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Original Article

Identification of Potential COX-2 Inhibitors from *Eleusine indica* Through In Vitro Cyclooxygenase Assay, LC-MS/MS-based Profiling, Molecular Docking, and Molecular Dynamics Simulation

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ABSTRACT

Introduction: Cyclooxygenase-2 (COX-2) is a key enzyme involved in the production of pro-inflammatory mediators and is an important target for anti-inflammatory drug development. This study investigated the anti-inflammatory potential of *Eleusine indica* through in vitro COX inhibition, LC–MS-based phytochemical profiling, and computational analyses to identify potential COX-2 inhibitory metabolites. **Methods:** The methanolic leaf extract of *E. indica* was evaluated for COX-1 and COX-2 inhibitory activities. Metabolites were identified using liquid chromatography–mass spectrometry (LC–MS) and screened for drug-likeness, ADMET, and toxicity properties using SwissADME and ProTox-3.0. Selected metabolites were subjected to molecular docking against COX-2 (PDB ID: 4COX), and the top-ranked compound was further evaluated through a 100 ns molecular dynamics simulation. **Results:** The extract exhibited significantly greater inhibition of COX-2 (IC_{50} = 4.0 µg/mL) than COX-1 (IC_{50} = 12.5 µg/mL; p = 0.0005), indicating preferential COX-2 inhibition. LC–MS analysis tentatively identified ten metabolites, among which diosmetin, sinapine, and senkyunolide I showed favorable drug-likeness, pharmacokinetic, and toxicity profiles. Molecular docking revealed that diosmetin exhibited the strongest binding affinity toward COX-2 (–7.8 kcal/mol), followed by senkyunolide I (–6.6 kcal/mol) and sinapine (–6.3 kcal/mol). Molecular dynamics simulations confirmed the stability of the diosmetin–COX-2 complex through reduced structural fluctuations, maintained protein compactness, and persistent hydrogen-bond interactions. **Conclusions:** Diosmetin emerged as the most promising metabolite from *E. indica*, exhibiting favorable pharmacokinetic, safety, docking, and molecular dynamics profiles. These findings suggest that diosmetin is a promising natural COX-2 inhibitor that warrants further experimental validation.

Keywords: anti-inflammation, COX2 inhibition, diosmetin, drug discovery, Eleusine indica, molecular docking, molecular dynamics

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INTRODUCTION

Inflammation is a complex physiological response that protects the body against infection, injury, and tissue damage[1]. However, persistent or dysregulated inflammation contributes to the pathogenesis of numerous chronic diseases, including arthritis, cardiovascular disorders[2], neurodegenerative diseases[3], and cancer[4]. Among the key mediators involved in inflammatory processes is cyclooxygenase-2 (COX-2), an inducible enzyme responsible for the conversion of arachidonic acid into prostaglandins that promote pain, swelling, and inflammation. Owing to its central role in inflammatory signaling, COX-2 has become an important therapeutic target for the development of anti-inflammatory agents[5]. Although synthetic nonsteroidal anti-inflammatory drugs (NSAIDs) effectively inhibit cyclooxygenase activity, their prolonged use has been associated with adverse effects such as gastrointestinal irritation, renal dysfunction, and cardiovascular complications[5,6]. These limitations have stimulated interest in the discovery of safer and more effective anti-inflammatory compounds from natural sources.

Medicinal plants have long served as valuable sources of bioactive compounds for the treatment of inflammatory disorders. Plant-derived secondary metabolites, including flavonoids, phenolic acids, alkaloids, terpenoids, and glycosides, have demonstrated the ability to modulate inflammatory pathways through inhibition of pro-inflammatory enzymes, suppression of cytokine production, and regulation of oxidative stress[7-9]. Advances in phytochemical profiling and computational drug discovery have facilitated the rapid identification and evaluation of bioactive natural products, enabling the prioritization of promising compounds before labor-intensive biological testing. In particular, the integration of liquid chromatography–mass spectrometry (LC–MS)[10-12], molecular docking, pharmacokinetic prediction, and molecular dynamics simulation[13] has emerged as a powerful strategy for identifying potential therapeutic leads from medicinal plants[14].

Eleusine indica, commonly known as goosegrass, is a widely distributed grass species traditionally used in several Asian countries for the management of various ailments[8]. Ethnopharmacological reports have described its use in treating fever, hypertension, urinary disorders, infections, and inflammatory conditions. Previous phytochemical and pharmacological investigations have revealed that *E. indica* contains diverse bioactive constituents, including flavonoids, phenolic compounds, alkaloids, and fatty acid derivatives, which contribute to its antioxidant, antimicrobial, antidiabetic, and anti-inflammatory activities. Extracts of *E. indica* have been reported to reduce inflammatory

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responses and oxidative stress, supporting its potential as a source of anti-inflammatory agents[15].

Despite the growing evidence supporting the pharmacological properties of *E. indica*[16], information regarding the specific metabolites responsible for its anti-inflammatory activity and their molecular interactions with COX-2 remains limited. Most previous studies have focused on crude extracts or general biological activities[15], while comprehensive investigations integrating phytochemical profiling, enzyme inhibition assays, and computational analyses remain scarce. Identification of the metabolites that contribute to COX-2 inhibition is essential for understanding the mechanistic basis of the plant's anti-inflammatory effects and for facilitating the development of bioactive lead compounds.

