Original Research Article

MODULATORY EFFECTS OF MORPHINE *AND XYLOPIA AETHIOICA* EXTRACT ON KAPPA OPIOD RECEPTORS (KOR), Δ OPIOID RECEPTOR (DOR), PAIN HYPERSENSITIVITY AND MOTOR FUNCTIONS IN WSTAR RATS

Comment [BO1]: Replace this with Delta

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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 with electroconvulsive stimuli and a hot plate, and a *Xylopia aethiopica* group receiving either 25 mg/kg or 50 mg/kg of hydromethanolic extract following similar pain induction. Pain perception was quantified using the tail flick test and anagelsymeter 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. 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. Future research should focus on optimizing the pharmacological profiles of identified compounds for clinical use.

Comment [BO3]: Mention how the docking studies was conducted and how you evaluated the binding affinities

Comment [BO4]: What of Morphine?

Keywords: *Xylopia aethiopica*, opioid receptors, analgesic, motor function, Wistar rats.

1. Introduction

Pain is a complex and multifaceted experience that can significantly impact quality of life. It is both a physiological response and a subjective sensation that involves various pathways and receptor systems in the body [1]. Opioid receptors, including kappa (KOR), delta (DOR), and mu (MOR) receptors, play a crucial role in the modulation of pain perception [2]. The mu opioid receptor is often the focus in pain management due to the analgesic effects of opioid drugs such

as morphine. However, the use of morphine is coupled with several limitations, including tolerance, dependence, and increased pain sensitivity, known as opioid-induced hyperalgesia [2]. This highlights the need for alternative approaches to pain relief that may circumvent these issues.

Kappa and delta opioid receptors are of particular interest due to their unique pharmacological profiles. Kappa receptors are primarily associated with analgesia, but their activation can also lead to side effects such as sedation and dysphoria [3]. Conversely, delta receptors have been implicated in modulating emotional responses to pain and may play a role in pain relief without the same adverse effects typically seen with mu receptor activation [4]. Understanding the balance and interaction between these receptors is essential for developing effective pain management strategies.In the quest for alternative analgesics, plants used in traditional medicine have garnered attention for their potential therapeutic properties [5]. One such plant is *Xylopia aethiopica*, commonly known as African guinea pepper or West African pepper [6]. This perennial shrub has been utilized for centuries in various cultures for its medicinal properties, including analgesic, anti-inflammatory, and antimicrobial effects [7]. Preliminary studies have indicated that extracts from *Xylopia aethiopica* may influence pain pathways [8], making it a candidate for further investigation as a natural adjunct or alternative to conventional opioid treatments.

The rise in the global prevalence of chronic pain conditions, alongside the escalating opioid epidemic, underscores the urgent need for the development of safer and more effective pain management strategies [9]. Chronic pain affects millions of individuals worldwide and significantly impacts their quality of life, leading to increased healthcare costs and a burden on mental health [10].Despite the traditional usage of *Xylopia aethiopica*, there remains a considerable gap in the scientific understanding of its mechanisms of action and therapeutic

potential in pain management [11]. Recent advances in pharmacology and neuroscience provide an opportunity to rigorously investigate the effects of *Xylopia aethiopica* extracts on pain modulation, including their interactions with opioid receptors [12]. Specifically, exploring how these extracts influence kappa (KOR) and delta (DOR) opioid receptors pathways could yield valuable insights into their analgesic efficacy and potential as adjuncts to conventional therapies.

The interplay between natural plant extracts and synthetic opioids presents a fascinating area of by examining the modulatory effects of morphine in comparison to *Xylopia aethiopica* extracts on pain hypersensitivity and motor functions in Wistar rats, this study aims to explore their respective roles in pain and its associated motor dysfunction management. Through this exploration, we hope to advance our understanding of alternative analgesic therapies, ultimately contributing to the reduction of opioid misuse and providing safer, effective strategies for managing chronic pain.

