Asian Journal of Research in Biochemistry

Volume 14, Issue 5, Page 122-133, 2024; Article no.AJRB.123601
ISSN: 2582-0516

Phytochemical Identification of Methanolic Extract of Leaves *Hernandia peltata* Meisn and Evaluation of Anti-Inflammatory Activity *In silico*

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Authors' contributions

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

Article Information

DOI: https://doi.org/10.9734/ajrb/2024/v14i5318

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/123601

Original Research Article

Received: 11/07/2024
Accepted: 13/09/2024
Published: 21/09/2024

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Cite as: *P., Siyana Saleem, Anoopa Satheesan, Suresh N. Nair, Sujith S, Suja Rani S, Nisha A.R., and Sajitha I.S. 2024. "Phytochemical Identification of Methanolic Extract of Leaves Hernandia Peltata Meisn and Evaluation of Anti-Inflammatory Activity In Silico". Asian Journal of Research in Biochemistry 14 (5):122-33. https://doi.org/10.9734/ajrb/2024/v14i5318.*

ABSTRACT

Aim: To identify the phytochemicals present in the methanolic extract of *Hernandia peltata* Meisn and to assess their potential anti-inflammatory activity using in silico methods**.**
Place and Duration of Study: Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, between February 2024 and March 2024.
Methodology: The chemical profiling of the methanolic leaf extract of *Hernandia peltata* Meisn. was performed using GC-MS analysis. In silico molecular docking studies were carried out using AutoDock4 to evaluate the binding affinities of these phytochemicals to the active sites of four key proteins involved in anti-inflammatory activity: COX-1, COX-2, 5-LOX, and NOS. Furthermore, the pharmacokinetic properties of the active compounds were analysed using the admetSAR software.
Results: Among the compounds identified through GC-MS analysis, 11 demonstrated promising binding scores. Predictions from admetSAR indicated that these phytoconstituents have favourable absorption, distribution, and metabolism profiles. However, toxicity assessments revealed that most compounds exhibit mutagenic properties at higher doses, and some may have potential carcinogenic effects.
Conclusion: The study effectively identified a variety of bioactive phytochemicals in the methanolic extract of *Hernandia peltata* and demonstrated their potential anti-inflammatory properties through in silico analysis. These results underscore the therapeutic potential of *Hernandia peltata* as a source of natural anti-inflammatory agents, encouraging further in vitro and in vivo research to validate these findings.

Keywords: Hernandia peltata; phytochemical; GC-MS; anti-inflammatory; In silico docking.

1. INTRODUCTION

Inflammation is a protective strategy that has evolved in higher organisms to respond to detrimental insults, such as microbial infections, tissue injuries and other harmful conditions. It is a critical immune response by the host that facilitates the removal of harmful stimuli and promotes the healing of damaged tissue [1].

Proinflammatory mediators, which are the primary orchestrators of the inflammatory response, are produced either by tissue cells or by endogenous leukocytes, such as macrophages, monocytes, dendritic cells, or lymphocytes, in response to the insult. These mediators initiate the recruitment of neutrophils, followed by monocytes and lymphocytes, to the sites of injury and induce the systemic responses commonly associated with classical inflammation [2].

Eicosanoids, a group of 20-carbon lipids, are broad bioactive lipid mediators involved in various pathophysiological processes, including inflammation and host defence. Arachidonic acid, a common endogenous precursor, is rapidly converted by cyclooxygenases, lipoxygenases, or epoxygenases into potent lipid mediators such as prostaglandins, leukotrienes, and endoperoxides, each specific to different cell types. [3]. The arachidonic acid pathway synthesises pro-inflammatory lipids, such as prostaglandin (PG) E2 and D2, as well as pro-resolving bioactive lipid mediators like lipoxins, resolvins, and protectins during the resolution phase. Differential gene regulation of enzymes involved in arachidonic acid metabolism has been observed in M1- and M2-polarised human macrophages. M1 macrophages exhibit a significant increase in COX2 and a decrease in COX1, leukotriene A4 hydrolase, thromboxane A synthase 1, and arachidonate 5-lipoxygenase, whereas M2 macrophages show upregulation of arachidonate 15-lipoxygenase and COX1 [4].

Hernandia peltata Meisn., an evergreen tree from the Hernandiaceae family, is native to the coastal regions of tropical islands in the Indian and western Pacific Oceans. The Hernandiaceae family is known for its diverse phytochemical content, including alkaloids, flavonoids, terpenoids, and lignans. Among these, lignans are the predominant class of chemical constituents. Phytochemical research has identified approximately 128 alkaloids within this family, classified into seventeen different structural types [5]. The alkaloids present in Hernandiaceae species display various biological activities, such as analgesic, anti-inflammatory, antipyretic, antibacterial, anticonvulsant, and cytotoxic effects.

