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Original Research Article

Inhibitory effect of *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt on prostaglandin/leukotrienes synthesis, and *in silico* analysis of HPLC-identified compounds

Joel O Onoja[1-4]*, Joseph C Unamba[1], Catherine C Eleje[1], Mathieu JM Tjegbe[3]
[1]Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, [2]Institute of Drug-Herbal Medicine-Excipient Research and Development, University of Nigeria, Nsukka, Nigeria, [3]Center for Drug Discovery, University of Buea, Buea, Cameroon, [4]Department of Chemistry, Faculty of Sciences, Chulalongkorn University, Bangkok, Thailand

For correspondence:* ***Email:* *joel.onoja@unn.edu.ng;* ***Tel:*** *+234-806 2872 407*

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Abstract

Purpose: *To investigate the lipoxygenase (LOX) and cyclooxygenase (COX) inhibitory effects of Siphonochilus aethiopicus (Schweinf) B.L. Burtt (Zingiberaceae) rhizome, and to undertake in silico studies of identified compounds.*
Methods: *The ground rhizome powder of S. aethiopicus was extracted sequentially using n-hexane, ethyl acetate and methanol. Inhibitory effects on cyclooxygenase 2 (Cox-2) and lipoxygenase (LOX) were determined using a Cox-2 test kit and spectrophotometric analysis, respectively, with Ibuprofen and quercetin as standards. High-performance liquid chromatography with diode array detection (HPLC-DAD) was utilized for qualitative analysis and identification of bioactive compounds. Molecular docking was carried out using Maestro software. Ligands were docked with PDB ID: 4PH9 and 1JNQ for Cox-2 (Mus musculus) and LOX-3 (Glycine max), respectively.*
Results: *Ethyl acetate extract showed the highest Cox-2 inhibitory activity (66.62 ± 6.50 %, IC_{50} = 0.241 ± 0.02 mg/mL) and LOX inhibitory activity (65.87 ± 7.75 %, IC_{50} = 0.190 ± 0.00 mg/mL) at 1 mg/mL. Polyphenolic compounds identified via HPLC-DAD were tannic acid, gallic acid, naringenin/caffeic acid, and quercetin. Molecular docking revealed hydrogen bonding and π-π interactions between the ligands and active sites of enzymes.*
Conclusion: *S. aethiopicus inhibits Cox-2 and LOX enzymes. The presence of quercetin and caffeic acid in S. aethiopicus rhizome highlights its potential as a therapeutic agent in inflammatory diseases and warrants further investigation for drug development.*

Keywords: *Siphonochilus aethiopicus, Anti-inflammatory, Lipoxygenase, Cyclooxygenase, Zingiberaceae, HPLC-DAD, Molecular docking*

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INTRODUCTION

The most important building block of immune responses and inflammatory pathways is arachidonic acid (AA). Arachidonic acid is an important fatty acid and a precursor in the biosynthesis of prostaglandins (PGs), thromboxanes, and leukotrienes (LTs). Arachidonic acid is quickly transformed into active metabolites by lipoxygenases (LOXs) to

make leukotrienes and cyclooxygenases (COXs) to produce prostaglandins, prostacyclin, and thromboxanes [1].

Leukotrienes are chemical messengers that powerfully activate an immune response. Excessive synthesis of cysteinyl leukotrienes (CysLTs), as seen in severe asthma (bronchoconstriction), has been linked to dysregulated metabolism of the AA LOXs pathway. Additionally, leukotrienes (LTs) play pivotal roles in allergic diseases, allergic rhinitis, rheumatoid arthritis and colitis ulcerosa [2]. On the other hand, disruptions in the synthesis and/or metabolism of prostaglandins are implicated in many diseases. Patients who overproduce prostaglandins may experience a variety of symptoms, such as pain, opening and closing of blood vessels, fever, inflammation, diarrhea, and menstrual cramps. Prostaglandin synthesis inhibitors (COX-2 inhibitors) are used to treat individuals with prostaglandin-related symptoms in certain disease situations. Use of COX inhibitors is associated with gastrointestinal risks, ranging from mild irritation to severe complications such as bleeding and perforation [3]. Targeting lipoxygenase and cyclooxygenase (COX-2), which are primary mediators of inflammation, inhibits the production of LTs and PGs, thereby alleviating inflammatory responses and related disorders [4].

Natural substances, particularly polyphenols and their derivatives are novel anti-inflammatory agents. It has been demonstrated that chebulagic acid, a benzopyran tannin obtained from *Terminalia chebula*, inhibits LOX and COX enzymes [5]. Flavonoids belonging to the flavones, flavonols, flavanones, and chalcones subclasses have been found to inhibit both COX and LOX enzymes, highlighting their therapeutic potential [6]. Alkaloids, including demethyleneberberine, palmatine, and berberine, have been found to suppress the expression of COX-2 and 5-LOX proteins [7]. Clerodane diterpenes (16-hydroxy-cleroda-4, 13-dien-16, 15-olide) from the seeds of *Polyalthia longifolia* and sesquiterpenes (Buddledin A), diterpenes (Teucrin A, Lagascatrieol), and triterpenoids (Ursolic acid) are examples of terpenoid-based anti-inflammatory agents that have demonstrated potent inhibition against COX and LOX [8]. By simulating the atomic-level interactions between small molecules and proteins using molecular docking, it is possible to uncover the underlying biochemical processes and define the binding behavior of small molecules at target protein sites, facilitating a better understanding of protein-ligand interactions. Nowadays, docking supports various drug discovery tasks, including target identification, drug repositioning, polypharmacology, and adverse effect prediction [9].

Siphonochilus aethiopicus (Schweinf.) B.L. Burtt (Zingiberaceae) is also known as African ginger or wild ginger. Large leaves on this deciduous plant grow yearly from a tiny, cone-shaped rhizome. In traditional medicine, freshly chopped roots and rhizomes are highly valued in southern Africa. Traditionally, *S. aethiopicus* has been used as a remedy for various ailments, including cough, colds, influenza, and other ailments [10]. Therefore, this study investigated the COX and LOX inhibitory properties of *Siphonochilus aethiopicus* extract employing both experimental and computational methods, aiming to unveil its mechanism of action in the treatment of inflammatory diseases and substantiate its ethnomedicinal applications in managing inflammation.