2. Materials and Methods

Experimental animals weighing between 80–100g obtained from the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt were used for this study and they were provided with standard laboratory rat feeds and water *ad libitum*. The experiment was structured into three distinct groups, each subjected to different treatment protocols to evaluate their responses to pain and motor functions tests. Group 1 served as the control group, with subjects in Control 1 administered distilled water and maintained in a stress-free environment throughout the experiment. They were then exposed to cognito-motor tests. In Control 2, subjects were subjected to pain using electroconvulsive unit to deliver 3A of electricity, hot plate, and anagelsymeterand were exposed to various tests without any drug treatment, facilitating a comparison for the effects of other treatments. Group 2, the

Comment [BO5]: Restructure this. Example " "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."

morphine group, received repetitive pain stimuli through the use of electroconvulsive unit and hot plate and thereafter treated with a low dose of morphine (5 mg/kg) or a high dose (10 mg/kg). Following treatment, subjects were evaluated through various cognitomotor tests. Similarly, Group 3, the *Xylopia aethiopica* group, was administered (25 mg/kg) and (50 mg/kg) doses of hydromethanolic extract, with the animals undergoing the same set of pain sensitivity and cognitomotor tests after treatment. This structured approach allowed for systematic investigation of pain sensitivity and motor responses across different treatments, providing valuable insight into the efficacy of morphine and *Xylopia aethiopica* in managing pain and pain-induced motor dysfunction. The study involved a comprehensive analysis of *Xylopia aethiopica* compounds, using GC/MS. Data acquisition included scanning methods and integration via ChemStation, identifying the unknown spectrum as Apex through NIST14.L libraries. The experiments included several tests: the Rotarod test measured coordination and balance, the Climbing/Beam Walk test evaluated fine motor coordination, the Handgrip test evaluated 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 Opiod Receptors receptors,were retrieved from the Protein Data Bank, with ligands sourced from PubChem and converted to the appropriate formats [14]. 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 [13]. Additionally, pharmacokinetic properties such as molecular weight and logP were calculated for selected compounds based on Lipinski's rule of five [15], while statistical

analysis employed one-way ANOVA with Newman-Keuls post-hoc tests to determine significant differences among treatment groups. Ethical approval for the study was granted by the University of Port Harcourt.

Comment [BO6]: Break down the text into distinct sections with subheadings for better structure (e.g., Experimental Animals, Treatment Groups, Behavioral Tests, Chemical Analysis, In Silico Studies, and Statistical Analysis).

Comment [BO7]: Add the reference number for the ethical approval. You also need to mention in the method section that the animals were handled using standard procedure. The housing conditions and feeding of the animal model should be mentioned as well.

3. Results

Table 1: Identified chemical compounds in *Xylopia aethiopica*

Comment [BO8]: There should be little explanation for this table

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.	Undec10-ynoicacid,undecylester	14.164	$C_{22}H_{40}O_2$	336.5518	2.11
5.	Hexadecanoicacid,methylester	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.	Heptadecanoicacid,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-ynoicacid,nonylester	16.883	$C_{20}H_{36}O_2$	308.4986	4.02
13.	Undec10-ynoicacid,undecylester	17.127	$C_{22}H_{40}O_2$	336.5518	3.72
14.	2-Decen-1-ol, (E)-	17.439	-	-	4.78

