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Modulatory Effects of Morphine and *Xylopia aethioica* Extract on Kappa Opiod Receptors (KOR), Delta Opioid Receptor (DOR), Pain Hypersensitivity and Motor Functions in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study investigates the modulatory effects of morphine and *Xylopia aethiopica* extract on kappa opioid receptors (KOR), delta opioid receptors (DOR), pain hypersensitivity, and motor functions in Wistar rats. We utilized three experimental groups: a control group receiving distilled water, a morphine group receiving either low (5 mg/kg) or high (10 mg/kg) doses after inducing pain, *Xylopia aethiopica* group received either 25 mg/kg or 50 mg/kg of hydromethanolic extract following similar

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pain induction. Pain perception was quantified using the tail flick test and Analgesy-Meter while motor functions were assessed through the Rotarod and Climbing/Beam Walk tests. Additionally, molecular docking studies were performed on selected compounds from *Xylopia aethiopica* to determine their binding affinities to opioid receptors using Vina. Results demonstrated that morphine and *Xylopia aethiopica* significantly increased tail flick response times, indicating notable analgesic effects, while improving motor functions particularly in animals treated with higher doses of *Xylopia aethiopica*. Molecular analysis revealed potential interactions between bioactive compounds and opioid receptors, suggesting further therapeutic applications. These findings highlight the potential of *Xylopia aethiopica* as a natural analgesic and its implications in managing pain and associated motor deficits, the findings further revealed that Morphine and not *Xylopia aethiopica* is implicated in pain hypersensitivity after long term exposure. Further research should pay attention on improving the pharmacological profiles of the identified compounds for clinical use.

Keywords: Xylopia aethiopica; analgesic, opioid receptors; motor functions; wistar rats.

1. INTRODUCTION

Pain is a complicated event that can have a significant effect on the quality of life. The experience is at the same time a physiological response and a subjective perception, which activates various pathways and receptor systems in the body. Opioid receptors, in particular kappa, delta-, and mu-receptors, regulate our experience of pain. In view of the analgesic effect of opioids like morphine, pain therapy focuses on a target at the mu opioid receptor. However, several undesirable effects have been associated with the administration of morphine, including the progression of tolerance, dependence, and a state of hyper-sensitization to pain, otherwise known as opioid-induced hyperalgesia [1,2]. The study of this type of effect outlines the important need for the identification of new and more innovative strategies of pain relief. The current work has focused mostly on Kappa and Delta opioid receptors because of their peculiar pharmacological features. The Kappa receptors have an established role for reducing pain. However, stimulating these receptors is known to create sedation or dysphoria [3]. Delta receptors have a role in the emotional component of pain and can induce analgesia without the common adverse effects associated with mu-receptor agonists [4]. Underpinning the development of appropriate pain management measures is the requirement for critical understanding of the relative balances and interactions amongst these receptors. Traditional medicinal herbs have recently gained increasing popularity as a prospective alternative avenue for pain relief following their potential therapeutic properties. *Xylopia aethiopica* is a tree native to West Africa and commonly referred to as Guinea Pepper or African Guinea Pepper. This perennial shrub has long been recognized by the different cultures using it for its medicinal value for pain-relieving, anti-inflammatory, and antimicrobial benefits. Preliminary studies have indicated that extracts from Xylopia aethiopica may present potential in the modulation of pain pathways, which would potentially allow it to be used as either a natural supplement or alternative to current opioid treatments. The growing global burden of chronic pain conditions and the rising opioid crisis have only emphasized the need for more effective solutions to manage the painful condition [5]. Indeed, chronic pain is a global problem that significantly diminishes the QoL of many while, at the same time, increasing health expenditure and the burden of mental health [6]. In connection with the advances in pharmacology and neurology, it was possible to have an extensive study into the effects of *Xylopia aethiopica* extracts on pain regulation and especially how they interact with opioid receptors [7]. A study on the interaction of these extracts in the pathway of kappa and delta opioid receptors would thus be very useful in seeing their analgesic capacity and its compatibility with other therapeutic approaches. The present study has been conducted to see the changes in pain hypersensitivity and motor functions in Wistar rats with different modes of morphine and *Xylopia aethiopica* extract administrations. This study tries to delineate the possible effects of these substances in the management of pain and associated motor dysfunction.

2. MATERIALS AND METHODS

30 experimental animals, weighing between 80 and 100 grams, were sourced from the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt. They were provided with standard laboratory rat feed and water ad libitum. The

