ORIGINAL ARTICLE

ISSN: 0331 - 670X

https://doi.org/10.51412/psnnjp.2025.042

The Nigerian Journal of PHARMACY
AS MEN OF HONOUR WE JOIN HANDS
THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927

Potentials of Bioactive Compounds of the Essential Oil of *Monodora myristica* as Inhibitors of COX-2: A Molecular Docking and In Silico Toxicity Assessment for Anti-Inflammatory Properties

Afolabi Felix Olaide[1]*, Apata Joseph Tosin.[2], Oyemitan Idris Ajayi[3], Elusiyan Christianah Abimbola[4], Afolabi Titilayo Oluwapamilerin[5], Akanmu Moses Atanda[3]

[1]Department of Pharmacology, Faculty of Basic Medical Sciences, Federal University of Health Sciences, Ila-Orangun, Nigeria
[2]Department of Biochemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
[3]Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
[4]Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
[5]Department of Preventive Dentistry, Obafemi Awolowo University Teaching Hospital, Ile-Ife, Osun State, Nigeria

ARTICLE INFO

Article history:

Received 30th September 2025
Revised 19th October 2025
Accepted 21st October 2025
Online Published

Keywords:

Anti-inflammatory,
Monodora myristica,
Cyclooxygenase-2,
Molecular docking,
ADMET

*Corresponding Author:
Dr. F. O. Afolabi
Email: felix.afolabi@fuhsi.edu.ng
Tel: +2347063491265

ABSTRACT

Background: The seed extract from *Monodora myristica* has traditionally been employed in anti-inflammatory therapies. In light of the escalating costs and side effects associated with existing drugs for managing inflammatory conditions, the pursuit of potential novel pharmacological agent is imperative. Cyclooxygenase, especially the COX-2 isoform, emerges as a key target owing to its critical involvement in inflammatory processes.
Methods: This investigation utilized computational modeling techniques to evaluate the binding affinities and interaction profiles of compounds derived from the dried seeds essential oil of *Monodora myristica* with COX-2.
Results: The leading three compounds exhibited binding affinities between -6.7 and -6.9 kcal/mol, which were notably superior to those of the reference ligands [diclofenac (–6.6 kcal/mol) and ibuprofen (-6.8 kcal/mol)]. Notably, naphthalene (-6.9 kcal/mol) and 2,3,5,6-tetramethylphenol (-6.9 kcal/mol) demonstrated the highest binding affinities, indicating their promise as COX-2 inhibitors. ADMET profiling revealed favorable physicochemical and pharmacodynamic characteristics for these compounds.
Conclusion: This study advances the field of innovative anti-inflammatory drug development and establishes a foundation for subsequent research on precision-targeted strategies against inflammatory disorders.

INTRODUCTION

Pain and inflammation represent two primary causes for visits to clinics and emergency departments in hospitals[1]. Pain is a multifaceted sensory and emotional phenomenon generally linked to tissue injury or potential harm. It functions as a defensive mechanism, signaling the body to recognize injury or illness, thereby encouraging actions to prevent further harm[2]. Pain is one of the symptoms of inflammation and plays a pivotal role in inflammatory processes. Inflammation is a fundamental biological response that occurs in the body when tissues are injured or infected[2]. It serves as a protective mechanism designed to eliminate harmful stimuli, initiate healing, and restore homeostasis. It could be acute or chronic. While acute inflammation is a vital part of the immune response, chronic inflammation can lead to various diseases, including arthritis, heart disease, diabetes, and cancer[3]. Elucidating the mechanisms of inflammation provides critical insights into the root causes of numerous diseases and guides the development of therapeutic interventions. From conventional pharmacological treatments to innovative biologics and lifestyle adjustments, effective inflammation management offers significant potential for enhancing health outcomes[4]. Several polyphenols are known to exert anti-inflammatory effects through modulation of different signaling pathways such as arachidonic acid (AA) metabolism, nuclear factor kappa B (NFKB), and TNF-α4. In the AA-dependent pathway, the anti-inflammatory effect of plant polyphenols is related to their ability to inhibit cyclooxygenase (COX), which converts AA into prostaglandins. Virtually every branch of medicine involves managing and controlling inflammation to some extent[5]. While numerous medications are currently in use, many are associated with significant side effects, and others pose risks of dependence and addiction. Novel drugs derived from flora and fauna offer natural, biodegradable alternatives with minimal or no adverse effects[6]. One such prominent plant is *Monodora myristica* (*M. myristica*).

Monodora myristica, a tropical species of the *Annonaceae* family, is widely distributed in West Africa and valued for both its culinary and medicinal uses. Traditionally, it has been employed in managing ailments such as dysentery, toothache, fever, constipation, and various skin disorders [7]. Despite these reported uses, only a limited number of them have been subjected to scientific validation. In a study by Afolabi et al.[7], the acute toxicity of the seed oil was evaluated, and the findings indicated that the oil exhibited moderate safety. Eguvbe et al.[8] explored the potential application of the bioactive constituents of *M. myristica* as dietary supplements. Their findings suggested that these compounds could be moderately incorporated into food formulations. Furthermore, the phenolic composition, antioxidant properties, and anti-nephrolithiasis potential of *M. myristica* seeds were examined, along with the influence of endogenous proteins and lipids. The findings indicated that proteins and lipids may augment the biological activities of the plant[9]. The methanol seed extract of *M. myristica* was evaluated for its anti-inflammatory properties and effects on hematological alterations. The study revealed that the extract mitigated hematological disturbances and inhibited lead acetate–induced leukocytosis[10]. Studies on the essential oil of *M. myristica* have reported evidence of antioxidant, antidiabetic, and antibacterial activities[11,12]. Despite the substantial scientific evidence available on the seed extract of *M. myristica*, no prior studies have validated the mechanism of its bioactive constituents from the dried seed essential oil against cyclooxygenase-2 (COX-2). Consequently, this study sought to evaluate the potential of bioactive compounds in the essential oil of *M. myristica* as COX-2 inhibitors through molecular docking, with the aim of confirming their anti-inflammatory activity and elucidating their underlying mechanisms. The *in silico* toxicity profile will also be investigated. The findings are expected to provide a scientific foundation for the isolation of safe active compounds from the dried seed essential oil of *M. myristica* for future research as promising cyclooxygenase inhibitors.

MATERIALS AND METHODS

Seed collection and identification

The dried seeds of *M. myristica* were collected and authenticated by Mr. I. I. Ogunlowo, Herbarium Officer, Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, where a voucher specimen was deposited. The Faculty of Pharmacy Herbarium (FPI) is listed in the online edition of the Index Herbariorum under the accession number FPI 2111.

Extraction and distillation procedure

Hydro-distillation was carried out using a Clevenger-type apparatus at the Postgraduate Toxicology Laboratory, Department of Biochemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria. A total of 567 g of dried *M. myristica* seeds was subjected to hydro-distillation for approximately 4 h. The essential oil obtained was collected, dried over anhydrous sodium sulfate, and stored

in an amber-colored bottle at 4 °C. The density of the oil was determined, and the percentage yield was subsequently calculated as follows:

$$\text{Percentage yield of the oil} = \frac{\text{Volume of oil obtained}}{\text{Weight of dried seed}} \times 100\%$$

Gas chromatography-mass spectrophotometric analysis.