Therefore, the present study aimed to evaluate the anti-inflammatory potential of *E. indica* through a combined experimental and computational approach. The methanolic leaf extract was assessed for cyclooxygenase inhibitory activity, followed by LC–MS-based metabolite profiling. Identified compounds were subsequently evaluated using drug-likeness, ADMET, and toxicity prediction tools, while selected metabolites were subjected to molecular docking against COX-2 and molecular dynamics simulations to assess binding stability. This integrated workflow was employed to identify potential COX-2 inhibitory metabolites and provide a molecular basis for the anti-inflammatory activity of *E. indica*.

MATERIALS AND METHODS

Sample preparation

Fresh mature leaves of *Eleusine indica* were collected from Barangay Tinga Labac, Batangas City, Batangas, Philippines (UTM Easting: 292819.80; UTM Northing: 1524889.93). Plant authentication was conducted by the Bureau of Plant Industry, Department of Agriculture, Manila, Philippines, and a certificate of authentication (PLT-ID-CRPSD-540-23) was issued. The collected leaves were washed with distilled water and air-dried at room temperature until a constant weight was achieved. Dried samples were pulverized using a mechanical grinder and passed through a 60-mesh sieve.

The powdered leaf material was extracted by maceration in 80% (v/v) methanol for 72hrs at room temperature[17]. The extract was filtered through Whatman No. 1 filter paper (Whatman, Maidstone, Kent, UK), concentrated, and subsequently lyophilized at −80°C to obtain the crude methanolic extract.

In vitro Cyclooxygenase Inhibition Assay

The cyclooxygenase inhibitory activity of the E. indica methanolic extract against COX-1 and COX-2 was evaluated using an ELISA-based assay following the method of Opog and Amor[18] with minor modifications. A stock solution of the dried extract (10,000 µg/mL) was prepared in dimethyl sulfoxide (DMSO) and diluted to the desired working concentrations.

Briefly, the enzyme–cofactor mixture was prepared according to the assay protocol, and 120 µL was dispensed into wells containing 50 µL of TRIS buffer. Subsequently, 10 µL of either the extract solution, DMSO (negative control), or indomethacin (160 mM; positive control) was added to the respective wells. The reaction mixtures were incubated at 25°C for 15 min, followed by the addition of 10 µL of 200 µM Amplex Red reagent and 10 µL of 2,000 µM arachidonic acid. The reaction mixture was purged with nitrogen gas (N_2) prior to analysis.

Fluorescence measurements were recorded for 2 min using a CLARIOstar® multifunctional microplate reader (BMG LABTECH, Ortenberg, Germany) at excitation and emission wavelengths of 535 and 590 nm, respectively. Fluorescence intensity was monitored at 12-s intervals, and the average slope of each replicate was used to calculate the percentage inhibition of COX-1 and COX-2 activity. The selectivity of the extract was assessed using the COX-2/COX-1 inhibition ratio.

All assays were performed in triplicate, and results were expressed as mean ± standard deviation (SD).

Phytochemical Profiling

The lyophilized *E. indica* extract was reconstituted in methanol, filtered through a 0.22 µm membrane filter, and subjected to LC–MS/MS analysis using a Waters ACQUITY I-Class UPLC system coupled to a Xevo G2-S QTOF mass spectrometer (Waters Corporation, Milford, MA, USA). Chromatographic separation was achieved on an ACQUITY UPLC HSS C18 reverse-phase column (2.1 × 100 mm, 1.8 µm; Waters Corporation) using a gradient elution system consisting of (A) ultrapure water containing 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid at a flow rate of 0.25 mL/min.

Metabolite annotation was performed using accurate mass measurements together with MS/MS fragmentation matching against the Waters CONNECT™ (UNIFI) Screening Library and Traditional Medicine Library. Authentic reference standards were not included in the present study; therefore, the reported metabolites are considered putative annotations (MSI Level 2) in accordance with the Metabolomics Standards Initiative (MSI).

In Silico Drug Likeness, Pharmacokinetics, and Toxicity Prediction

The drug-likeness properties of the LC–MS/MS-identified compounds from *E. indica* were evaluated based on Lipinski's Rule of Five[19] using the SwissADME online platform[20]. The absorption, distribution, metabolism, excretion (ADME) profiles of the phytochemical constituents of *E. indica* were predicted using the SwissADME[20] and pkCSM[21] web servers. The LD50 and organ toxicity predictions were determined using the ProTox online platform[22].

Protein and Ligand Preparation

The three-dimensional (3D) structures of diosmetin, sinapine, senkyunolide I, and the reference ligand indomethacin were retrieved from the PubChem database in Structure Data File (SDF) format[23]. Ligands were prepared using Avogadro software by adding hydrogen atoms and performing geometry optimization through energy minimization.

The crystal structure of cyclooxygenase-2 (COX-2; PDB ID: 4COX) was obtained from the Protein Data Bank[24]. Protein preparation was carried out using UCSF ChimeraX[25] and AutoDock Tools, which involved the removal of water molecules and co-crystallized ligands, addition of polar hydrogen atoms, assignment of Kollman charges, and conversion of the protein structure into PDBQT format for molecular docking.

Molecular Docking

Molecular docking was performed using AutoDock Vina[26] to evaluate the binding affinity of the selected metabolites toward COX-2. Ligands were converted to PDBQT format, and rotatable bonds were assigned by defining torsional degrees of freedom. The docking grid was centered on the native ligand-binding site at coordinates X = 26.739, Y = 15.723, and Z = 20.302, with dimensions of 15 × 30 × 30 $Å^3$ to encompass the active-site cavity.