EXPERIMENTAL

Collection and identification of plant materials

Rhizome of *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt was collected during the rainy season, in August 2021 from local forests in Nsukka (Opi), Enugu State, Nigeria. Mr. Nwafor Felix, a taxonomist in the Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, identified the plant, and a voucher specimen was deposited in the herbarium (voucher no. PCG/UNN/0417).

Extraction technique

The *Siphonochilus aethiopicus* rhizome was sliced into thin pieces, air-dried at room temperature for 3 weeks, ground into fine powder using a miller, and stored in an air-tight container. The plant material was then subjected to successive extraction, a technique that employs solvents of increasing polarity to isolate desired phytochemicals. The powdered rhizome of *Siphonochilus aethiopicus* (227.55g), weighed with electronic weighing balance (M411L, M-Metlar, Nigeria) was macerated in 3000 mL n-hexane in a maceration jar, agitated gently and left to stand for 72 h with continuous agitation every 8 h. After 72 h, the mixture was filtered with No. 1 Whatman filter papers to obtain the filtrate. The marc was air-dried and macerated in 3000 mL ethyl acetate for 72 h.

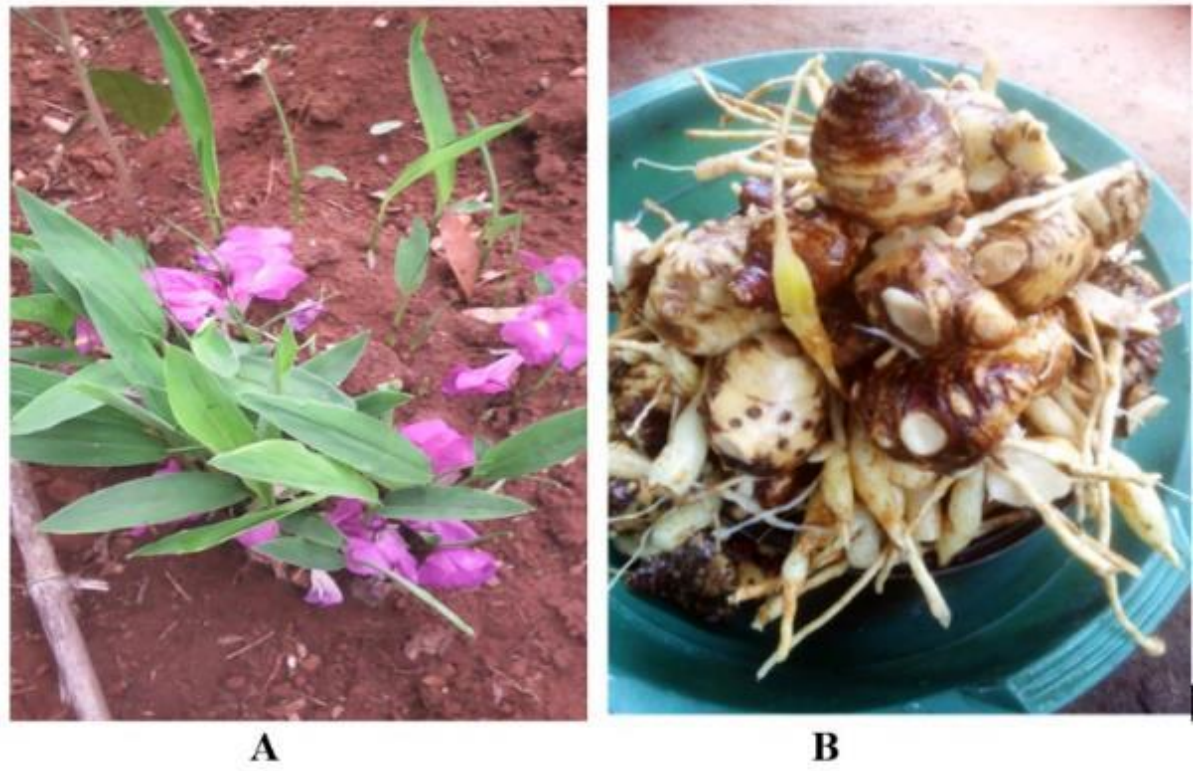


Figure 1: *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt. A = Aerial part (leaf and flower), B = Rhizome. Sources: Local forest Nsukka Opi in Enugu State, 2021

After filtration, the marc was further macerated in 3000 mL methanol for 72 h, with intermittent stirring every 8 h, and then filtered. The filtrates were subsequently concentrated at 40 °C under reduced pressure using a rotary evaporator (Buchi, R-300). The concentrated extracts were then weighed, sealed in airtight containers, and stored in a refrigerator at 4 °C until needed.

Cyclooxygenase (COX) inhibition assay

The COX-2 inhibitory activities of the extracts were evaluated using COX-2 test kit (Catalogue No. 560131, Cayman Chemical, Ann Arbor, MI, USA) while Ibuprofen (UniCure Ibuten) was used as positive control [11]. The fractions were dissolved in dimethyl sulfoxide and assayed at a final concentration of 1mg/mL - 0.03125 mg/mL. The mixture contained 0.96 mL buffer (0.1 M Tris-HCl, pH 8, containing 5 mM EDTA, and 2 mM phenol), enzyme (0.01 mL), heme (0.01 mL) and 0.01 mL of each test sample. This was incubated for 10 min at 37 ^{o}C and arachidonic acid solution (0.01 mL) was added. The reaction was terminated with 0.05 mL HCl (1.0 M). Thereafter, reduction of PGH 2 to PGF 2α by stannous chloride (0.1 mL) was measured by enzyme immunoassay (EIA). The distinct yellow product obtained as a result of the enzymatic reaction was determined spectrophotometrically at 412 nm on a microplate reader (BioTek, EPOCH/2). The negative control was prepared with COX-2 inactivated in boiling water for 3 min. Percentage inhibition was calculated using Eq 1. The IC_{50} of each sample was extrapolated from the concentration inhibition response curve using regression analysis.

Inhibition (%) = {(AEA–AIA)/AEA}100 …… (1)

Where: AEA is the activity of the enzyme test absorbance, and AIA is the activity inhibition test absorbance.