experiment was structured into three distinct groups with 5 animals per group. Each of the groups were subjected to different treatment protocols to assess their responses to pain and motor functions. Group 1 served as the control group 1, with animals in Control group administered with distilled water and maintained in a stress-free environment throughout the experiment. They were then exposed to cognito-motor tests. Control Group 2 animals were exposed to pain using hot plate, tail immersion and Analgesy-Meter and were exposed to the various neurobehavioural tests without any drug administration, allowing a comparison for the effects of different treatments. Group 2, the morphine group, had repetitive pain applied and thereafter treated with 5 mg/kg or 10 mg/kg of morphine. Following administration of the various treatment, animal were assessed through several cognitomotor tests. Also, Group 3, "the *Xylopia aethiopica* group" was treated with (25 mg/kg) and (50 mg/kg) doses of the extract, with the animals exposed to the same set of pain sensitivity and cognitomotor tests. These systematic investigations were conducted in a structured way that allowed for comparative analysis of pain sensitivity and motor responses among several treatments, providing valuable understanding into the effectiveness of *Xylopia aethiopica* in the management of pain and pain-induced motor dysfunction. This research explored *Xylopia aethiopica* compounds completely using GC/MS. Data acquisition involved scanning method and integration through ChemStation; unknown spectrum identified as Apex by NIST14.L libraries. Several tests were done such as: Rotarod test measuring coordination and balance, Climbing/Beam Walk test assessing fine motor coordination while Handgrip measured grip strength. Each test employed specific protocols to measure performance, helping to gauge the efficacy of the treatments on coordination, strength, and fine motor functions in rat models.

In silico studies was carried out and this involved the preparation of protein and ligand structures for molecular docking analysis. Crystal structures of various proteins, including delta opioid and Kappa Opioid receptors, were retrieved from the Protein Data Bank, with ligands sourced from PubChem and converted to the appropriate formats [8]. Docking was executed using Vina, assessing ligand binding affinities across multiple protein targets with specific grid parameters. A cluster analysis was performed based on RMSD values to identify the lowest energy conformations, followed by analyzing molecular interactions using Discovery Studio Visualizer [9]. Additionally, pharmacokinetic properties such as molecular weight and logP were calculated for selected compounds based on Lipinski's rule of five [10], while statistical analysis employed one-way ANOVA with Newman-Keuls post-hoc tests to determine significant differences among treatment groups.

2.1 Experimental Protocols

2.1.1 Hot plate

We utilised a hot plate from Ugo Basile Srl with a pre-set plate temperature of 52.5°C, which is the recommended temperature for rats [11]. Upon placing the rat on the hot plate, the duration (in seconds) of its response, such as licking, shaking, or stepping of the hindpaws, was promptly recorded. The rat was then promptly removed from the hot plate. A time limit of 60 seconds was implemented to reduce the risk of harm to the skin tissue [12].

2.1.2 The tail flick method

This animal model is commonly used to assess analgesic activity in rats. When a rat's tail comes into contact with heat or thermal stimuli, the animal instinctively tries to withdraw its tail or flick it away from the source of the stimuli. This demonstrates the typical response time for pain perception and is regarded as the final point [13].

2.1.3 Randall-selitto test

The Randall-Selitto or paw pressure test is a valuable tool for evaluating response thresholds to mechanical pressure stimulation. It is widely recognised as a measure of mechanical hyperalgesia [14]. This experiment required the application of a gradually increasing mechanical force to the surface of the paw or tail until the subject either withdrew or vocalised. Practically speaking, this test proves to be valuable for evaluating nociceptive thresholds in rats [15]. We conducted the experiment using the bench-top Ugo Basile Analgesy-Meter to perform the Randall-Selitto test.

2.1.4 The Rotarod

Also known as the Rotarod test, is used as a basic assessment tool for coordination and balance in rodents and provides one measure of

locomotors ability as originally described by Crawley et al. [16].

2.1.5 Climbing/beam walk test

The beam walking assay was used to evaluate fine motor coordination and balance. The objective of this experiment was to observe the rodent's ability to maintain balance and navigate across a narrow elevated beam to reach a secure platform. This experiment spans across a period of three consecutive days, with two days dedicated to training and one day solely focused on testing. Measuring the performance on the beam involves tracking the time it takes for the mouse to cross the beam and keeping count of any paw slips that occur along the way. The Protocol used was derived from previous studies [17,18].

2.2 In silico Studies

2.2.1 Protein preparation

The crystal structures of DOR [19] and KOR [20] were obtained from the protein databank (www.rcsb.org). The crystal structures were prepared individually by removing existing ligands and water molecules, and missing hydrogen atoms were added using the Autodock v4.2 program from the Scripps Research Institute. Afterwards, non-polar hydrogens were combined while polar hydrogens were incorporated into each enzyme. The process was repeated for all proteins and then saved into a PDBQT file for molecular docking.

2.2.2 Preparation of ligands

The structures of the reference compounds and phytochemical ligands identified in *Xylopia aethiopica* were obtained from the PubChem database (www.pubchem.ncbi.nlm.nih.gov). The compounds were transformed into mol2 chemical format using Open babel [21]. Hydrogens with different properties were treated differently, with polar hydrogens being added and non-polar hydrogens being combined with the carbons. Additionally, the internal degrees of freedom and torsions were adjusted accordingly. The protein and ligand molecules were converted to the PDBQT file using Autodock tools.

