World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Bryophyllum pinnatum methanolic extract and flavonoid-rich fraction regulate antioxidant/neurotransmitter levels in MSG-induced rat neurotoxicity

LOLA ADEOLA ADELAKIN [1, 2, *], PONLE BAMIDELE FAKUNLE [2], DORCAS OLUBUNMI TAIWO-OLA [2], KHADIJAT MOTUNRAYO OLADEJO [2], ISA SULEIMAN [2], MOTUNRAYO EUNICE ORESANYA [2], EZEKIEL ABIOLA OLUGBOGI [1] and JOSHUA OLADELE OWOLABI [1, 3]

[1] *Department of Anatomy, Ben Carson College of Health & Medical Sciences, Babcock University, Ogun State, Nigeria.*
[2] *Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria.*
[3] *Philadelphia College of Osteopathic Medicine, United States of America.*

World Journal of Advanced Research and Reviews, 2024, 23(02), 2711–2724

Publication history: Received on 19 July 2024; revised on 28 August 2024; accepted on 30 August 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.23.2.2575

Abstract

Introduction: *Bryophyllum pinnatum* (*B. pinnatum*/BP), a medicinal plant known for its diverse pharmacological properties, has been traditionally used for treating various ailments. This study investigated its potential therapeutic effects against MSG-induced oxidative stress and neurotoxicity in Wistar rats, employing both in vivo and in-silico approaches.

Methods: Forty-eight adult Wistar rats (n=48) were divided into six groups: control group, MSG group, and treatment groups receiving BP extract and *B.pinnatum* flavonoid-rich fractions. Biochemical markers such as LDH, MDA, CAT, GST, and AChE were assessed. Neurotransmitter levels of glutamate and GABA were also measured. Molecular docking and MM GBSA analyses were conducted using Maestro 12.8v to evaluate the binding affinities of 10 *B.pinnatum* compounds with Nrf2 protein, NMDA receptor, and acetylcholinesterase. ADMET profiling was performed using SwissADME and ADMETlab 3.0.

Results: MSG increased LDH and MDA levels significantly, while decreasing CAT, GST, and AChE levels, indicating oxidative stress and neurotoxicity. Treatment with *B.pinnatum* extracts and fractions mitigated these effects, restoring antioxidant enzymes/neurotransmitter levels and reducing lipid peroxidation. In silico studies showed *B.pinnatum* compounds exhibiting substantial binding affinities for Nrf2, NMDA receptor and acetylcholinesterase, correlating with their neuroprotective effects observed in vivo. ADMET profiling confirmed the drug-like properties of these compounds.

Conclusions: *B. pinnatum* methanolic extract and its flavonoid-rich fraction demonstrated significant antioxidant and neuroprotective effects against MSG-induced toxicity in Wistar rats. There was significant difference in the activity levels of the flavonoid fractions as compared to the crude extract in LDH and AChE, however, the difference was only relative in MDA, CAT and GST.

Keywords: *Bryophyllum pinnatum*; MSG; Oxidative stress; Neurotoxicity; Neurotransmitters; Antioxidants.

1. Introduction

The zest for signature tastes in food consumption has brought about the production and use of various food additives, of which Monosodium glutamate (MSG) dates back, between the early 1900s and 60s [1-3]. As an enhancer of flavor in cooking, it is frequently used in savory snacks, gravy, stews, soups, ramen, stock (bouillon) cubes, and sauces [4].

* Corresponding author: ADELAKIN LOLA ADEOLA

Various forms of concomitant toxicity have also been reported in studies involving MSG ingestion [5]. It is associated with obesity, metabolic diseases, and the "Chinese restaurant" syndrome [5]. Monosodium glutamate derivatives include Ajinomoto, Vedan, Spicity, A-one, Maggi etc.

Recent studies have revealed that MSG causes disruption of cells and tissues, and is one of the most commonly used food additives in commercial meals. It works by producing oxidative stress, and increasing the brain's production of reactive oxygen species (ROS) [6]. According to [7], MSG affects the cerebellar cortex in the brain and spinal cord. [8], showed MSG to be neurotoxic, causing neurons and astrocytes to die off in the cerebellar cortex of albino rats. Consuming MSG has also been linked to increased liver lipid peroxidation and lower levels of Superoxide dismutase (SOD), Glutathione-S-Transferase (GSH), and Catalase (CAT) [6]. Notably, low intake of MSG was linked to higher levels of oxidative stress in the liver of rats, while high intake of MSG has been linked to the buildup of glutamate [9]. This makes glutamate receptors, especially N-methyl-D-aspartate receptors (NMDA receptors), which are the most open to calcium ions, overwork (excitotoxicity). Glutamate- induced excitotoxicity is when the body's antioxidants can't fight off the ROS that are made. This leads to Ca2+ influx, lipid peroxidation, and neurodegeneration [10].

As a perennial, medicinal and edible herb, *B. pinnatum* is also referred to as miracle leaf [11] and is used around the world for medicinal purposes [12]. The plant is made up of bioactive compounds which include alkaloids, triterpenes, lipids, flavonoids, glycosides, bufadienolides, phenols, and also contains organic acids [13].

Figure 1 *Bryophyllum pinnatum* plants, Professor Grace Tayo Botanical Garden, Babcock University, Ogun State, Nigeria, 2023.

B. pinnatum is utilized in treating asthma, palpitations, headache, vomiting, gastric ulcers and fevers [9], and the flavonoids have been reported to ameliorate Aluminum chloride (AlCl3) induced oxidative imbalance and lipid peroxidation in the hippocampus and cortex of treated rats [14].

In the human brain, Purkinje cells are the second-largest inhibitory neurons after Betz cells [15]. Their dendritic spines create synapses at the outer molecular layer of the cerebellum, while their axons penetrate deeply into the granular layer to generate synapses in the cortex [15]. They are located in the purkinje layer of the cerebellum [16]. Balance and posture, mental function, voluntary movement, motor learning and coordination, and vision are all impacted by the cerebellum, hence proper motor coordination and movement will be disrupted in the case of trauma or damage to the neurons [17; 18].

Bryophyllum pinnatum's biological activity and pharmacological properties have been tested in silico [19; 20]. Recent advancements in Computer-Aided Drug Design (CADD) have opened new avenues for exploring its bioactive compounds' therapeutic potentials. This study employed CADD techniques to investigate the binding affinities and molecular interactions of *Bryophyllum pinnatum* phytochemicals with key protein targets implicated in oxidative stress and neurodegenerative diseases. By integrating molecular docking and MM GBSA analysis, this research investigated the antioxidant/neuroprotective functions of *Bryophyllum pinnatum* crude extracts and flavonoid fractions [21; 22], and also for further development as therapeutic agents.

2. Materials and methods

2.1. Ethical Clearance

Ethical approval was obtained from Babcock University Health Research Ethical Committee (BUHREC) with BUHREC number: 329/24.

2.2. Animal Procurement

Forty-eight (n=48) adult male Wistar rats weighing 150g averagely were procured from Babcock University Animal Facility. They were housed in plastic cages, provided with pelletized feed, and given distilled water *ad libitum* throughout the experimental period.

2.3. Bryophyllum pinnatum

Leaf Methanolic Extract Preparation: ~200 g of fresh *B.pinnatum* leaves were properly dried through evaporation and dissolved over the night in 100 mL of 100% methanol (MeOH). The extracts were sieved through muslin cloth, moved to clean jars and dried by evaporation [23]. Extracts were reconstituted in water before use.

