# *Bryophylumpinnatum* extract modulates BDNF expression:The potential implications for oxidative stressregulationand cognitive functions in pain-induced Wistar rats


## Abstract

This research work investigated modulatory effects of *Bryophylumpinnatum* extract on BDNF expression, and cognitive functions in repetitive pain-induced oxidative stress in Wistar rats. Animals weighing between 80–100g were acquired from the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, and all animals received standard laboratory rat feeds and water ad libitum. The study was designedto assess the time dependent effects with a total of 30 rats divided into 6 groups. Group 1(Control), Group 2 (Pain Only), Group 3 (Pain + 5mg/kg Morphine), Group 4 (Pain + 10mg/kg Morphine), Group (Pain + 25mg/kg BryophylummPinnatum), Group 6 (Pain + 50mg/kg BryophylummPinnatum), Hydromethanolic extracts was prepared accordingly, and Gas Chromatography Mass Spectrometry (GC/MS) analysis were carried out. Neurobehavioral studies were conducted weekly to assess the effects of the interventions on cognitive and neurological parameters, using radial maze and navigational maze test. Assay of BDNF was done using the Elisa method. Through a comprehensive analysis of pharmacological and experimental data, it was found that Morphine and *Bryophyllumpinnatum* demonstrated significant increase in BDNF expression, antioxidant effects, cognitive improvement, and potential pain relief mechanisms, suggesting their therapeutic potential in managing chronic pain and oxidative stress. Furthermore, investigations into the pharmacokinetics, binding affinities, and drug-likeness properties of active compounds from these plant extracts revealed favorable characteristics for oxidative stress management and cognitive functions in pain conditions.



Keywords: BDNF Expression, Cognitive functions, chronic pain, oxidative stress, *Bryophylumpinnatum*




## 1. Introduction:

The management of chronic pain conditions remains a significant challenge in the field of healthcare, with a growing need for innovative and effective treatment strategies [1]. the exploration of natural plant-derived compounds has garnered increasing attention due to their potential therapeutic benefits and relatively lower risk of adverse effects compared to conventional pharmacological interventions [2]. One such promising candidate is the extract of

Bryophyllumpinnatum, a succulent plant native to Madagascar, which has been traditionally used in various traditional medicinal systems. Bryophyllumpinnatum (also known *as Kalanchoe pinnata*) is a medicinal plant traditionally used in various cultures for its pharmacological properties, including anti-inflammatory, analgesic, and antioxidant effects[3]. Recent scientific investigations have demonstrated the potential neuroprotective and cognitive-enhancing properties of *Bryophyllumpinnatum* extract, suggesting its therapeutic relevance in managing neurodegenerative disorders and cognitive dysfunction [3].Brain-derived neurotrophic factor (BDNF) is a crucial neurotrophin that plays a significant role in regulating neuronal growth, development, and plasticity [4]. Deregulation of brain-derived neurotrophic factor (BDNF) levels is associated with various neurological conditions. Low levels of BDNF have been linked to cognitive decline, increased risk of psychiatric disorders, and sensory impairments [5]. Conditions such as chronic pain and oxidative stress may also influence BDNF levels and contribute to cognitive deficits and other neurological issues such as anxiety disorders and depression. Brain-derived neurotrophic factor (BDNF) is a crucial neurotrophin that plays a vital role in the modulation of neuronal plasticity, synaptic function, and cognitive processes. Emerging evidence suggests that alterations in BDNF expression and signaling pathways are closely associated with the pathogenesis of various pain conditions, including neuropathic pain, inflammatory pain, and chronic pain syndromes [6].Emerging evidence suggests that natural compounds with neuroprotective properties, such as *Bryophyllumpinnatum* extract, may modulate BDNF expression and signaling pathways, thus influencing oxidative stress regulation and cognitive functions in animal models [7].

In the context of pain-induced conditions, the interplay between neuronal plasticity, oxidative stress, and cognitive functions is complex and multifaceted [8]. Understanding the molecular

mechanisms involved in BDNF modulation by *Bryophyllumpinnatum* extract could provide valuable insights into the potential therapeutic benefits of this natural compound in alleviating pain, reducing oxidative stress, and improving cognitive abilities in animal models of chronic pain.

This research article aims to investigate the effects of *Bryophyllumpinnatum* extract on BDNF expression and its potential implications for oxidative stress regulation and cognitive functions in pain-induced rats. By elucidating the underlying mechanisms of action of *Bryophyllumpinnatum* extract at the molecular level, this study seeks to contribute to the growing body of literature on natural products with neuroprotective properties and their therapeutic potential in pain management and cognitive enhancement.

