | Conventional Hydrogen Bond: HIS278<br>Pi-Akyl Bond: TYR271<br>Akyl Bond: ALA66, VAL87, PHE171, ILE274 |

**Table 2.** *End.*

| Protein | Compounds | Binding affinity (KCal/Mol) | Interactions |
|---|---|---|---|
| | n-Hexadecanoic acid | -6.23 | Conventional Hydrogen Bond: GLU170<br>Carbon Hydrogen Bond: LYS173<br>Pi-Akyl Bond: ALA8A, CYS85, LEU88, ILE175<br>Akyl Bond: CYS85, LEU88, ILE89 |
| | dl-Threitol | -4.20 | Conventional Hydrogen Bond: TYR12, ASN70, GLU170<br>Pi-Donor Hydrogen Bond: PHE171 |
| | *1,3-dipropyl-8-cyclopentylxanthine (DPCPX) | -8.04 | Conventional Hydrogen Bond: ASN254<br>Pi-Sigma Bond: ILE274<br>Pi-Pi Stacked: PHE171<br>Pi-Akyl Bond: LEU250<br>Akyl Bond: ILE69, LEU250, HIS251 |
| Serotonin transporter | 9,12,15-Octadecatrienoic acid (Z,Z,Z) | -5.63 | Conventional Hydrogen Bond: THR225<br>Pi-Akyl Bond: VAL21, HIS223<br>Akyl Bond: TYR46, TYR51 |
| | n-Hexadecanoic acid | -5.00 | Carbon Hydrogen Bond: HIS223<br>Akyl Bond: VAL21 |
| | dl-Threitol | -3.05 | Conventional Hydrogen Bond: GLN22, TYR46<br>Carbon Hydrogen Bond: GLN22 |
| | *L-Tryptophan | -5.01 | Conventional Hydrogen Bond: TYR127<br>Carbon Hydrogen Bond: TYR51<br>Pi-Hydrogen Bond: THR47<br>Pi-Pi T-Shaped: TYR46 |
| Dopamine transporter | n-Hexadecanoic acid | -6.75 | Conventional Hydrogen Bond: ASP79, PHE320<br>Pi-Akyl Bond: PHE326<br>Akyl Bond: VAL152, MET427, VAL430, LEU150 |
| | dl-Threitol | -4.90 | Carbon Hydrogen Bond: GLY153 |
| | *Dopamine | -6.82 | Carbon Hydrogen Bond: SER149<br>Pi-Donor Hydrogen Bond: TYR156<br>Pi-Akyl Bond: ALA423 |

*Reference compound

## Conclusion

The study outcome revealed that *Strychnos innocua* leaf extract elicits potent antinociceptive and antidepressant activities in mice. The extract likely exerted its antinociceptive activity through interactions with the ATP-sensitive potassium channel, adrenergic and serotonergic pathways, while its antidepressant activity may result from inhibitory interactions with serotonin and dopamine transporters. These findings suggest the extract as a candidate for further therapeutic development targeting these effects.

### Abbreviations

ASA: Aspirin
AMP: Adenosine monophosphate
ATP: Adenosine triphosphate
GC-MS: Gas chromatography-Mass spectrometry
NSAIDs: Non-steroidal anti-inflammatory drugs
PASS: Prediction of Activity Spectra for Substances
SAR: Structure-activity relationships

### Authors' Contribution

CE, DO, IOF, OPO and SOA designed, collected data and prepared manuscript; EE, UEE, MEA, EAO, ECN and JOE reviewed the manuscript.

### Acknowledgments

The authors are grateful to staff members of Department of Pharmacology, Novena University for the technical support during the study.