***In vitro* lipoxygenase (LOX) inhibitory assay**

A UV/visible light spectrophotometer was used to assess lipoxygenase inhibitory activities of plant fractions using linoleic acid as substrate, following the method of Malterud and Rydland [12] with some modifications. A range of extract concentrations (1 – 0.015625 mg/mL) was tested. The reaction mixture consisted of 50 µL extracts solution, 150 µL phosphate borate buffer (2M, pH 9.0), 50 µL lox enzyme (*Glycine max*, SIGMA) solution (167 U/mL in phosphate borate), and 250 µL of substrate solution (0.15 mM in the same buffer) were added to start the reaction. The enzymatic kinetic assay was performed at wavelength of maximum absorbance (A) of 234 nm for 5 min. An extract solution was not included in the 1 % methanol

solution that was used as negative control. Quercetin was used as positive control, and every experiment was done in triplicate. The percentage inhibition of lipoxygenase was calculated using Eq 2.

$$\text{Inhibition (\%)} = \{(A_{control} - A_{sample})/A_{control}\}100 \quad \ldots (2)$$

Standards and sample preparation for HPLC-DAD analysis

A mixed standard solution containing 50 ug/L of caffeic acid, coumeric acid, malic acid, gallic acid, tannic acid, rutin, saponin, naringenin, quercetin, and glutathione was used for calibration, based on the equipment-generated standard curve. The sample (1 g) was accurately weighed, dissolved in 1000 µL of methanol, and transferred to a vial for HPLC diode array detector analysis (Agilent 1260 Infinity II LC series). Duplicate runs of the sample were performed alongside calibrated standards [13].

Molecular modeling

The Maestro graphical user interface created by Schrödinger, New York, NY, USA, was used for virtual screening [14]. The molecules under study, i.e., COX-2 and LOX crystal structures, are found in the Protein Data Bank (PDB), accessible through the Research Collaboratory for Structural Bioinformatics (RCSB) website. These PDB IDs are 4PH9 (Ibuprofen bound to cyclooxygenase-2) and 1JNQ (lipoxygenase-3 (*Glycine max*) complexed with Epigallocatechin (EGC). The protein structure underwent a comprehensive optimization and minimization process using the OPLS3 force field. The receptor grids were constructed using prepared protein structures, and these grids were centered on specific binding pockets identified for each protein using Maestro's Glide module.

Crystal structures were painstakingly preprocessed with the help of Schrodinger Maestro's Protein Preparation Wizard tool. Polar hydrogens were incorporated, and non-essential water molecules were excluded from the structures. The Maestro Confgen and Ligprep were used to generate conformers and prepare ligands, respectively. The docking tool was utilized in Extra Precision mode (XP) to facilitate the docking process. Confgen for the ligands and the glide grid for the proteins were docked. The docked pose with the most favorable binding energy, as indicated by the lowest negative XP GScore and dG Bind (MM-GBSA) value, was selected for each target.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). Results were expressed in mean ± standard deviation (SD; n = 3), and compared using analysis of variance (ANOVA) followed by Dunnett's Multiple Comparisons test. $P < 0.05$ was considered statistically significant. Microsoft Excel was used to create standard curves and calculate IC_{50} values.

RESULTS

Cyclooxygenase (COX) inhibitory activity

Ethyl acetate extract (ESA) demonstrated the strongest inhibitory activity (IC_{50} = 0.241 ± 0.02 mg/mL, 66.62 ± 6.50 %) at 1mg/mL, outperforming methanol (MSA; IC_{50} = 0.280 ± 0.01 mg/mL, 74.93 ± 1.52 %) and n-hexane (HSA; IC_{50} = 0.976 ± 0.05 mg/mL, 50.41 ± 2.21 %) extracts (Table 1).

Lipoxygenase inhibitory activity

Ethyl acetate extract exhibited the highest inhibitory potential against lipoxygenase (IC_{50} = 0.190 ± 0.00 mg/mL, 65.87 ± 7.75 %) enzyme at 1 mg/mL, followed by the methanol extract (IC_{50} = 0.326 ± 0.00 mg/mL), while n-hexane extract exhibited the lowest activity (IC_{50} = 0.458 ± 0.05 mg/mL), compared to quercetin at 0.1 mg/mL (IC_{50} = 0.026 ± 0.00 mg/mL; Table 2).

Table 1: Cyclooxygenase inhibitory activities of extracts of *S. aethiopicus* rhizome at 1mg/mL

Conc (mg/mL)	Inhibition (%; mean ± SD)			
	HAS	***ESA***	***MSA***	***Ibuprofen***
1	50.41±2.21	66.62±6.50	74.93±1.52	67.31±1.66
0.5	33.65±0.13	62.88±4.70	70.36±3.87	47.22±1.80
0.25	31.85±1.10	54.15±0.41	58.31±1.52	27.83±1.80
0.125	23.26±2.49	49.16±0.13	27.83±2.35	17.31±0.69
0.0625	17.45±0.55	33.10±0.69	27.00±1.52	11.63±0.55
0.03125	6.60±1.06	6.37±0.83	10.52±0.55	5.26±0.55
IC_{50} (mean ± SD)	0.976±0.05**	0.241±0.02*	0.280±0.01*	0.065±0.00

HSA – n-Hexane extract, ESA – Ethyl acetate extract, MSA – Methanol extract. *$P < 0.05$, **$p < 0.05$ vs Ibuprofen

Table 2: Lipoxygenase inhibitory activity of extracts of *S. aethiopicus* rhizome

Conc. (mg/mL)	Inhibition (%; mean ± SD)			
	HSA	ESA	MSA	Quercetin
1	61.31±3.35	65.87±7.75	48.28±7.75	70.67±0.41
0.5	58.20±0.87	60.28±6.31	42.69±6.31	61.55±0.44
0.25	42.77±0.47	55.64±2.31	38.70±0.28	55.76±2.16
0.125	41.65±6.76	44.45±4.39	26.86±4.39	44.74±0.39
0.0625	37.58±1.99	36.22±1.27	18.63±1.27	34.58±1.10
0.03125	26.31±3.51	32.54±0.79	14.95±0.79	28.76±0.97
IC_{50} (mean ± SD)	0.458±0.05**	0.190±0.00*	0.326±0.00**	0.026±0.00

HSA – n-hexane extract, ESA – Ethyl acetate extract, MSA – Methanol extract. *P < 0.05, **p < 0.05 vs quercetin