## 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 experimental study design was categorized into three phases: Phase 1 (chronic study) where drugs were administered for fourteen days, Phase 2 (sub-chronic study) with a 35-day administration, and Phase 3 (chronic study) lasting 105 days. The animals were grouped as follows: The experiment was structured into three distinct groups, each subjected to different treatment protocols to evaluate their responses to pain and cognitive 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 cognitomotor tests. In Control 2, subjects were also placed under stress-free conditions but were

exposed to various tests without any drug treatment, facilitating a comparison for the effects of other treatments. Group 2, the 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 Bryophylumpinnatumgroup, was administered low (25 mg/kg) and high (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 and cognitive responses across different treatments, providing valuable insight into the efficacy of morphine and *Bryophylumpinnatum* in managing pain and pain-inducedneurological functions.The study involved a comprehensive analysis of Bryophilumpinnatum compounds, using GC/MS. Data acquisition included scanning methods and integration via ChemStation, identifying the unknown spectrum as Apex through NIST14.L libraries. Neurobehavioral studies were conducted weekly on test groups treated with various substance doses, featuring three trials per week to assess outcomes related to neuro biomarkers like BDNF, and Nitric oxide. The experiments included several tests: the Rotarod test measured coordination and balance, the Inverted Screen test assessed muscle strength and endurance, the Climbing/Beam Walk test evaluated fine motor coordination, the Handgrip test evaluated grip strength, and the Barnes Maze test focused on cognitive deficits and spatial learning. Each test employed specific protocols to measure performance, helping to gauge the efficacy of the treatments on coordination, strength, and cognitive functions in rodent models.At the end of each phases, BDNF, and Nitric oxide were assayed using the Elisa method. The protocol involves collecting rat brain tissue samples, which are flushed with cold PBS, minced, homogenized, freeze-thawed, and centrifuged to obtain a supernatant for assay.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 NMDA receptors, were retrieved from the Protein Data Bank, with ligands sourced from PubChem and converted to the appropriate formats. 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. Additionally, pharmacokinetic properties such as molecular weight and logP were calculated for selected compounds based on Lipinski's rule of five, 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.



## 3. Results

**Table 1: Oxidative stress markers**

| Groups/Treatments | GPX (ug/ml) | MDA (mmo/l) | GSH (ug/ml) | CAT (mmo/l) | SOD (mmo/l) |
|---|---|---|---|---|---|
| **Group 1 (Control)** | 0.078#± 0.002 | 0.43#±0.02 | 2.88±0.24 | 2.98#±0.07 | 0.28±0.04 |
| **Group 2 (Pain Only)** | 0.059*±0.001 | 0.53*±0.01 | 1.97*±0.02 | 2.03*±0.05 | 0.24±0.01 |
| **Group 3 (Pain + 5mg/kg Morphine)** | 0.067*#±0.002 | 0.45±0.02 | 2.23#±0.06 | 1.86*±0.16 | 0.34#±0.00 |
| **Group 4 (Pain + 10mg/kg** | 0.076#±-0.000 | 0.45±0.01 | 2.56#±0.02 | 2.26±0.04 | 0.33±0.02 |

| | | | | | |
|---|---|---|---|---|---|
| **Morphine)** | | | | | |
| **Group 5 (Pain + 25mg/kg BryophylummPinnatum)** | 0.083*#±0.000 | 0.57#±0.01 | 2.79#±0.01 | 2.94#±0.13 | 0.20±0.01 |
| **Group 6 (Pain + 50mg/kg BryophylummPinnatum)** | 0.064*#±0.003 | 0.46#±0.01 | 2.85#±0.10 | 2.88#±0.18 | 0.46#±0.01 |

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



**Table 2: Result of cognitive activities using Navigational Maze**

| Groups | Week2 Time (s) | Week 9 Time (s) | Week 15 Time (s) |
|---|---|---|---|
| **Group 1(Control)** | 91.840±8.16 | 53.20#±39.42 | 46.56#±21.98 |
| **Group 2(Pain Only)** | 233.20±9.28 | 299.80±61.38 | 300*±0.00 |
| **Group 3 (Pain + 5mg/kg Morphine)** | 218.84±8.7 | 57.20*#±17.09 | 108.20*#±0.42 |
| **Group 4 (Pain + 10mg/kg Morphine)** | 110.84#±7.21 | 188.04*#±47.42 | 208.80*#±0.42 |
| **Group 5** | 128.20#±6.42 | 185.92*#±53.15 | 213.60*#±0.42 |

| | | | |
|---|---|---|---|
| **(Pain + 25mg/kg *BryophylummPinnatum)*** | | | |
| **Group 6 (Pain + 50mg/kg *BryophylummPinnatum)*** | 112.44*#±8.71 | 300.00*±0.00 | 114.20*#±0.42426 |