Molecular docking of phytochemicals involves the computational simulation of how these plant-derived compounds interact with specific target proteins at the molecular level. This technique is vital for drug discovery and development, as it helps predict the binding affinity and orientation of phytochemicals within the active sites of proteins. By elucidating these interactions, researchers can identify potential therapeutic agents and optimise their efficacy. Molecular docking offers valuable insights into the mechanisms of action of phytochemicals, aiding in the design of more effective and targeted treatments for various diseases. ADMETSAR (ADMET Structure-Activity Relationship) is an online platform designed to predict the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of chemical compounds. This tool utilizes a comprehensive database of experimentally measured ADMET properties and employs machine learning algorithms to generate predictive models.

This study aims to explore the anti-inflammatory effects of phytochemicals, to uncover their potential as natural alternatives or complements to conventional treatments. By investigating the mechanisms through which these plant-derived compounds exert their anti-inflammatory properties, we seek to pave the way for innovative therapeutic approaches. The findings from this research could enhance our understanding of phytochemicals and provide new, effective solutions for managing inflammatory conditions with fewer adverse effects.

2. MATERIALS AND METHODS

2.1 Preparation of Methanolic Extract of leaves of *Hernandia peltata* Meisn

The leaves of *Hernandia peltata* Meisn. was collected from Kalpeni Island, Lakshadweep (10.069034°N, 73.640644° E) during December 2023. The leaves were dried at room temperature and then coarsely ground using an electric pulveriser. The resulting powder was extracted with methanol using a Soxhlet extraction apparatus. The methanol was removed from the extract using a rotary vacuum evaporator under reduced pressure and temperature [6].

2.2 Gas Chromatography-Mass Spectroscopy Analysis

The GC-MS analysis of the *Hernandia peltata* crude extract was conducted at the Centre for Analytical Instrumentation-Kerala (CAI-K) of the Kerala Forest Research Institute (KFRI) in Peechi, Kerala. The analysis was performed using a Shimadzu Nexus GC-2030 Gas Chromatography Mass Spectrometer, with a mass range of 1.5–1000 m/z. Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was programmed to start at 60°C and increase to 280°C over 5 minutes, with the injector temperature set at 260°C. The total analysis duration was 50 minutes. After establishing a clear baseline, 0.4 µL aliquots of the extract were injected into the chromatographic column, and the major components were identified using the NIST 20 mass spectrum library [7].

2.3 Preparation of Receptor and Ligand

The ligand structures were sourced from the PubChem Compound Database (National Center for Biotechnology Information; https://pubchem.ncbi.nlm.nih.gov/) in Spatial Data File (.SDF) format. These structures were then processed using Marvin View 17.25.0 (www.chemaxon.com) and converted into the Tripos Mol2 format. With the help of modifying tools of AutoDock Tools, the ligands were adjusted by detecting and expanding roots, as well as selecting the number of rotatable bonds. Following these preparations, the ligand molecules were converted to PDBQT format for use in AutoDock4 [8].

The receptor structures for cyclooxygenase 1 (Cox-1) (AlphaFold ID: Q63921 for rat), cyclooxygenase 2 (Cox-2) (AlphaFold ID: P35355 for rat), lipoxygenase (Lox5) (AlphaFold ID: P12527 for rat), and nitric oxide synthase (NOS) (AlphaFold ID: Q9R0W4 for rat) were obtained in PDB format from the AlphaFold Protein Structure Database [9]. The structures were prepared for further processing and docking using Accelrys Discovery Studio Visualizer 3.5.0.12158. (Copyright © 2005-12, Accelrys Software Inc). Following this, the macromolecules were processed with MGL tools 1.5.7 (Molecular Graphics Laboratory tools, www.mgltools.scripps.edu), following the standard protocol and parameters outlined in the Auto Dock Tools (ADT) tutorial [10]

2.4 Docking Methodology

Docking studies were conducted using AutoDock4, created by the Scripps Research Institute (La Jolla, CA, www.autodock.scripps.edu). The grid map for the

study was generated with AutoDock4. To identify active sites in the proteins, the Computed Atlas of Surface Topography of Proteins (CASTp) server (http://cast.engr.uic.edu) was utilized. By submitting the target protein to the CASTp server, key amino acids involved in binding interactions were predicted, which assisted in determining ligand binding sites and supported the docking studies [11]. The grid centre for the x, y, and z axes of cox1 was set to 1.153498, 15.60914 and -17.5556 respectively. The grid centre for the x, y, and z axes of cox2 was set to 1.450852, 17.4337 and -9.19963 respectively. The grid centre for the x, y, and z axes of 5-LOX was set to -5.15626, 17.59771 and 7.718147 respectively. The grid centre for the x, y, and z axes of NOS was set to 3.70794, 3.89704 and 2.02787 respectively. The processed file was saved in the grid parameter file (gpf) format. Using parameters optimized by ADT, a docking parameter file (dpf) was created. All docking simulations were conducted using the Lamarckian genetic algorithm. The docking log (dlg) file, which included an RMSD table, reported the binding energy (Kcal/mol) for the best-docked configurations of each molecule.

2.5 Preparation of Ligand for admetSAR Prediction

Ligands were obtained from PubChem in SMILES format [12]. These SMILES representations of the selected ligands were then submitted to the AdmetSAR program to evaluate their toxicity [13].