The constituents of the crude oil extract done at the Central Research laboratory, Ilorin were characterized using GC-MS (QP2010) done at the coupled with an MS-5973-634071 Series under the following analytical conditions: the column oven temperature was initially set at 70.0 °C and increased to 280 °C within 5 minutes at a flow rate of 10 ml/min. The injection was performed at 250.0 °C using split flow injection with linear velocity flow control. The column pressure was maintained at 116.9 kPa, with a total flow rate of 40.8 ml/min, a column flow of 1.80 ml/min, and a linear velocity of 49.2 cm/s. A purge flow of 3.0 ml/min and a split ratio of 20.0 were employed. The ion source temperature was set at 200.0 °C, while the interface temperature was maintained at 250.0 °C, with a solvent cut time of 2.5 minutes. Mass spectrometric analysis commenced at 3.0 minutes and ended at 24.0 minutes, with a scan event time of 0.5 seconds and a scan speed of 666. The scan range was set between m/z 30.00 and 350.00. The compounds were identified and characterized using their gas chromatography retention times, while mass spectra were matched through computer-assisted comparison with reference standards from the NIST14 compound library.

***In silico* evaluation**

Ligand and target protein selection

The twelve ligands identified through GC–MS analysis of the essential oil from dried seeds of *M. myristica*, along with the standard drugs ibuprofen and diclofenac, were retrieved from the PubChem database (http://pubchem.ncbi.nlm.nih.gov) and converted from Structured Data File (SDF) to Protein Data Bank (PDB) format. The crystal structure of the target protein, cyclooxygenase-2 (PDB ID: 4PH9), was obtained from the Protein Data Bank (https://www.rcsb.org) and stored in PDB format.

Molecular docking

The molecular docking analysis was carried out as previously described[13] using AutoDock (v4.2) integrated within the PyRx platform (v0.8, Windows). Docking was performed with an exhaustiveness parameter of 8, generating nine binding conformations for each protein. The conformation with the lowest binding energy (most negative value) was selected to represent the ligand–receptor interactions. Visualization of the docking results was performed with Biovia Discovery Studio (v21.1.0.20289). The three ligands with the most favorable binding energies, along with the reference drugs ibuprofen and diclofenac, were further analyzed and illustrated in both 2D and 3D formats.

ADMET evaluation

The GC-MS identified compounds alongside ibuprofen and diclofenac were subjected to ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) evaluation. ADMET predictions were conducted using the ADMETlab 2.0 web server (http://lmmd.ecust.edu.cn/admetsar2)[14].

Results

Percentage yield of *M. myristica* dried seed

The extraction of the powdered *M. myristica* seed (567 g) yielded 110.24 g of the essential oil, corresponding to a percentage yield of 19.4%. (Table 1).

Gas chromatography-mass spectrometric analysis (GCMS)

Table 2 summarizes the retention times, peak areas, and systematic names of the compounds identified from the dried seed extract of *M. myristica* by GC–MS analysis. A total of twelve phytoconstituents were detected, with *p*-cymene (31.16%), tricyclene (17.35%), α-phellandrene (13.07%), and linalool (11.45%) as the predominant components. Minor constituents, occurring at less than 6%, included α-thujene, (±)-β-pinene, α-terpineol, caryophyllene, naphthalene, 4-carene, and tetramethylphenol. Furthermore, the total ion chromatogram (TIC) displayed several distinct peaks, each corresponding to volatile constituents eluting at specific retention times (Fig. 1). Also, prominent peaks were observed at retention times 6.363, 9.328, 10.404, 13.751, 17.047, 24.147, 26.787, 27.174, 27.487, 38.226, and 39.469 minutes, indicating the presence of multiple abundant compounds. The most dominant peak occurred at 10.404 minutes, suggesting that this compound constitutes the major component of the essential oil. Additional prominent peaks at 9.328 and 13.751 minutes point to other compounds present in appreciable amounts, whereas

smaller peaks distributed throughout the chromatogram represent minor phytochemical constituents (Fig. 1).

Molecular docking analysis

The molecular docking study of bioactive compounds identified in the essential oil of *Monodora myristica* seeds revealed notable binding interactions with the target protein, with docking scores ranging between –5.5 and –6.9 kcal/mol (Table 3). Among the tested bioactive compounds, naphthalene (–6.9 kcal/mol) and 2,3,5,6-tetramethylphenol (–6.9 kcal/mol) exhibited the strongest binding affinities, which were comparable to ibuprofen (–6.8 kcal/mol) and superior to diclofenac (–6.6 kcal/mol) (Table 4). Furthermore, moderately strong binding affinities were observed for α-phellandrene (–6.7 kcal/mol), p-cymene (–6.5 kcal/mol), α-terpineol (–6.5 kcal/mol), 4-carene (–6.5 kcal/mol), 2-cyclohexen-1-ol (–6.5 kcal/mol), and β-pinene (–6.4 kcal/mol), all of which fall within the same binding range as the standard reference drugs. On the other hand, linalool (–5.5 kcal/mol) demonstrated the lowest docking score, suggesting weaker interaction with the receptor relative to other constituents (Table 3).

Table 1: Percentage yield of dried seed of *M. myristica*

	Powder plant material	Yield obtained
Mass (g)	567	110.24
Percentage yield (%)		19.4

Table 2: Compounds identified by GC-MS analysis of dried seed extract of *M. myristica*

S/No	Retention Time	% Peak area	Systematic name	Compound name
1	6.069	5.88	Bicyclo[3.1.0] hex-2-ene, 4-methyl-(1-methylethyl)-	alpha-thujene
2	6.363	17.35	Tricyclo[2.2.1.0(2,6)] heptane, 1,7,7-trimethyl-	Tricyclene
3	7.939	1.38	Bicyclo [3.1.1] heptane, 6,6-dimethy 15908 018172-67-3 97 l-2-methylene-, (1S)-	(±)-β-Pinene
4	9.328	13.07	alpha.-Phellandrene	alpha.-Phellandrene
5	10.404	31.16	p-Cymene	p-Cymene
6	13.751	11.45	1,6-Octadien-3-ol, 3,7-dimethyl -	Linalool
7	14.282	1.65	(1R,4R) -1-methyl -4-propan -2-ylcyclohex-2-en-1-ol	2-Cyclohexen-1-ol
8	17.047	2.58	Alpha-Terpineol	α-Terpineol
9	24.141	1.28	Caryophyllene	Caryophyllene
10	26.787	1.03	Naphthalene	Naphthalene
11	38.228	1.40	4-Carene, (1S,3R,6R)-(-)-	4-Carene
12	38.490	2.15	Phenol,2,3,5,6-tetramethyl-	Phenol