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



**Table 3: Result of cognitive Function Test using Radial Maze**

| Groups/Treatment | Week 2 | Week 9 | Week 15 |
|---|---|---|---|
| **Group 1(Control)** | 2.20±0.92 | 3.60#±1.60 | 4.60#±0.51 |
| **Group 2(Pain Only)** | 1.00*±0.78 | 0.60*±0.40 | 0.80*±1.02 |
| **Group 3 (Pain + 5mg/kg Morphine)** | 0.00±0.00 | 3.80#±0.92 | 2.60*±1.08 |
| **Group 4 (Pain + 10mg/kg Morphine)** | 0.00±0.00 | 2.40±0.60 | 3.20#±1.02 |
| **Group 5 (Pain + 25mg/kg BryophylummPinnatum)** | 1.60#±1.17 | 3.40#±0.75 | 1.20*±0.20 |

| | | | |
|---|---|---|---|
| **Group 6 (Pain + 50mg/kg BryophylummPinnatum)** | 0.80±0.80 | 3.00#±1.09 | 3.80#±1.11 |

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



**Table 4: Result of BDNF**

| Groups/treatment | 2 weeks | 9 weeks | 15 weeks |
|---|---|---|---|
| **Group 1 (Control)** | 296.00±13.85 | 252.50**b**±24.53 | 265.00*#±5.6 |

| | | | |
|---|---|---|---|
| **Group 2 (Pain Only)** | 391.50±58.02 | 595.00*±17.17 | 505.00*±6.1 |
| **Group 3 (Pain + 5mg/kg Morphine)** | 304.00±66.97 | 560.500±14.8 | 469.00*±4.8 |
| **Group 4 (Pain + 10mg/kg Morphine)** | 1380.00*±139.14 | 1079.00*b±17.3 | 801.50*#±7.1 |
| **Group 5 (Pain + 25mg/kg BryophylummPinnatum)** | 123.50b±11.25 | 344.00±12.70 | 259.50*±6.4 |
| **Group 6 (Pain + 50mg/kg BryophylummPinnatum)** | 874.00*b±42.72 | 907.50*±27.01 | 1392.50*#±10.6 |

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.



**Table 5: Identified chemical compounds in *Bryophylumpinnatum***

| S/N | Name Of Compound | Retention Time (RT) (Minutes) | Molecular Formular | Molecular Weight (g/mol) | Area% |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| **1.** | Phenol,2,6-bis(1,1-dimethylethyl) | 10.118 | $C_{14}H_{22}O$ | 220.35 | 3.38 |
| **2.** | Benzene,(2-methylpropoxy)- | 14.616 | $C_{10}H_{14}O$ | 150.2176 | 5.49 |
| **3.** | 3-Tridecen-1-yne,(E)- | 14.985 | $C_{13}H_{22}$ | 178.31 | 11.56 |
| **4.** | 1H-Pyrrole-2,5 dione,2,5-dihydro-1 (3,5-dimethylphenyl)- | 15.156 | $C_{14}H_{18}O$ | 202.29 | 10.30 |
| **5.** | 2-Methyl-Z,Z-3,13-octadecadienol | 16.411 | $C_{19}H_{36}O$ | 280.5 | 3.66 |
| **6.** | 9-Oxabicyclo[6.1.0]nonane | 16.686 | $C_8H_{14}O$ | 126.1962 | 10.16 |
| **7.** | Bicyclo[2.2.2]octane,2-chloro- | 16.884 | C9H14O2 | 144.642 | 2.57 |
| **8.** | 9-octadecanoicacid,2,2,3,3,4,4,4-heptafluorobutylester | 17.268 | $C_{22}H_{35}F_7O_2$ | 464.5 | 6.80 |
| **9.** | cis-7-Oxabicyclo[4.3.0]nonan-8-one | 18.414 | $C_8H_{12}O_2$ | 140.18 | 24.53 |
| **10.** | 2-Butynedioicacid,di-2-propenyl ether | 23.561 | - | - | 21.55 |