### Conflict of interest

The authors declare no conflict of interest

**Article history:**

Received: 30 October 2025
Received in revised form: 1st December 2025
Accepted: 7 December 2025
Available online: 7 December 2025

## References

1. Enegide C, Akah PA, Ezike AC, Ameh SF, Ezenyi IC, Okhale SE. 2024. Evaluation of the antioxidant, antinociceptive, and anti-inflammatory activities of *Combretum nigricans* ethanolic leaf extract. *Trop J Nat Prod Res*. 8(6):7554–60. https://doi.org/10.26538/tjnpr/v8i6.35
2. Pandelani FF, Nyalunga SLN, Mogotsi MM, Mkhatshwa VB. 2023. Chronic pain: its impact on the quality of life and gender. *Front Pain Res*. 4:1253460. https://doi.org/10.3389/fpain.2023.1253460
3. Cao B, Xu Q, Shi Y, Zhao R, Li H, Zheng J, Liu F, Wan Y, Wei B. 2024. Pathology of pain and its implications for therapeutic interventions. *Sig Transduct Target Ther*. 9:155. https://doi.org/10.1038/s41392-024-01845-w
4. Colceriu-Șimon IM, Feștilă D, Emoke H, Pancsur A, Șimon MȘ, Olteanu CD, Păstrav M, Bunta O, Ghergie M. 2025. The effects of non-steroidal anti-inflammatory drugs used for orthodontic pain management on tooth movement: a comprehensive review of the literature. *J Clin Med*. 14(9):2920. https://doi.org/10.3390/jcm14092920
5. Enegide C, Akah PA, Ofili CC, Agatemor UM, Ameh SF, Jacob DL, Onah AI. 2021. Evidence supporting the use of Combretum nigricans as an antimalarial agent in ethnomedicine. *Int J Current Res Physio Pharmacol*. 5(2):13–20.
6. El-Saadony MT, Saad AM, Mohammed DM, Korma SA, Alshahrani MY, Ahmed AE, Ibrahim EH, Salem HM, Alkafaas SS, Saif AM, Elkafas SS, Fahmy MA, Abd El-Mageed TA, Abady MM, Assal HY, El-Tarabily MK, Mathew BT, AbuQamar SF, El-Tarabily KA, Ibrahim SA. 2025. Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits—a comprehensive review. *Front Immunol*. 16:1491777. https://doi.org/10.3389/fimmu.2025.1491777
7. Enegide C, Ezeanochie U, Efejene IO, Nwegbu E, Osifo O, Ehimare E, Ossai E, Okwaji N. 2025. In vivo and in silico studies on the nootropic effect of

Strychnos innocua Del. (Loganiaceae) leaf ethanol extract. *Trends Nat Prod Res*. 6:215–23. https://doi.org/10.61594/tnpr.v6i3.2025.135