2.6 Visualisation of Results

Post-docking analysis involved identifying binding site locations, hydrogen-bond interactions, hydrophobic interactions, and bonding distances using LigPlot and Discovery Studio Visualizer. The most optimal and energetically favourable conformations of each ligand were determined by evaluating their binding poses and detailing their interactions with the protein [10].

3. RESULTS

Fig. 1. shows the chromatogram obtained for the plant extract. The phytochemicals obtained on GC-MS analysis are listed in Table 1. Ligands were docked against different proteins of anti-inflammation such as COX-1, COX-2, 5-LOX and NOS. The binding energies of different ligands obtained from the RMSD table are given in Table 2 and Table 3.

Among the 28 compounds analysed, 11 exhibited moderately higher binding energies against all four receptors. Of these, Methyl-3-(5-formylfuran-2-yl) benzoate demonstrated the lowest binding energy with all four receptors. Nine phytochemicals formed hydrogen bonds with COX-1, seven with COX-2, ten with 5-LOX, and seven with NOS. The docked pictures of Methyl-3-(5-formylfuran-2-yl) benzoate against with different receptors are given in Fig. 2.

The *In-silico* analysis of pharmacokinetics and toxicity profiles for selected ligands, presented in Table 4 and Table 5, indicates that Methyl-3-(5-formylfuran-2-yl) benzoate exhibits a high affinity for the four receptors and has an absorption rate of 0.946 through the blood-brain barrier. Furthermore, the intestinal absorption rates for most compounds range from 0.9 to 1, suggesting they possess good bioavailability.

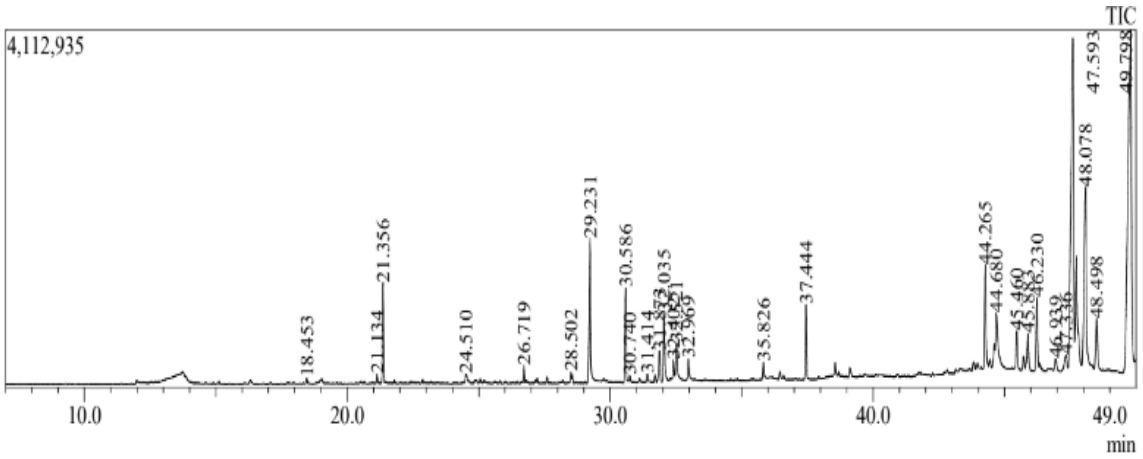

Fig. 1. Chromatogram of *Hernandia peltata* Meisn

Table 1. Phytochemicals screened on GC-MS analysis of *Hernandia peltata* Mesin

Sl. No.	Ligand
1	3,4-Dimethoxybenzenecarbonal
2	3,4,5-Trimethoxybenzaldehyde
3	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol
4	Neophytadiene
5	n-Hexadecanoic acid
6	2-Heptanone,6-(3,5-dimethyl-2-furanyl)-6-methyl-
7	Furan-2(3H)-one,4,5-dihydro-5-(2,4-dimethoxybenzyl)-
8	Phytol
9	9,12-Octadecadienoicacid (Z, Z)-
10	Octadecanoic acid
11	Methyl-3-(5-formylfuran-2-yl) benzoate

Table 2. Binding energy of different phytochemicals against COX-1 and COX-2

Sl. No.	Ligand	COX-1			COX-2		
		Binding energy	No. of hydrogen bond	Amino acid involved in hydrogen bond	Binding energy	No. of hydrogen bond	Amino acid involved in hydrogen bond
1	3,4-Dimethoxybenzenecarbonal	-5.08	2	Cys49(A), Gln46(A)	-4.91	1	Ser516(A)
2	3,4,5-Trimethoxybenzaldehyde	-5.35	2	Cys49(A), Arg471(A)	-5.23	1	Ser516(A)
3	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	-5.54	4	Gln46(A), Cys49(A), Cys43(A), Asn45(A)	-5.81	2	Gln178(A), Phe504(A)
4	Neophytadiene	-5.17	0		-4.77	0	
5	n-Hexadecanoic acid	-4.77	1	Ser128(A)	-3.74	3	Lys68(A), Glu510(A), Phe456(A)
6	2-Heptanone,6-(3,5-dimethyl-2-furanyl)-6-methyl-	-4.78	1	Cys49(A)	-5.24	1	Arg106(A)
7	Furan-2(3H)-one,4,5-dihydro-5-(2,4-dimethoxybenzyl)-	-6.31	1	Arg471(A)	-5.88	2	Arg106(A), Lys68(A)
8	Phytol	-5.41	1	Gln463(A)	-4.69	0	
9	9,12-Octadecadienoicacid (Z, Z)-	-4.75	0		-4.40	0	
10	Octadecanoic acid	-3.99	1	Thr62(A)	-2.93	0	
11	Methyl 3-(5-formylfuran-2-yl) benzoate	-6.33	1	Gln46(A)	-5.89	1	Arg106(A)