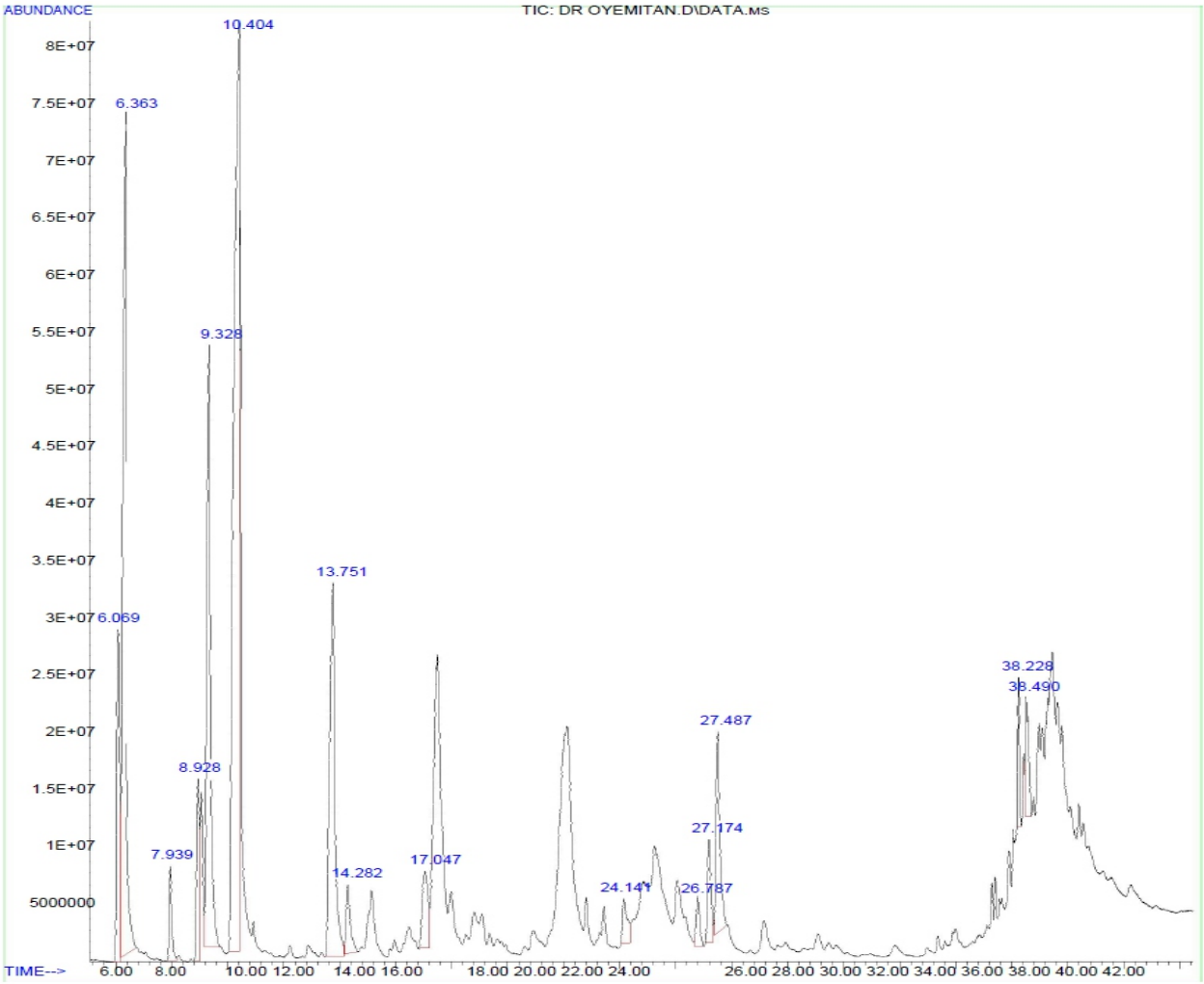

Fig. 1: GC-MS chromatogram of bioactive compounds identified from the dried seed of *M. myristica*

Table 3. The Docking Score (kcal/mol) of the Bioactive Compounds Identified in Essential Oil of Dried Seed of *Monodora Myristica*

S/N	Systemic Name	Compound Name	PubChem CID	Docking Score (kCal/mol)
1	Bicyclo[3.1.0]hex-2-ene, 4-methyl-(1-methylethyl)-	alpha-thujene	17868	-6.2
2	Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	Tricyclene	79035	-6.3
3	Bicyclo[3.1.1]heptane, 6,6-dimethy 15908 018172-67-3 97 l-2-methylene-, (1S)-	(±)-β-Pinene	14896	-6.4
4	alpha-Phellandrene	alpha.-Phellandrene	7460	-6.7
5	p-Cymene	p-Cymene	7463	-6.5
6	1,6-Octadien-3-ol, 3,7-dimethyl-	Linalool	6549	-5.5
7	(1R,4R)-1-methyl-4-propan-2-ylcyclohex-2-en-1-ol	2-Cyclohexen-1-ol	122485	-6.5

8	Alpha-Terpineol	α-Terpineol	17100	-6.5
9	Caryophyllene	Caryophyllene	5281515	-6.3
10	Naphthalene	Naphthalene	931	-6.9
11	4-Carene, (1S,3R,6R)-(-)-	4-Carene	530422	-6.5
12	Phenol,2,3,5,6-tetramethyl-	Phenol	10694	-6.9
		Standard Drug		
1	Diclofenac	Diclofenac	3033	-6.6
2	Ibuprofen	Nurofen	3672	-6.8

Table 4: The binding energy of the top three compounds identified in essential oil of dried seed of *Monodora Myristica*

S/N	Systemic Name	Compound Name	PubChem CID	Docking Score (kCal/mol)
1	alpha-Phellandrene	alpha.-Phellandrene	7460	-6.7
2	Naphthalene	Naphthalene	931	-6.9
3	Phenol, 2,3,5,6-tetramethyl-	Phenol	10694	-6.9
		Standard Drug		
1	Diclofenac	Diclofenac	3033	-6.6
2	Ibuprofen	Nurofen	3672	-6.8

Post-docking analysis

The post-docking analysis illustrated the binding interactions between the alpha-phellandrene and the active site residues of the target protein (COX-2) (Fig. 2). The 3D visualization revealed hydrogen bond donor and acceptor regions, confirming stable interactions within the binding pocket. The 2D interaction diagram further showed that the alpha-phellandrene engaged in multiple non-covalent interactions with key amino acid residues. These included van der Waals interactions with GLN42, ASN39,43, CYS41, GLY45, LYS469, ARG44,470, and GLU466. Additionally, alkyl interactions were observed with LEU153 and PRO154, which contribute to hydrophobic stabilization of the ligand–protein complex.

Furthermore, the interaction between COX-2 amino acid residues and naphthalene is depicted in Fig. 3. The 3D representation (left) highlights the binding pocket where naphthalene associates with specific amino acid residues of the target protein, whereas the 2D representation (right) depicts the detailed bonding interactions within the binding site. Naphthalene was observed to interact with key residues, including MET523, TRP388, VAL524,350, and LEU353, through Pi–Sulfur, Pi–Pi T-shaped, and Pi–Alkyl interactions.