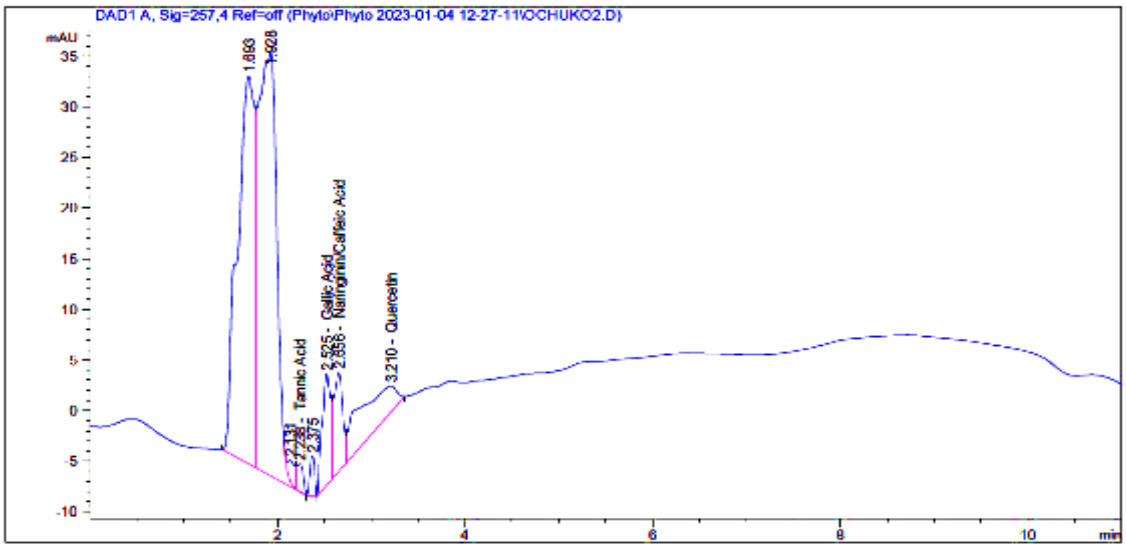


Figure 1: HPLC-DAD chromatogram of ethyl acetate extract of *Siphonochilus aethiopicus*

Table 3: HPLC-DAD of ethyl acetate extract of *Siphonochilus aethiopicus* rhizome

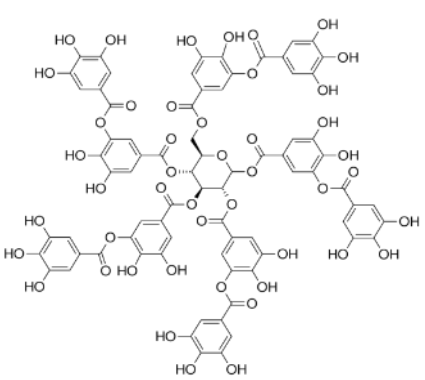
Peak (s)	Retention time (min)	Width (min)	Area (mAU*s)	Area (%)	Name
1	1.693	0.1790	489.65836	36.6296	-
2	1.928	0.1819	566.33466	42.3655	-
3	2.131	0.0823	12.02558	0.8996	-
4	2.238	0.0718	12.56449	0.9399	Tannic acid
5	2.375	0.0594	14.12506	1.0566	-
6	2.525	0.0859	69.54553	5.2025	Gallic acid
7	2.656	0.0963	65.55952	4.9043	Naringinin/Caffeic acid
8	3.210	0.5370	106.96988	8.0020	Quercetin
9	3.703	0.0000	0.00000	0.0000	Maleic acid
10	6.204	0.0000	0.00000	0.0000	Saponin

Ethyl acetate extract of *Siphonochilus aethiopicus* rhizome

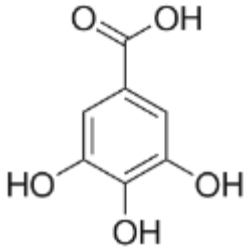
Ethyl acetate extract of *Siphonochilus aethiopicus* rhizome was subjected to HPLC-DAD analysis, resulting in the identification and quantification of several phytochemicals including tannic acid (RT: 2.238, Area %: 0.9399), gallic acid (RT: 2.525, Area %: 5.2025), naringenin/caffeic acid (RT: 2.652, Area %: 4.9043), quercetin (RT: 3.210, Area %: 8.0020; Figure 1, Table 3 and Table 4). The structures of identified compounds are represented in Figure 2.

Table 4: Concentrations of phytochemicals identified in ethyl acetate extract of *Siphonochilus aethiopicus* rhizome using HPLC-DAD

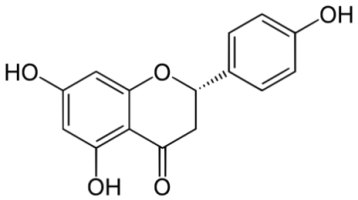
Phytochemical	Conc (mg/mL)
Tannic acid	0.004
Naringenin	0.007
Gallic acid	0.012
Quercetin	0.010



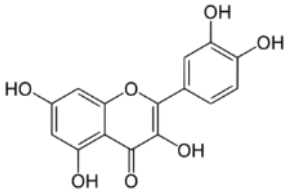
Tannic acid



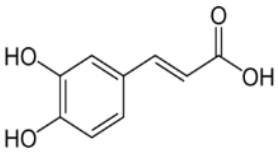
Gallic acid



Naringenin



Quercetin



Caffeic acid

Figure 2: Structures of phytochemicals identified from ethyl acetate extract of *Siphonochilus aethiopicus* rhizome using HPLC-DAD