Binding affinity scores and ligand-binding conformations were generated for each protein–ligand complex. The docking protocol was validated through redocking of the co-crystallized ligand, indomethacin.

Protein–ligand interactions, binding poses, and interacting amino acid residues were subsequently analyzed and visualized using BIOVIA Discovery Studio Visualizer (BIOVIA, San Diego, CA, USA).

Molecular Dynamics Simulation

To further evaluate the stability of the diosmetin–COX-2 complex, a 100 ns molecular dynamics (MD) simulation was performed using GROMACS version 2023[9]. The CHARMM36 force field was applied to the protein, while ligand parameters were generated using the CHARMM General Force Field (CGenFF).

The protein–ligand complex was subjected to energy minimization using the steepest descent algorithm to eliminate unfavorable steric interactions. The minimized system was placed in a cubic simulation box with a minimum distance of 0.5 nm from the box edges and solvated using the SPC water model. Counter ions (Na^+ and Cl^-) were added to neutralize the system and achieve a physiological salt concentration of 0.15 M.

Following system preparation, the system was equilibrated under constant volume and temperature (NVT) and constant pressure and temperature (NPT) ensembles for 100 ps each. Temperature was maintained at 300 K using the V-rescale thermostat, while pressure was maintained at 1 bar using the Parrinello–Rahman barostat. Long-range electrostatic interactions were calculated using the Particle Mesh Ewald (PME) method with a cutoff distance of 1.2 nm for both electrostatic and van der Waals interactions. Covalent bonds involving hydrogen atoms were constrained using the LINCS algorithm, allowing an integration time step of 2 fs. Coordinates were recorded every 10 ps throughout the simulation. Production MD simulations were subsequently performed for 100 ns under periodic boundary conditions. Simulation convergence was assessed by monitoring the stabilization of the root mean square deviation (RMSD) throughout the production run. Structural stability and conformational behavior of the complex were further evaluated using the root mean square fluctuation (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA), and protein–ligand hydrogen bond occupancy.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 7.05. A 2-tailed Student's *t*-test was used for comparison between two groups. Error bars for all graphs represented the mean ± standard deviation (S.D.). A $p < 0.05$ was considered statistically significant (n.s., not significant).

RESULTS AND DISCUSSION

Cyclooxygenase Inhibitory Activity

The E. indica extract exhibited significantly greater inhibitory activity against COX-2 than COX-1 (Figure 1). The extract showed an IC_{50} value of approximately 4.0 µg/mL for COX-2, which was significantly lower than the IC_{50} value of 12.5 µg/mL observed for COX-1 ($p = 0.0005$). These findings indicate a preferential inhibition of the inducible COX-2 isoform, suggesting a selective anti-inflammatory effect.

Selective COX-2 inhibition is considered desirable because COX-2 is primarily involved in inflammatory processes, whereas COX-1 plays important physiological roles in gastric and renal protection. Similar observations have been reported for several medicinal plant extracts rich in flavonoids and phenolic compounds, which exhibit preferential inhibition of COX-2 and reduced inflammatory responses through modulation of prostaglandin biosynthesis[27]. The observed activity may therefore be attributed to the presence of bioactive metabolites in E. indica, supporting its traditional use in inflammatory conditions.

Phytochemical Profiling

LC–MS/MS analysis of the methanolic extract of *Eleusine indica* putatively identified ten metabolites belonging to various chemical classes, including flavonoids, alkaloids, phenolics, fatty acids, and glycosides (Table 1). Although the metabolite annotations are considered putative (MSI Level 2) pending confirmation using authentic reference standards, the low mass error values (−1.96 to −4.31 ppm) indicate excellent mass accuracy and support the reliability of the proposed metabolite assignments.

Among the detected compounds, terrestribisamide, oliveramine, 6-gingerol, and rhein 8-glucoside exhibited the highest responses, suggesting their relative abundance in the extract. Notably, several identified metabolites, including diosmetin, fisetin, 6-gingerol, sinapine, and senkyunolide I, have been previously reported to possess anti-inflammatory properties. Diosmetin and fisetin, in particular, have been shown to suppress pro-inflammatory mediators and inhibit NF-κB signaling pathways, whereas 6-gingerol exhibits anti-inflammatory activity through modulation of cyclooxygenase and cytokine production[28-29]. The presence of these metabolites provides a plausible phytochemical basis for the COX-2 inhibitory activity observed in the present study.

Drug likeness, Pharmacokinetics and Toxicity Profile

The drug-likeness, pharmacokinetic, and toxicity profiles of the metabolites identified from E. indica were evaluated to prioritize candidates for molecular docking studies (Tables 2–4). Based on Lipinski's Rule of Five, nine of the ten compounds satisfied the criteria for oral drug-likeness, with only rhein 8-glucoside exhibiting two violations. Overall, the identified metabolites demonstrated favorable physicochemical properties, including acceptable molecular weights, lipophilicity, hydrogen-bonding capacity, and molecular flexibility.