2.4. Bryophyllum pinnatum

Leaf Polar Extract Preparation: Fresh leaves were air-dried at ±16°C, crushed, and soaked in 1% HCL for 24 hours, then sieved. The extract were partitioned with chloroform to remove non-polar compounds and separated using a separating funnel. Both fractions were vacuum dried and tested for flavonoids and terpenes. Flavonoids were quantified using a spectrophotometer following reactions with aluminum chloride (AlCl3) [24]. Care was taken to ensure no polar-solvent extractable material was in the chloroform fraction, and no terpenoids were in the 1% HCL fraction. Extracts were reconstituted in water before use.

2.4.1. Furin Inhibition Procedure and Glycosylation Effect

Furin Inhibition Procedure was done following [25], while Glycosylation Effect on Furin Inhibition was done following [26].

2.5. Drug Procurement and Administration

Monosodium glutamate was purchased from Central Drug House (P) Ltd. Corp. Office 7/28 Vardaan House, Daryaganj, Delhi, India. Drug administration followed the protocol outlined in Table 1 below:

2.6. Experimental design

Table 1 Table of Experimental design, showing the administration of MSG and *Bryophyllum pinnatum*

GROUPS	ANIMALS	TREATMENT	DURATION
1	8	Normal rat chow with distilled water only, *ad libitum*	14 days
2	8	MSG only (4g/kg body weight) [6]	14 days
3	8	MSG (14 days) + *B. pinnatum* Methanolic extract (14 days)	14 + 14 =28 days
4	8	MSG (14 days) + *B. pinnatum.* Flavonoid fraction (14days)	14 + 14 =28 days
5	8	*B. pinnatum.* Methanolic extract (120mg/Kg body weight), according to [14; 27]	14 days
6	8	*B. pinnatum.* Flavonoid fraction (120mg/Kg body weight), according to [28]	14 days

2.7. In silico methods

A library of 30 bioactive compounds derived from *Bryophyllum pinnatum* were analyzed, but this study documented 10 of the compounds with the most favorable drug-like properties and minimal toxicity. The study employed molecular docking and MMGBSA (Molecular Mechanics Generalized Born Surface Area) analysis using Maestro 12.8v. Standard protein preparation protocols were strictly followed, including proper alignment, optimization, and energy minimization of the protein structures. Molecular docking was then performed to predict the binding orientations and affinities of the compounds, yielding docking scores that indicate the strength of interaction between the ligands and the protein targets.

Subsequent to docking, MMGBSA calculations were conducted to estimate the binding free energies and stability of the ligand-protein complexes. Additionally, ADMET profiling was carried out using SwissADME and ADMETlab 3.0 to evaluate the pharmacokinetic and toxicity properties of the compounds. This profiling included prediction for absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters, based on their blood-brain barrier (BBB) permeability, molecular mass, Lipinski's rule violations, and gastrointestinal (GI) absorption, ensuring that the selected compounds have favorable drug-like properties and minimal toxicity. This facilitated the identification of promising *Bryophyllum pinnatum* compounds with potential efficacy and safety for further development in therapeutic applications [20; 21].

2.8. Statistical Analysis

GraphPad Prism software (version 9.0, GraphPad Software, Inc.) was used for data analysis. All data are reported as mean values with standard deviation (mean ± SD). One-way analysis of variance (ANOVA) followed by Tukey post hoc test was employed for multiple comparisons between groups. Significant difference was set at $P < 0.05$.

3. Results and discussion

3.1. Measures of Oxidative Analysis

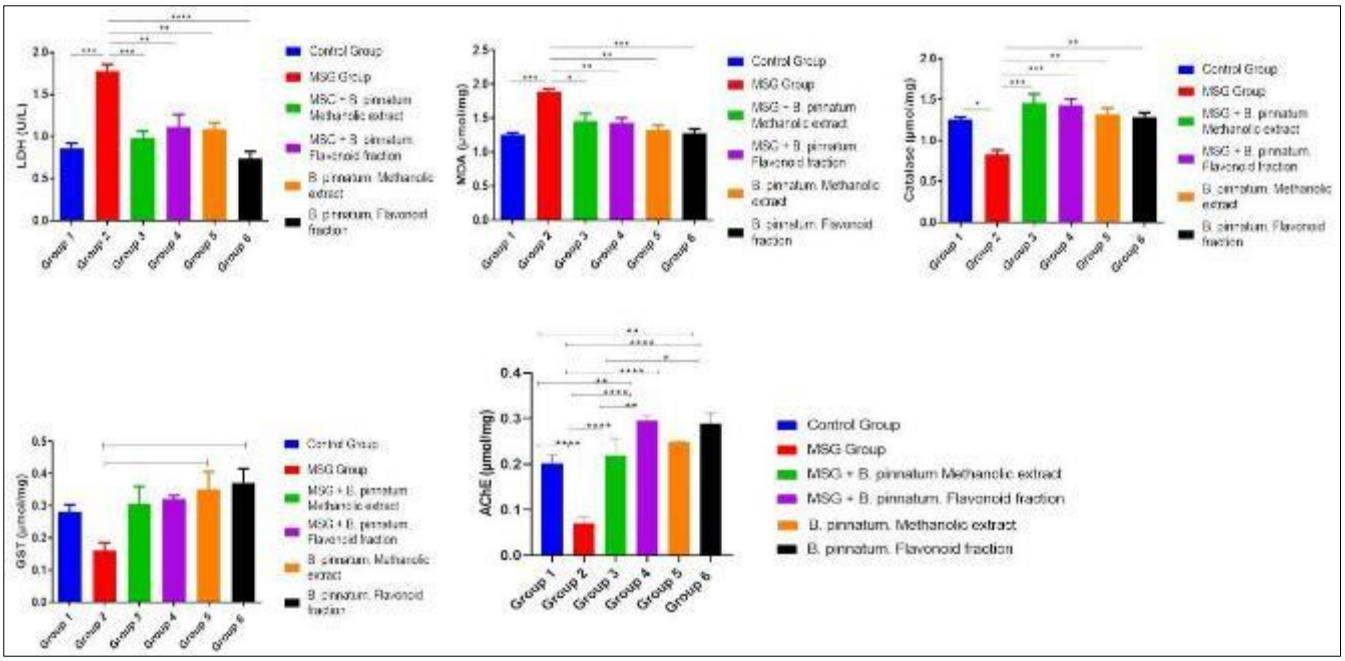

Figure 2 LDH levels (U/L), MDA levels (µmol/mg), CAT levels (µmol/mg), GST levels (µmol/mg) and AChE levels (µmol/mg) in MSG-induced neurotoxic rats, following treatments. The control group exhibited baseline levels across the experimental groups of stress markers.

LDH and MDA levels significantly increased, while CAT, GST and AChE levels reduced significantly, in group 2 (MSG-treated) (***p<0.001) compared to the control. Treatment with *Bryophyllum pinnatum* methanolic extract and flavonoid-rich fraction significantly reduced LDH and MDA levels, and increased significantly, CAT, GST and AChE levels, across the treated groups (3-6), compared to group 2, showing their potential protective effects. Data are presented as mean ± SEM.