Table 3. Binding energy of different phytochemicals against 5-LOX and NOS

Sl. No.	Ligand	5-LOX			NOS		
		Binding energy	No. of hydrogen bond	Amino acid involved in hydrogen bond	Binding energy	No. of hydrogen bond	Amino acid involved in hydrogen bond
1	3,4-Dimethoxybenzenecarbonal	-3.98	1	Gln611(A)	-3.56	0	
2	3,4,5-Trimethoxybenzaldehyde	-4.07	3	Lys183(A), Gln611(A), Asn180(A)	-3.86	0	
3	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	-4.53	5	Glu612(A), Gln611(A), Trp14(A), Glu72(A), Asn613(A)	-4.46	4	Lys814(A), Asp813(A), Asp804(A), Tyr809(A)
4	Neophytadiene	-3.21	0		-3.24	0	
5	n-Hexadecanoic acid	-3.31	3	Gln611(A), Glu612(A), Asn613(A)	-2.78	1	Glu805(A)
6	2-Heptanone,6-(3,5-dimethyl-2-furanyl)-6-methyl-	-4.52	1	Gln611(A)	-4.10	1	Lys814(A)
7	Furan-2(3H)-one,4,5-dihydro-5-(2,4-dimethoxybenzyl)-	-4.91	1	Asn613(A)	-4.54	0	
8	Phytol	-3.41	1	Phe610(A)	-2.77	1	Leu803(A)
9	9,12-Octadecadienoicacid (Z, Z)-	-2.47	1	Glu612(A)	-3.02	1	Tyr809(A)
10	Octadecanoic acid	-2.36	2	Gln611(A), Asn180(A)	-2.07	1	Glu805(A)
11	Methyl 3-(5-formylfuran-2-yl) benzoate	-5.24	3	Asn613(A), Glu612(A), Lys183(A)	-4.73	2	Ser806(A), Glu805(A)

Table 4. Pharmacokinetic properties of phytochemicals obtained from admetSAR

Ligands	3,4-Dimethoxybenzenecarbonal	3,4,5-Trimethoxybenzaldehyde	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	Neophytadiene	n-Hexadecanoic acid	2-Heptanone, 6-(3,5-dimethyl-2-furanyl)-6-methyl-	Furan-2(3H)-one,4,5-dihydro-5-(2,4-dimethoxybenzyl)-	Phytol	9,12-Octadecadienoicacid (Z, Z)-	Octadecanoic acid	Methyl 3-(5-formylfuran-2-yl) benzoate
ABSORPTION											
Blood-Brain Barrier	0.8687	0.8988	0.5335	0.9425	0.9488	0.9924	0.9642	0.9375	0.9838	0.9488	0.946
Human Intestinal Absorption	1	0.9949	0.9938	0.975	0.9888	0.9943	0.9604	0.9846	0.9941	0.9888	0.9936
Caco-2 Permeability	0.9253	0.8538	0.8124	0.6849	0.8326	0.7223	0.7237	0.6445	0.8177	0.8326	0.564
P-glycoprotein Substrate	0.6844	0.675	0.5901	0.6	0.6321	0.5757	0.6345	0.5851	0.6747	0.6321	0.7297
P-glycoprotein Inhibitor	0.7523	0.626	0.7839	0.6247	0.9598	0.602	0.7653	0.8865	0.8472	0.9598	0.7131
P-glycoprotein Non-Inhibitor	0.8929	0.7966	0.5112	0.5993	0.9277	0.698	0.5	0.5696	0.678	0.9277	0.7308
Renal Organic Cation Transporter	0.8458	0.8995	0.8273	0.8361	0.9266	0.8379	0.768	0.8179	0.8934	0.9266	0.8924
DISTRIBUTION											
Subcellular localization	0.9245	0.8592	0.8365	0.6326	0.5152	0.6679	0.8577	0.5576	0.6788	0.5152	0.8471