Table 6: Binding affinity of ligands to Neurotrophin Receptor P75 (p75NTR)and **the tropomycin receptor kinase B (TrkB)**

| | | **Binding affinity (Kcal/mol)** | |
|---|---|---|---|
| **S/N** | Compounds | p75NTR | TRKB |

| | | | |
|---|---|---|---|
| **R** | Lamotrigine | -6.2 | |
| **R** | LM22A-4 | -7.0 | -7.7 |
| **R** | Morphine | -7.2 | -8.2 |
| **1** | Phenol,2,6-bis(1,1-dimethylethyl) | -7.2 | -8.1 |
| **2** | Benzene,(2-methylpropoxy)- | -7.0 | -6.7 |
| **3** | 3-Tridecen-1-yne,(E)- | -5.0 | -7.9 |
| **4** | 1H-Pyrrole-2,5-dione,2,5-dihydro | -5.3 | -5.3 |
| **5** | 2-Methyl-Z,Z-3,13-octadecadienol | -6.6 | -8.1 |
| **6** | 9-Oxabicyclo[6.1.0]nonane | -3.9 | -6.1 |
| **7** | Bicyclo[2.2.2]octane,2-chloro- | -3.1 | -6.3 |
| **8** | 9-octadecanoicacid | -6.3 | -9.1 |
| **9** | cis-7-Oxabicyclo[4.3.0]nonan-8-one | -5.6 | -6.5 |
| **10** | 2-Butynedioicacid,di-2-propenyl | -5.3 | -7.7 |

## 4. Discussion

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 modulatory effects of hydromethanolic extract of *Bryophylumpinnatum*on oxidative stress markers, brain derived neurotropic factor (BDNF) and cognitive functions in repetitive pain-induced Wistar rats.

Table 1 presents the oxidative stress markers measured across different treatment groups, highlighting the effects of pain and various interventions on oxidative stress indicators such as GPX, MDA, GSH, CAT, and SOD. The control group (Group 1) shows the highest levels of GPX (0.078 µg/ml) and GSH (2.88 µg/ml), with the lowest MDA (0.43 mmo/l) and SOD (0.28 mmo/l). In contrast, the pain-only group (Group 2) exhibits lower GPX (0.059 µg/ml) and GSH levels (1.97 µg/ml), along with significant increase in MDA level (0.53 mmo/l), indicating

elevated oxidative stress. Morphine treatment (Groups 3 and 4) yields mixed results, with Group 3 (5 mg/kg) showing improved GPX but reduced CAT activity, and Group 4 (10 mg/kg) maintaining GPX levels but not reducing MDA effectively. *The Bryophyllumpinnatum* treatments (Groups 5 and 6) in varying doses show significant antioxidant effects, with Group 5 (25 mg/kg) reaching the highest GPX (0.083 µg/ml) and GSH (2.79 µg/ml) levels, indicating potential protective effects against oxidative stress. This information is crucial as oxidative stress plays a significant role in various physiological and pathological processes, including pain perception and management. For instance, the observed increase in Glutathione Peroxidase (GPX) levels is indicative of enhanced antioxidant defense mechanisms against reactive oxygen species (ROS) generated during pain conditions. This is consistent with previous research demonstrating the involvement of GPX in reducing oxidative stress and inflammation in pain models [9]. Conversely, the increase in Malondialdehyde (MDA) levels in the pain only group suggests elevated lipid peroxidation and oxidative damage, which could contribute to pain sensitivity and inflammation. Several studies have linked increased MDA levels with pain states and neuroinflammation [10]. The changes in Glutathione (GSH) levels observed in the *Bryophyllumpinnatum*-treated group further emphasize the role of this antioxidant in mitigating oxidative stress and maintaining cellular homeostasis. Similar findings have been reported in studies demonstrating the neuroprotective and anti-inflammatory properties of GSH in pain conditions [11]. Moreover, the alterations in Catalase (CAT) and Superoxide Dismutase (SOD) levels across different treatment groups reflect the dynamic interplay between antioxidant enzymes and ROS regulation in pain modulation. Previous research has highlighted the importance of CAT and SOD in scavenging ROS and reducing oxidative damage in pain-related disorders [12].The results from the cognitive function tests using the Radial Maze revealed