3.2. Neurotransmitters Results

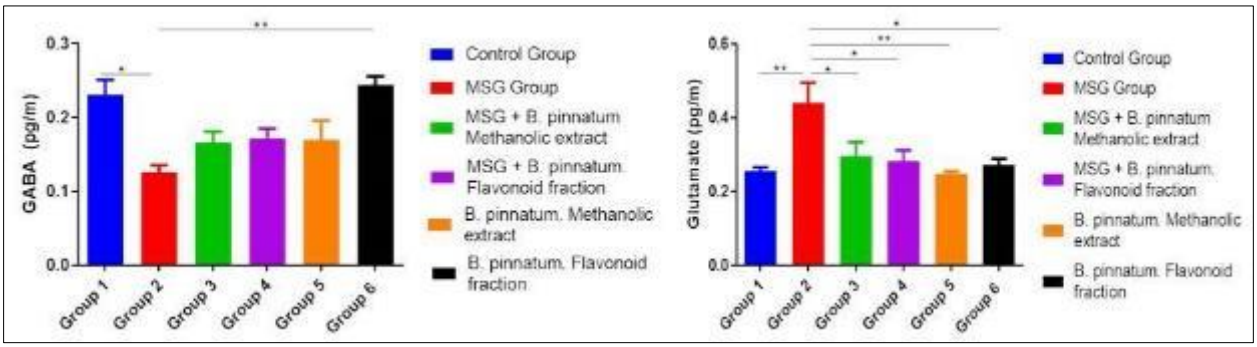

Figure 3 GABA levels (pg/m) and GLU levels (pg/m) in MSG-induced neurotoxic rats, following treatments. The control group exhibited baseline glutamate and GABA levels across the experimental groups.

Group 2 (MSG-treated) showed a significant increase in glutamate levels (***p<0.001), with a concomitant decrease in GABA levels (*p<0.05) compared to the control. *Bryophyllum pinnatum* methanolic extract and flavonoid-rich fraction treatments significantly reduced glutamate levels in the treated groups (3-6), compared to Group 2 (MSG-treated), while it increased GABA levels, indicating their potential effects against excitotoxicity. Data are presented as mean ± SEM.

3.3. In Silico Results

Molecular Docking and Binding-Free Energy Analysis

The docking results of *Bryophyllum pinnatum* ligands against Nuclear factor erythroid 2-related factor 2 (PDB ID: 5CGJ) demonstrate excellent binding affinities as displayed in Table 2 (A-C). Astragalin, Linarin, and Quercitrin showed the highest docking scores of -9.707, -9.198, and -8.996 kcal/mol, respectively, indicating strong interactions with the target protein. Interestingly, Linarin exhibited the most favorable binding free energy (MMGBSA) of -67.02 kcal/mol, surpassing even the cognate ligand (-77.44 kcal/mol), suggesting a stable and energetically favorable complex formation.

Quercitrin (-11.507 kcal/mol) and Quercetin (-11.119 kcal/mol) exhibited the highest docking scores against N-methyl-D-aspartate (NMDA) receptor (PDB ID: 3QEL), indicating strong binding affinities to the receptor's active site; followed closely by Luteolin (-10.988 kcal/mol), which also showed favorable binding free energy (MMGBSA: -45.93 kcal/mol), suggesting stable complex formation. Linarin exhibited the highest docking score of -14.004 kcal/mol and a favorable MMGBSA value of -58.19 kcal/mol against acetylcholinesterase (PDB ID: 7E3H), indicating robust interactions and complex stability. Myricetin 7-rhamnoside (-13.323 kcal/mol) and Quercetin (-12.33 kcal/mol), also demonstrated strong binding, with corresponding MMGBSA values of -47.03 and -50.62 kcal/mol, respectively.

Nuclear factor erythroid 2-related factor 2 5CGJ		
COMPOUND	DOCKING Score(kcal/mol)	MMGBSA(kcal/mol)
Astragalin	-9.707	-39.62
Linarin	-9.198	-67.02
Quercitrin	-8.996	-51.07
Quercetin	-8.114	-35.3
Luteolin	-7.672	-33.99
Kaempferol	-6.891	-36.14
17alpha-Hydroxyprogesterone	-5.109	-49.14
Lycopersicosol	-4.791	-46.35
alpha-Dihydroartemisinin	-4.56	-37.6
4-Hydroxyestrone	-3.666	-35.45
Cognate Ligand	-6.909	-77.44

A

N-methyl-D-aspartate receptor (3QEL)		
COMPOUND	DOCKING Score(kcal/mol)	MMGBSA(kcal/mol)
Quercitrin	-11.507	-42.96
Quercetin	-11.119	-49.75
Luteolin	-10.988	-45.93
3,8-Dimethoxy-4',5,7-trihydroxyflavone	-8.888	-54.98
Retinoic acid	-8.556	-7.05
Casticin	-7.438	-41
Asacoumarin	-7.027	-39.31
Eupatilin	-6.591	-43
Artemetin	-6.488	-43.23
Methyl-delta-ionone	-4.264	-14.01
Cognate Ligand	-10.401	-68.87

B

Acetylcholinesterase (7E3H)		
COMPOUND	DOCKING Score(kcal/mol)	MMGBSA(kcal/mol)
Linarin	-14.004	-58.19
Myricetin 7-rhamnoside	-13.323	-47.03
Quercetin	-12.33	-50.62
Luteolin	-11.919	-43.1
Myricitrin	-11.265	-58.76
Eupatilin	-11.256	-58.9
Kaempferol	-10.812	-38
Cubebininolide	-10.326	-58.4
Retinoic acid	-9.484	-53.01
Asacoumarin	-5.147	-48.38
Cognate Ligand	-9.649	-63.14

C

Table 2 (A-C) Docking Scores and MMGBSA Binding Free Energies of the Top Ten *Bryophyllum pinnatum* Compounds (from the 90 docked ligands), and Cognate Ligand, against Three Key Proteins: Nuclear Factor Erythroid 2-Related Factor 2 (5CGJ) (A), N-methyl-D-aspartate (NMDA) Receptor (3QEL) (B) and Acetylcholinesterase (7E3H) (C).

3.4. Pharmacokinetic and Drug-Likeness Profiling

Ligands	BBB permeant	Mass	Lipinski	Gi-Absorption
Astragalin	No	448.38 g/mol	2	Low
Linarin	No	592.55g/mol	3	Low
5CGJ_Cognate Ligand	No	452.57 g/mol	0	High
Myricetin 7-rhamnoside	No	464.38 g/mol	2	Low
7E3H_Cognate Ligand	Yes	379.49 g/mol	0	High
Quercitrin	No	448.38 g/mol	2	Low
Quercetin	No	302.24g/mol	0	High
3QEL_Cognate Ligand	Yes	325.44g/mol	0	High

Table 3 Comparative analysis of the Pharmacokinetic and Drug-Likeness Profiles of Selected *Bryophyllum pinnatum* Ligands and Cognate Ligands, based on their blood-brain barrier (BBB) permeability, molecular mass, Lipinski's rule violations, and gastrointestinal (GI) absorption.

Bioavailability Radar Plots for the top scoring *Bryophyllum pinnatum* ligands in this study:

The bioavailability radar expressed the pharmacokinetic properties of the analyzed ligands based on six key attributes: lipophilicity (LIPO), size, polarity (POLAR), solubility (INSOLU), flexibility (FLEX), and saturation (INSATU). The red lines represent the ideal ranges for drug-like properties, while the radar plots depict the actual profiles of the ligands. The left plot shows a ligand with balanced lipophilicity and size but suboptimal solubility and polarity, indicating potential absorption issues. The middle plot illustrates a compound that fits well within the ideal ranges for most attributes, suggesting favorable bioavailability. The right plot demonstrates a ligand with high lipophilicity but potential drawbacks in flexibility and solubility, which could affect its absorption and distribution. Overall, these radar plots help visualize the strengths and weaknesses of each ligand's pharmacokinetic profile, guiding further optimization for better drug-like behavior.