In addition, Fig. 4 shows the molecular interaction of amino acids of COX-2 with phenol, 2,3,5,6-tetramethyl-. The 3D diagram on the left illustrates the binding pocket of COX-2, showing how phenol, 2,3,5,6-tetramethyl, interacts with key amino acid residues of the target protein, while the 2D representation on the right provides a detailed view of the molecular interactions within the active site. The compound established interactions with critical residues, including VAL89, ARG121, and GLU525**,** mediated through π–sigma, π–cation, and conventional hydrogen bonds**.** Notably, ARG121 engaged in both π-cation and conventional hydrogen bonding, underscoring its role in ligand stabilization. These interaction patterns resemble those of standard nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and diclofenac, which also exploit conventional hydrogen bonding with native amino acid residues to achieve effective binding.

Fig. 5 illustrates the molecular interactions between COX-2

amino acid residues and the reference drug ibuprofen. The 3D structure highlights the binding pocket, showing the arrangement of native residues that accommodate the ligand, while the 2D diagram provides a detailed view of residue-specific contacts. Ibuprofen, a well-established NSAID, was found to interact with TYR (A:123) through both π–π T-shaped and π–alkyl interactions, thereby enhancing its stability and affinity within the active site. Additionally, conventional hydrogen bonds were formed with SER122, LYS533, and GLN373**,** further supporting its strong binding orientation and potential to induce conformational changes in COX-2. Similarly, docking analysis revealed that diclofenac engaged comparable amino acid residues within the COX-2 active site; however, the interaction was mediated through distinct bond types, including π–π stacking, amide–π stacking, carbon–hydrogen interactions, and conventional hydrogen bonds (Fig. 6). The observed interactions confirm the anti-inflammatory potential of ibuprofen and diclofenac through COX-2 inhibition, a mechanism that was similarly replicated by the top three bioactive compounds with the highest negative binding energies.

ADMET analysis of the identified compounds

Table 5 summarizes the ADMET predictions of compounds identified from the GC–MS analysis of *Monodora myristica* seed extract, alongside the reference drugs diclofenac and ibuprofen. All compounds demonstrated good intestinal absorption, with Caco-2 permeability values ranging from

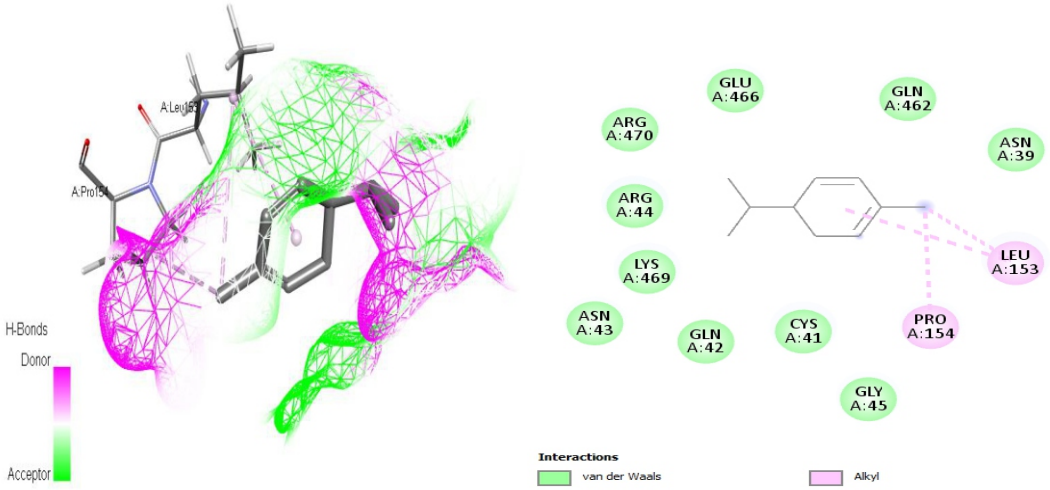

Fig. 2: The molecular interaction of amino acids residues of cyclooxygenase-2 (4PH9) with alpha-Phellandrene (7460), 3D left, 2D right

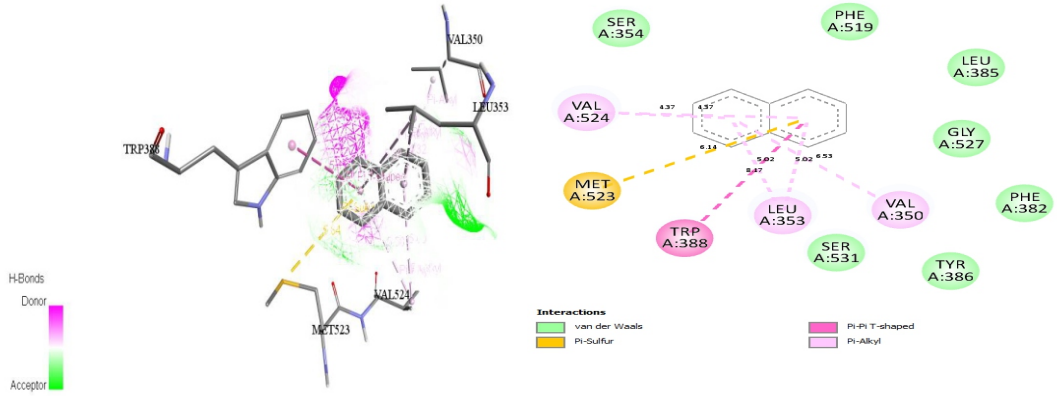

Fig. 3: The molecular interaction of amino acids residues of cyclooxygenase-2 (4PH9) with naphthalene (CID:931), 3D left, 2D right

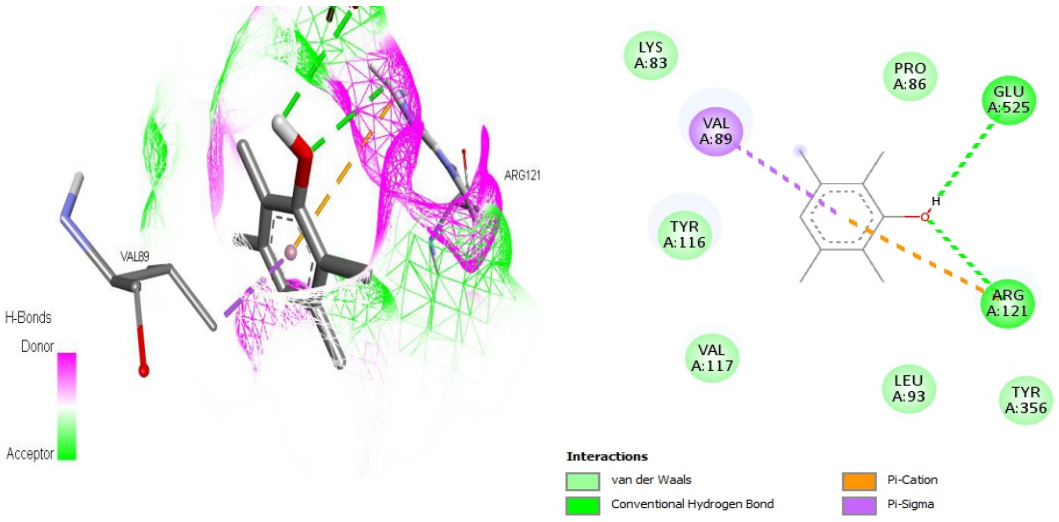

Fig. 4: The molecular interaction of amino acids residues of cyclooxygenase-2 (4PH9) with Phenol, 2,3,5,6-tetramethyl- (10694), 3D left, 2D right