significant differences across the treatment groups over the three weeks. The Control group demonstrated consistent improvement in performance, starting at 2.20 in Week 2 and reaching 4.60 by Week 15. In contrast, the Pain Only group exhibited the poorest performance, with scores decreasing from 1.00 to 0.60 and then slightly recovering to 0.80, indicating continued cognitive impairment. Treatment with Morphine at both 5mg/kg and 10mg/kg yielded variable outcomes: the 5mg/kg dose improved performance notably by Week 9 (3.80), while the 10mg/kg dose showed minimal progression. *BryophyllumPinnatum* (25mg/kg and 50mg/kg) showed significant improvements by Week 9 (3.40 and 3.00, respectively). Notably, the treatments resulted in statistically significant improvements compared to the Pain Only group, particularly in Weeks 9 and 15. The role of *Bryophyllumpinnatum and morphine*on cognitive activities observed in our study are consistent with existing literature highlighting the diverse mechanisms through which these treatments can influence pain perception and memory processes [13]. Previous studies have also implicated the role of opioid receptors, signaling, and plant-derived compounds in modulating pain-induced behaviors, memory formation, and cognitive functions [14].The interpretation of the results from the navigational maze tasks in the study sheds light on the potential effects of different interventions on cognitive function in the presence of pain. Firstly, the Control group (Group 1) consistently demonstrated improved maze completion times over the weeks, implying stable cognitive function without external factors affecting performance. Conversely, the Pain Only group (Group 2) experienced increased maze completion times, indicating a potential negative impact of pain on cognitive abilities. Groups receiving morphine showed varying results, with (Pain + 10mg/kg Morphine) exhibiting significant improvement in cognitive performance. This suggests a potential positive effect of morphine on cognitive function in the presence of pain, consistent with previous studies

highlighting the role of opioids in cognitive enhancement under specific conditions [15]. Similarly, groups administered *Bryophyllumpinnatum* demonstrated improvements in maze completion times, suggesting potential beneficial effects on cognitive function. These results align with research highlighting the neuroprotective and cognitive benefits of phytochemical compounds found in *Bryophyllumpinnatum* [16].Brain-Derived Neurotrophic Factor (BDNF) can be tied to its levels in different treatment groups and their implications for neuronal function and pain modulation.The study demonstrated that certain treatments led to a significant increase in BDNF levels compared to control groups. For example, Group (Pain + 10mg/kg Morphine) consistently showed a substantial elevation in BDNF levels across different phases of the study. This suggests that Morphine administration may potentiate the production or release of BDNF, which could have implications for neuronal survival, growth, and synaptic plasticity.BDNF is known to promote the growth and differentiation of neurons, as well as synaptic plasticity, which are essential for maintaining neuronal function and connectivity [17]. The increased BDNF levels in response to Morphine suggest a potential mechanism by which Morphine exerts its effects on neuronal health. Group (Pain + 25mg/kg *Bryophyllumpinnatum*) also exhibited significant elevations in BDNF levels, particularly in Phase 3 of the study. This indicates that *Bryophyllumpinnatum* may possess neuroprotective properties by promoting the expression of BDNF. Neurotrophic factors like BDNF play a crucial role in enhancing neuronal resilience and protecting against damage or degeneration [18], suggesting a potential therapeutic benefit of *Bryophyllumpinnatum* in preserving neuronal health.The observed changes in BDNF levels in response to different treatments, such as Morphine and *Bryophyllumpinnatum*, suggest a link between BDNF and pain modulation. BDNF is involved in synaptic plasticity and can enhance the transmission of pain signals in the central nervous system [19]. The alterations in BDNF

levels induced by these treatments may influence neuronal pathways related to pain perception and processing, highlighting the complex interplay between neurotrophic factors and pain signaling [20]. Brain-Derived Neurotrophic Factor (BDNF) is a pivotal neurotrophin that plays a crucial role in neuronal survival, differentiation, and synaptic plasticity [21]. It is widely expressed in the brain and peripheral tissues, exerting diverse functions in neural development, neuronal maintenance, and response to injury or stress [22].Altered BDNF levels have been implicated in various neurological disorders, including depression, resulted from chronic pain [23]. Studies have suggested that dysregulation of BDNF signaling pathways contributes to the pathogenesis of these disorders and that targeting BDNF may offer therapeutic benefits [24]. BDNF plays a crucial role in synaptic plasticity, the ability of synapses to strengthen or weaken in response to neural activity. By enhancing synaptic transmission and promoting neuronal connectivity, BDNF contributes to learning and memory processes as seen in the result of the study.Emerging evidence suggests that BDNF plays a role in pain modulation by influencing nociceptive signaling pathways in the central nervous system. Studies have shown that alterations in BDNF levels can impact pain sensitivity and contribute to chronic pain conditions [25].The analysis of chemical compounds in Bryo*phyllumpinnatum* reveals a diverse array of 10 identified compounds, each characterized by unique molecular formulas, weights, and retention times, indicating a complex chemical profile. Notably, "cis-7-Oxabicyclo[4.3.0]nonan-8-one" emerges as the most abundant compound, comprising 24.53% of the sample, followed by "2-Butynedioic acid, di-2-propenyl ether" at 21.55%, and "9-Oxabicyclo[6.1.0]nonane" at 14.83%. Other compounds include "Bicyclo[2.2.2]octane, 2-chloro-" at 2.57%, "9-octadecanoic acid, 2,2,3,3,4,4,4-heptafluorobutyl ester" at 4.37%, and "2,3-Dihydro-1H-pyrrolo[3,4-b]quinolin-1-one" at 4.12%. The molecular weights of these compounds range from 126.20 g/mol to 464.5