METABOLISM											
CYP450 2C9 Substrate	0.8113	0.8195	0.749	0.8645	0.7886	0.7765	0.807	0.791	0.8474	0.7886	0.7889
CYP450 2D6 Substrate	0.7941	0.7622	0.8448	0.8111	0.8956	0.8251	0.8022	0.8278	0.8876	0.8956	0.9182
CYP450 3A4 Substrate	0.5834	0.5424	0.6559	0.5525	0.6982	0.5838	0.5	0.527	0.6171	0.6982	0.7099
CYP450 1A2 Inhibitor	0.7171	0.6851	0.5361	0.7161	0.8326	0.5069	0.7297	0.9046	0.5466	0.8326	0.7835
CYP450 2C9 Inhibitor	0.9554	0.99	0.7752	0.8903	0.8808	0.7687	0.6845	0.9071	0.9433	0.8808	0.7289
CYP450 2D6 Inhibitor	0.9658	0.9597	0.9453	0.9474	0.9554	0.8948	0.8805	0.923	0.953	0.9554	0.9559
CYP450 2C19 Inhibitor	0.513	0.618	0.6161	0.8891	0.9578	0.6895	0.8625	0.9026	0.9415	0.9578	0.5801
CYP450 3A4 Inhibitor	0.9153	0.7935	0.7997	0.9627	0.9484	0.8615	0.6243	0.9088	0.9705	0.9484	0.96
CYP Inhibitory Promiscuity	0.6546	0.6388	0.5589	0.7252	0.9647	0.698	0.6848	0.765	0.8518	0.9647	0.5256
TOXICITY											
AMES Toxicity	0.9133	0.9114	0.8418	0.9494	0.9865	0.9362	0.798	0.9132	0.9321	0.9865	0.8534
Carcinogens	0.8474	0.8132	0.869	0.5698	0.6452	0.7035	0.9324	0.5055	0.5217	0.6452	0.8141
Fish Toxicity	0.7675	0.8421	0.5869	0.9955	0.9144	0.5793	0.945	0.9236	0.958	0.9144	0.9232
Tetrahymena Pyriformis Toxicity	0.7926	0.9255	0.5403	0.9952	0.999	0.9881	0.9741	0.9864	0.9958	0.999	0.9527
Honey Bee Toxicity	0.8096	0.8336	0.745	0.8387	0.6691	0.6422	0.7429	0.8229	0.7976	0.6691	0.6664
Biodegradation	0.7256	0.6844	0.6226	0.7175	0.8795	0.5605	0.6373	0.8931	0.8105	0.8795	0.8444
Acute Oral Toxicity	0.91	0.6519	0.7514	0.9077	0.6378	0.601	0.5748	0.8552	0.639	0.6378	0.6807
Carcinogenicity (Three-class)	0.494	0.602	0.6485	0.4862	0.7057	0.5268	0.7143	0.6507	0.7273	0.7057	0.4517

Table 5. Toxicity profile of phytochemicals obtained from admetSAR

	`ADMET Predicted Profile --- Regression										
Model	**3,4-Dimethoxybenzenecarbonal**	**3,4,5-Trimethoxybenzaldehyde**	**(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol**	**Neophytadiene**	**n-Hexadecanoic acid**	**2-Heptanone,6-(3,5-dimethyl-2-furanyl)-6-methyl-**	**Furan-2(3H)-one,4,5-dihydro-5-(2,4-dimethoxybenzyl)-**	**Phytol**	**9,12-Octadecadienoicacid (Z, Z)-**	**Octadecanoic acid**	**Methyl-3-(5-formylfuran-2-yl) benzoate**
ABSORPTION											
Aqueous solubility (Log S)	-1.8753	-2.2844	-1.443	-5.2549	-3.5022	-2.7917	-2.5431	-2.472	-3.8977	-3.5022	-1.6464
Caco-2 Permeability (Log Papp,cm/s)	1.6082	1.4795	1.0783	1.3417	1.395	1.6705	0.9064	1.2481	1.2275	1.395	0.7191
TOXICITY											
Rat Acute Toxicity (LD50,mol/kg)	1.9517	2.3809	1.5616	1.472	1.3275	1.9373	2.5939	1.6146	1.7357	1.3275	2.3940
Fish Toxicity (pLC50,mg/L)	1.87	1.5693	1.6928	-0.8334	1.892	0.9649	-0.0332	0.6732	0.3195	1.892	0.9692
Tetrahymena Pyriformis Toxicity (plGC50,µg/L)	-0.4376	-0.0055	0.063	0.9633	0.3852	0.5461	0.7455	1.0249	1.1527	0.3852	0.2109