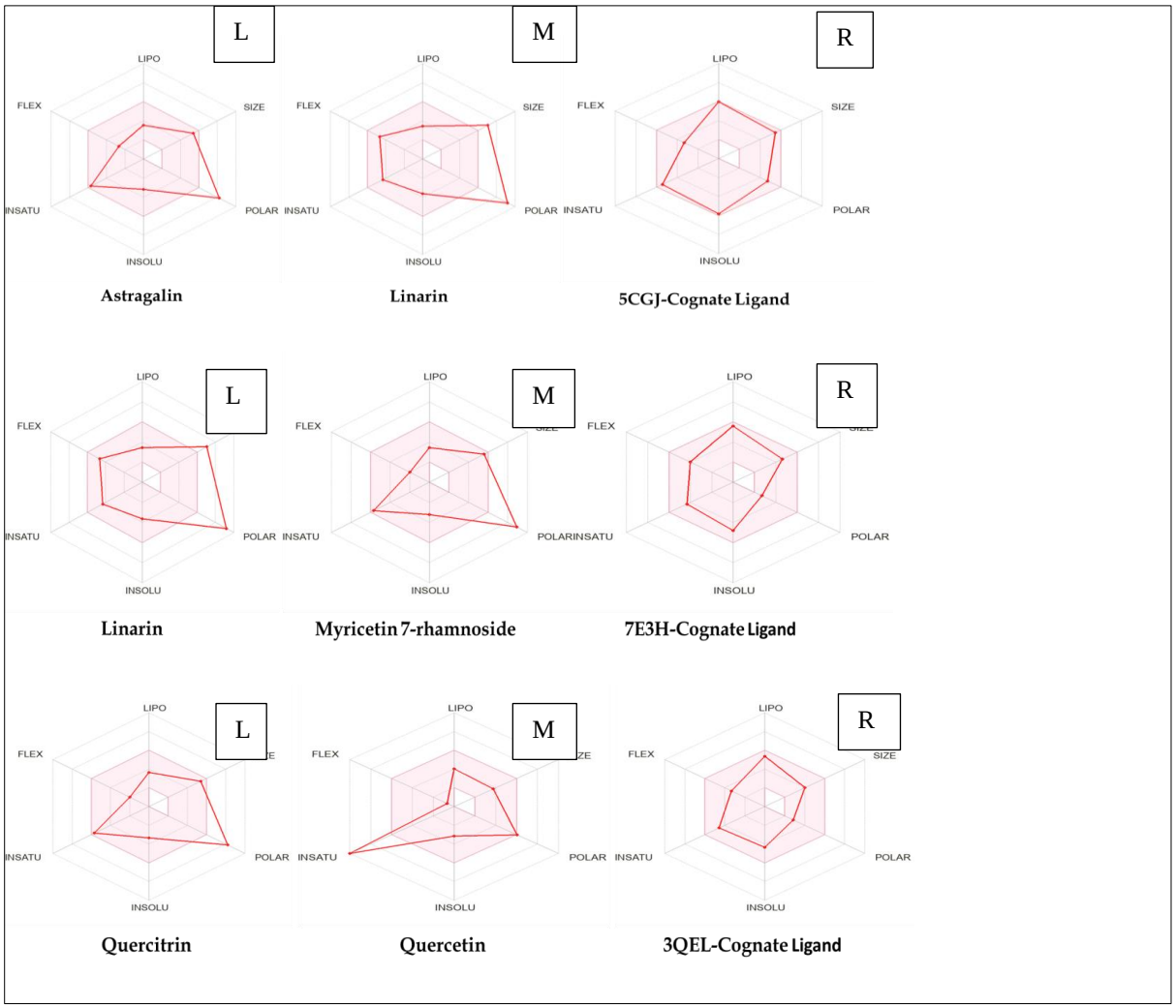

Figure 4 Bioavailability Radar Charts for Astragalin, Linarin, and 5CGJ Cognate Standard Ligand; Linarin, Myricetin-7-rhamnoside and 7E3H-Cognate Ligand; and Quercitrin, Quercetin and 3QEl-Cognate Ligand (L-Left, M-Middle, R-Right).

Astragalin shows good alignment with lipophilicity and size criteria, but is suboptimal in solubility and polarity, indicating potential absorption challenges. Linarin, with a high molecular weight and less optimal flexibility, also has issues in polarity and solubility, suggesting limited oral bioavailability. In contrast, the 5CGJ Cognate Ligand fits well within most optimal ranges, indicating favorable pharmacokinetics, which is essential in drug development. Quercitrin demonstrates a balanced profile in terms of lipophilicity and flexibility but shows suboptimal solubility, which may affect its bioavailability. Myricetin 7-rhamnoside presents significant deviations in polarity and solubility, indicating potential challenges in absorption and distribution, likely requiring formulation adjustments for improved pharmacokinetics. The 7E3H Cognate Ligand, on the other hand, aligns well within the optimal ranges for most properties, suggesting it possesses favorable drug-like characteristics.

Interactions demonstrating Hydrogen Bonds and Hydrophobic Interactions of Specific Ligands with Key Residues of Nuclear Factor Erythroid 2-Related Factor 2 (5CGJ), NMDA receptor and Acetylcholinesterase.

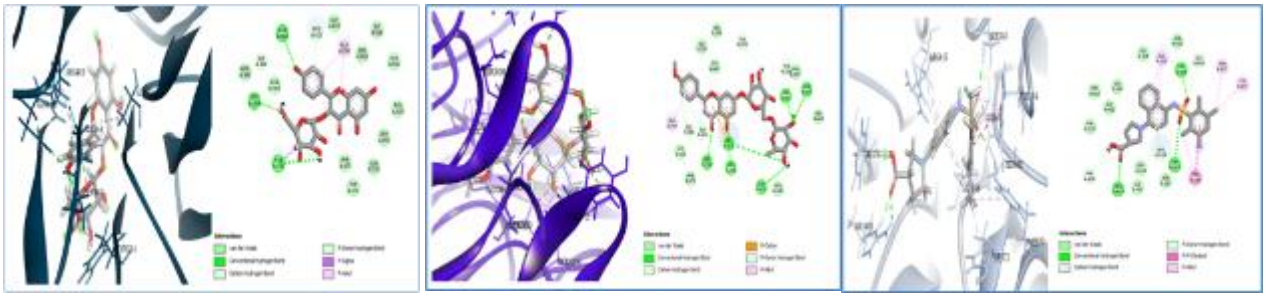

Figure 5 Interaction Profile of Astragalin (left), Linarin (middle) and Cognate Ligand (right) at the binding pocket of Nrf2 Protein (PDB ID: 5CGJ)

The interaction analyses of Astragalin, linarin and cognate ligand bound to the Nrf2 protein (Fig. 3.4 a) show significant stabilizing interactions that align with the reported docking scores (-9.707 kcal/mol) and MMGBSA scores (-39.62 kcal/mol), (-9.198 kcal/mol) and highly favorable MMGBSA binding energy (-67.02 kcal/mol), and (-6.909 kcal/mol) and highly favorable MMGBSA binding energy (-77.44 kcal/mol), respectively.