g/mol, reflecting a variety of structural complexities that may contribute to the plant's pharmacological properties. This diversity in chemical composition underscores the potential of *Bryophyllumpinnatum* for further research into its therapeutic applications, as the different compounds may possess unique biological activities that warrant exploration in medicinal contexts. Hence the molecular docking of the identified compound to BDNF receptors was carried out in our research.The binding affinity of various ligands to the Neurotrophin Receptor P75 (p75NTR) and the tropomycin receptor kinase B (TrkB) is summarized in Table 6. The most potent binding affinities observed for p75NTR are from Morphine and Phenol, 2,6-bis(1,1-dimethylethyl), both showing values of -7.2 kcal/mol, closely followed by LM22A-4 at -7.0 kcal/mol. For TrkB, Morphine also displays a strong affinity at -8.2 kcal/mol, with LM22A-4 showing a competitive affinity at -7.7 kcal/mol. Other notable compounds include 2-Methyl-Z,Z-3,13-octadecadienol, which binds strongly to both receptors (-6.6 kcal/mol for p75NTR and -8.1 kcal/mol for TrkB), and 9-octadecanoic acid, which exhibits a stronger affinity to TrkB (-9.1 kcal/mol) compared to its p75NTR affinity (-6.3 kcal/mol). The data highlights the varying affinities of different compounds for each receptor, with some compounds preferring one receptor over the other. This information can be instrumental for further research into the therapeutic implications of these ligands in modulating neurotrophic signaling pathways.The binding affinities of ligands to the Neurotrophin Receptor P75 (p75NTR) and the tropomycin receptor kinase B (TrkB) have significant implications for Brain-Derived Neurotrophic Factor (BDNF) expression and its associated biological functions. BDNF is a critical neurotrophin involved in neuronal survival, growth, differentiation, and synaptic plasticity, and it exerts its effects primarily through TrkB receptors.Overall, this result may be implicated in the following:

TrkB Activation: Compounds with high binding affinity for TrkB, such as 9-octadecanoic acid (-9.1 kcal/mol), may enhance BDNF signaling by promoting TrkB receptor activation. This activation can lead to increased BDNF expression, which is essential for neuroprotection and cognitive functions. Enhanced TrkB signaling can also facilitate neurogenesis and synaptic plasticity, which are vital for learning and memory [26].

p75NTR Role: The p75NTR receptor, while having a lower binding affinity for most compounds, plays a crucial role in modulating the effects of BDNF. It can act as a co-receptor with TrkB, influencing the signaling pathways activated by BDNF. Compounds that bind to p75NTR may alter the balance of signaling pathways, potentially leading to different outcomes in neuronal survival and differentiation [27]. For instance, while TrkB activation promotes survival and growth, p75NTR can mediate apoptosis under certain conditions. Therefore, the interaction of ligands with p75NTR could modulate the overall effects of BDNF.

Therapeutic Potential: The varying affinities of these compounds for p75NTR and TrkB suggest that they could be explored as potential therapeutic agents for conditions associated with BDNF dysregulation, such as depression, neurodegenerative diseases, and cognitive disorders [28]. Compounds that selectively enhance TrkB signaling while minimizing p75NTR-mediated negative effects could provide a targeted approach to boost BDNF expression and its neuroprotective effects.



## 5. Conclusion

The emerging evidence on the effects of *Bryophyllumpinnatum* extract in pain-induced rat models highlights its promising potential as a therapeutic agent for the management of chronic

pain conditions. The extract's ability to modulate BDNF expression, regulate oxidative stress, and potentially support cognitive functions suggests a multifaceted approach to addressing the complex pathophysiology underlying various pain syndromes. The binding affinities of ligands of Bryophylumpinnatum extract to p75NTR and TrkB have important implications for BDNF expression and signaling, highlighting potential therapeutic avenues for enhancing neuroprotection and cognitive function through modulation of neurotrophic pathways.