ADME analysis further differentiated the compounds according to their pharmacokinetic behavior. Diosmetin, sinapine, and senkyunolide I exhibited favorable intestinal absorption (>79%) and were not predicted to inhibit CYP2D6 or CYP3A4, suggesting a low risk of metabolism-related drug interactions. Among these compounds, senkyunolide I displayed the highest Caco-2 permeability, indicating enhanced membrane transport potential. Similar pharmacokinetic characteristics have been reported for flavonoid-based natural products, which commonly exhibit favorable oral absorption and metabolic profiles suitable for drug development[20].

Toxicity prediction further supported the suitability of these metabolites as lead candidates. Diosmetin, sinapine, and senkyunolide I were predicted to be non-hepatotoxic, non-neurotoxic, and non-nephrotoxic, with relatively high oral LD_{50} values compared with several other identified compounds. In contrast, 6-gingerol, fisetin, oliveramine, terrestribisamide, and ginkgolic acid exhibited nephrotoxicity alerts, indicating potentially less favorable safety profiles.

Considering their favorable drug-likeness, pharmacokinetic properties, and predicted safety, diosmetin, sinapine, and senkyunolide I were selected for subsequent molecular docking studies. Notably, these compounds have also been reported to possess anti-inflammatory activities in previous studies, further supporting their potential as COX-2 inhibitory candidates[29,30]. Among the three candidates, diosmetin exhibited the most balanced overall profile, combining favorable absorption, safety, and previously reported anti-inflammatory activity, which justified its further evaluation through molecular dynamics simulation.

Table 1. **Phytochemical compounds identified in *Eleusine indica* methanolic extract**

No	Name	Expected mass (Da)	Observed m/z	Mass error (ppm)	RT (min)	Response
1	Diosmetin	300.06339	301.06977	-2.967	2.64	1534.852
2	Sinapine	309.15762	310.16405	-2.73	2.315	1176.727
3	Senkyunolide I	192.11503	193.12168	-3.229	8.291	383.357
4	Terrestribisamide	440.19474	441.20115	-1.962	3.236	3450.269
5	Ginkgolic Acid	346.2508	347.25694	-3.244	7.847	748.954
6	Rhein 8-glucoside	446.08491	447.09131	-1.966	2.27	2743.014
7	Oliveramine	352.14231	353.14854	-2.958	3.265	2804.311
8	6-Gingerol	294.18311	295.18941	-3.305	6.095	2744.012
9	Fisetin	286.04774	287.05378	-4.305	3.064	459.294
10	Cimicifugic Acid C	418.09	419.09645	-1.961	3.564	820.905

Table 2. **Drug likeness prediction of identified compounds in *Eleusine indica* methanolic extract**

No	Isolated Compounds	Molar Mass (g/mol)	Rotatable Bonds	H-bond acceptors	H-bond donors	LOGP	Violation	Drug likeness
1	Diosmetin	300.26	2	6	3	2.5854	0	Yes
2	Sinapine	310.37	8	5	1	1.672	0	Yes
3	Senkyunolide I	192.25	3	2	0	2.69	0	Yes
4	Terrestribisamide	440.49	13	6	4	2.8542	0	Yes
5	Ginkgolic Acid	346.5	14	3	2	6.5001	1	Yes
6	Rhein 8-glucoside	446.36	4	11	6	-0.9555	2	No
7	Oliveramine	352.38	4	6	0	2.6387	0	Yes
8	6-Gingerol	294.39	10	4	2	3.2338	0	Yes
9	Fisetin	286.24	1	6	4	2.2824	0	Yes
10	Cimicifugic Acid C	418.35	9	10	6	0.8714	1	Yes

Table 3. **ADME prediction of identified compounds in *Eleusine indica* methanolic extract**

No	Compound	Adsorption		Distribution		Metabolism		Excretion
		Intestinal absorption (human) (% Absorbed)	Caco2 permeability (log Papp in 10-6 cm/s)	VDss (human) (log L/kg)	BBB permeability (log BB)	CYP2D6 inhibitor (Yes/No)	CYP3A4 inhibitor (Yes/No)	Total Clearance (log ml/min/kg)
1	Diosmetin	79.898	0.326	**0.709**	-0.954	No	No	-2.316
2	Sinapine	95.901	0.466	0.311	-0.288	No	No	0.857
3	Senkyunolide I	96.595	1.611	0.314	0.588	No	No	1.422
4	Terrestribisamide	65.942	0.011	**-0.084**	-1.03	No	Yes	0.462
5	Ginkgolic Acid	94.702	1.048	**-1.232**	-0.278	No	No	1.514
6	Rhein 8-glucoside	22.868	-0.686	**-0.715**	-1.341	No	No	-0.405
7	Oliveramine	99.04	1.382	**-0.198**	-0.675	No	Yes	0.815
8	6-Gingerol	92.416	0.94	**0.524**	-0.727	No	No	1.339
9	Fisetin	83.752	0.058	0.718	-1.039	No	No	0.421
10	Cimicifugic Acid C	7.466	-1.008	**-0.303**	-1.442	No	No	0.301

Table 4. **Toxicity prediction of identified compounds in *Eleusine indica* methanolic extract**

No	Compound	Oral Rat Acute Toxicity LD50 (mg/kg)	Hepatotoxic	Neurotoxic	Nephrotoxic
1	Diosmetin	3919	No	No	No
2	Sinapine	978	No	No	No
3	Senkyunolide I	2000	No	No	No
4	Terrestribisamide	1180	No	No	Yes
5	Ginkgolic Acid	1000	No	No	Yes
6	Rhein 8-glucoside	3000	No	No	Yes
7	Oliveramine	500	No	No	Yes
8	6-Gingerol	250	No	No	Yes
9	Fisetin	159	No	No	Yes
10	Cimicifugic Acid C	5000	No	No	Yes