2.2.3 Exploring molecular docking

The ligands were docked to different protein targets and their binding affinities were determined using Vina [22]. The proteins and ligands were placed into their respective columns by dragging the PDBQT file. The coordinates for the docking grid centre were found to be X = 47.08, Y = 38.44, Z = 14.84. The dimensions of the grid box were measured to be 83.39 x 60.94 x 102.87 for DOR and X = 15.26, Y = 17.84, Z = 4.63. The dimensions of the grid box for KOR were 44.74 x 52.16 x 78.12. Additionally, X = 7.87 was recorded. Afterwards, the software was executed and a cluster analysis was conducted using root mean square deviation (RMSD) values, comparing them to the initial geometry. The lowest energy conformation of the most prevalent cluster was then deemed the most reliable solution. The recorded data includes the binding affinities of compounds for the three targets. The compounds were then evaluated based on their affinity scores. Afterwards, the molecular interactions between the proteins and compounds with a higher binding affinity than the reference compounds or phytochemical ligands were observed using Discovery Studio Visualiser, BIOVIA, 2020.

3. RESULTS

The Table 1 presents comprehensive information on the discovered chemicals compounds in the plant, including their retention durations, molecular formulae, molecular weights, and peak area percentages. The most abundant compounds identified is 1-Dodecanol, 2-methyl-, (S)- With Peak Area (%): 23.59, while the least identified compound is Undec 10 -ynoic acid, undecyl ester with Peak Area (%): 2.11.

The Table 2 revealed the results of tail flick test in the first phase of the work which lasted for 14 days. (Pain + 5mg/kg Morphine) and (Pain + 10mg/kg Morphine) showed significant increases in tail flick time compared to the control group in Week 1. This indicates that the administration of morphine led to an increase in tail flick response time. (Pain + 25mg/kg *Xylopia aethiopica*) and (Pain + 50mg/kg *Xylopia aethiopica*) demonstrated significant increases in tail flick time compared to the Pain Only group in Week 2. This implies that the administration of *Xylopia aethiopica* at these doses led to an increase in tail flick response time. Therefore, in these cases, the significant differences observed indicate an increase in tail flick response time in the experimental groups compared to the control or Pain Only groups.

Table 1. Identified chemical compounds in *Xylopia aethiopica*

S/N	Name Of Compound	Retention Time (RT) (Minutes)	Molecular Formular	Molecular Weight (g/mol)	Peak Area%
1.	Phenol, 2,6-bis(1,1-dimethylethyl)	10.117	$C_{14}H_{22}O$	220.35	2.59
2.	Heneicosane	12.359	$C_{21}H_{44}$	296.5741	3.24
3.	1-Docosene	13.246	$C_{22}H_{44}$	308.5848	2.44
4.	Undec 10-ynoic acid, undecyl ester	14.164	$C_{22}H_{40}O_2$	336.5518	2.11
5.	Hexadecanoic acid, methyl ester	14.595	$C_{17}H_{34}O_2$	270.4507	11.52
6.	1-Dodecanol, 2-methyl-, (S)-	15.015	-	-	23.59
7.	Cyclododecane, ethyl-	15.270	-	-	2.05
8.	9-Octadecenal, (Z)-	16.292	$C_{18}H_{34}O$	266.4620	3.06
9.	7-Oxabicyclo[4.1.0]heptane, 1,5-di methyl-	16.416	$C_{28}H_{58}O$	410.7595	17.24
10.	Heptadecanoic acid, 16-methyl-, methyl ester	16.530	$C_{19}H_{38}O_2$	298.5038	5.24
11.	9,17-Octadecadienal, (Z)-	16.681	$C_{18}H_{32}O$	264.4461	14.40
12.	Undec-10-ynoic acid, nonyl ester	16.883	$C_{20}H_{36}O_2$	308.4986	4.02
13.	Undec 10-ynoic acid, undecyl ester	17.127	$C_{22}H_{40}O_2$	336.5518	3.72
14.	2-Decen-1-ol, (E)-	17.439	-	-	4.78

Table 2. Pain perception using tail flick test

Groups	Week 1 Time (s)	Week 2 Time (s)
(Control)	1.60±0.25	1.20±0.37
(Pain Only)	1.30±0.25	1.40±0.25
(Pain + 5mg/kg Morphine)	2.60±0.45*	2.20±0.20*
(Pain + 10mg/kg Morphine)	3.00±0.25	2.20±0.25*
(Pain + 25mg/kg *Xylopia aethiopica*)	2.40±0.40	2.60±0.25*#
(Pain + 50mg/kg *Xylopia aethiopica*)	2.40±0.40	2.63±0.25*#

*Values are presented in mean ± sem, n= 5. * Means values are statistically significant (p≤0.05) when compared to the control, # means values are statistically significant (p≤0.05) when compared to Pain Only group.*

Table 3. Pain perception using tail flick test

Groups	Week 3 Time (s)	Week 4 Time (s)	Week 5 Time (s)	Week 6 Time (s)	Week 7 Time (s)	Week 8 Time (s)	Week 9 Time (s)
(Control)	1.00±0.32	2.20±0.20#	2.20±0.20#	2.60±0.25#	2.00±0.32	2.00±0.00	2.00±0.00
(Pain Only)	1.60±0.25	1.40±0.25*	1.40±0.25*	1.60±0.00*	1.70±0.20	1.60±0.25	2.10±0.00
(Pain + 5mg/kg Morphine)	1.80±0.45*	1.80±0.32	2.00±0.32#	2.00±0.00*#	1.80±0.20	1.80±0.20	1.80±0.20
(Pain + 10mg/kg Morphine)	2.00±0.45*	2.00±0.25*	1.90±0.25*	2.00±0.00*#	2.20±0.25#	2.20±0.20#	2.20±0.20
(Pain + 25mg/kg *Xylopia aethiopica*)	1.80±0.20	1.60±0.25	2.00±0.00#	1.90±0.25*#	1.80±0.20*#	2.00±0.00	3.00±0.00*#
(Pain + 50mg/kg *Xylopia aethiopica*)	1.80±0.20	1.60±0.25	2.80±0.01#	1.60±0.25*#	2.26±0.20*#	2.00±0.00	3.00±0.00*#