**References**


1. Themelis, K., & Tang, N. K. Y. (2023). The Management of Chronic Pain: Re-Centring Person-Centred Care. *Journal of clinical medicine*, *12*(22), 6957. https://doi.org/10.3390/jcm12226957
2. Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., &Bezirtzoglou, E. (2021). Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms, 9(10), 2041. https://doi.org/10.3390/microorganisms9102041
3. Araújo, E. R. D., Xavier-Santos, J. B., da Silva, V. C., de Lima, J. B. F., Schlamb, J., Fernandes-Pedrosa, M. F., da Silva Júnior, A. A., de Araújo Júnior, R. F., Rathinasabapathy, T., Moncada, M., Esposito, D., Guerra, G. C. B., &Zucolotto, S. M. (2023). Gel formulated with Bryophyllumpinnatum leaf extract promotes skin wound healing in vivo by increasing VEGF expression: A novel potential active ingredient for pharmaceuticals. Frontiers in pharmacology, 13, 1104705. https://doi.org/10.3389/fphar.2022.1104705
4. Martins Fernandes Pereira, K., Calheiros de Carvalho, A., André Moura Veiga, T., Melgoza, A., Bonne Hernández, R., Dos Santos Grecco, S., Uchiyama Nakamura, M., & Guo, S. (2022). The psychoactive effects of Bryophyllumpinnatum (Lam.) Oken leaves in young zebrafish. *PloS one*, *17*(3), e0264987. https://doi.org/10.1371/journal.pone.0264987
5. Correia, A. S., Cardoso, A., & Vale, N. (2023). BDNF Unveiled: Exploring Its Role in Major Depression Disorder Serotonergic Imbalance and Associated Stress Conditions. *Pharmaceutics*, *15*(8), 2081. https://doi.org/10.3390/pharmaceutics15082081
6. Salim S. (2017). Oxidative Stress and the Central Nervous System. *The Journal of pharmacology and experimental therapeutics*, *360*(1), 201–205. https://doi.org/10.1124/jpet.116.237503

7. Pisani, A., Paciello, F., Del Vecchio, V., Malesci, R., De Corso, E., Cantone, E., &Fetoni, A. R. (2023). The Role of BDNF as a Biomarker in Cognitive and Sensory Neurodegeneration. *Journal of personalized medicine*, *13*(4), 652. https://doi.org/10.3390/jpm13040652

8. De Sousa, L. P., Rosa-Gonçalves, P., Ribeiro-Gomes, F. L., & Daniel-Ribeiro, C. T. (2023). Interplay Between the Immune and Nervous Cognitive Systems in Homeostasis and in Malaria. *International journal of biological sciences*, *19*(11), 3383–3394. https://doi.org/10.7150/ijbs.82556

9. Pei, J., Pan, X., Wei, G., & Hua, Y. (2023). Research progress of glutathione peroxidase family (GPX) in redoxidation. *Frontiers in pharmacology*, *14*, 1147414. https://doi.org/10.3389/fphar.2023.1147414

10. Cordiano, R., Di Gioacchino, M., Mangifesta, R., Panzera, C., Gangemi, S., &Minciullo, P. L. (2023). Malondialdehyde as a Potential Oxidative Stress Marker for Allergy-Oriented Diseases: An Update. *Molecules (Basel, Switzerland)*, *28*(16), 5979. https://doi.org/10.3390/molecules28165979

11. Rehman, M. U., Wali, A. F., Ahmad, A., Shakeel, S., Rasool, S., Ali, R., Rashid, S. M., Madkhali, H., Ganaie, M. A., & Khan, R. (2019). Neuroprotective Strategies for Neurological Disorders by Natural Products: An update. *Current neuropharmacology*, *17*(3), 247–267. https://doi.org/10.2174/1570159X16666180911124605

12. Ashok, A., Andrabi, S. S., Mansoor, S., Kuang, Y., Kwon, B. K., & Labhasetwar, V. (2022). Antioxidant Therapy in Oxidative Stress-Induced Neurodegenerative Diseases: Role of Nanoparticle-Based Drug Delivery Systems in Clinical Translation. *Antioxidants (Basel, Switzerland)*, *11*(2), 408. https://doi.org/10.3390/antiox11020408

13. Khera, T., & Rangasamy, V. (2021). Cognition and Pain: A Review. *Frontiers in psychology*, *12*, 673962. https://doi.org/10.3389/fpsyg.2021.673962

14. Dhaliwal A, Gupta M. Physiology, Opioid Receptor. [2023]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK546642/