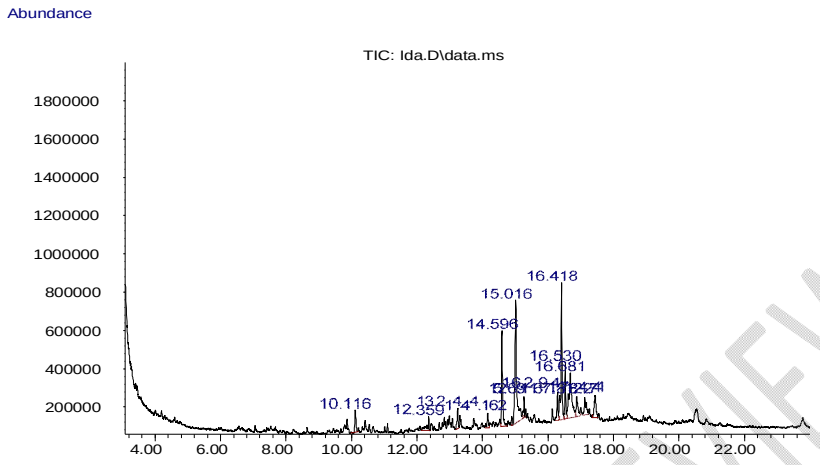

Figure 1: Chromatogram from GC-MS screening of the Extract of *Xylopia aethiopica*

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Table 2. Pain perception using Tail flick test

Groups	Week 1 Time (s)	Week 2 Time (s)
Group 1 (Control)	1.60±0.25	1.20±0.37
Group 2 (Pain Only)	1.30±0.25	1.40±0.25
Group 3 (Pain + 5mg/kg Morphine)	2.60*±0.45	2.20*±0.20
Group 4 (Pain + 10mg/kg Morphine)	3.00±0.25	2.20*±0.25
Group 9 (Pain + 25mg/kg *Xylopiaeathiopica*)	2.40±0.40	2.60*#±0.25
Group 10 (Pain + 50mg/kg *Xylopiaeathiopica*)	2.40±0.40	2.63*#±0.25

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

Comment [BO10]: use this "*#" after the Standard deviation and make is superscript.

The table above revealed the results of tail flick test in the first phase of the work which lasted for 14 days. (Pain + 5mg/kg Morphine) and G (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 *Xylopiaeathiopica*) and (Pain + 50mg/kg *Xylopiaeathiopica*) demonstrated significant increases in tail flick time compared to the Pain Only group in Week 2. This implies that the administration of *Xylopiaeathiopica* 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 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	2.00a±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 *Xylopiaeathiopica*)	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 *Xylopiaeathiopica*)	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 ($p \leq 0.05$) when compared to the control, # means values are statistically significant ($p \leq 0.05$) 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 *Xylopiaeathiopica*) and Group 10 (Pain + 50mg/kg *Xylopiaeathiopica*) 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 *Xylopiaeathiopica* in modulating pain responses in this experimental context.

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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 + 23mg/kg *Xylopiaeathiopica*)	10.46±2.73	16.58#±3.23
(Pain 50mg/kg *Xylopiaeathiopica*)	11.46±2.73	16.58#±3.23

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

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 + *Xylopiaeathiopica*) displayed significant increases in pain threshold values in Week 2 compared to the Pain Only group, implying a potential analgesic effect of *Xylopiaeathiopica* treatment.

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

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 *Xylopiaeathiopica*)	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 *Xylopiaeathiopica*)	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

Test

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 *Xylopiaeathiopica*) and Group 10 (Pain + 50mg/kg *Xylopiaeathiopica*) both demonstrated improvements in pain threshold values over the weeks, with significant differences compared to the Pain Only group in multiple instances. Group 11 (Pain + *Xylopiaeathiopica* + *Bryophyllumpinnatum*) and Group 12 (Pain + *Xylopiaeathiopica* +

Bryophyllumpinnatum + Morphine) showed varying effects on pain threshold values, with weeks 6,7,8,9 indicating significant differences compared to the Pain Only group.

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 *Xylopiaeathiopica*)	126.00[*#]±0.12	204.28[*#]±59.05
(Pain + 50mg/kg *Xylopiaeathiopica*)	180.00[*#]±0.00	224.28[*#]±59.05

Values are presented in mean ± sem, n= 5. * Means values are statistically significant ($p \leq 0.05$) when compared to the control, # means values are statistically significant ($p \leq 0.05$) when compared to 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 + *Xylopiaeathiopica*) displayed significant improvements in both weeks compared to the Pain Only group. the results suggest that the treatments involving *Xylopiaeathiopica* 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.