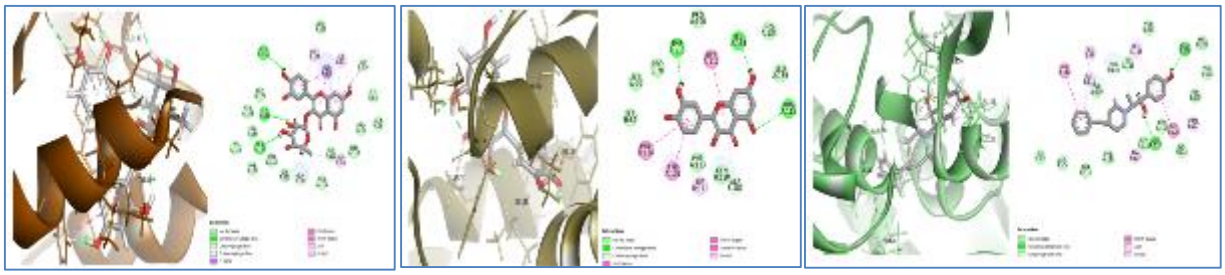

Figure 6 Interaction Profile of Quercitrin (left), Quercetin (middle) and the Cognate Ligand (right) at the binding pocket of NMDA Receptor (PDB ID: 3QEL)

The interaction profiles of quercitrin, quercetin and cognate ligand, bound to the NMDA receptor in Figure 3.4b reveal significant stabilizing interactions that align with their strong docking scores (-11.507 kcal/mol) and favorable MMGBSA binding energy (-42.96 kcal/mol), (-11.119 kcal/mol) and favorable MMGBSA binding energy (-49.75 kcal/mol), and (-10.401 kcal/mol) and highly favorable MMGBSA binding energy (-68.87 kcal/mol), respectively.

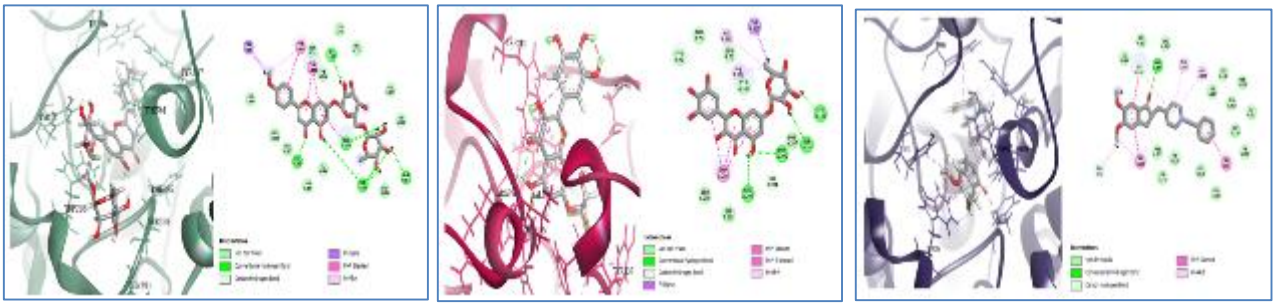

Figure 7 Interaction Profile of Linarin, Myricetin 7-rhamnoside and the Cognate Ligand at the binding pocket of Acetylcholinesterase (AChE, PDB ID: 7E3H)

The interaction profiles of Linarin, Myricetin 7-rhamnoside and cognate ligand bound to AChE in Figure 3.4c show significant stabilizing interactions that correspond with the excellent docking score (-14.004 kcal/mol) and favorable MMGBSA energy (-58.19 kcal/mol); strong docking score (-13.323 kcal/mol) and favorable MMGBSA binding energy (-47.03 kcal/mol); and docking score (-9.649 kcal/mol) and highly favorable MMGBSA binding energy (-63.14 kcal/mol), respectively.

4. Discussion

4.1. Effect of *Bryophyllum pinnatum* Methanolic and Flavonoid Fractions on Oxidative Stress Markers

The data presented in Figure 2 highlight the biochemical implications of different treatments on lactate dehydrogenase (LDH), malondialdehyde (MDA), catalase, glutathione S-transferase (GST), and acetylcholinesterase (AChE) levels in the context of MSG-induced toxicity and the potential protective effects of *Bryophyllum pinnatum* methanolic extract and its flavonoid-rich fraction. LDH is an enzyme released during tissue damage and cellular stress, making it a key indicator of cell damage [29]. The significant increase in LDH levels in the MSG group (***$p<0.001$) compared to the control indicates that MSG induced substantial tissue damage. However, the reduction in LDH levels upon treatment with *Bryophyllum pinnatum* extract and the flavonoid-rich fraction suggests their ability to mitigate tissue damage, reflecting their cytoprotective properties [30; 31]. MDA is a marker of lipid peroxidation, which occurs when free radicals attack lipids in cell membranes, leading to oxidative stress [32]. The significant increase in MDA levels in the MSG group (***$p<0.001$) indicates heightened oxidative stress due to MSG exposure. The reduction in MDA levels following treatment with *Bryophyllum pinnatum* extract and the flavonoid-rich fraction highlights their potential antioxidant properties, as they can neutralize free radicals and reduce lipid peroxidation. This protective effect against oxidative stress is crucial in preventing further cellular damage and maintaining cell integrity [33].

Catalase and GST are essential antioxidant enzymes that preserve cellular equilibrium through detoxification of harmful chemicals and breaking down of hydrogen peroxide, thereby, protecting cells from oxidative damage [34]. The significant decrease in catalase levels (**$p<0.01$) and GST levels in the MSG group compared to the control suggests that MSG exposure compromises the antioxidant defense system, rendering cells more vulnerable to oxidative damage [35]. The subsequent increase in catalase and GST levels upon treatment with *Bryophyllum pinnatum* extract and the flavonoid-rich fraction indicates a restoration of the antioxidant defense mechanisms, thereby enhancing the cell's ability to cope with oxidative stress [36]. AChE is an enzyme essential for the breakdown of acetylcholine in the nervous system, and its activity is crucial for proper neuronal function. The significant decrease in AChE levels in the MSG group (***$p<0.001$) suggests that MSG negatively impacts neuronal function, potentially leading to neurotoxicity [37]. Treatment with *Bryophyllum pinnatum* extract and the flavonoid-rich fraction significantly increased AChE levels, indicating their neuroprotective effects. This restoration of AChE activity could help maintain neuronal communication and prevent neurodegenerative changes associated with MSG exposure. Overall, the data emphasize the protective and restorative effects of *Bryophyllum pinnatum* methanolic extract and its flavonoid-rich fraction against MSG-induced biochemical alterations [38; 39].

4.2. *Bryophyllum pinnatum* Methanolic/Flavonoid Extracts Potentiate ability to Regulate Neuronal Transmission and Excitoxicity

Glutamate is a major excitatory neurotransmitter in the central nervous system, playing a critical role in synaptic transmission, plasticity, and overall brain function. However, excessive glutamate can lead to excitotoxicity, a process that damages or kills neurons through overactivation of glutamate receptors, leading to increased calcium influx and subsequent cellular damage [40]. In Figure 3, the MSG group showed a significant increase in glutamate levels (***$p<0.001$) compared to the control, indicating potential excitotoxic damage caused by MSG. Treatment with *Bryophyllum pinnatum* methanolic extract and its flavonoid-rich fraction significantly reduced glutamate levels, suggesting their protective effects against excitotoxicity. This reduction in glutamate levels can prevent neuronal damage and maintain neuronal health by reducing excitatory stress [41].

Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the brain, essential for maintaining the balance between excitation and inhibition in the nervous system; adequate levels of GABA are therefore crucial for preventing hyperexcitability and neurotoxicity [42]. The MSG-treated group exhibited a significant decrease in GABA levels (*$p<0.05$) compared to the control, indicating a disruption in inhibitory neurotransmission and potential neurotoxicity. Treatment with *Bryophyllum pinnatum* methanolic extract and its flavonoid-rich fraction significantly increased GABA levels in the intervention groups (Groups 3-6), compared to the MSG-treated group (Group 2), indicating their neuroprotective effects. By restoring GABA levels, these treatments help to re-establish the inhibitory tone in the brain, protecting against neurotoxicity and associated neurological disorders such as Alzheimer's disease, Parkinson's disease, and epilepsy [43].

4.3. In Silico Study: Molecular Docking Results of *Bryophyllum pinnatum* Ligands

The docking results of *Bryophyllum pinnatum* ligands against Nuclear factor erythroid 2-related factor 2 (PDB ID: 5CGJ) showed excellent binding affinities as displayed in Table 2 (A-C). Astragalin, Linarin, and Quercitrin showed the highest docking scores of -9.707, -9.198, and -8.996 kcal/mol, respectively, indicating strong interactions with the target

protein. Interestingly, Linarin exhibited the most favorable binding free energy (MMGBSA) of -67.02 kcal/mol, surpassing even the cognate ligand (-77.44 kcal/mol), suggesting a stable and energetically favorable complex formation. These findings indicate that these ligands may have superior inhibitory potential compared to the standard. Additionally, compounds like Quercetin and Kaempferol, known for their bioactivity, also displayed favorable binding energies. The lower docking scores coupled with highly negative MMGBSA values underscore the strong binding affinity and stability of these phytocompounds, highlighting their potential as promising candidates for therapeutic applications targeting the Nrf2 pathway [19; 20].

The docking analysis of *B. pinnatum* ligands against the N-methyl-D-aspartate (NMDA) receptor (PDB ID: 3QEL) reveals promising interactions as displayed in Table 2 (A-C). Quercitrin (-11.507 kcal/mol) and Quercetin (-11.119 kcal/mol) exhibited the highest docking scores, indicating strong binding affinities to the receptor's active site; followed closely by Luteolin (-10.988 kcal/mol), which also showed favorable binding free energy (MMGBSA: -45.93 kcal/mol), suggesting stable complex formation. Notably, some of these compounds outperformed the cognate ligand (-10.401 kcal/mol, -68.87 kcal/mol), particularly in docking scores, highlighting their potential as effective NMDA receptor inhibitors. Interestingly, while compounds like 3,8-Dimethoxy-4',5,7-trihydroxyflavone showed moderate docking scores (-8.888 kcal/mol), their MMGBSA values (-54.98 kcal/mol) indicate enhanced binding stability. These findings suggest that the identified phytocompounds have strong potential as NMDA receptor inhibitors, with implications for neurological conditions associated with excitotoxicity and synaptic dysfunction. The ability of these compounds to bind favorably positions them as candidates for further exploration in managing NMDA receptor-mediated pathologies [19; 20].

The docking analysis of *B. pinnatum* ligands against acetylcholinesterase (PDB ID: 7E3H) reveals promising inhibitory potential, as summarized in Table 2 (A-C). Linarin exhibited the highest docking score of -14.004 kcal/mol and a favorable MMGBSA value of -58.19 kcal/mol, indicating robust interactions and complex stability. Myricetin 7-rhamnoside (-13.323 kcal/mol) and Quercetin (-12.33 kcal/mol), also demonstrated strong binding, with corresponding MMGBSA values of -47.03 and -50.62 kcal/mol, respectively. The docking scores and MMGBSA energies indicate that these phytocompounds have comparable or better binding affinities than the cognate ligand (-9.649 kcal/mol, -63.14 kcal/mol), suggesting that they could be potent acetylcholinesterase inhibitors. The high binding affinity of these ligands highlights their potential therapeutic application in managing neurological conditions like Alzheimer's disease, where acetylcholinesterase inhibition is a key strategy [19; 20].

4.4. Pharmacokinetic and Drug-Likeness Profiles of *Bryophyllum pinnatum* Leaves Compounds

Table 3 presents a comprehensive comparative analysis of the Pharmacokinetic and Drug-Likeness Profiles of Selected *Bryophyllum pinnatum* Ligands and Cognate Ligands, based on their blood-brain barrier (BBB) permeability, molecular mass, Lipinski's rule violations, and gastrointestinal (GI) absorption. Among the 90 docked ligands, only the cognate ligands for 7E3H (Acetylcholinesterase) and 3QEL (NMDA receptor) are predicted to be BBB permeant, a critical feature for targeting central nervous system (CNS) disorders. BBB permeability indicates the ability of a compound to cross into the brain, which is essential for therapeutic agents aiming to treat neurodegenerative diseases. In contrast, phytocompounds like Astragalin, Linarin, and Quercitrin are not BBB permeant, which limits their direct applicability for CNS targeting, despite their demonstrated binding affinities. This differential BBB permeability emphasizes the challenges of translating these naturally derived ligands into CNS-active drugs.

Regarding molecular mass and Lipinski's rule of five, most ligands fall within the drug-like molecular weight range (below 500 g/mol), except for Linarin (592.55 g/mol), which exceeds this threshold, contributing to its three Lipinski violations. The higher molecular weight and multiple violations suggest that Linarin may have poor oral bioavailability and limited absorption, despite strong binding properties observed in docking studies. The GI absorption data further highlight this challenge in compounds such as Astragalin, Linarin, and Myricetin 7-rhamnoside display low GI absorption, suggesting limited effectiveness if administered orally. In contrast, the cognate ligands for 5CGJ (Nrf2), 7E3H (AChE), and 3QEL (NMDA) demonstrate high GI absorption, reinforcing their suitability for oral delivery. These findings underscore that while phytocompounds may exhibit potent in silico binding, their pharmacokinetic limitations, including low GI absorption and poor BBB permeability, could hinder their drug development, emphasizing the need for further optimization or formulation strategies to enhance their bioavailability and CNS penetration. Overall, the table illustrates the diverse physicochemical pharmacokinetic profiles and drug-likeness profiles, highlighting the importance of these parameters in drug development and safety assessments [20; 44].

4.5. Bioavailability Radar Charts of *Bryophyllum pinnatum* Ligands

The bioavailability radar charts in Figure 4 are a visual representation of the drug-likeness and pharmacokinetic properties of the analyzed ligands (Astraglin, Linarin, 5CGJ Cognate Ligand, Myricetin 7-rhamnoside, and the 7E3H

Cognate Ligand, Quercetin and 3QEl-Cognate Ligand) based on six key attributes: lipophilicity (LIPO), size, polarity (POLAR), solubility (INSOLU), flexibility (FLEX), and saturation (INSATU).

Astragalin showed good alignment with lipophilicity and size criteria but is suboptimal in solubility and polarity, indicating potential absorption challenges. Linarin, with a high molecular weight and less optimal flexibility, also had issues in polarity and solubility, suggesting limited oral bioavailability. In contrast, the 5CGJ Cognate Ligand fitted well within most optimal ranges, indicating favorable pharmacokinetics, which is essential in drug development. These plots highlight that while natural compounds like Astragalin and Linarin have therapeutic potential, their pharmacokinetic limitations necessitate further optimization, such as improving solubility or enhancing formulation strategies, to achieve effective drug-like properties [22].