Virtual screening analyses of compounds

Molecular docking approach was employed to study the binding between cyclooxygenase-2 protein (PDB ID: 4PH9) and ligands identified from *Siphonochilus aethiopicus* rhizome, including calculation of the estimated free energy of binding (ΔG) to gain insight into their interactions. Caffeic acid, a hydroxycinnamic acid docked into cyclooxygenase-2 active site, exhibiting hydrogen bond interactions with Ser 531, Arg 121 and Tyr 356 with the estimated free energy of binding (ΔG_{bind}) of -7.135 kcal/mol (XP G score) and -20.17 kcal/mol (Molecular mechanics with generalized Born and surface area solvation (MM-GBSA)). The docking results showed that Ser 531 formed a hydrogen bond with the hydroxyl group of the phenyl ring, and the carbonyl group at position 9 was involved in hydrogen bonding with Arg 121 and Tyr 356 (Figure 3, Table 5). Gallic acid, which is a trihydroxybenzoic acid, interacted with the protein through hydrogen bonds with Ser 531 and Met 523. The hydroxyl groups at positions 3 and 5 were involved in these interactions, with Ser 531 and Met 523 binding to positions 3 and 5, respectively. The estimated free energy of binding (ΔG_{bind}) was -4.444 kcal/mol (XP G score) and -5.908 kcal/mol (MM-GBSA; Figure 3, Table 5). Naringenin, a flavanone, interacted with the protein through a hydrogen bond between its hydroxyl group at position 7 on ring (A) and Ser 531, as well as π-π stacking interactions between ring (B) attached to the heterocyclic pyrene (C) and Tyr 356. The estimated free energy of binding (ΔG_{bind}) was -8.226 kcal/mol (XP G score) and -20.253 kcal/mol **(**MM-GBSA; Figure 3, Table 5). Quercetin, a flavonol, binding mode involving π-π stacking interactions with Tyr 356 and a hydrogen bond with Arg 121. The hydrogen bond was formed between Arg 121 and the hydroxyl group at position 3 on ring B, while Tyr 356 engaged in π-π stacking with the same ring. The estimated free energy of binding (ΔG_{bind}) was -9.162 kcal/mol (XP G score) and -23.533 kcal/mol (MM-GBSA; Figure 4, Table 5). Ibuprofen ((*RS*)-2-(4-(2-methylpropyl)phenyl)propanoic acid), the co-crystallized ligand, formed hydrogen bonds with Tyr 356 and Arg 121, as well as a salt bridge with Arg 121. Specifically, the hydroxyl group interacted with Tyr 356 through hydrogen bond, while the carbonyl group formed a hydrogen bond and a salt bridge with Arg 121. The estimated free energy of binding (ΔG_{bind}) was -

9.243 kcal/mol (XP G score) and -49.514 kcal/mol (MM-GBSA; Figure 4, Table 5). Virtual screening analysis identified quercetin and caffeic acid as potential cyclooxygenase-2 inhibitors, given their ability to bind to crucial residues (Tyr 356, Arg 121) in a manner similar to the co-crystallized ligand.

To elucidate the binding mechanism, molecular docking studies were performed between ligands identified from *Siphonochilus aethiopicus* rhizome and lipoxygenase-3 protein (PDB ID: 1JNQ). Docking analysis revealed that caffeic acid interacted with the lipoxygenase-3 active site through a hydrogen bond with Gln 716 and a salt bridge with Fe2 858. Specifically, the hydroxyl group at position 4 on the phenyl ring formed a hydrogen bond with Gln 716, while the hydroxyl group of the acid at position 9 engaged in a salt bridge with Fe2 858. The estimated free energy of binding (ΔG_{bind}) was -6.001 kcal/mol (XP G score) and -11.977 kcal/mol **(**MM-GBSA; Figure 5, Table 6). Gallic acid interacted with Fe2 858 through a salt bridge, mediated by the hydroxyl groups of the acid attached to the benzene ring at position 1. The estimated free energy of binding (ΔG_{bind}) was -7.842 kcal/mol (XP G score) and -11.759 kcal/mol (MM-GBSA; Figure 5, Table 6).

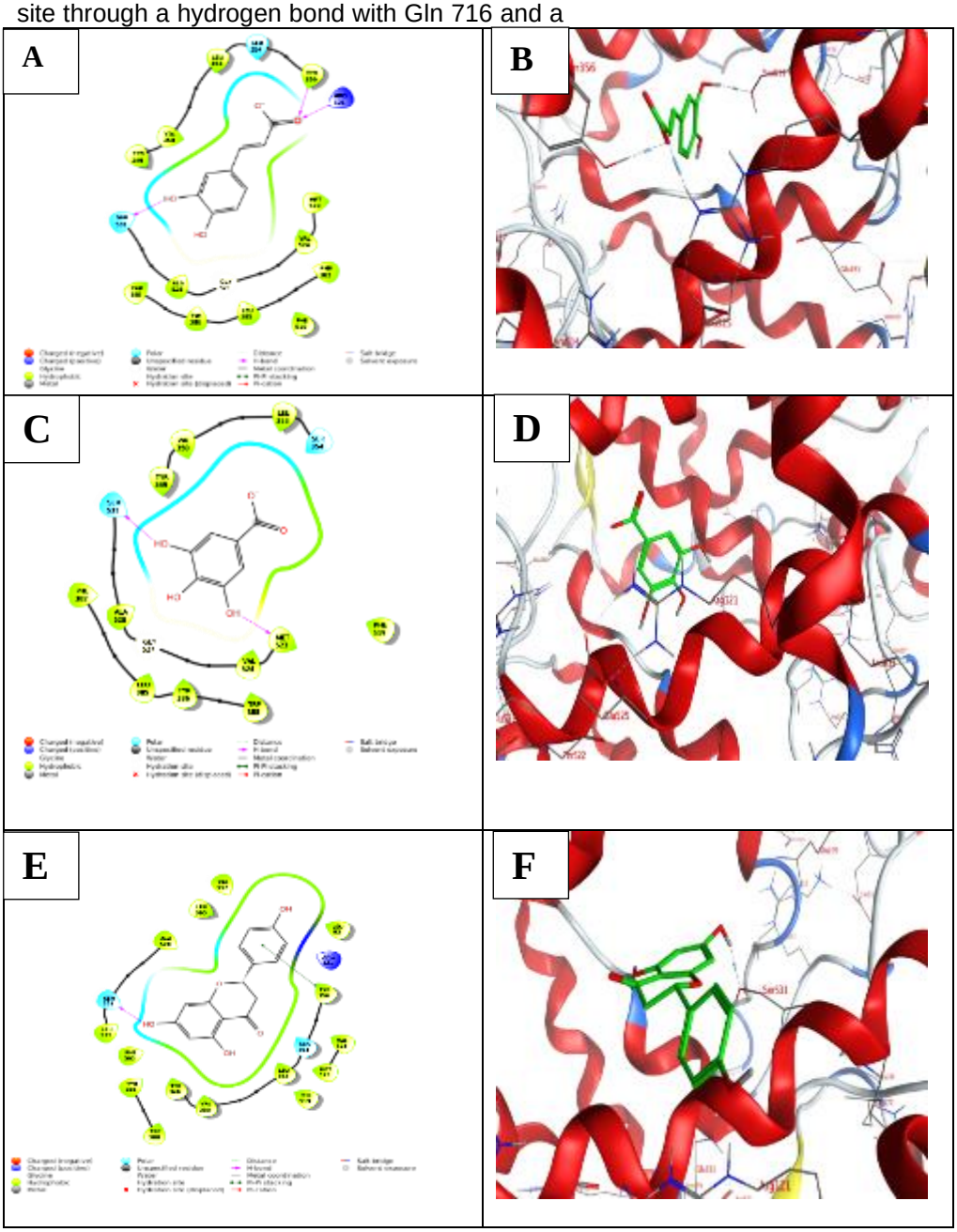


Figure 3: 2D and 3D binding interaction of caffeic acid (A and B), gallic acid (C and D), naringenin (E and F) with COX-2 active site (PDB ID: 4PH9)