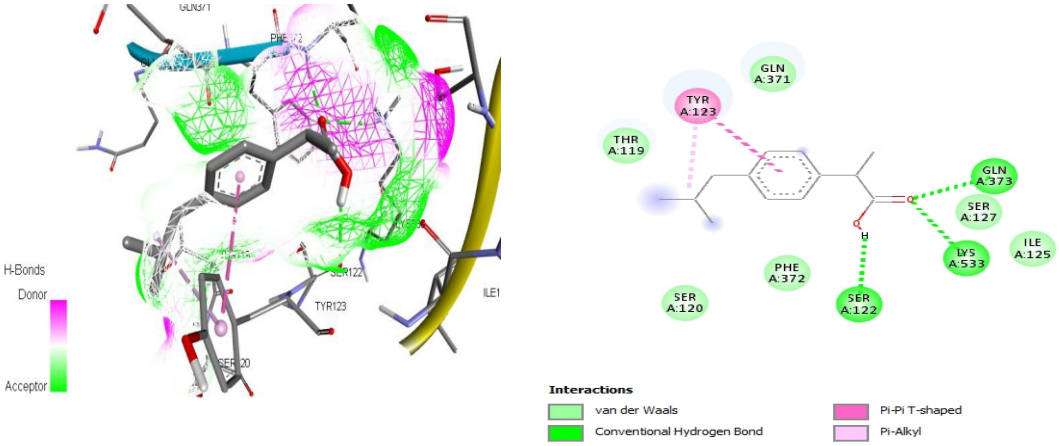

Fig. 5: The molecular interaction of amino acids residues of cyclooxygenase-2 (4PH9) with ibuprofen (3672), 3D left, 2D right

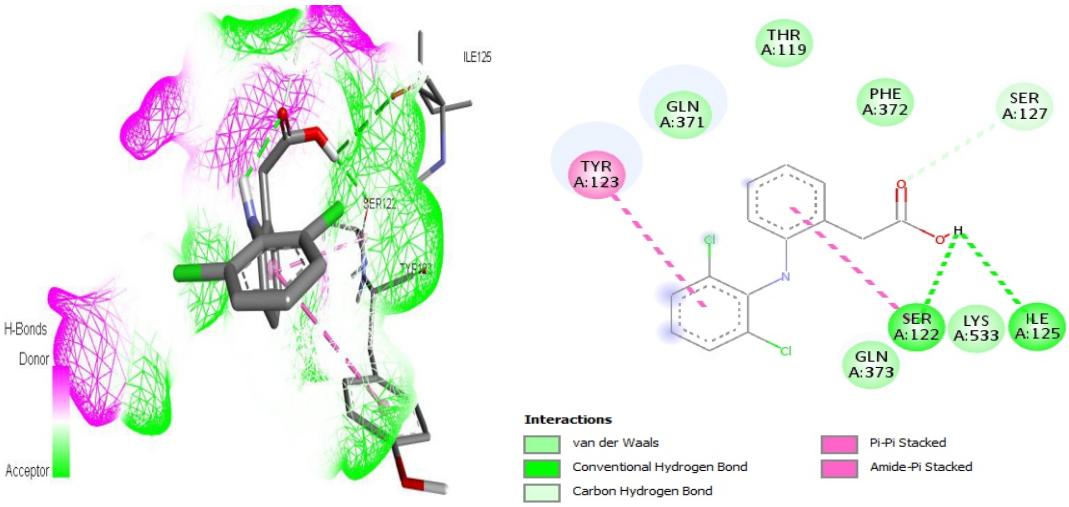

Fig. 6: The molecular interaction of amino acids residues of cyclooxygenase-2 (4PH9) with diclofenac (3033), 3D left, 2D right

Fig. 4: The molecular interaction of amino acids residues of cyclooxygenase-2 (4PH9) with Phenol, 2,3,5,6-tetramethyl- (10694), 3D left, 2D right

S/N	Compound name	Absorption		Distribution	Metabolism		Excretion	Toxicity	
		Caco-2 permeability	BBB	Subcellular localization	CYP4502C9	CYP4503A4	Renal OCT	AMES Toxicity	Carcinogenicity
		Probability			Substrate/Non-substrate		Inhibitor/Non-inhibitor	Yes/No	Yes/No
1	Bicyclo[3.1.0]hex-2-ene, 4-methyl-(1-methylethyl)-	0.6114	0.9550	Lysosome	Non-substrate	Substrate	Non-inhibitor	No	No
2	Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	0.6515	0.9827	Lysosome	Non-substrate	Substrate	Non-inhibitor	No	No
3	Bicyclo[3.1.1]heptane, 6,6-dimethy 15908 018172-67-3 97 l-2-methylene-, (1S)-	0.6648	0.9229	Lysosome	Non-substrate	Substrate	Non-inhibitor	No	No
4	**alpha-Phellandrene**	**0.7702**	**0.9049**	**Lysosome**	**Non-substrate**	**Non-substrate**	**Non-inhibitor**	**No**	**Yes**
5	p-Cymene	0.8762	0.9677	Lysosome	Non-substrate	Non-substrate	Non-inhibitor	No	Yes
6	1,6-Octadien-3-ol, 3,7-dimethyl-	0.7201	0.9699	Lysosome	Non-substrate	Substrate	Non-inhibitor	No	No
7	(1R,4R)-1-methyl-4-propan-2-ylcyclohex-2-en-1-ol	0.7985	0.9741	Mitochondria	Non-substrate	Non-substrate	Non-inhibitor	No	No
8	Alpha-Terpineol	0.7505	0.9568	Lysosome	Non-substrate	Substrate	Non-inhibitor	No	No
9	Caryophyllene	0.6327	0.9536	Lysosome	Non-substrate	Substrate	Non-inhibitor	No	No
10	**Naphthalene**	**0.8537**	**0.9728**	**Lysosome**	**Non-substrate**	**Non-substrate**	**Non-inhibitor**	**Yes**	**No**
11	4-Carene, (1S,3R,6R)-(-)-	0.6904	0.9824	Lysosome	Non-substrate	Substrate	Non-inhibitor	No	No
12	**Phenol,2,3,5,6-tetramethyl-**	**0.6988**	**0.5951**	**Mitochondria**	**Non-substrate**	**Substrate**	**Non-inhibitor**	**No**	**No**
13	*Diclofenac	0.8867	0.9541	Mitochondria	Non-substrate	Non-substrate	Non-inhibitor	No	No
14	*Ibuprofen	0.8866	0.9619	Mitochondria	Non-substrate	Non-substrate	Non-inhibitor	No	Yes