Quercitrin demonstrated a balanced profile in terms of lipophilicity and flexibility but showed suboptimal solubility, which may affect its bioavailability. Myricetin 7-rhamnoside presents significant deviations in polarity and solubility, indicating potential challenges in absorption and distribution, likely requiring formulation adjustments for improved pharmacokinetics. The 7E3H Cognate Ligand, on the other hand, aligns well within the optimal ranges for most properties, suggesting it possesses favorable drug-like characteristics. Overall, these radar plots help visualize the strengths and weaknesses of each ligand's pharmacokinetic profile, guiding further optimization for better drug-like behavior [22].

5. Conclusion

This study explored the therapeutic potential of *Bryophyllum pinnatum* methanolic extract and its flavonoid-rich fraction in mitigating MSG-induced oxidative stress and neurotoxicity in Wistar rats. The biochemical analysis revealed that MSG exposure significantly increased oxidative stress markers, including LDH and MDA, while decreasing essential antioxidant enzymes like catalase and GST. Treatment with *B. pinnatum* extracts effectively reversed these alterations, highlighting their antioxidant and cytoprotective properties. Additionally, the extracts modulated neurotransmitter levels, reducing glutamate-induced excitotoxicity and restoring GABA levels, thereby providing neuroprotective benefits. In silico analyses supported these findings, demonstrating significant binding affinities of *B. pinnatum* compounds with key protein targets such as the NMDA receptor, Acetylcholinesterase and Nuclear factor erythroid 2-related factor 2. Molecular docking and MM GBSA calculations indicated that these compounds could potentially modulate neurotransmitter activity and enhance cholinergic function, aligning with their observed protective effects in vivo. Pharmacokinetic profiling further validated the drug-like properties of these compounds, suggesting favorable pharmacokinetic and safety profiles. Overall, the findings that *Bryophyllum pinnatum* methanolic extract and its flavonoid-rich fraction can modulate these neurotransmitter levels underscore their potential as neuroprotective agents. The combination of wet-lab and in-silico approaches provides a comprehensive understanding of the molecular mechanisms underlying the protective effects of *Bryophyllum pinnatum*, paving the way for future drug development and clinical applications.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared no conflict of interest to be disclosed.

References

[1] Omowumi T Kayode, Jemilat A Bello, Jamiu A Oguntola, Abolanle AA Kayode, and Daniel K Olukoya. The Interplay between Monosodium Glutamate (MSG) Consumption, and Metabolic Disorders. 2023. 9(9): e19675.

[2] Sarah A Evbuomwan, Omolola E Omotosho, Olusegun O Akinola. MONOSODIUM GLUTAMATE: HEALTH RISKS, CONTROVERSIES AND FUTURE PERSPECTIVES. 2023. Researchgate. www.researchgate.net/publication/371637831

[3] Husarova V, Ostatnikova D. Monosodium Glutamate Toxic Effects and their Implications for Human Intake. *IBIM;* 2013. 1.

[4] Takashi Yamamoto, Chizuko Inui-Yamamoto. The flavor-enhancing action of glutamate and its mechanism involving the notion of kokumi. *NPJ Sci Food.*; 2023. 7(1):3.

[5] Niaz, K, Zaplatic, E, & Spoor, J. Extensive use of monosodium glutamate: A threat to public health? *EXCLI journal*; 2018. 17, 273.

[6] Mohammad Mahdi Hajihasani, Vahid Soheili, Mohammad Reza Zirak, Amirhossein Sahebkar, Abolfazl Shakeri. Natural products as safeguards against monosodium glutamate-induced toxicity. *Iranian Journal of Basic Medical Sciences; 2020.* 23(4):416-430.

[7] Abd El-mawla et al.. Effect of Monosodium Glutamate on the Cerebellar Cortex of Male Albino Rat and Protective Role of Vitamin C. *Zagazig University Medical Journal;* 2019. 2, 250-260.

[8] Mondona S McCann, Kathleen A Maguire-Zeiss, (2021). Environmental toxicants in the brain: A review of astrocytic metabolic dysfunction. *Environ Toxicol Pharmacol*; 2021. 84:103608.

[9] Folake Olubukola Asejeje, Gladys Omolola Gabriel & Michael Abayomi Abiola. Monosodium glutamate aggravates lipopolysaccharide-induced liver injury via inflammation and oxidative stress in rats. *Nutrire;* 2023. 48 (5).

[10] Noemi Nicosia, Mattia Giovenzana, Paulina Misztak, Jessica Mingardi, and Laura Musazzi. Glutamate-Mediated Excitotoxicity in the Pathogenesis and Treatment of Neurodevelopmental and Adult Mental Disorders. *Int J Mol Sci;* 2024. 25(12): 6521.

[11] Abida Latif, Kanwal Ashiq, Mehwish Qayyim, Sana Ashiq, Ejaz Ali. PHYTOCHEMICAL AND PHARMACOLOGICAL PROFILE OF THE MEDICINAL HERB: *BRYOPHYLLUM PINNATUM. The Journal of Animal and Plant Sciences*; 2019. 29(6):1528-1534

[12] Fernandes, JM, Cunha, LM, Azevedo, EP, Lourenço, EM, Pedrosa, M.F., Zucolotto, SM. Kalanchoe laciniata and *Bryophyllum pinnatum:* an updated review about ethnopharmacology, phytochemistry, pharmacology, and toxicology. *Rev. Brasi de Farmacog*; 2019. 29(4): 529-558.

[13] Anderson Wilbur Lopes Andrade, Gerlane Coelho Bernardo Guerra, Daline Fernandes de Souza Araújo, Raimundo Fernandes de Araújo Júnior, Aurigena Antunes de Araújo, Thaís Gomes de Carvalho. Anti-Inflammatory and Chemopreventive Effects of *Bryophyllum pinnatum* (Lamarck) Leaf Extract in Experimental Colitis Models in Rodents. *Front Pharmacol;* 2020. 11: 998.

[14] Joyce Oloaigbe Ogidigo, Chioma Assumpta Anosike, Parker Elijah Joshua, Collins U Ibeji, Bennett C Nwanguma, Okwesili Fred Chiletugo Nwodo. Neuroprotective effect of *Bryophyllum pinnatum* flavonoids against aluminum chloride-induced neurotoxicity in rats. *Toxicol Mech Methods*; 2022. 32(4):243-258..

[15] Stefano Masoli, Diana Sanchez-Ponce, Nora Vrieler, Karin Abu-Haya, Vitaly Lerner, Tal Shahar. Human Purkinje cells outperform mouse Purkinje cells in dendritic complexity and computational capacity. *Communications: Biology*; 2024. 7(5).

[16] Purves D, Augustine GJ, Fitzpatrick D, Hall WC, LaMantia A, McNamara JO, and White LE. Neuroscience. 4th ed. Sinauer Associates; 2008. 432–4.

[17] Witter L, De Zeeuw CI. Regional functionality of the cerebellum. *Curr Opin Neurobiol*; 2015. 33:150-5.

[18] Roostaei T, Nazeri A, Sahraian MA, Minagar A. The human cerebellum: a review of physiologic neuroanatomy. *Neurol Clin*; 2014. 32(4):859-69.

[19] Olugbogi, EA, Arobadade, OA, Bodun, DS, Omoseeye, SD, Omirin, ES, Fapohunda, O, ... & Omotuyi, OI. Identification of apposite antagonist for androgen receptor in prostate cancer: an in silico study of fenugreek compounds. *Journal of Biomolecular Structure and Dynamics;* 2023. 1-17.

[20] Olugbogi, E, Arobadade, O, Balogun, S, Akingbola, H, Adelakun, O, Popoola, T, ... & Arietarhire, L. Application of In-silico Methodologies in Exploring the Antagonistic Potential of Trigonella foenum-graecum on Cyclooxygenase-2 (Cox-2) in Cancer Treatment. IPS *Journal of Molecular Docking Simulations*; 2023. 2(1), 26-36.