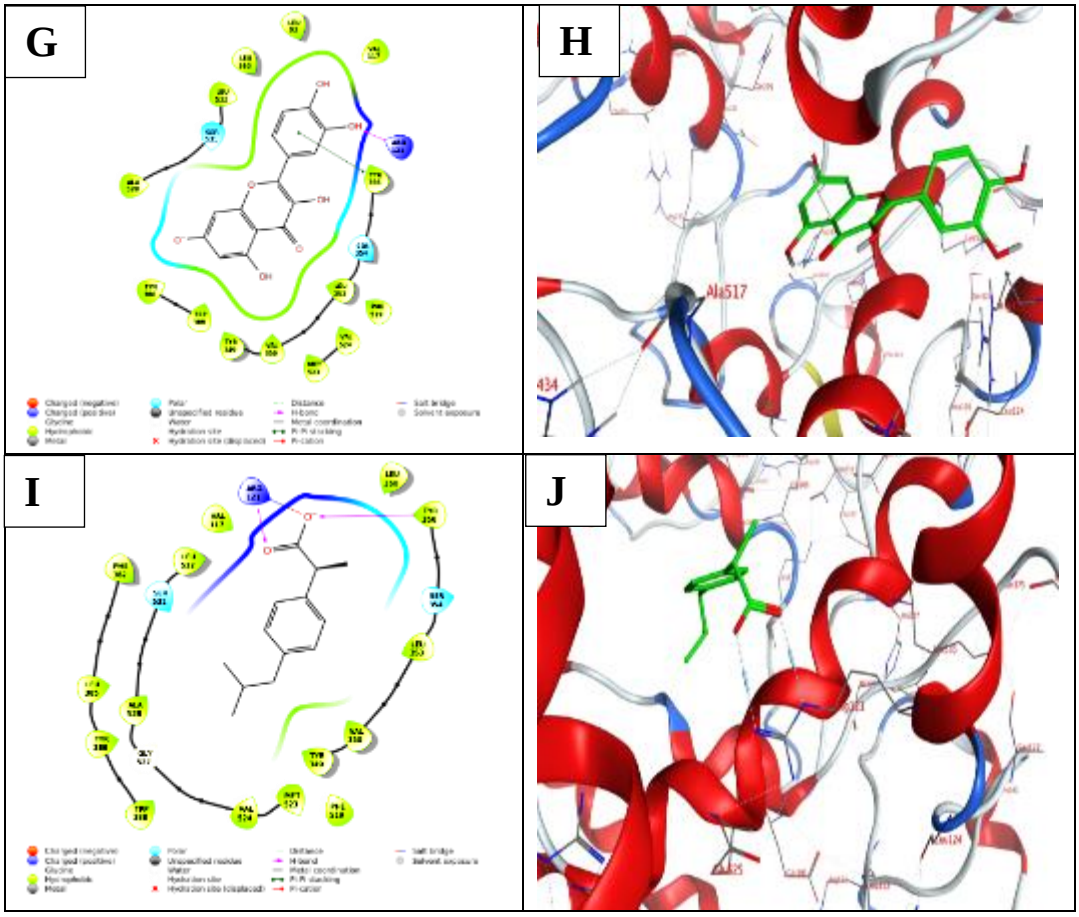


Figure 4: 2D and 3D binding interaction of quercetin (G and H), and ibuprofen (I and J) with COX-2 active site (PDB ID: 4PH9)

Table 5: Estimated free energy binding scores and ligand interactions with COX-2 active site (PDB ID: 4PH9)

Ligand	XP G Score (kcal/mol)	(ΔG_{bind}; MM-GBSA; kcal/mol)	Ligand interactions
Ibuprofen	-9.243	-49.514	Hydrogen bond with Tyr 356, Arg 121, Salt bridge with Arg 121
Naringenin	-8.226	-20.253	Hydrogen bond with Ser 531, π-π interaction with Tyr 356
Quercetin	-9.162	-23.533	π-π interaction with Tyr 356, hydrogen bond with Arg 121
Caffeic acid	-7.135	-20.17	Hydrogen bond with Ser 531, Arg 121, Tyr 356
Gallic acid	-4.444	-5.908	Hydrogen bond with Ser 531, Met 523

Naringenin interacted with the protein through a hydrogen bond with Ile 357 and π-π stacking interactions with His 518 and Phe 576. Specifically, the hydroxyl group at position 7 on ring (A) formed a hydrogen bond with Ile 357, while ring A interacted with His 518 and ring B attached to the heterocyclic pyrene (C) interacted with Phe 576 through π-π stacking.

The estimated free energy of binding (ΔG_{bind}) was -5.925 kcal/mol (XP G score) and -6.198 kcal/mol (MM-GBSA; Figure 5, Table 6). The binding mode of quercetin involved hydrogen bonds with Hie 513 (ring B hydroxyl group at position 4) and Ile 857 (ring A hydroxyl group at position 7), and π-π stacking interactions with His 518 (ring A and C) and Phe 576 (ring B). The estimated free energy of binding (ΔG_{bind}) was -8.045 kcal/mol (XP G score) and -9.61 kcal/mol (MM-GBSA; Figure 6, Table 6).

The co-crystallized ligand Epigallocatechin (EGC), a flavan-3,3',4',5',5'7-hexol and a catechin binding mode involved hydrogen bonds with Gln 716, His 518, Ile 857, and a π-π stacking interaction with His 523 at ring A. The hydrogen bond was formed between Ile 857 and the hydroxyl group at position 7 attached to ring

A and between Gln 716 and His 518 and the hydroxyl groups at position 4' and position 5', respectively, attached to ring B. The estimated free energy of binding (ΔG_{bind}) was -10.004 kcal/mol (XP G score) and -35.37 kcal/mol **(**MM-GBSA; Figure 6, Table 6).