Key: *Represents the standard drug

0.6114 to 0.8867, comparable to the standards diclofenac (0.8867) and ibuprofen (0.8866). Most compounds exhibited a high probability of BBB penetration (>0.90), indicating potential central nervous system activity, except Phenol, 2,3,5,6-tetramethyl- (0.5951), which showed relatively lower permeability. With respect to subcellular distribution, the majority of the phytochemicals localized within the lysosome, whereas Phenol, 2,3,5,6-tetramethyl-, diclofenac, and ibuprofen were predicted to localize within the mitochondria, suggesting possible differences in intracellular targeting. In terms of metabolism, several compounds (Bicyclo[3.1.0]hex-2-ene, 4-methyl-(1-methylethyl)-, Tricyclo[2.2.1.0(2,6)]heptane,1,7,7-trimethyl-, Bicyclo[3.1.1]heptane, 6,6-dimethy 15908 018172-67-3 97 l-2-methylene- (1S)-, 1,6-Octadien-3-ol, 3,7-dimethyl-, alpha-Terpineol, Caryophyllene, 4-Carene, Phenol, 2,3,5,6-tetramethyl-) were predicted to be CYP3A4 substrates, whereas others (alpha-phellandrene, p-Cymene, Naphthalene, (1R,4R)-1-methyl-4-propan-2-ylcyclohex-2-en-1-ol, diclofenac, and ibuprofen) were classified as non-substrates. Importantly, none of the compounds were predicted as CYP2C9 inhibitors, minimizing the risk of drug–drug interactions via this pathway. For excretion, all compounds were predicted as non-substrates of renal OCT transporters, indicating low potential for renal transporter-mediated clearance issues. Toxicity screening revealed that most compounds were non-mutagenic (AMES negative) and non-carcinogenic, with the exceptions of Naphthalene (AMES positive), alpha-Phellandrene and p-Cymene (carcinogenic), and ibuprofen (carcinogenic). Diclofenac showed a favorable safety profile (non-mutagenic and non-carcinogenic).

Discussion

The extraction of the dried seed powder of *Monodora myristica* yielded 110.24 g essential oil from the starting 567 g of plant material, equivalent to a percentage yield of 19.4%. This relatively high yield indicates that the seeds are rich in extractable constituents, particularly volatile and non-volatile phytochemicals, which is consistent with earlier reports highlighting the abundance of essential oils, alkaloids, and phenolic compounds in *M. myristica* seeds [9,15]. The yield percentage is also within the range typically obtained from plant seed extractions, suggesting that the extraction process employed was efficient and suitable for recovering bioactive compounds. A high extraction yield is important, as it reflects both the availability of secondary metabolites and the potential for subsequent bioassays, phytochemical profiling, and pharmacological evaluations.

Gas Chromatography–Mass Spectrometry (GC–MS) analysis of the dried seed extract of *Monodora myristica* revealed twelve phytochemical constituents with varying peak areas, indicating differences in their relative abundance (Table 2). The most abundant compounds were p-cymene (31.16%), tricyclene (17.35%), α-phellandrene (13.07%), and linalool (11.45%), which together accounted for more than 70% of the total composition. These major constituents have been widely reported in essential oils of aromatic plants and are known to possess important biological and pharmacological properties. p-Cymene, a monoterpene hydrocarbon, is recognized for its anti-inflammatory, antioxidant, and antimicrobial activities[16], while linalool is a well-documented terpenoid alcohol with significant sedative, analgesic, and anti-anxiety effects[17]. The presence of α-phellandrene and tricyclene also suggests potential antioxidant, insecticidal, and antimicrobial applications, consistent with earlier reports on the pharmacological relevance of *M. myristica* essential oils[18].

The molecular docking analysis of bioactive compounds identified in the essential oil of dried seeds of *Monodora myristica* revealed binding affinities ranging between –5.5 and –6.9 kcal/mol against COX-2 (Table 3). Among the twelve compounds tested, naphthalene (–6.9 kcal/mol) and phenol, 2,3,5,6-tetramethyl- (–6.9 kcal/mol) demonstrated the strongest binding affinities, which were comparable to the standard drug ibuprofen (–6.8 kcal/mol) and slightly stronger than diclofenac (–6.6 kcal/mol). This suggests that these phytochemicals could potentially contribute to the anti-inflammatory properties traditionally associated with *M. myristica*[19]. Other notable compounds such as α-phellandrene (–6.7 kcal/mol), p-cymene (–6.5 kcal/mol), α-terpineol (–6.5 kcal/mol), and 4-carene (–6.5 kcal/mol) also exhibited favorable docking scores, indicating their possible role in modulating COX-2 activity[20]. Interestingly, linalool (–5.5 kcal/mol) showed the weakest binding interaction, which may limit its contribution to COX-2 inhibition despite its known bioactivity in other pharmacological contexts.

The fact that several essential oil constituents demonstrated docking scores within the same range as conventional NSAIDs highlights the therapeutic potential of *M. myristica* as a natural source of anti-inflammatory agents. The structural diversity of these compounds, including monoterpenes (α-thujene, β-pinene, tricyclene), sesquiterpenes (caryophyllene), and aromatic derivatives (naphthalene, phenolics), may act synergistically to enhance biological efficacy[21].

In addition to these predominant compounds, several minor constituents were detected, including α-thujene (5.88%), α-terpineol (2.58%), phenol derivatives (2.15%), and caryophyllene (1.28%). Although present in smaller proportions, these compounds are noteworthy, as they contribute synergistically to the overall bioactivity of the extract. For instance, α-terpineol is known for its anti-inflammatory and anticancer potential, while caryophyllene, a bicyclic sesquiterpenes, has been associated with anti-inflammatory and analgesic effects through cannabinoid receptor (CB2) binding[22]. The detection of naphthalene (1.03%) is also interesting, as this aromatic hydrocarbon has been previously reported in natural plant extracts, though its pharmacological implications in medicinal plants remain under investigation[23].

The analysis of the free energy of the top three compounds from *Monodora myristica* essential oil against COX-2 revealed promising inhibitory potentials (Table 4). Naphthalene (–6.9 kcal/mol) and phenol, 2,3,5,6-tetramethyl- (–6.9 kcal/mol) displayed the strongest binding affinities, while α-phellandrene (–6.7 kcal/mol) also showed a favorable interaction. Notably, the free binding energies of these compounds were comparable to those of the reference drugs ibuprofen (–6.8 kcal/mol) and diclofenac (–6.6 kcal/mol), suggesting that the bioactive constituents of *M. myristica* possess COX-2 inhibitory potential that could rival conventional NSAIDs.