*Values are presented in mean ± sem, n= 5. * Means values are statistically significant (p≤0.05) when compared to the control, # means values are statistically significant (p≤0.05) when compared to Pain Only group*

Table 4. Pattern of noxious sensitivity and response in the test and control rats using analgesy-meter test

Groups/Treatment	Week 1	Week 2
(Control)	9.82±2.36	10.08±1.09
(Pain Only)	8.86±2.89	8.62±0.68
(Pain + 5mg/kg Morphine)	10.10±2.39	12.40±2.49
(Pain + 10mg/kg Morphine)	13.78±3.08	10.50±3.69
(Pain + 25mg/kg *Xylopia aethiopica*)	10.46±2.73	16.58±3.23#
(Pain 50mg/kg *Xylopia aethiopica*)	11.46±2.73	16.58±3.23#

*Values are presented in mean ± sem, n= 5. * Means values are statistically significant (p≤0.05) when compared to the control, # means values are statistically significant (p≤0.05) when compared to Pain Only group*

Analyzing the tail flick test results from the phase 2 of the study across the treatment groups:

The Control group exhibited relatively consistent response times across the study period, with minor fluctuations observed. (Pain Only) consistently exhibited reduced response times compared to the Control group throughout the study period, indicating the presence of sustained pain perception. (Pain + 10mg/kg Morphine) exhibited consistent improvements in pain perception with significant increase in response times compared to the Pain Only group. (Pain + 25mg/kg *Xylopia aethiopica*) and Group 10 (Pain + 50mg/kg *Xylopia aethiopica*) showed varying effects on response times, with some improvements noted in weeks 5, 6, 7 and 8 compared to the Pain Only group. Generally, the results suggest diverse effects of the administered treatments on pain perception across the different treatment groups, highlighting the potential analgesic properties of *Xylopia aethiopica* in modulating pain responses in this experimental context.

Based on the pain threshold results of the phase 1 as presented above, we observed the following: The control group showed relatively stable pain threshold values across both weeks, indicating that the experimental conditions did not significantly affect the pain response in this group. The Pain Only group displayed consistently lower pain threshold values compared to the control group, suggesting that the induction of pain in this group resulted in a decrease in pain tolerance. When comparing the groups receiving different treatments, several interesting trends emerge: (Pain + 10mg/kg Morphine) showed an increase in pain threshold values in Week 1 compared to the Pain Only group, indicating the analgesic effect of the morphine treatment. (Pain + *Xylopia aethiopica*) displayed significant increases in pain threshold values in Week 2 compared to the Pain Only group, implying a potential analgesic effect of *Xylopia aethiopica* treatment.

The results of the pain threshold and sensitivity measurements using the Analgesy-Meter in Phase 2 of the experiment are as follows: The Control group (Group 1) showed relatively consistent pain threshold values across the weeks, with a slight decline towards the later weeks. The Pain Only group displayed variable pain threshold values over the weeks but generally stayed within a certain range (8-11). (Pain + 5mg/kg Morphine) showed fluctuations in pain threshold values, with weeks 3, 5, 6 displaying statistically higher values compared to both the Control and Pain Only groups. Group 4 (Pain + 10mg/kg Morphine) exhibited varying pain threshold values, with weeks 4,5,6,7 and 9 showing significant increases compared to the Pain Only group. (Pain + 25mg/kg *Xylopia aethiopica*) and Group administered (Pain + 50mg/kg *Xylopia aethiopica*) both demonstrated improvements in pain threshold values over the weeks, with significant differences compared to the Pain Only group in multiple instances. (Pain + *Xylopia aethiopica* + *Bryophyllum pinnatum*) and (Pain + *Xylopia aethiopica* + *Bryophyllum pinnatum* + Morphine) showed varying effects on pain threshold values, with weeks 6,7,8,9 indicating significant differences compared to the Pain Only group.

Based on the results presented on the table above, Motor coordination and balance, as measured by Beam walking in Phase 1, showed significant differences among the various treatment groups. (Pain + *Xylopia aethiopica*) displayed significant improvements in both weeks compared to the Pain Only group. the results suggest that the treatments involving *Xylopia aethiopica* have significant impact on motor coordination and balance in the experimental model used, as indicated by the

improvements observed compared to the Pain Only group.