The binding of caffeic acid to Gln 716, similar to the co-crystallized ligand, suggests its potential as a LOX inhibitor. MM-GBSA analysis further supports its stability within the active site.

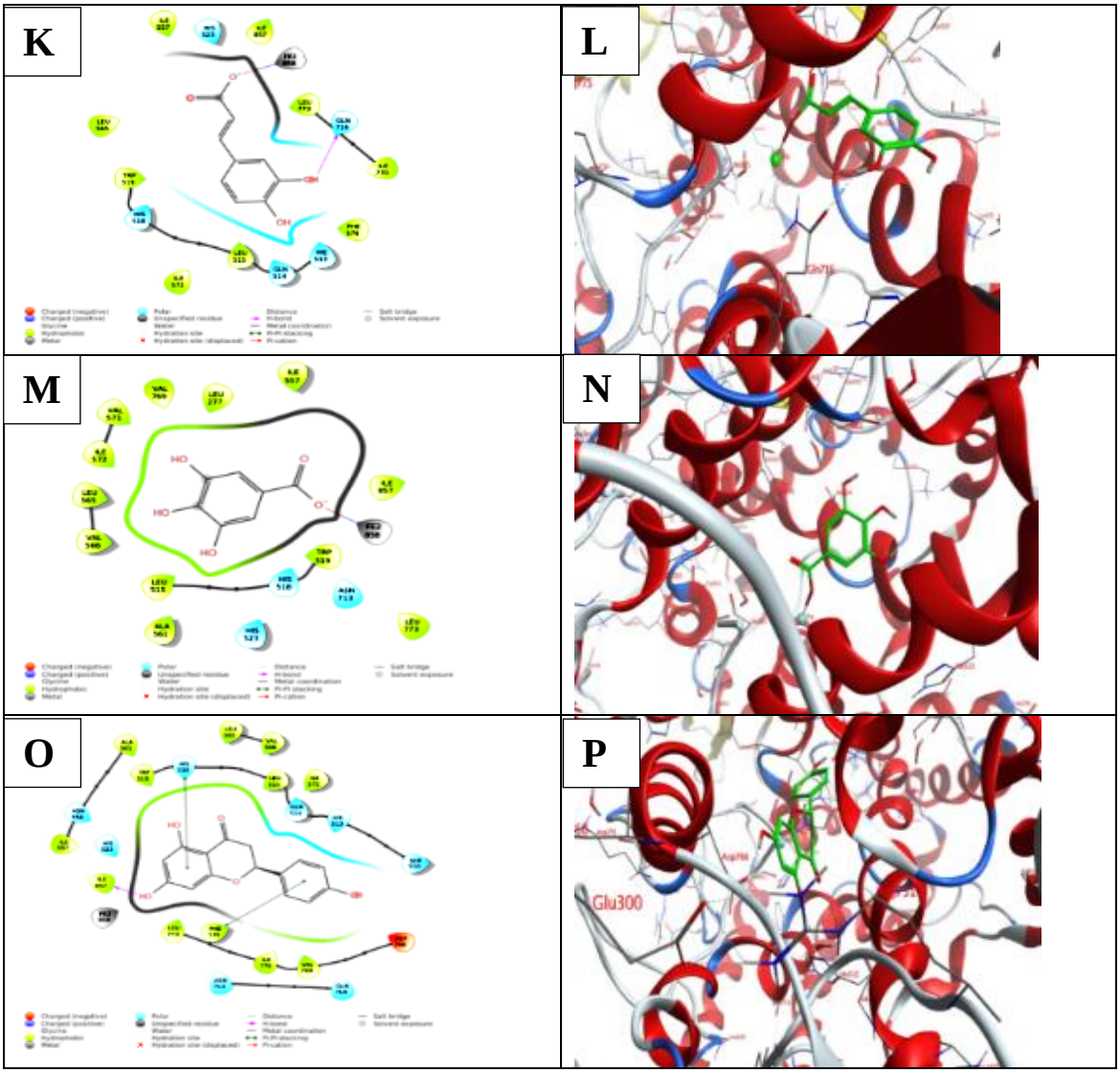


Figure 5: 2D and 3D Binding interaction of caffeic acid (K and L), gallic acid (M and N), naringenin (O and P) with LOX-3 active site (PDB ID: 1JNQ)

Table 6: Estimated free energy binding scores and ligand interactions with LOX-3 active site (PDB ID: 1JNQ)

Ligand	XP G Score (kcal/mol)	(ΔG_{bind}; MM-GBSA; kcal/mol)	Ligand interactions
Epigallocatechin (EGC)	-10.004	-35.37	Hydrogen bond with Gln 716, His 518, Ile 857, π-π interaction with His 523
Naringenin	-5.925	-6.198	Hydrogen bond with Ile 357, π-π interactions with His 518, Phe 576
Quercetin	-8.045	-9.61	Hydrogen bond with Hie 513, Ile 857, π-π interactions with His 518, Phe 576
Caffeic acid	-6.001	-11.977	Hydrogen bond with Gln 716, Salt bridge with Fe^2 858
Gallic acid	-7.842	-11.759	Salt bridge Fe^2 858