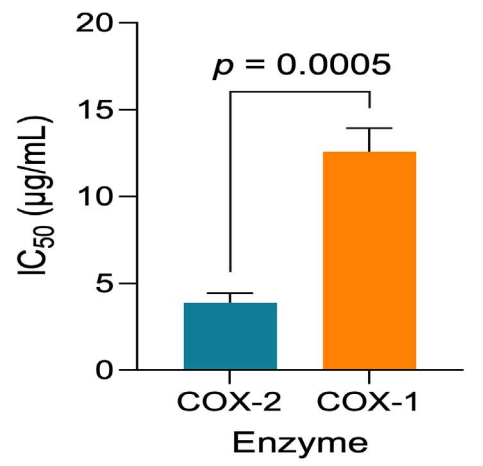

Figure 1. *In vitro* cyclooxygenase inhibitory activity of the *E. indica* extract

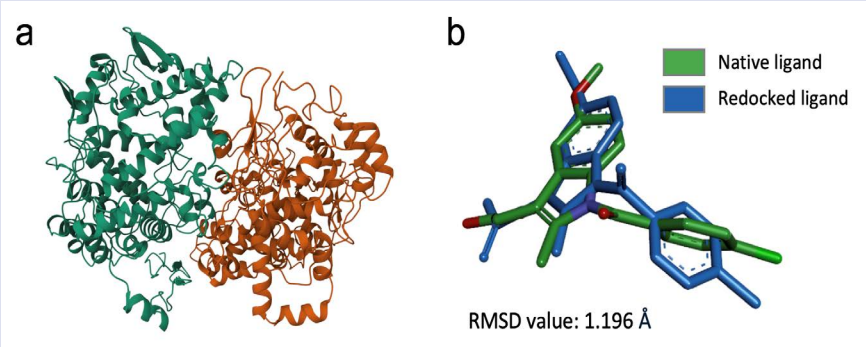

Figure 2. *In silico* validation of molecular docking analysis of COX-2. **a.** Three-dimensional structure of the COX-2 Enzyme (PDB ID: 4COX), **b.** Superimposition of the co-crystallized (green) and redocked (blue) ligand, indomethacin, within the active site of COX-2.

Docking Protocol Validation

The docking protocol was validated by redocking the co-crystallized ligand, indomethacin, into the active site of COX-2 (PDB ID: 4COX). The superimposition of the native and redocked ligand poses showed a close overlap, with an RMSD value of 1.196 Å (Figure 2). Since RMSD values below 2.0 Å indicate reliable docking performance, the result confirms the accuracy of the docking parameters and their suitability for predicting ligand–COX-2 interactions[31]. The validated protocol was subsequently used for docking the selected *E. indica* metabolites.

Molecular docking analysis

Molecular docking was performed to evaluate the binding affinity and interaction profiles of diosmetin, sinapine, and senkyunolide I within the active site of COX-2 using indomethacin as the reference inhibitor (Table 5, Figures 3 and 4). As expected, indomethacin exhibited the strongest binding affinity (−9.0 kcal/mol), reflecting its established inhibitory activity against COX-2. Among the screened metabolites, diosmetin showed the most favorable binding energy (−7.8 kcal/mol), followed by senkyunolide I (−6.6 kcal/mol) and sinapine (−6.3 kcal/mol). While diosmetin demonstrated a lower predicted binding affinity than indomethacin, the observed difference alone does not necessarily translate into proportional differences in biological activity or therapeutic performance, as docking scores provide only relative estimates of protein–ligand interactions rather than direct measures of pharmacological potency.

Diosmetin formed three conventional hydrogen bonds with Tyr355, Tyr385, and Ser530, together with hydrophobic interactions involving key active-site residues such as Arg120 and Val116. These residues are known to play important roles in ligand recognition and stabilization within the COX-2 binding pocket. Previous studies have reported that diosmetin possesses anti-inflammatory activity through the suppression of pro-inflammatory mediators and modulation of inflammatory signaling pathways[32,33]. Furthermore, molecular docking investigations have demonstrated favorable interactions between diosmetin and inflammation-related targets, supporting its potential as a bioactive anti-inflammatory flavonoid[34-35].

Although sinapine formed four conventional hydrogen bonds, its weaker binding affinity suggests less favorable overall ligand–protein interactions. Senkyunolide I exhibited moderate binding affinity and interacted primarily through hydrophobic contacts without forming conventional hydrogen bonds. Notably, all three metabolites shared interactions with important COX-2 residues involved in indomethacin binding, including Tyr355, Arg120, Val89, Val116, and Glu524, indicating their ability to occupy the enzyme's active site.

Among the tested metabolites, diosmetin demonstrated the most favorable overall profile, combining the strongest predicted binding affinity with key stabilizing interactions and favorable ADMET characteristics. Collectively, these findings identified diosmetin as the most promising candidate for subsequent molecular dynamics simulations to further evaluate the stability of its interaction with COX-2 under dynamic conditions.