The results of motor coordination and balance using Beam walking in Phase 2 show the following key points: (Control) maintained stable performance throughout the weeks, with consistent low times indicating good motor coordination. Group 2 (Pain Only) consistently showed the highest times across all weeks, indicating impaired motor coordination and balance due to pain. (Pain + 5mg/kg Morphine) showed improvements in Weeks 1, 2, 6, and 7 compared to the Pain Only group, suggesting a positive effect of morphine on motor coordination. (Pain + 10mg/kg Morphine) displayed mixed results but generally showed improvements compared to the Pain Only group in some weeks. (Pain + *Xylopia aethiopica*) also exhibited improvements in various weeks compared to the Pain Only group.

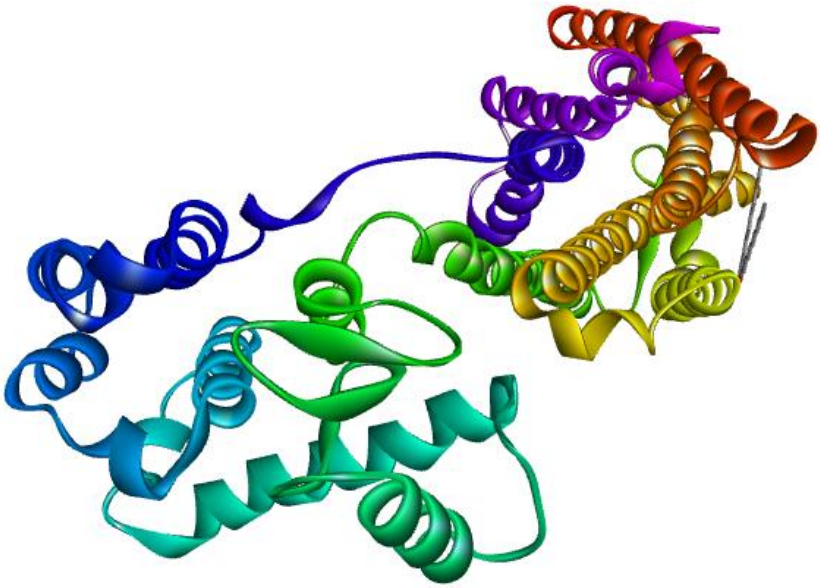

Fig. 1. 3D view of the binding of undec 10-ynoic acid, undecyl ester to DOR

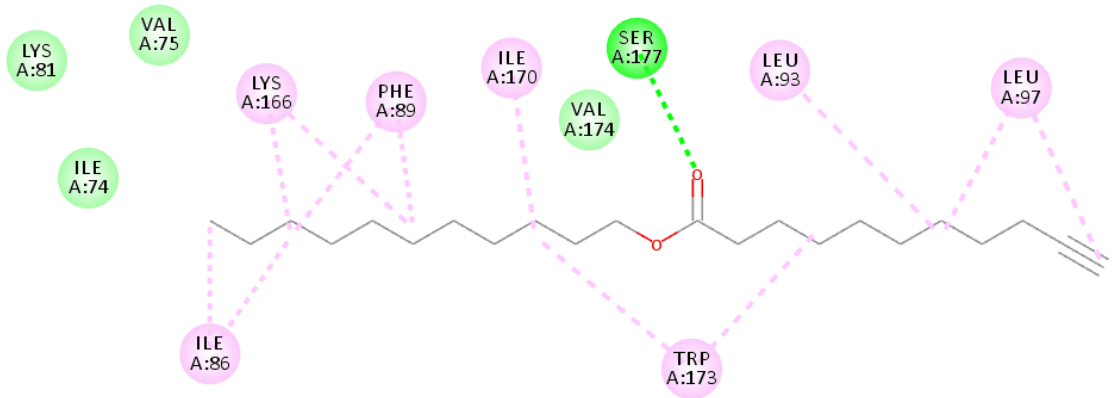

Fig. 2. 2D view of the interaction between undec 10-ynoic acid, undecyl ester and amino acids in the binding site of DOR

Table 5. Pattern of noxious sensitivity and response in the test and control rats Using Analgesy-meter Test

Groups	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
(Control)	12.76±2.42	10.42±3.11	9.22±3.02	9.10±2.03	9.64±1.79	9.02±3.97	9.28±1.97
(Pain Only)	11.62±2.57	10.36±1.98	9.54±2.34	8.40±1.41	10.48±2.21	10.58±1.85	9.70±2.24
(Pain + 5mg/kg Morphine)	16.68±2.21#	13.52±2.05	13.54±1.85*#	13.24±3.68#	11.48±3.33	11.82±2.31	10.22±0.87
(Pain + 10mg/kg Morphine)	12.80±1.70	17.80±1.82#	14.52±.42*#	14.72±2.70#	13.78±.95#	14.12±3.66	14.98±2.62*#
(Pain + 25mg/kg *Xylopia aethiopica*)	11.90±2.33	12.60±2.24	11.72±2.58	10.38±1.07	13.22±3.02#	15.08±1.09#	14.24±1.13*#
(Pain + 50mg/kg *Xylopia aethiopica*)	11.90±2.33	12.60±2.24	11.72±2.58	15.38±1.07#	15.22±3.02#	15.08±1.09#	15.24±1.13*#