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 *Xylopiaeathiopica*)	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 *Xylopiaeathiopica*)	120.00*#±0.42	146.00*±0.42	300.00±0.00	188.00±0.00	167.00b±0.00	162.00*#±0.00	157.92a±25.98

Values are presented in mean ± sem, n= 5. * Means values are statistically significant ($p \leq 0.05$) when compared to the control, # means values are statistically significant ($p \leq 0.05$) when compared to 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 + *Xylopiaeathiopica*) also exhibited improvements in various weeks compared to the 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	Undec10-ynoicacid,undecylester	-7.9	-7.0
5	Hexadecanoicacid,methylester	-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	Heptadecanoicacid,16-methyl-	-7.2	-6.9
11	9,17-Octadecadienal,(Z)-	-6.0	-6.3
12	Undec-10-ynoicacid,nonylester	-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.

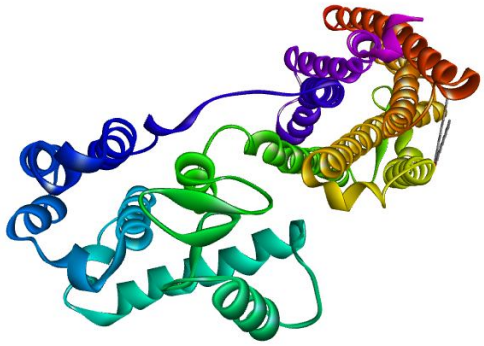

Figure 2: 3D view of the binding of undec10-ynoicacid,undecylester to DOR

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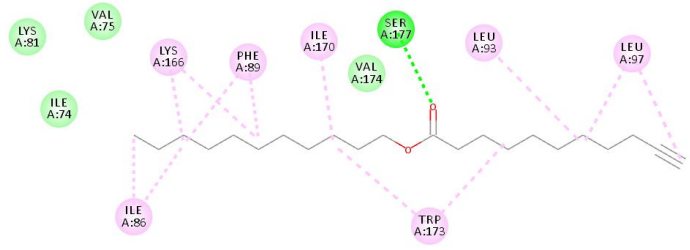

Figure 3: 2D view of the interaction between undec10-ynoicacid,undecylester and amino acids in the binding site of DOR

Figure 4: 3D view of the binding of phenol,2,5-bis(1,1-dimethylethyl) to KOR

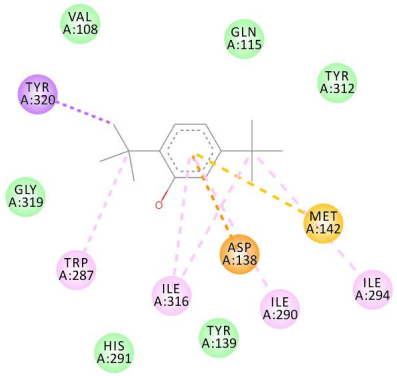

Figure 5: 2D view of the interaction between phenol,2,5-bis(1,1-dimethylethyl) and amino acids in the binding site of KOR

4. Discussion of Findings

Pain management remains a critical challenge in the field of medicine, with researchers continually seeking effective and safe therapeutic interventions. The present study explores the analgesic pattern of *Xylopia aethiopica* over time. The tail flick test, a widely recognized method

Comment [BO13]: Try to limit the repetition on the discussion of results which you have started in the result section.
Also, try to have a more elaborate discussion by making references to other works agreement or disagreement with your study. Also, state the reasons of such agreement/disagreement.