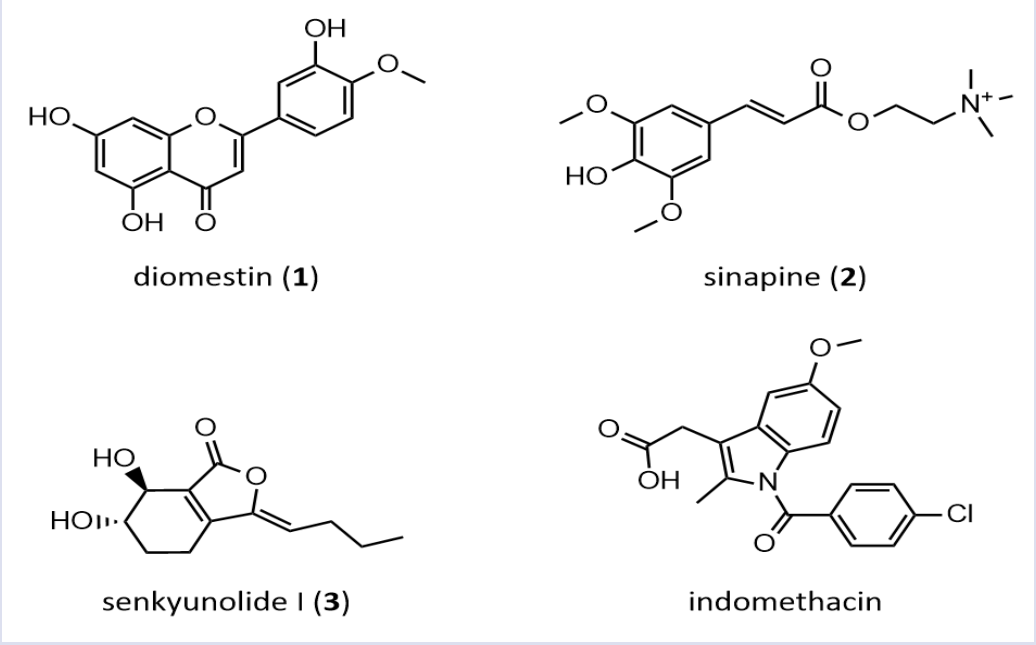

Figure 3. Chemical structures of the three (3) screened compounds from *E. indica* and a known COX-2 inhibitor, indomethacin

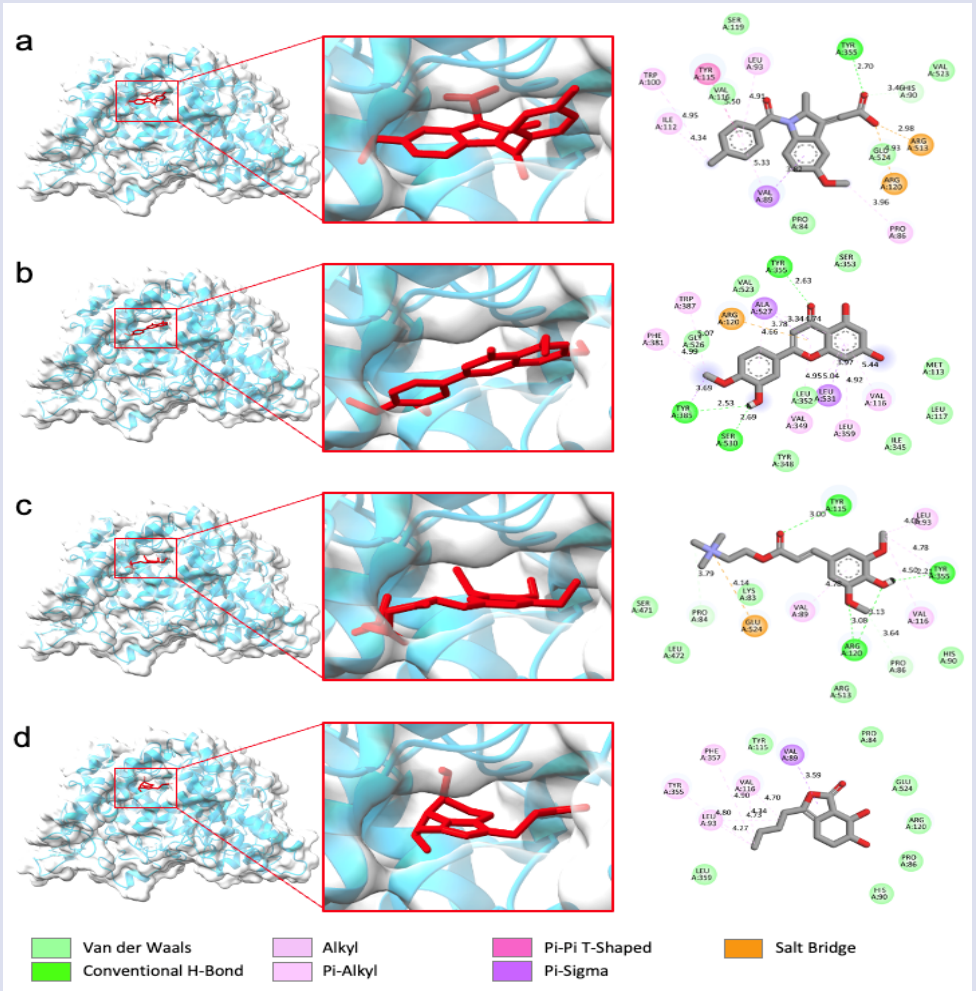

Figure 4. Key interactions of screened compounds 1 to 3 bound within the COX-2 active site. Docked poses of known inhibitor **(a)** indomethacin and screened ligands **(b)** diosmetin, **(c)** sinapine, and **(d)** senkyunolide I and their interacting amino acid residues within the COX-2 active site