Table 6. Result of motor coordination and balance using beam walking

Groups/Treatment	Week 1 Time (s)	Week 2 Time (s)
(Control)	100.00±0.00$^{\#}$	99.60±62.47$^{\#}$
(Pain Only)	125.08±60.16*	145.60±54.40*
(Pain + 5mg/kg Morphine)	199.80±61.66$^{*\#}$	110.00±0.00*
(Pain + 10mg/kg Morphine)	205.00±58.82$^{*\#}$	191.00±66.88*
(Pain + 25mg/kg *Xylopia aethiopica*)	126.00±0.12$^{*\#}$	204.28±59.05$^{*\#}$
(Pain + 50mg/kg *Xylopia aethiopica*)	180.00±0.00$^{*\#}$	224.28±59.05$^{*\#}$

*Values are presented in mean ± sem, n= 5. * Means values are statistically significant (p≤0.05) when compared to the control, # means values are statistically significant (p≤0.05) when compared to Pain Only group*

Fig. 3. 3D view of the binding of phenol, 2,5-bis(1,1-dimethylethyl) to KOR

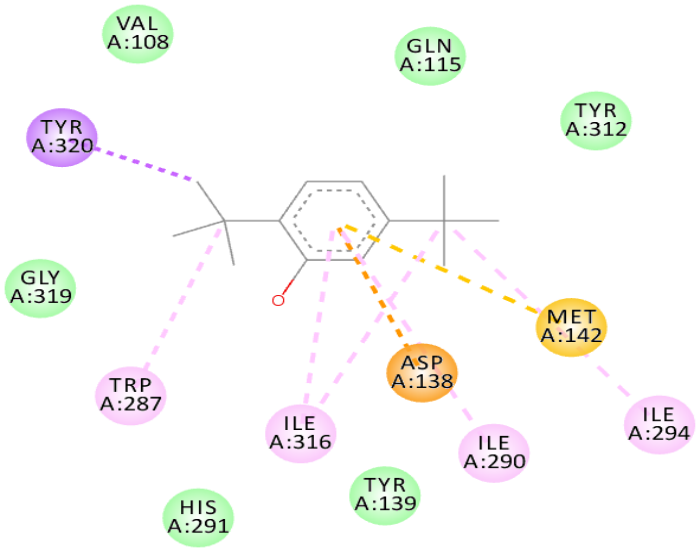

Fig. 4. 2D view of the interaction between phenol, 2,5-bis(1,1-dimethylethyl) and amino acids in the binding site of KOR

Table 7. Result of motor coordination and balance using beam walking

Groups/Treatment	Week 3 Time (s)	Week 4 Time (s)	Week 5 Time (s)	Week 6 Time (s)	Week 7 Time (s)	Week 8 Time (s)	Week 9 Time (s)
Group 1 (Control)	49.00±0.41*	48.00±0.42#	30.00±0.12#	36.00±51.43#	30.00±0.32#	31.20±0.18#	41.20±0.42#
Group 2 (Pain Only)	190.00±0.23*	163.00±0.31*	196.40±63.57	300.00±0.00	246.60a±62.82	290.00±0.21	284±49.91*#
Group 3 (Pain + 5mg/kg Morphine)	110.00±0.32*#	161.00±0.13*	152.12±61.46	142.00±0.32*#	199.48±61.87	123.41±0.34*#	121.96±10.68*#
Group 4 (Pain + 10mg/kg Morphine)	144.20±55.80*#	145.00±55.60*	207.00±57.39	196.08±64.13	206.48±57.73	195.68±64.41	121.52±24.91*#
Group 9 (Pain + 25mg/kg *Xylopia aethiopica*)	121.00±4.12*#	140.00±5.23*	156.00±4.80	99.00±5.12*#	162.00b±5.20	138.00±8.32*#	157.92±25.98*
Group 10 (Pain + 50mg/kg *Xylopia aethiopica*)	120.00±0.42*#	146.00±0.42*	300.00±0.00	188.00±0.00	167.00b±0.00	162.00±0.00*#	157.92±25.98*

*Values are presented in mean ± sem, n= 5. * Means values are statistically significant (p≤0.05) when compared to the control, # means values are statistically significant (p≤0.05) when compared to Pain Only group.*

Table 8. Binding affinity of ligands to DOR and KOR

		Binding affinity (Kcal/mol)	
S/N	**Compounds**	**DOR**	**KOR**
R	Morphine	-7.7	-7.5
1	Phenol, 2,5-bis(1,1-dimethylethyl)	-7.6	-7.7
2	Heneicosane	-7.4	-6.8
3	1-Docosene	-7.4	-6.8
4	Undec 10-ynoic acid, undecyl ester	-7.9	-7.0
5	Hexadecanoic acid, methyl ester	-6.6	-6.1
6	1-Dodecanol, 2-methyl-, (S)-	-5.6	-5.8
7	Cyclododecane, ethyl-	-6.6	-7.0
8	9-Octadecenal, (Z)-	-6.5	-7.4
9	7-Oxabicyclo[4.1.0]heptane, 1,5-di methyl-	-5.0	-5.1
10	Heptadecanoic acid, 16-methyl-	-7.2	-6.9
11	9,17-Octadecadienal, (Z)-	-6.0	-6.3
12	Undec-10-ynoic acid, nonyl ester	-6.8	-7.0
13	2-Decen-1-ol, (E)-	-4.8	-5.3