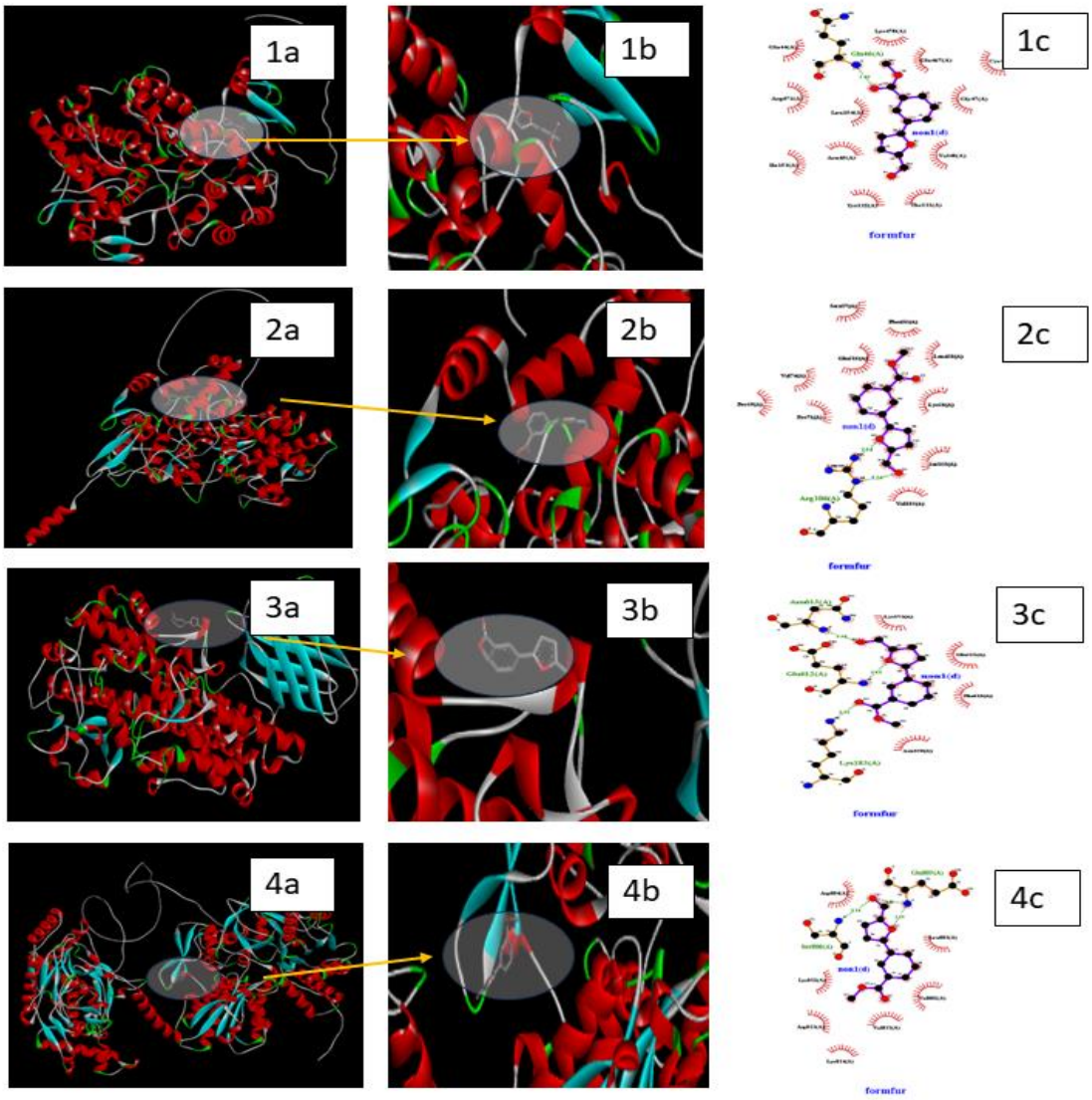

Fig. 2. Post-docking interaction of Methyl 3-(5-formylfuran-2-yl) benzoate against COX-1, COX-2, 5-LOX and NOS. 1a and 1c: In situ docked Methyl 3-(5-formylfuran-2-yl) benzoate against COX-1 and Ligplot showing their interaction in the docked pose. 2a and 2c: In situ docked Methyl 3-(5-formylfuran-2-yl) benzoate against COX-2 and Ligplot showing their interaction in the docked pose. 3a and 1c: In situ docked Methyl 3-(5-formylfuran-2-yl) benzoate against 5-LOX and Ligplot showing their interaction in the docked pose. 4a and 1c: In situ docked Methyl 3-(5-formylfuran-2-yl) benzoate against NOS and Ligplot showing their interaction in the docked pose. 1b, 2b, 3b and 4b: Magnified view illustrating the ligand binding pocket within the receptor molecule

4. DISCUSSION

GC-MS analysis of the methanolic extract from fresh *Hernandia peltata* Meisn. leaves identified 28 compounds, with 11 major components exhibiting significant pharmacological activity. The genus Hernandia has been ethnobotanically utilized for various medicinal purposes, with several species exhibiting notable biological activities such as antiplasmodial, cytotoxic, and antiplatelet effects. Detailed phytochemical investigations have revealed that the genus primarily produces isoquinoline alkaloids and lignans, which are responsible for these observed pharmacological properties [14]. The members of this family possess 128 identified compounds across 17 structural types, primarily isolated from species in diverse geographical regions such as Africa, Asia, Europe, and the Americas [15].

There is scientific validation for the traditional use of *Hernandia peltata* bark in combination with other plants in treating inflammation, pain and fever by demonstrating their significant analgesic and anti-inflammatory activities [16,17].