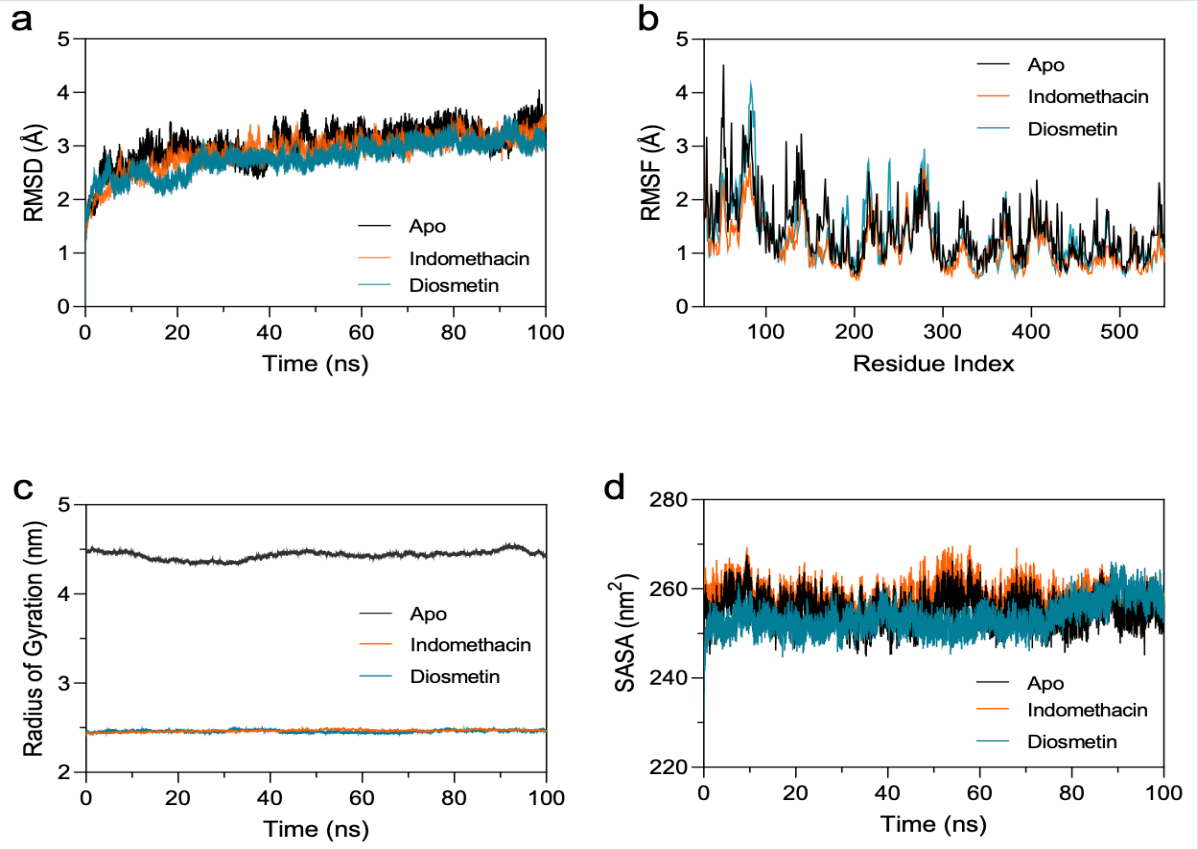

Figure 5. Structural stability of COX-2 complexes. (a) RMSD over 100 ns MD simulations; (b) residue index-based RMSF; (c) radius of gyration; and (d) solvent accessible surface area. Apo (gray), indomethacin (orange) and diosmetin (blue)