8. Enegide C, Divine, Efejene IO, Okwaji N, Ogio Q, Eugene, Ossai E. 2025. Evaluation of the antioxidant, anti-proliferative and anti-seizure effects of ethanol leaf extract of Strychnos innocua Del. *Trends Nat Prod Res*. 6:136–44. https://doi.org/10.61594/tnpr.v6(2).2025.128
9. Handa SS, Khanuja SPS, Longo G, Rakesh DD. 2008. Extraction technologies for medicinal and aromatic plants. International Centre for Science and High Technology ICS UNIDO, Trieste, Italy.
10. NIH. 1985. Guide for the care and use of laboratory animals (Revised). Washington: NIH Publication; p. 83–123.
11. Woode E, Ameyaw EO, Abotsi WK, Boakye Gyasi E. 2015. An isobolographic analysis of the antinociceptive effect of xylopic acid in combination with morphine or diclofenac. *J Basic Clin Pharm*. 6(4):103–8.
12. Hunskaar S, Hole K. 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain*. 30:103–14.
13. Amoateng P, Adjei S, Osei-Safo D, Kukuia KK, Kretchy IA, Sarkodie JA, N'Guessan BB. 2017. Analgesic effects of a hydro-ethanolic whole plant extract of Synedrella nodifora (L.) Gaertn in paclitaxel-induced neuropathic pain in rats. *J Pain Res*. 10:226.
14. Brenner DS, Golden JP, Vogt SK, Gereau RW IV. 2015. A simple and inexpensive method for determining cold sensitivity and adaptation in mice. *J Vis Exp*. 97:52640. https://doi.org/10.3791/52640
15. Enegide C, Eyinmisan OM, Oguzie EN, Efejene IO, Ewode AG, Akinsola AO, Okwaji NO, Okpoghono J. 2025. Preclinical investigation on the antinociceptive, antipyretic, anti-inflammatory and antioxidant activities of Ananas comosus leaf extract. *J Basic Appl Pharmacol*. 5:1–15.
16. Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, Gould TD. 2012. The tail suspension test. *J Vis Exp*. 59:e3769. https://doi.org/10.3791/3769
17. Bunman S, Muengtaweepongsa S, Piyayotai D, Charlermroj R, Kanjana K, Kaew-amdee S, Makornwattana M, Kim S. 2023. Analgesic and anti-inflammatory effects of 1% topical cannabidiol gel in animal models. *Cannabis Cannabinoid Res*. 9(3):740–50. https://doi.org/10.1089/can.2023.0070
18. Yam MF, Loh YC, Oo CW, Basir R. 2020. Overview of neurological mechanism of pain profile used for animal "pain-like" behavioral study with proposed analgesic pathways. *Int J Mol Sci*. 21(12):4355. https://doi.org/10.3390/ijms21124355
19. Hirota I, Koyama Y, Shimada S. 2023. Histochemical analysis of the biphasic properties of formalin pain-induced behavior. *Biochem Biophys Rep*. 34:101467. https://doi.org/10.1016/j.bbrep.2023.101467
20. Sammons M, Raval P, Davey P, Rogers D, Parsons A, Bingham S. 2000. Carrageenan-induced thermal hyperalgesia in the mouse: Role of nerve growth factor and the mitogen-activated protein kinase pathway. *Brain Res*. 876:48–54. https://doi.org/10.1016/S0006-8993(00)02596-8
21. Luo J, Feng J, Liu S, Walters ET, Hu H. 2015. Molecular and cellular mechanisms that initiate pain and itch. *Cell Mol Life Sci*. 72(17):3201–23. https://doi.org/10.1007/s00018-015-1904-4
22. Karim N, Khan I, Khan W, Khan I, Khan A, Halim SA, Khan H, Hussain J, Al-Harrasi A. 2019. Anti-nociceptive and anti-inflammatory activities of asparacosin A involve selective cyclooxygenase 2 and inflammatory cytokines inhibition: an in vitro, in vivo, and in silico approach. *Front Immunol*. 10:581. https://doi.org/10.3389/fimmu.2019.00581
23. Beich SS, Kokoti L, Al-Karagholi MA. 2025. Activation of KATP channels in pain modulation: a systematic review of preclinical studies. *Front Physiol*. 16:1444270. https://doi.org/10.3389/fphys.2025.1444270
24. Giovannitti JA Jr, Thoms SM, Crawford JJ. 2015. Alpha-2 adrenergic receptor agonists: a review of current clinical applications. *Anesth Prog*. 62(1):31–9. https://doi.org/10.2344/0003-3006-62.1.31
25. Bahari Z, Meftahi GH. 2019. Spinal alpha-2-adrenoceptors and neuropathic pain modulation; therapeutic target. *Br J Pharmacol*. 176(14):2366–81. https://doi.org/10.1111/bph.14580
26. Vincenzi F, Pasquini S, Borea PA, Varani K. 2020. Targeting adenosine receptors: a potential pharmacological avenue for acute and chronic pain. *Int J Mol Sci*. 21(22):8710. https://doi.org/10.3390/ijms21228710
27. Horiuchi H, Ogata T, Morino T, Yamamoto H. 2010. Adenosine A1 receptor agonists reduce hyperalgesia after spinal cord injury in rats. *Spinal Cord*. 48(9):685–90. https://doi.org/10.1038/sc.2009.194
28. O'Leary OF, Cryan JF. 2009. The tail-suspension test: A model for characterizing antidepressant activity in mice. In: Gould TD, editor. Mood and anxiety related phenotypes in mice: characterization using behavioral tests. Humana Press/Springer Nature. p.119–37. https://doi.org/10.1007/978-1-60761-303-9_7
29. Nguyen H, Cheng MH, Lee JY, Aggarwal S, Mortensen OV, Bahar I. 2024. Allosteric modulation of serotonin and dopamine transporters: new insights from computations and experiments. *Curr Res Physiol*. 7:100125. https://doi.org/10.1016/j.crphys.2024.100125