In silico analysis of these phytoconstituents using AutoDock Tool revealed binding energies ranging from -0.2 to -0.7 kcal/mol. Among these compounds, Methyl-3-(5-formylfuran-2-yl) benzoate showed the highest binding affinity towards COX-1, COX-2, 5-LOX, and NOS, with binding energies of -6.33, -5.89, -5.24, and -4.73 kcal/mol, respectively. Additionally, 4,5-dihydro-5-(2,4-dimethoxybenzyl)-furan-2(3H)-one, (E)-4-(3-hydroxyprop-1-en-1-yl)-2-methoxyphenol, phytol, 3,4-di methoxy benzene carbonal, and neophytadiene also demonstrated good binding affinity. Among the 11 compounds, most formed hydrogen bonds with receptors associated with anti-inflammatory action, specifically bonding with residues such as Cys49, Gln46, Arg471, Gln611, Asn613, and Glu612.

The *In-silico* pharmacokinetics and toxicity profiles of selected ligands revealed that the phytoconstituents exhibit blood-brain barrier absorption rates between 0.8 and 0.9, indicating their lipophilic nature and suggesting potential use in central nervous system conditions such as meningitis. Many compounds demonstrated high intestinal absorption, indicating favourable bioavailability. Depending on the dosage, these compounds may act as P-glycoprotein substrates, inhibitors, or non-inhibitors. Notably, n-hexadecanoic acid, octadecanoic acid, 9,12-octadecadienoic acid (Z, Z)-, and phytol could inhibit the bioavailability of other chemicals, potentially increasing toxicity by enhancing P-glycoprotein activity. Additionally, some compounds might disrupt sodium, potassium, and calcium homeostasis by affecting renal organic cation transporters. Several compounds also influence the metabolism of other chemicals by acting as substrates for CYP450 2C9 and inhibitors of CYP450 3A4 and CYP450 2D6.

Toxicity assessments indicated that most compounds exhibit mutagenic properties at higher doses and some have potential carcinogenic effects. Since these conclusions are preliminary based on substructure search, these findings need to be validated initially by *in vitro* and to be confirmed *in vivo* methods. Additionally, these compounds are ecotoxic, particularly affecting fish and *Tetrahymena pyriformis*, although they pose relatively low risks to arthropods, which also need to be validated *in vivo*.

Phytochemical screening followed by docking studies is a critical approach in drug discovery from plants, providing a systematic method to identify and evaluate bioactive compounds. Initially, phytochemical screening allows researchers to isolate and characterize various compounds present in plant extracts, assessing their potential therapeutic effects. This screening often involves techniques such as chromatography and spectrometry to determine the chemical profiles of the extracts, which can reveal compounds with significant biological activities, such as antimicrobial, anti-inflammatory, or anticancer properties [18]. Once promising phytochemicals are identified, molecular docking studies are employed to predict how these compounds interact with specific biological targets, such as enzymes or receptors implicated in disease processes. This computational technique simulates the binding of phytochemicals to target proteins, allowing researchers to evaluate binding affinities and conformational changes that occur during the interaction. By utilizing software like AutoDock, researchers can analyze the energy profiles of these interactions, identifying which phytochemicals exhibit the strongest affinities and thus the greatest potential as therapeutic agents. Together, these methodologies not only streamline the drug discovery process but also enhance the likelihood of developing effective, plant-derived pharmaceuticals with favourable pharmacokinetic properties [19,20]. Furthermore, the screening process often includes assessing the drug-likeness of these compounds using criteria such as Lipinski's rule of five, which evaluates properties like molecular weight, hydrogen bond donors and acceptors, and lipophilicity. Compounds that meet these criteria are more likely to exhibit favourable pharmacokinetic profiles, making them suitable candidates for further development [20].

In silico toxicity testing has become increasingly relevant in drug discovery from plant-based molecules, as it offers a rapid and cost-effective means to assess the safety profiles of phytochemicals before they undergo extensive experimental validation [21]. By employing computational tools such as admetSAR, researchers can predict various toxicological endpoints based on the chemical structure of the compounds, facilitating the early identification of potential adverse effects. This approach not only enables the screening of large libraries of plant-derived compounds but also helps prioritize candidates that demonstrate favourable pharmacokinetic and toxicological properties. Moreover, in silico methods can elucidate the

mechanisms of toxicity by simulating interactions between phytochemicals and biological targets, providing insights into their safety profiles. The integration of in silico toxicity predictions with traditional experimental assays streamlines the drug discovery process, reduces reliance on animal testing, and enhances the likelihood of developing safe and effective therapeutics from natural sources [22]. Overall, the adoption of in silico methodologies is expected to play a significant role in regulatory assessments and the future of drug development, as they align with the growing emphasis on ethical and efficient testing practices in the pharmaceutical industry.