The table provides the binding affinities of various ligands to the DOR (Delta opioid receptor) and KOR (Kappa opioid receptor). These receptors play crucial roles in the opioid system and are important targets for pain management and drug development. When analyzing the data, we can observe the relative binding strengths of different compounds to the DOR and KOR receptors, with Morphine serving as a reference point. Some key observations from the table include: Phenol, 2,5-bis(1,1-dimethylethyl) demonstrates comparable binding affinities to both DOR and KOR receptors compared to Morphine. Undec 10-ynoic acid, undecyl ester exhibits higher binding affinity to DOR compared to KOR.- 1-Dodecanol, 2-methyl-, (S)- and 7-Oxabicyclo [4.1.0] heptane, 1,5-dimethyl show lower binding affinities to both DOR and KOR receptors compared to other compounds in the list.

4. DISCUSSION OF FINDINGS

Pain management remains a critical challenge in the field of medicine, with researchers continually seeking effective and safe therapeutic interventions. This present study is aimed at investigating the pattern of analgesic activity of *Xylopia aethiopica* over time. Tail flick tests, which are well-established methods for studying pain perception in animal models, were used to assess the efficacy of the compound. The studies were performed in two phases, each yielding information regarding the pain-modulating properties of the test substances. The results for phase I showed that morphine, at 5 mg/kg and 10 mg/kg, significantly increased the tail flick response time, hence showing reduced perception of pain. On the other hand, both the 25mg/kg and 50mg/kg of *Xylopia aethiopica* extract had a clear increase in the tail flick time compared with the pain-induced-only group. The results were an indication that these compounds have some analgesic activity, attenuating the pain response. The second phase elaborated on how the pain-modulating properties of the substances had evolved over time. The animals in the control group still continued with almost constant response times, while the "pain-only group" experienced relatively shorter response times consistently, confirming the sustainability of pain. More interestingly, after the different periods, they showed different effects on sensitivity to pain but with significant improvements in the response times, compared to animals in the pain-only group. This was in view of the observed remarkable increase in the tail flick response time for the morphine-treated groups at 10 mg/kg and the groups treated with *Xylopia aethiopica* at different phases of the experimental period. In the light of the foregoing, these findings demonstrate that such complexes have potential analgesic activities capable of modulating pain responses. The study agreed with that of Woode et al. [23], who reported that xylopic acid displayed remarkable analgesic activity against acetic acid-induced visceral nociception, formalin-induced neurogenic, and inflammatory paw pain, thermal pain and carrageenan-induced mechanical and thermal hyperalgesia in rats. The activities that affect the perception of pain, especially pain hypersensitivity, were elicited by various treatments, as shown by the results in the study above. Pain hypersensitivity is characterized by heightened sensitivity to pain stimuli and is a

common feature of various conditions, notably including chronic pain, neuropathic pain, and inflammatory pain. Thus, understanding how the different interventions modulate pain responses becomes very important in the development of an efficient strategy for the management of pain hypersensitivity. Such treatments were able to influence the tail flick response times, hence their potential in modulating pain sensitivity and reducing pain perception in this experimental setup. Studies have shown that *Xylopia aethiopica* plants may be used for pain-related conditions according to the research conducted by. The natural compounds demonstrated anti-inflammatory and analgesic properties contributing to the attenuation of pain hypersensitivity in the results of the study. Pain and pain hypersensitivity, especially hyperalgesia, are complex phenomena involving the perception and modulation of pain signals within the body. The lessons learned from obtained experimental results, using the Analgesy-Meter on pain threshold values in pain-induced conditions with various treatments, contribute greatly towards the pain modulation process and the efficacies of various treatments. Different kinds of responses, obtained from various treatment groups under the pain stimuli given in the study [24], throw light on the complex nature of pain hypersensitivity. In the last phase of the experiment, the large differences in the values of the pain threshold were noted across the treatment groups, indicating the involvement of morphine and *Xylopia aethiopica* in modulating sensitivity to pain. In previous studies, these findings are consistent with those found in hyperalgesic alleviation and enhancement of strategies in the management of pain.