The free energy of binding of naphthalene and tetramethyl phenol can be attributed to their aromatic structures, which facilitate hydrophobic interactions and π–π stacking with key amino acid residues within the COX-2 binding pocket. Likewise, the monoterpene α-phellandrene showed effective binding, consistent with previous reports of monoterpenes as anti-inflammatory agents due to their ability to modulate cyclooxygenase activity.

The similarity in docking scores between these phytochemicals and the standard drugs underscores the pharmacological relevance of *M. myristica*. Given their natural abundance in the essential oil and their comparable activity to NSAIDs, these compounds may contribute significantly to the traditional use of the plant in managing inflammatory disorders[24].

The ADMET analysis of the phytoconstituents identified from the essential oil of *Monodora myristica* dried seed provides key insights into their pharmacokinetic and toxicological properties in comparison with the standard NSAIDs, diclofenac and ibuprofen. Most of the compounds exhibited favorable intestinal absorption, with Caco-2 permeability values above 0.6. Notably, *p*-cymene (0.8762) and naphthalene (0.8537) showed absorption values comparable to diclofenac (0.8867) and ibuprofen (0.8866). Blood–brain barrier (BBB) penetration was high across the majority of phytochemicals (>0.90), suggesting potential central activity but also indicating a possible risk of CNS-related adverse effects. Subcellular localization predictions showed that most metabolites accumulated in the lysosome, whereas diclofenac and ibuprofen localized to the mitochondria, which may influence their intracellular pharmacodynamics[25]. The cytochrome P450 interaction profile indicated that diclofenac and ibuprofen were non-substrates for CYP2C9 and CYP3A4, consistent with their established metabolic stability[26]. In contrast, several phytochemicals, including tricyclene, α-terpineol, caryophyllene, and 4-carene, were predicted substrates of CYP3A4, suggesting greater susceptibility to hepatic metabolism and possible first-pass elimination[27]. Importantly, none of the compounds, including the standards, were predicted to act as CYP inhibitors, minimizing the risk of drug–drug interactions. Furthermore, all compounds were non-inhibitors of renal organic cation transporters (OCT), implying favorable renal clearance.

Toxicity predictions demonstrated some variability. Diclofenac was non-mutagenic and non-carcinogenic, whereas ibuprofen was non-mutagenic but predicted to be carcinogenic, aligning with reports of long-term safety concerns[28]. Among the phytochemicals, α-phellandrene and *p*-cymene were predicted to be carcinogenic, while naphthalene exhibited mutagenic but non-carcinogenic potential. Conversely, tricyclene, linalool, α-terpineol, caryophyllene, and 4-carene were both non-mutagenic and non-carcinogenic, indicating a relatively safer profile than ibuprofen.

Conclusion

The essential oil of *Monodora myristica* dried seeds contains bioactive compounds with promising anti-inflammatory potential. Molecular docking showed that naphthalene and 2,3,5,6-tetramethylphenol exhibited strong COX-2 free energy of binding (–6.9 kcal/mol), comparable to ibuprofen and diclofenac. ADMET profiling revealed favorable absorption, distribution, and safety profiles for most compounds, though some displayed mutagenic or carcinogenic alerts. These findings support the ethnomedicinal use of *M. myristica* and justify further in vitro and in vivo studies to validate its therapeutic potential.

Authors contribution

Conceptualization and Data curation: AFO, ATO & OIA
Formal analysis: AFO, ATO & AJT
Methodology: AJT, AFO, ECA & AMA
Writing – original draft: AJT, AFO, OIA, ECA & AMA
Writing – review and editing: AJT, AFO, OIA, ECA & AMA

Funding

The author(s) received no specific funding for this work.

CRediT authorship contribution statement

Afolabi F. O: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis. **Oyemitan I.A:** Visualization, Formal analysis, Data curation, Conceptualization. **Apata J.T:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Oyemitan I.A:** Visualization, Formal analysis, Data curation, Conceptualization. **Afolabi T.O:** Writing – review & editing, Writing – original draft, project administration, Investigation, Data curation. **Akanmu M.A:** Writing – original draft, Validation, Resources, Investigation, Formal analysis, Methodology & Conceptualization

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

REFERENCES

1. Zanza C, Romenskaya T, Zuliani M, Piccolella F, Bottinelli M, Caputo G, Rocca E, Maconi A, Savioli G, Longhitano Y. Acute traumatic pain in the emergency department. Diseases. 2023;11(1):45. https://doi.org/10.3390/diseases11010045
2. Budnik JV, Higgins TF, Malfait AM, Weinrich JA, Basbaum AI, Hsu JR, Morshed S, Bahney CS. New paradigms in pain management after skeletal trauma: Orthopaedic Trauma Association's 2023 Basic Science Focus Forum Symposium. OTA International. 2025;8(2S): e352. https://doi.org/10.1097/OI9.0000000000000352
3. Medzhitov R. The spectrum of inflammatory responses. Science. 2021;374(6571):1070-1075. DOI: https://doi.org/10.1126/science.abi5200
4. Britto N, Alavala RR, Brijesh S. Rethinking arthritis: exploring its types and emerging management strategies. Inflammopharmacology. 2025; 1-7. https://doi.org/10.1007/s10787-025-01833-8
5. Convertino I, Lopes LC, Pratt N, Ingrasciotta Y, Tuccori M. Biologic drugs in immune-mediated inflammatory diseases, validation, drug utilization, effectiveness, regulation, costs, and safety in the real world. Frontiers in Pharmacology. 2025; 15:1542453. https://doi.org/10.3389/fphar.2024.1542453
6. Acharya B, Behera A, Dilnawaz F, Chowdhury B, Behera S. Ethnogynecological properties of some selected local herbal plants from western Odisha: an ethnobotanical survey. Environment, Development and Sustainability. 2025 27(2):4849-4879. https://doi.org/10.1007/s10668-023-04103-1
7. Afolabi FO, Akanmu MA, Elusiyan CA, Oyemitan IA. Anticonvulsant, anxiolytic and hypnotic effect of *Monodora myristica* (Gaertn.) Dunal dried seed essential oil in mice. Nigerian Journal of Pharmacy, 2024;58(1) https://doi.org/10.51412/psnnjp.2024.14
8. Okpoghono J, Isoje EF, Igbuku UA, Ekayoda O, Uwague A, Ojebah KC, Eguvbe PM, Omoike GO, Adonor TO, Igue UB, Okom SU. Potentialities of Monodora myristica active components as functional ingredients in food formulation. Food Chemistry Advances. 2025; 6:100884.
9. Irondi EA, Aroyehun TM, Anyiam AF, Lal MK. Phenolics profile, anti-nephrolithiasis, and antioxidant activities of *Monodora myristica* seed: impact of endogenous proteins and lipids. Food Production, Processing and Nutrition. 2023;5(1):52. https://doi.org/10.1186/s43014-023-00167-8
10. Aikpitanyi I, Ebomoyi MI. Methanol extract of *Monodora myristica* seeds ameliorates lead acetate-induced leucocytosis and hematological alterations. FUOYE Journal of Biomedical Research. 2024;1(2):131-139.
11. Awojide SH, Akinlade B, Oyewole KA, Adeyemo AG, Adeniyi EO, Fadunmade OE, Anifowose AJ. Synergistic and antagonistic medicinal activities of essential oil of *Monodora myristica*. CTU