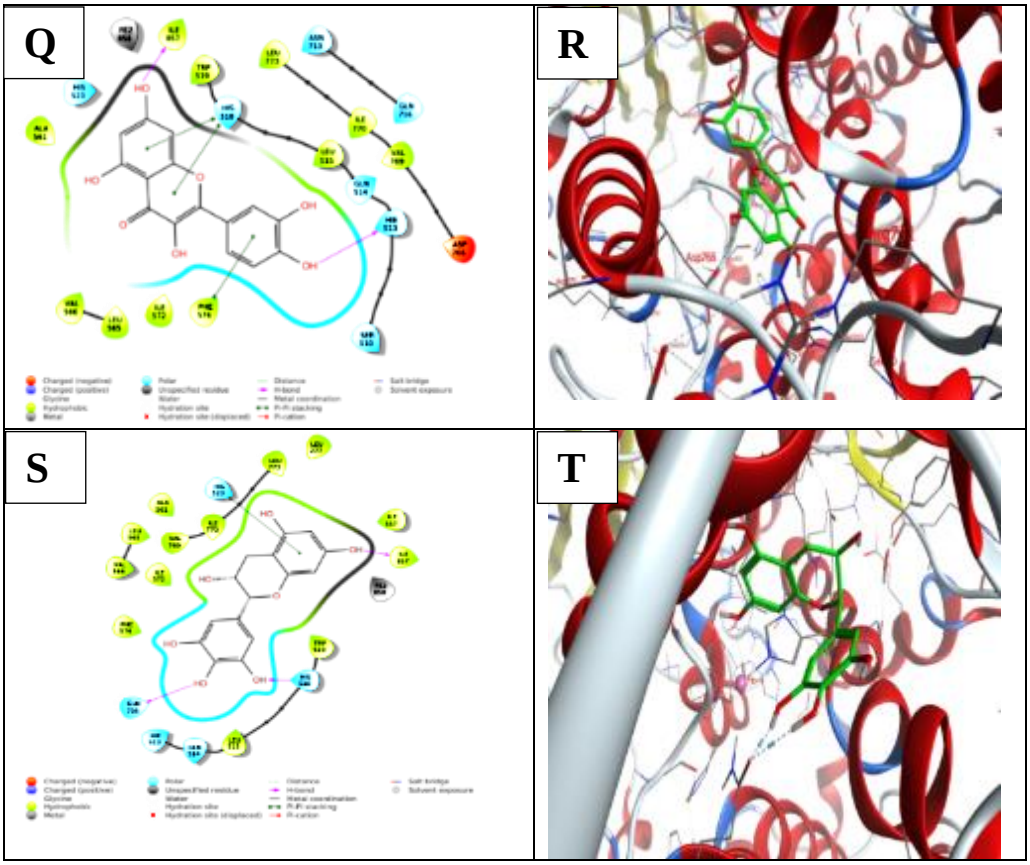


Figure 6: 2D and 3D Binding interaction of quercetin (Q and R), and Epigallocatechin (EGC; S and T) with LOX-3 active site (PDB ID: 1JNQ)

DISCUSSION

Nature-derived compounds are being investigated as potential selective COX-2 and LOX inhibitors for anti-inflammatory effects. The anti-inflammatory activity of extracts of the leaves of *Siphonochilus aethiopicus* has been studied [15]. Polyphenols have been reported to exhibit anti-inflammatory properties by suppressing pro-inflammatory cytokines and cyclooxygenase-2 (COX-2) enzyme activity. Compounds from the Zingiberaceae family, including 10-gingerol, 8-shogaol, and 10-shogaol from ginger (*Zingiber officinale*), and xanthorrhizol, beta-turmerone, and ar-turmerone from turmeric (*Curcuma xanthorrhiza* and *Curcuma zedoaria*), show potential as COX-2 inhibitors, likely reducing inflammation by blocking prostaglandin synthesis [16].

Targeting lipoxygenase pathway inhibition may offer benefits in managing inflammatory diseases, including asthma and other related diseases such as cancer, allergy, arthritis, COVID-19 and cardiovascular disease. Natural compounds from the Zingiberaceae family, such as gingerols and diaryl heptanoids isolated from the rhizome of *Zingiber officinale*, Curcumin from *Curcuma longa*, luteolin and kaempferol found in *Boesenbergia albosanguinea,* exhibit lipoxygenase inhibitory activity, and as a result, are promising agents for reducing inflammation by blocking leukotriene production [17].

The cyclooxygenase and lipoxygenase inhibitory activity of *Siphonochilus aethiopicus* rhizome may be linked to the presence of phenolic metabolites identified by HPLC. Plant-derived flavonoids reduce cellular ROS levels by acting as antioxidants. The structure of Naringenin which features hydroxyl groups at position 5 and 7 on ring A and a carbonyl group at position 4 on ring C, enables the chelation of iron and copper ions, facilitating free radical neutralization.

Studies suggest that naringenin has the potential to shield cells from damage triggered by oxidative stress and inflammation [18]. Gallic acid and caffeic acid are potent apoptosis inducers, strong antioxidants and lipoxygenase and cyclooxygenase inhibitors [19,20]. The goal of protein-ligand docking is to predict how a ligand (a small molecule) binds to a protein, including its position and orientation. Docking techniques are used in pharmaceutical research for several applications, prominent amongst which is the virtual screening of vast chemical

databases to identify promising candidates for drugs. Potential therapeutics must be able to enter the site of action or the biological target's active site. The molecule needs to stay long enough to interact with its targets in addition to reaching the therapeutic concentration to elicit the desired effect. For this to occur, a medication molecule needs to have an adequate ratio of hydrophilicity to hydrophobicity [21].

Conventional hydrogen bonds in protein-ligand complexes involve interactions between hydrogen bond donors and acceptors, influencing complex stability and specificity [22]. Also, salt bridges may form between ligands of small molecules and proteins. Salt bridges are frequently observed in drug-protein interactions. It has been shown that over 1100 distinct protein-ligand complexes from the Protein Databank (PDB) form salt bridges with protein targets. Salt bridges are crucial for maintaining protein-ligand complex stability because they function as molecular clips to maintain the shape of the protein and increase inhibitor activity hundreds of times.

Therefore, adding salt bridges between ligands and their protein targets may be a useful strategy in the development of sensible drugs [23]. Aromatic interactions are the third most common type of protein-ligand interactions in the PDB. In chemical and biological systems, interactions involving aromatic rings are common and can be viewed as a specific type of hydrophobic interaction. The aromatic ring of both phenylalanine and a ligand is involved in over half of all π-stacking interactions, followed by tyrosine, tryptophan and histidine [24,25]. Aromatic ring interactions play a significant role in both drug design and protein-ligand recognition. The binding affinity of inhibitors is strengthened via π-π stacking interactions with the target [24].

CONCLUSION

The rhizome of *Siphonochilus aethiopicus* contains polyphenolic molecules with the potential of inhibiting cyclooxygenase (COX-2) and lipoxygenase (LOX) responsible for inflammatory diseases, possibly via antioxidant mechanisms. Quercetin and caffeic acid identified in *Siphonochilus aethiopicus* rhizome may inspire the discovery of new anti-inflammatory drugs. However, *in vivo* studies are required to determine its safety profile and therapeutic efficacy. Isolation and characterization of bioactive compounds responsible for these effects are recommended.

DECLARATIONS

Acknowledgment/Funding

None.

Ethical approval

None provided.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Onoja O Joel designed the experiment, wrote the first draft of the manuscript and supervised the project. Mathieu JM and Onoja OJ performed the in silico studies. Joseph C Unamba and Catherine C Eleje collected and extracted the plants and performed the bio-assay in vitro. All authors read and approved the manuscript.

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