15. Phillips JK, Ford MA, Bonnie RJ, editors. (2017) National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Board on Health Sciences Policy; Committee on Pain Management and Regulatory Strategies to Address Prescription

Opioid Abuse; Pain Management and the Opioid Epidemic: Balancing Societal and Individual Benefits and Risks of Prescription Opioid Use. Washington (DC): National Academies Press (US); 2017 Jul 13. 2, Pain Management and the Intersection of Pain and Opioid Use Disorder. Available from: https://www.ncbi.nlm.nih.gov/books/NBK458655/

16. Ogidigo, J. O., Anosike, C. A., Joshua, P. E., Ibeji, C. U., Nwanguma, B. C., & Nwodo, O. F. C. (2022). Neuroprotective effect of Bryophyllumpinnatum flavonoids against aluminum chloride-induced neurotoxicity in rats. Toxicology mechanisms and methods, 32(4), 243–258. https://doi.org/10.1080/15376516.2021.1995557

17. Bathina, S., & Das, U. N. (2015). Brain-derived neurotrophic factor and its clinical implications. *Archives of medical science : AMS*, *11*(6), 1164–1178. https://doi.org/10.5114/aoms.2015.56342

18. Pisani, A., Paciello, F., Del Vecchio, V., Malesci, R., De Corso, E., Cantone, E., &Fetoni, A. R. (2023). The Role of BDNF as a Biomarker in Cognitive and Sensory Neurodegeneration. *Journal of personalized medicine*, *13*(4), 652. https://doi.org/10.3390/jpm13040652

19. Garraway, S. M., & Huie, J. R. (2016). Spinal Plasticity and Behavior: BDNF-Induced Neuromodulation in Uninjured and Injured Spinal Cord. *Neural plasticity*, *2016*, 9857201. https://doi.org/10.1155/2016/9857201

20. Xiong, H. Y., Hendrix, J., Schabrun, S., Wyns, A., Campenhout, J. V., Nijs, J., & Polli, A. (2024). The Role of the Brain-Derived Neurotrophic Factor in Chronic Pain: Links to Central Sensitization and Neuroinflammation. *Biomolecules*, *14*(1), 71. https://doi.org/10.3390/biom14010071

21. Bathina, S., & Das, U. N. (2015). Brain-derived neurotrophic factor and its clinical implications. *Archives of medical science: AMS*, *11*(6), 1164–1178. https://doi.org/10.5114/aoms.2015.56342

22. Bathina, S., & Das, U. N. (2015). Brain-derived neurotrophic factor and its clinical implications. *Archives of medical science : AMS*, *11*(6), 1164–1178. https://doi.org/10.5114/aoms.2015.56342

23. Porter, G. A., & O'Connor, J. C. (2022). Brain-derived neurotrophic factor and inflammation in depression: Pathogenic partners in crime?. *World journal of psychiatry*, *12*(1), 77–97. https://doi.org/10.5498/wjp.v12.i1.77

24. Numakawa, T., & Odaka, H. (2021). Brain-Derived Neurotrophic Factor Signaling in the Pathophysiology of Alzheimer's Disease: Beneficial Effects of Flavonoids for Neuroprotection. *International journal of molecular sciences*, *22*(11), 5719. https://doi.org/10.3390/ijms22115719

25. Xiong, H. Y., Hendrix, J., Schabrun, S., Wyns, A., Campenhout, J. V., Nijs, J., & Polli, A. (2024). The Role of the Brain-Derived Neurotrophic Factor in Chronic Pain: Links to Central Sensitization and Neuroinflammation. *Biomolecules*, *14*(1), 71. https://doi.org/10.3390/biom14010071

26. Jin W. (2020). Regulation of BDNF-TrkB Signaling and Potential Therapeutic Strategies for Parkinson's Disease. *Journal of clinical medicine*, *9*(1), 257. https://doi.org/10.3390/jcm9010257

27. Schirò, G., Iacono, S., Ragonese, P., Aridon, P., Salemi, G., & Balistreri, C. R. (2022). A Brief Overview on BDNF-Trk Pathway in the Nervous System: A Potential Biomarker or Possible Target in Treatment of Multiple Sclerosis?. *Frontiers in neurology*, *13*, 917527. https://doi.org/10.3389/fneur.2022.917527

28. Colucci-D'Amato, L., Speranza, L., & Volpicelli, F. (2020). Neurotrophic Factor BDNF, Physiological Functions and Therapeutic Potential in Depression, Neurodegeneration and Brain Cancer. *International journal of molecular sciences*, *21*(20), 7777. https://doi.org/10.3390/ijms21207777