Journal of Innovation and Sustainable Development. 2023;15(3):1-11. https://doi.org 10.22144/ctujoisd.2023.046

12. Okechukwu QN, Ugwuona FU, Ofoedu CE, Juchniewicz S, Okpala CO. Chemical composition, antibacterial efficacy, and antioxidant capacity of essential oil and oleoresin from *Monodora myristica* and *Tetrapleura tetraptera* in Southeast Nigeria. Scientific reports. 2022;12(1):19861. https://doi.org/10.1038/s41598-022-23161-5
13. Adesina AF, Apata JT, Babalola OO, Otuechere CA, Adekola MB, Ogunleye GS, Asaolu F. Hepatoprotective activity of *Alstonia boonei* (De Wild) stem bark in isoniazid-induced Wistar rats: Antioxidant, anti-inflammatory, and in silico evaluations. Pharmacological Research-Modern Chinese Medicine. 2025; 14:100558. https://doi.org/10.1016/j.prmcm.2024.100558
14. Adekola MB, Ojelade OC, Olurode SA, Adebowale TO, Akinde AO, Apata JT, Adesina AF, Oyeyemi P, Alichi CU, Ajala OT. The protective potential of *Blighia sapida* on the behavioural and hematobiochemical disruption in kerosene-exposed Clarias gariepinus: *In vivo* and *in silico* evaluation. Pharmacological Research-Reports. 2025; 3:100036. https://doi.org/10.1016/j.prerep.2025.100036
15. Cameselle C, Zou P, Jia Z, Chipaca-Domingos HS, Zeye CK, Pessela BC, Costas C, Otero P, Simal-Gandara J. Chemical and nutritional characterization of edible *Heinsia crinita*, *Xylopia aethiopica, Piper guineense, Monodora myristica* and *Dorstenia convexa* plants from Angola. Food Bioscience. 2025; 67:106336. https://doi.org/10.1016/j.fbio.2025.106336
16. Baginska S, Golonko A, Swislocka R, Lewandowski W. Monoterpenes as medicinal agents: Exploring the pharmaceutical potential of p-cymene, p-cymenene, and γ-terpinene. Acta Pol. Pharm.—Drug Res. 2023; 80:879-92. http://dx.doi.org/10.32383/appdr/178242
17. Alfieri A, Di Franco S, Maffei V, Sansone P, Pace MC, Passavanti MB, Fiore M. Phytochemical Modulators of Nociception: A Review of Cannabis Terpenes in Chronic Pain Syndromes. Pharmaceuticals. 2025;18(8):1100. https://doi.org/10.3390/ph18081100
18. Poudel DK, Rokaya A, Ojha PK, Timsina S, Satyal R, Dosoky NS, Satyal P, Setzer WN. The chemical profiling of essential oils from different tissues of *Cinnamomum camphora* L. and their antimicrobial activities. Molecules. 2021;26(17):5132. https://doi.org/10.3390/molecules26175132
19. Chandrasekaran M, Sharma PM, Yadav A, Pragadheesh VS. Comprehensive analysis of the chemical compositions, chiral profile, and physical properties of *Myristica fragrans* fruit essential oils and oleoresins. European Food Research and Technology. 2025:1-2. https://doi.org/10.1007/s00217-025-04783-1
20. Ashokkumar K, Simal-Gandara J, Murugan M, Dhanya MK, Pandian A. Nutmeg (*Myristica fragrans* Houtt.) essential oil: A review on its composition, biological, and pharmacological activities. Phytotherapy Research. 2022;36(7):2839-51. https://doi.org/10.1002/ptr.7491
21. Tocmo R, Pena-Fronteras J, Calumba KF, Mendoza M, Johnson JJ. Valorization of pomelo (Citrus grandis Osbeck) peel: A review of current utilization, phytochemistry, bioactivities, and mechanisms of action. Comprehensive Reviews in Food Science and Food Safety. 2020;19(4):1969-2012. https://doi.org/10.1111/1541-4337.12561
22. Bilbrey JA, Ortiz YT, Felix JS, McMahon LR, Wilkerson JL. Evaluation of the terpenes β-caryophyllene, α-terpineol, and γ-terpinene in the mouse chronic constriction injury model of neuropathic pain: Possible cannabinoid receptor involvement. Psychopharmacology. 2022;239(5):1475-86. https://doi.org/10.1007/s00213-021-06031-2
23. Sharmin S, Muzahid AA, Islam MM, Yeasmin MS, Dey AK, Uddin MJ, Rana GM, Barmon J, Alam S, Bhuiyan MN, Ahmed NU. Preliminary Investigation of GC–MS Profiling and Antibacterial Activities of Different Solvent Extracts from Litchi chinensis Sonn. Seed. Scientifica. 2025;2025(1):7644558. https://doi.org/10.1155/sci5/7644558
24. Mohanty D, Padhee S, Sahoo C, Jena S, Sahoo A, Panda PC, Nayak S, Ray A. Integrating network pharmacology and experimental verification to decipher the multitarget pharmacological mechanism of *Cinnamomum zeylanicum* essential

oil in treating inflammation. Heliyon. 2024;10(2). https://doi.org/10.1016/j.heliyon.2024.e24120

25. Kato R, Zhang L, Kinatukara N, Huang R, Asthana A, Weber C, Xia M, Xu X, Shah P. Investigating blood–brain barrier penetration and neurotoxicity of natural products for central nervous system drug development. Scientific Reports. 2025;15(1):7431. https://doi.org/10.1038/s41598-025-90888-2

26. Wen C, Zhuang Z, Song H, Tong S, Wang X, Lin Y, Zhan H, Chen Z, Hu L. Metabolism of liver CYP450 and ultrastructural changes after long-term administration of aspirin and ibuprofen. Biomedicine & Pharmacotherapy. 2018; 108:208-15. https://doi.org/10.1016/j.biopha.2018.08.162

27. Haron MH, Dale O, Martin K, Avula B, Chittiboyina AG, Khan IA, Gurley BJ, Khan SI. Evaluation of the herb-drug interaction potential of commonly used botanicals on the US market with regard to PXR-and AhR-mediated influences on CYP3A4 and CYP1A2. Journal of dietary supplements. 2023;20(5):763-76. https://doi.org/10.1080/19390211.2022.2110351

28. Ghosh J, Repon MR, Pranta AD, Rupanty NS, Khan F, Noor T. Bioactive component integrated textiles: A promising source of medicine and healthcare. Journal of Engineered Fibers and Fabrics. 2025; 20:15589250241308561. https://doi.org/10.1177/15589250241308561