**Original Research Article**

# Identification of phytochemicals in methanolic extract of *Hernandia peltata* Meisn. and assessment of their anti-inflammatory activity *in silico*


## 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* 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 then 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 AutoDock 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

Figure 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.

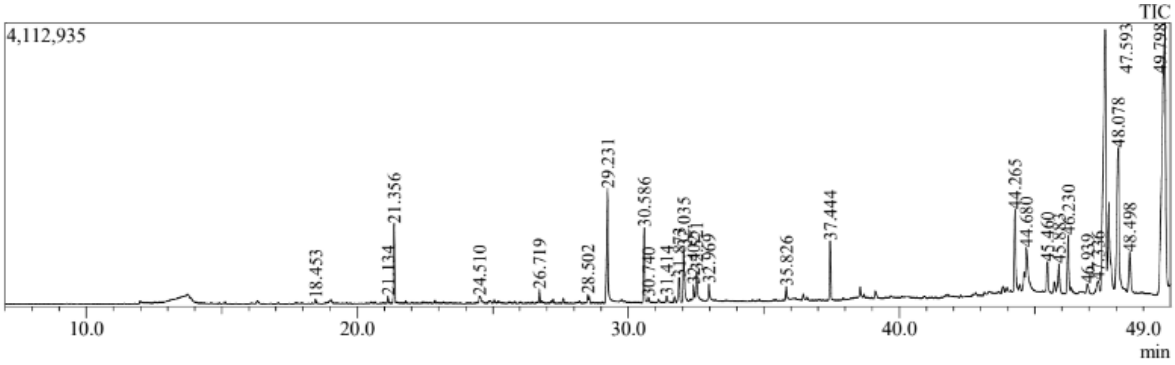


Fig. 1. Chromatogram of *Hernandia peltata* Meisn.

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 |

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 figure 2.

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) |

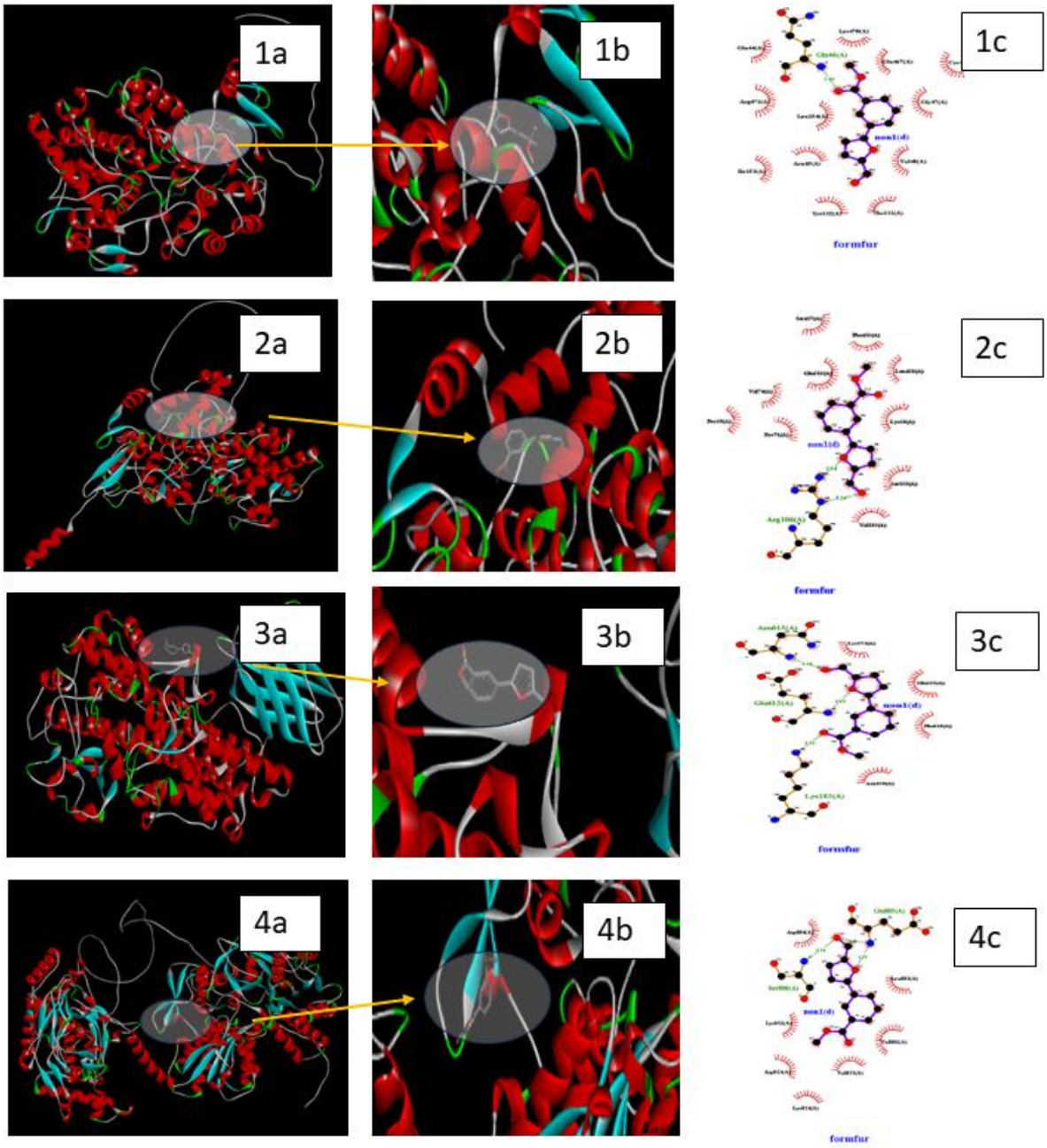


Fig. 2. Post-docking interaction of Methyl 3-(5-formylfuran-2-yl) benzoate against COX-1, COX-2, 5-LOX and NOS. 1a and 1c: In situ docked Methyl 3-(5-formylfuran-2-yl) benzoate against COX-1 and Ligplot showing their interaction in the docked pose. 2a and 2c: In situ docked Methyl 3-(5-formylfuran-2-yl) 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

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.

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 (pIGC50 ,µg/L) | -0.4376 | -0.0055 | 0.063 | 0.9633 | 0.3852 | 0.5461 | 0.7455 | 1.0249 | 1.1527 | 0.3852 | 0.2109 |



## 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. *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-dimethoxybenzenecarbonal, 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. Additionally, these compounds are ecotoxic, particularly affecting fish and *Tetrahymena pyriformis*, although they pose relatively low risks to arthropods.

## 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.



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