[21] Fasooto, TT, Ogundana, OR, Adebayo, AF, Olawade, DB, Olugbogi, EA, Fapohunda, O, & Akinmoladun, AC. In-silico and in-vivo comparative evaluation of the cardioprotective potential of Yellow turmeric and White turmeric in Mn-induced cardiac oxidative stress. *Pharmacological Research-Modern Chinese Medicine*; 2024. 10, 100399.

[22] Olanrewaju, J, Arietarhire, LO, Soremekun, OE, Olugbogi, EA, Afolabi, TO, Aribisala, PO, ... & Omotuyi, OI. Spondias mombin flavonoids showed super-binder ability with Downstream Molecular Targets of Parkinson's Disease: A Structural Study. *Informatics in Medicine Unlocked;* 2024. 101543.

[23] Samuel O Onoja, Ginika Q Ihejirika, Oluchi N Nwankudu, Yusuf N Omeh, and Maxwell I Ezeja. Antidiarrheal and Antioxidant Activities of Methanol Extract of *Bryophyllum pinnatum* Leaf Harvested from South-Eastern Nigeria in Mice. *J Pharm (Cairo).* 2018. 2018: 6810620.

[24] Rhayanne TM Ramos, Isabelle CF Bezerra, Magda RA Ferreira, and Luiz Alberto Lira Soares. Spectrophotometric Quantification of Flavonoids in Herbal Material, Crude Extract, and Fractions from Leaves of *Eugenia uniflora* Linn. *Pharmacognosy Res;* 2017. 9(3): 253–260.

[25] Kasi Pandima Devi, Mahmoud Reza Pourkarim, Marijn Thijssen, Antoni Sureda, Maryam Khayatkashani, Cosmin Andrei Cismaru, Ioana Berindan Neagoe. A perspective on the applications of furin inhibitors for the treatment of SARS-CoV-2. *Pharmacol Rep;* 2022. 74(2): 425–430.

[26] Shengjun Wang, Wei Ran, Lingyu Sun, Qingchi Fan, Yuanqi Zhao, Bowen Wang. Sequential glycosylations at the multibasic cleavage site of SARS-CoV-2 spike protein regulate viral activity. *Nature: Communications*; 2024. 15: 4162.

[27] Adelakin L A, Fabiyi S O, Edward O, Olatunji S Y, Olanrewaju J A, Owolabi J O. Kainic Acid Epilepsy Model in Wistar rats: The Comparative Ameliorate use of *Bryophyllum pinnatum,* Ketogenic diet and Carbamazepine on the frontal lobe cortex with selected behavioral parameters. *Journal of Scientific Research and Reports*; 2022. 28(3): 28-46.

[28] Taiwo O Elufioye, Adebola Omowunmi Oyedeji, Solomon Habtemariam. A Review of the Traditional Uses, Phytochemistry and Pharmacology of *Bryophyllum pinnatum* (Lam.) (Crassulaceae). *Journal of Biologically Active Products from Nature*; 2022. 12(4):1-34.

[29] Tiwari, BK, & Pandey, KB. Role of oxidative stress in cardiovascular diseases. *British Journal of Pharmacology*; 2012. 167(3), 493-504.

[30] Gharbi, M, Chograni, H, & Aidi Wannes, W. Antioxidant and anti-inflammatory activities of different extracts from aerial parts of *Bryophyllum pinnatum* (Lam.) Oken. *Journal of Food Biochemistry;* 2018. 42(5), e12603.

[31] George, BP, Abrahamse, H, & Hemmaragala, NM. Antioxidant and cytoprotective properties of *Bryophyllum pinnatum* (Lam.) Oken leaf extracts in human lymphocytes. *Journal of Ethnopharmacology*; 2018. 216, 159-165.

[32] Raffaele Cordiano, Mario Di Gioacchino, Rocco Mangifesta, Claudia Panzera, Sebastiano Gangemi, Paola Lucia Minciullo. Malondialdehyde as a Potential Oxidative Stress Marker for Allergy-Oriented Diseases: An Update. *Molecules*; 2023. 28(16):5979.

[33] Navid Omidifar, Amir Nili-Ahmadabadi, Amin Nakhostin-Ansari, Kamran Bagheri Lankarani, Mohsen Moghadami *et al*. The modulatory potential of herbal antioxidants against oxidative stress and heavy metal pollution: plants against environmental oxidative stress. *Environmental Science and Pollution*; 2021. 28, 61908–61918.

[34] Ankita Nandi, Liang-Jun Yan, Chandan Kumar Jana, Nilanjana Das. Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. *Oxid Med Cell Longev*; 2019. 9613090.

[35] Abdullah Yilgor & Canan Demir. Determination of oxidative stress level and some antioxidant activities in refractory epilepsy patients. *Scientific Reports*; 2024. 14: 6688.

[36] Halliwell, B, & Gutteridge, JMC. Free Radicals in Biology and Medicine. Oxford University Press.2015.

[37] Choi, JY, & Lee, YJ. Modulation of acetylcholinesterase activity by oxidative stress in vivo and in vitro. *Biochemical and Biophysical Research Communications;* 2012. 427(2), 259-264.

[38] Adeoye, AO, Lateef, KM, & Adekunle, AS. Acetylcholinesterase inhibition and antioxidant properties of *Bryophyllum pinnatum (Lam.)* Oken leaf extracts in vitro. *Journal of Ethnopharmacology*; 2011. 137(3), 1413-1418.

[39] Francis, PT, Palmer, A. M, & Snape, M. The cholinergic hypothesis of Alzheimer's disease: a review of progress. *Journal of Neurology, Neurosurgery & Psychiatry*; 1999. 66(2), 137-147.

[40] David R Andrew, Eszter Farkas, Jed A Hartings, K. C. Brennan, Oscar Herreras, Michael Müller. Questioning Glutamate Excitotoxicity in Acute Brain Damage: The Importance of Spreading Depolarization. *Neurocrit Care*; 2022. 37(1): 11–30.

[41] Vaarmann, A Kovac S, Holmström KM, Gandhi S & AY Abramov AY. Dopamine protects neurons against glutamate-induced excitotoxicity. *Nature: Cell Death & Disease;* 2013. 4, (455).

[42] Cutler, AJ, Mattingly, GW & Maletic, V. Understanding the mechanism of action and clinical effects of neuroactive steroids and GABAergic compounds in major depressive disorder. *Transl Psychiatry*; 2023. 13, 228.

[43] Koh, W, Kwak, H, Cheong, E *et al*. GABA tone regulation and its cognitive functions in the brain. *Nat. Rev. Neurosci*; 2023. 24, 523–539.

[44] Ogunbiyi, OE, Ogunbiyi, BT, Adeleke, SO, Arietarhire, LO, Alege, PE, Olugbogi, EA, & Olanrewaju, JA. Bioactive compounds found in Cucumis sativus demonstrate optimal binding affinity to PTP1B. *IPS Journal of Molecular Docking Simulations*; 2023. 2(1), 37-47.

[45] Bodun, DS, Omoboyowa, DA, Omotuyi, OI, Olugbogi, EA, Balogun, TA, Ezeh, CJ, & Omirin, ES. QSAR-based virtual screening of traditional Chinese medicine for the identification of mitotic kinesin Eg5 inhibitors. *Computational Biology and Chemistry*; 2023. 104, 107865.