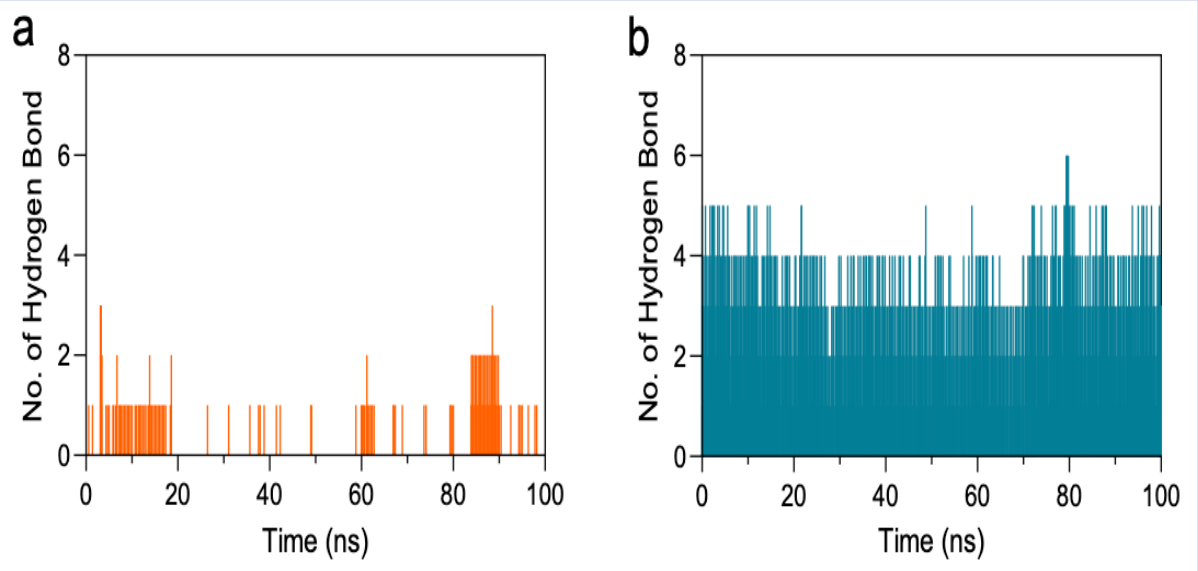

Figure 6. Hydrogen bond formation between COX-2 and (a) indomethacin and (b) diosmetin during docking process

Table 5. Summary of ligand binding energies and interacting amino acid residues with COX-2

Ligand	Binding Energy (kcal/mol)	Interacting Amino Acid Residues	Conventional H-Bonds	Unfavorable Bond
Indomethacin (control)	-9	TYR355, ARG513, GLU524, VAL89, TYR115, ILE112, TRP100, VAL116, LEU93, PRO86, ARG120, HIS90, SER119, VAL523, PRO84, THR85	1	0
Diosmetin	-7.8	**TYR355**, TYR385, SER530, **ARG120**, ALA527, LEU531, VAL349, LEU359, **VAL116**, TRP387, PHE381, GLY526, VAL523, SER353, LEU352, TYR348, ILE345, LEU117, MET113	3	0
Sinapine	-6.3	**TYR115**, **ARG120**, **TYR355**, **GLU524**, **VAL89**, LEU93, **VAL116**, PRO84, SER471, LEU472, LYS83, **ARG513**, PRO86, **HIS90**	4	0
Senkyunolide I	-6.6	**VAL89**, LEU93, **VAL116**, PHE357, **TYR355**, **TYR115**, PRO84, **GLU524**, **ARG120**, PRO86, **HIS90**, LEU359	0	0

Molecular Dynamics Analysis

To further assess the stability of the docked complex, 100 ns molecular dynamics simulations were performed for apo COX-2, indomethacin–COX-2, and diosmetin–COX-2 systems (Figure 5). The RMSD profiles indicated that all systems achieved equilibrium during the simulation, with the diosmetin–COX-2 complex exhibiting slightly lower fluctuations than the apo and indomethacin-bound forms, suggesting enhanced structural stability upon ligand binding.

RMSF analysis revealed similar residue flexibility patterns across all systems, with higher fluctuations predominantly localized in loop regions. However, the diosmetin-bound complex generally displayed reduced residue mobility relative to apo COX-2, indicating stabilization of flexible regions without inducing major conformational changes. Consistent with these observations, the radius of gyration (Rg) remained relatively constant throughout the simulation, demonstrating preservation of protein compactness in the ligand-bound systems.

The SASA profiles also remained stable over the 100 ns simulation period, indicating that diosmetin binding did not significantly alter protein solvent exposure or structural integrity. Furthermore, hydrogen bond analysis showed that diosmetin maintained approximately 3–5 hydrogen bonds throughout most of the simulation, with occasional formation of up to six hydrogen bonds. The persistence of these interactions suggests strong ligand retention within the COX-2 binding pocket and contributes to the overall stability of the complex.

The observed stability of the diosmetin–COX-2 complex is consistent with previous studies reporting that flavonoids can establish stable interactions with key COX-2 active-site residues through hydrogen bonding and hydrophobic contacts, thereby enhancing binding persistence during molecular dynamics simulations[34]. Similarly, it has been reported that natural anti-inflammatory compounds exhibiting low RMSD fluctuations, stable compactness, and sustained hydrogen-bond interactions are more likely to maintain favorable target engagement throughout simulation[35]. Collectively, the MD results support the docking findings and demonstrate that diosmetin forms a stable complex with COX-2, highlighting its potential as a natural anti-inflammatory lead compound.

CONCLUSIONS

The present study demonstrated the anti-inflammatory potential of *Eleusine indica* through a combined experimental and computational approach. The methanolic extract exhibited selective COX-2 inhibitory activity, while LC–MS profiling identified ten metabolites that may contribute to the observed bioactivity. Drug-likeness, ADME, and toxicity evaluations highlighted diosmetin, sinapine, and senkyunolide I as promising candidates for further investigation. Molecular docking revealed favourable interactions of these compounds with key COX-2 active-site residues, with diosmetin exhibiting the strongest binding affinity. Subsequent molecular dynamics simulations confirmed the stability of the diosmetin–COX-2 complex through sustained structural integrity and persistent hydrogen-bond interactions. Collectively, these findings identify diosmetin as a promising lead compound for the development of natural COX-2 inhibitors and provide a molecular rationale for the anti-inflammatory activity of *E. indica*. However, the molecular docking and molecular dynamics analyses presented herein represent computational predictions of protein–ligand interactions. Further experimental validation through enzyme kinetic studies, mechanistic cellular assays such as western blotting and cytokine analyses, and appropriate in vivo animal models is warranted to establish the selective COX-2 inhibitory activity of diosmetin.

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