4.1 Motor Functions

Motor coordination and balance are parameters of physical function that exert an impact on a good deal of how an individual's ability to perform daily tasks and, in fact, maintain overall well-being. The ability to move efficiently and balance is important in activities such as walking and running, even in simple activities like reaching for something or standing up from a sitting position. Impairment of motor coordination and balance can not only affect physical functioning but also increase falls and injuries, particularly in vulnerable populations like the elderly or people with medical conditions. The results presented in Table show the effect of various treatments on motor coordination and balance assessed by beam walking under different phases of the experimental model. *Xylopia aethiopica* demonstrated its efficiency in improving the motor deficits associated with chronic pain. Higher doses generally showed greater improvement, thus further supporting that these treatments actually do have potentials as interventions for persons experiencing impairments related to pain. The positive effects seen in the *Xylopia aethiopica* group add weight to its use in improving motor function in individuals experiencing pain-related impairment. These results are, therefore, in agreement with previous literature on the beneficial effects of *Xylopia aethiopica* on the management of pain [25]. As noted by the authors, such improvements in motor function for the treated group receiving the *Xylopia aethiopica* treatment underscore its potential to be of very valuable intervention addressing motor deficits associated with chronic pain. Morphine Withdrawal Morphine is an opioid analgesic drug with potent activity. Clinical use of the drug includes for the treatment of moderate to severe pain. During the phase of morphine withdrawal, it has been found that both groups receiving morphine (Pain + 5mg/kg Morphine and Pain + 10mg/kg Morphine) significantly reduced tail flick times when compared with the group Pain Only. Hence, their results have demonstrated an enhanced pain sensitivity or hypersensitivity during withdrawal from morphine. Although this was a sufficient pain control using morphine, considerations had to be taken into account for possible tolerance and dependence problems that could arise. *Xylopia aethiopica* Withdrawal: Although there was known medicinal value for *Xylopia aethiopica*, the study continued to look into its possible analgesic efficacy during withdrawal. Groups experiencing *Xylopia aethiopica* withdrawal, Pain + 25mg/kg *Xylopia aethiopica* and Pain + 50mg/*kg Xylopia aethiopica* did not demonstrate any significant change in tail flick times from the Pain Only group. This means that withdrawal of *Xylopia aethiopica* increased sensitivity to pain, indicative of its potency as a natural analgesic in managing pain hypersensitivity. Motor functions were generally sustained in the Morphine and *xylopia* group. It showed that the plant *Xylopia aethiopica* might be important in neurological disorders associated with pain: The drugs that have the capacity to improve motor functions may offer new therapeutic opportunities for diseases in patients suffering from neurological disorders such as Parkinson's disease, multiple sclerosis, stroke, or spinal cord injuries. Such drugs have the

potential to enhance mobility, coordination, and quality of life if they have the capacity to improve motor skills among affected individuals. Also, drugs improving motor functions can act through mechanisms promoting neuroplasticity: the brain's ability to reorganize and form new neural connections. This could provide opportunities to enhance the recovery from brain injuries, promote learning/skill acquisition, or support changes in the nervous system for adaptive purposes [26-28].

4.2 Molecular Interactions of Identified Compounds of *Xylopia aethiopica* with Opioid Receptors (Delta, and Kappa)

This interactions of compounds of *Xylopia aethiopica* to delta and kappa receptors enabled us to infer from our research findings about *Xylopia aethiopica* as a potential source of new drug agents targeting opioid receptors: The compounds obtained from the plant, such as Phenol, 2,5-bis(1,1-dimethylethyl), display opioid receptor binding activity comparable with Morphine, which could suggest the potential presence of analgesic effects. Other compounds with higher affinities, such as Undec 10-ynoic acid, undecyl ester and Heneicosane, could also be further researched in terms of selective targeting of specific opioid receptors. Compounds that bind with lower affinities could be further studied for their pharmacological relevance and how they can be developed for application in pain alleviation [29-31]. The data in general shows very useful insights about the binding affinities of various compounds isolated from *Xylopia aethiopica* towards opioid receptors, highlighting its potential as a plant with a source of new drug candidates for the modulation of the opioid system and pain management. DOR: Principally involved in the regulation of analgesia, mood modulation, and emotional responses. The DOR agonists have been shown to be helpful in pain relief without the usual side effects of conventional opioid medicines. It has been shown that DOR agonists are helpful in pain relief without usual side effects, as seen with conventional opioid medicines. Phenol, 2,5-bis(1,1-dimethylethyl), which is the most active compound identified in the plant, reveals a binding property comparable to morphine, thus showing a potential as an analgesic. These phenolic compounds are bioactive and can work by stabilizing receptor conformations that promote activation [32-34]. Undec-10-ynoic acid, undecyl ester seems to exhibit higher affinity for the DOR and thus may indicate its potential as a selective DOR agonist [35,36]. Such selectivity could be harnessed in designing new analgesics that engender fewer side effects relative to non-selective opioids. Compounds with extremely high affinities could result in strong analgesic effects, while compounds with less variance in affinities might more subtly affect the modulation of the receptor activity to alleviate pain without the concern of large side effects. Inevitably, some compounds would exhibit relatively low binding affinities, but this alone does not make them less pharmacologically relevant. These compounds can either act as allosteric modulators, altering receptor activity, or modulate the action of more potent ligands [37-40].

5. CONCLUSION

Xylopia aethiopica extract was found to be effective in the management of pain and pain-induced motor dysfunction with no implication in pain hypersensitivity, the analgesic effect of the extract as seen in the result is similar to that of morphine. Furthermore, the chemical compound “Undec 10-ynoic acid, undecyl ester” identified in the extract exhibited a better binding properties to opioid receptors further validating its efficacy in pain management. The potential of *Xylopia aethiopica* as a source of novel analgesic agents targeting opioid receptors is promising. The identified compounds may serve as a foundation for new drug developments aimed at modulating pain pathways, regulating pain-associated motor deficits while mitigating the risks associated with traditional opioid therapies. Future research should focus on elucidating and, optimizing lead compounds, and evaluating the therapeutic potential in clinical contexts.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval for the study was granted by the University of Port Harcourt with reference number UPH/CEREMAD/REC/MM74/009.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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