for assessing pain perception in animal models, to evaluate the efficacy of the compound. The study was conducted in two phases, each providing valuable insights into the pain-modulating properties of the test substances.In the first phase, the results revealed that the administration of morphine (at 5 mg/kg and 10 mg/kg) led to a significant increase in tail flick response time, indicating a reduction in pain perception. Similarly, *Xylopiaeathiopica*, when administered at 25 mg/kg and 50 mg/kg, also demonstrated a notable increase in tail flick time compared to the pain-only group. These findings suggest that these compounds possess analgesic properties, capable of attenuating pain responses in the experimental animals. The second phase of the study provided a more comprehensive understanding of the pain-modulating effects of the test substances. The control group exhibited relatively consistent response times, while the pain-only group consistently displayed reduced response times, confirming the sustained presence of pain. Interestingly, the various treatment groups exhibited diverse effects on pain perception, with some displaying significant improvements in response times compared to the pain-only group, while others showed more variable results. Notably, the groups receiving morphine (10 mg/kg), and combinations of *Xylopiaeathiopica* and demonstrated significant increases in tail flick response times at various stages of the study period. These findings suggest that these compounds hold promising analgesic potential, capable of modulating pain responses in this experimental context.The findings of the study presented above provide valuable insights into the effects of various treatments on pain perception, particularly in the context of pain hypersensitivity. Pain hypersensitivity refers to an increased sensitivity to pain stimuli, often seen in conditions such as chronic pain, neuropathic pain, and inflammatory pain. Understanding how different interventions modulate pain responses is crucial in developing effective strategies for managing pain hypersensitivity.These treatments were able to influence the tail flick response

Comment [BO14]: Rephrase to have more meaning

times, indicating their potential in modulating pain sensitivity and reducing pain perception in the experimental context.Studies by [16] have highlighted the potential pain-relieving properties of *Xylopiaeathiopica*, respectively. These natural compounds have been shown to exhibit anti-inflammatory and analgesic effects, which may contribute to their ability to alleviate pain hypersensitivity, as observed in the study's results.Pain and pain hypersensitivity, particularly hyperalgesia, are complex phenomena that involve the perception and modulation of pain signals in the body.The experimental findings from the study examining the impact of various treatments on pain threshold values in pain-induced conditions using the Analgesymeter offer valuable insights into pain modulation and treatment efficacy. The research conducted [17] provides a detailed analysis of the responses observed in different treatment groups exposed to pain stimuli, shedding light on the complex nature of pain hypersensitivity.In the final phase of the experiment, significant changes in pain threshold values continued to be observed across treatment groups, further emphasizing the role of morphine, and *Xylopiaeathiopica* in modulating pain sensitivity. These findings are in line with previous studies by [17] on the efficacy of these treatments in alleviating hyperalgesia and enhancing pain management strategies

Motor Functions

Motor coordination and balance are critical aspects of physical function that significantly impact an individual's ability to perform daily tasks and maintain overall well-being. The ability to move efficiently and maintain balance is essential for activities such as walking, running, and even simple actions like reaching for an object or standing up from a seated position. Impairments in motor coordination and balance can not only affect physical functioning but also increase the risk of falls and injuries, particularly in vulnerable populations such as the elderly or individuals with