5. CONCLUSION

The study indicates that phytochemicals in the methanolic leaf extract of *Hernandia peltata* Meisn. exhibit strong binding affinities to receptors associated with anti-inflammatory responses. Among the 28 tested ligands, Methyl-3-(5-formylfuran-2-yl) benzoate demonstrated the lowest binding energies against COX-1, COX-2, 5-LOX, and NOS. These *in silico* findings suggest that the methanolic leaf extract of *Hernandia peltata* Meisn. has the potential to modulate anti-inflammatory responses by interacting with key proteins involved in these pathways. However, further research, including both *in vitro* and *in vivo* studies, is required to validate the efficacy of these plant compounds in promoting anti-inflammatory activity.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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

ACKNOWLEDGEMENT

Financial and facility support provided by the Kerala Veterinary and Animal Sciences University vide order number. KVASU/DAR/ A1/2438/2023(1) dt.23/12/2023 is thankfully acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ahmed AU. An overview of inflammation: mechanism and consequences. Front Biol (Beijing). 2011;6(4):274–81.
2. Janssen WJ, Henson PM. Cellular regulation of the inflammatory response. Toxicol Pathol. 2012;40(2):166–73.
3. Freire MO, Van Dyke TE. Natural resolution of inflammation. Periodontol. 2013;63(1):149–64.
4. Ortega-Gómez A, Perretti M, Soehnlein O. Resolution of inflammation: an integrated view. EMBO Mol Med. 2013;5(5):661–74.
5. Conserva LM, Cynara de Araújo BP, Barbosa-Filho JM. Alkaloids of the Hernandiaceae: Occurrence and a compilation of their biological activities. Alkaloids Chem Biol. 2005;62:175–243.
6. Udayan D, Nair SN, Juliet S, Ravindran R, Athalathil S, Adarshkrishna TP, et al. Acaricidal activity of *Artemisia nilagirica* leaves against *Rhipicephalus (Boophilus) annulatus* ticks. Planta Med. 2020;86(18): 1335–44.
7. Mahesh DM, Juliet S, Suresh NN, Nisha AR, Ravindran R, Shijin MS, et al. *In vitro* analysis of 1, 8-cineole in the plasma of domestic fowl by Gas Chromatography–Mass Spectrometry. IJCS. 2019;7(1): 2249–53.
8. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem. 1998;19(14):1639–62.
9. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. Nature. 2021;596(7873): 583–9.
10. Raj A, Nair SN, Abdulvahab R, Ittoop G. *In-silico* modelling of interaction between environmental xenoestrogens and estrogen receptor of pacific oyster (Magallana gigas [Thunberg, 1793]) using Auto Dock; 2022;
11. Kausar MA, Ali A, Qiblawi S, Shahid SMA, Izhari MA. Molecular docking based design of Dengue NS5 methyltransferase inhibitors. Bioinformation. 2019;15(6): 394.
12. Moon A, Khan D, Gajbhiye P, Jariya M. *In silico* prediction of toxicity of ligands utilizing admetSAR. Int J Pharma Bio Sci. 2017;8:674–7.
13. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, et al. admetSAR: a comprehensive source and free tool for assessment of

chemical ADMET properties. ACS Publications; 2012.
14. Gu JQ, Kinghorn AD. Bioactive constituents of the genus Hernandia. Studies in Natural Products Chemistry. 2005;30:559-602.
15. Conserva LM, Cynara de Araújo BP, Barbosa-Filho JM. Alkaloids of the Hernandiaceae: Occurrence and a compilation of their biological activities. The Alkaloids: Chemistry and Biology. 2005; 62:175-243.
16. Das S, Mukherjee H, Ahmed SM, Haldar PK, Mandal AB, Mahapatra A, Mukherjee PK, Chakraborti S, Chattopadhyay D. Evaluation of an ethnomedicinal combination containing *Semecarpus kurzii* and *Hernandia peltata* used for the management of inflammation. Pharmaceutical Biology. 2013;51(6):677-85.
17. Chakraborty S, Chattopadhyay DD. Evaluation of an ethnomedicinal formulation containing *Semecarpus kurzii* and *Hernandia peltata* used for the management of inflammation.
18. Manojkumar S, Thandeeswaran M, Thangavel SK, Arjunan A, Muthuselvam M, Kalaiarasi G, Gnanajothi K. Phytochemical Screening, *In Silico* Molecular Docking, ADME Properties, and In Vitro Antioxidant, Anticancer, and Antidiabetic Activity of Marine Halophyte *Suaeda maritima* (L.) Dumort. ACS omega. 2024, 29;9(10): 11200-16.
19. Umesh HR, Ramesh KV, Devaraju KS. Molecular docking studies of phytochemicals against trehalose–6–phosphate phosphatases of pathogenic microbes. Beni-Suef University Journal of Basic and Applied Sciences. 2020, 9:1-4.
20. Mendie LE, Hemalatha S. Molecular docking of phytochemicals targeting GFRs as therapeutic sites for cancer: an *in silico* study. Applied biochemistry and biotechnology. 2022;194(1):215-31.
21. Raies AB, Bajic VB. *In silico* toxicology: computational methods for the prediction of chemical toxicity. Wiley Interdisciplinary Reviews: Computational Molecular Science. 2016 ;6(2):147-72.
22. Vedani A, Smiesko M. *In silico* toxicology in drug discovery—concepts based on three-dimensional models. Alternatives to Laboratory Animals. 2009;37(5): 477-96.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/123601