medical conditions. The results presented in the table highlight the effects of various treatments on motor coordination and balance, as assessed through Beam walking in different phases of the experimental model.*Xylopiaeathiopica* demonstrated their efficacy in ameliorating motor deficits associated with chronic pain, with higher dosages generally correlating with more pronounced improvements further supporting the notion that these treatments hold promise as interventions for individuals experiencing pain-related impairments. The positive effects observed in the group treated with *Xylopia aethiopica* support its role in enhancing motor function in individuals experiencing pain-related impairments. Furthermore, the results of the current study are in line with existing literature on the benefits of *Xylopiaeathiopica* in pain management[18]. The improvements in motor function seen in the group receiving *Xylopiaeathiopica* treatment underscore its potential as a valuable intervention for addressing motor deficits associated with chronic pain. These treatments offer valuable options for enhancing functional outcomes and quality of life for individuals experiencing chronic pain.Morphine Withdrawal: Morphine, a potent opioid analgesic, is often used to manage moderate to severe pain. During morphine withdrawal, groups subjected to morphine (Pain + 5mg/kg Morphine and Pain + 10mg/kg Morphine) exhibited significant reductions in tail flick times compared to the Pain Only group. These findings suggest that morphine withdrawal led to increased pain sensitivity or hypersensitivity. While morphine effectively controlled pain, considerations must be made for potential tolerance and dependence issues that may arise.*Xylopia aethiopica* Withdrawal: Xylopia Aethiopica, with its known medicinal properties, was also investigated for its potential analgesic effects during withdrawal. Groups undergoing *Xylopia aethiopica* withdrawal (Pain + 25mg/kg *Xylopia aethiopica* and Pain + 50mg/kg Xylopia Aethiopica) demonstrated no significant changes in tail flick times compared to the Pain Only group. This indicates that

Xylopia aethiopica withdrawal increased pain sensitivity, suggesting its potential as a natural analgesic for managing pain hypersensitivity.Motor functions were generally sustained in Morphine, and xylopia group. This revealed that *Xylopia aethiopica* could be useful in the treatment of neurological disorders associated with pain: Drugs that can improve motor functions may offer new therapeutic options for individuals with neurological disorders such as Parkinson's disease, multiple sclerosis, stroke, or spinal cord injuries. By enhancing motor skills, these drugs could help improve mobility, coordination, and overall quality of life for affected individuals [19].Furthermore, Drugs that improve motor functions may work through mechanisms that promote neuroplasticity, the brain's ability to reorganize and form new neural connections. This could offer opportunities for enhancing recovery after brain injuries, promoting learning and skill acquisition, and supporting adaptive changes in the nervous system [20].

Molecular Interactions of identified compounds of *Xylopia aethiopica* with opioid receptors (delta, and kappa)

In the context of evaluating *Xylopia aethiopica* as a potential source of novel drug agents targeting opioid receptors, we can potentially infer the following: Compounds like Phenol, 2,5-bis(1,1-dimethylethyl) from *Xylopia aethiopica* possess opioid receptor binding properties comparable to Morphine, suggesting potential analgesic effects. Additional compounds such as Undec 10-ynoic acid, undecyl ester with higher affinity for DOR, and Heneicosane could be further investigated for their selective targeting of specific opioid receptors. Compounds with lower binding affinities may warrant additional studies to understand their pharmacological significance and potential applications in pain management [21]. Overall, the data provides valuable insights into the binding affinities of various compounds from *Xylopia aethiopica* to

opioid receptors, indicating the potential of this plant as a source of novel drug candidates for modulating the opioid system and managing pain.

Delta Opioid Receptors (DOR): Primarily associated with analgesia, mood modulation, and the regulation of emotional responses. DOR agonists can be beneficial in pain relief without the same level of side effects commonly associated with traditional opioid medications [22, 23].

Phenol, 2,5-bis(1,1-dimethylethyl) which is the most active compound identified in the plant shows binding properties comparable to morphine, it indicates a potential as an analgesic. Such phenolic compounds are known for their bioactivity and may work by stabilizing receptor conformations conducive to activation [24].Undec 10-ynoic acid, undecyl esterappears to exhibit higher affinity for the DOR, which may suggest its potential as a selective DOR agonist [25]. Such selectivity could be harnessed in designing new analgesics with fewer side effects compared to non-selective opioids.High-affinity compounds may lead to stronger analgesic effects, while those with lower affinities could interact more subtly, modulating receptor activity to alleviate pain without the risk of significant side effects [26].While some compounds may exhibit lower binding affinities, their pharmacological relevance should not be overlooked. These compounds could potentially act as allosteric modulators, altering receptor activity or influencing the effect of more potent ligands [27].

5. Conclusion

Comment [BO15]: State the finding before resorting to the potentials of XA.

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.

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