

MULTI-TARGET IN VITRO ENZYME INHIBITION (5-LOX, COX-2, ACHE/BCHE), ANTI-INFLAMMATORY PROTEIN PROTECTION, AND IN SILICO COMPUTATIONAL VALIDATION OF NIGELLA SATIVA FOLIUM HYDROETHANOLIC EXTRACT

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ABSTRACT

Nigella sativa L. seeds are extensively studied for their bioactive compounds, whereas its polyphenol-rich foliar biomass remains largely underutilised. This study investigated the in vitro anti-inflammatory, antioxidant, and enzyme-inhibitory activities of a crude hydroethanolic *Nigella sativa* leaf extract (NSLE) using animal-free experimental and computational approaches. Shade-dried leaves were macerated in 70% ethanol. Total phenolic content (TPC) and total flavonoid content (TFC) were determined colourimetrically. Anti-inflammatory activity was determined using heat-induced egg albumin and bovine serum albumin (BSA) denaturation assays. Enzyme inhibition was evaluated against 5-lipoxygenase (5-LOX), cyclooxygenase-2 (COX-2), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE). Antioxidant activity was explored using DPPH, ABTS, and FRAP assays. Molecular docking of thymoquinone, rutin, quercetin, and kaempferol was performed against AChE (PDB: 4EY7), COX-2 (PDB: 5KIR), and 5-LOX (PDB: 6N2W). NSLE yielded 12.84% (w/w), with TPC and TFC values of 71.28 ± 2.14 mg GAE/g and 36.85 ± 1.62 mg QE/g, respectively. In the BSA denaturation assay, the IC_{50} was 114.6 ± 4.2 μ g/mL, compared with 78.2 ± 2.8 μ g/mL for aspirin. Concentration-dependent inhibition was observed for 5-LOX (IC_{50} : 42.18 ± 1.95 μ g/mL), COX-2 (68.34 ± 2.45 μ g/mL), AChE (84.12 ± 3.10 μ g/mL), and BChE (108.50 ± 4.20 μ g/mL). DPPH and ABTS IC_{50} values were 38.64 ± 1.42 and 29.15 ± 1.12 μ g/mL,

respectively. Rutin and quercetin exhibited docking energies of -8.2 to -9.6 kcal/mol. These findings suggest that *N. sativa* foliage may be a promising source of bioactive compounds with antioxidant, anti-inflammatory, and enzyme-inhibitory properties, warranting further investigation.

Keywords: molecular docking, 5-lipoxygenase, acetylcholinesterase, protein denaturation, *in vitro* anti-inflammatory, *Nigella sativa* leaves.

1. INTRODUCTION

Chronic inflammatory pathologies and progressive degenerative diseases are largely caused by oxidative stress in cells and dysregulation of critical catalytic enzymes (Leyane *et al.* 2022). Through parallel 5-lipoxygenase (5-LOX) and cyclooxygenase-2 (COX-2) pathways, arachidonic acid from the breakdown of membrane phospholipids is metabolised to pro-inflammatory eicosanoids, primarily leukotrienes and prostaglandins, which cause vascular permeability, tissue oedema, mucosal damage, and hyperalgesia (Ekin 2023). Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) break down acetylcholine, which is crucial for the parasympathetic nervous system and functions as an anti-inflammatory in the body. The cholinergic system simultaneously influences the peripheral and central inflammatory response (Li *et al.* 2025). When used clinically for extended periods of time, conventional therapeutic interventions based on non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors are frequently linked to serious side effects, including peptic ulceration, nephrotoxicity, and adverse cardiovascular events (Khalil *et al.* 2024). An attractive therapeutic option is a dual- or multi-target inhibitor derived from natural medicinal flora that can stabilise structural proteins, block eicosanoid-producing enzymes, and quench reactive oxygen species (ROS).

Much research has examined the therapeutic qualities of *Nigella sativa* L. seeds. A well-researched byproduct of commercial *Nigella sativa* seed harvesting is *Nigella sativa* folium (Danish and Alam 2024). Phytochemical research shows that vegetative leaves produce secondary metabolites, including polyphenols, flavonol glycosides, and volatile chemicals, that differ from those in seed oils (Ahlawat *et al.* 2024).

Furthermore, the 3Rs (Replacement, Reduction, and Refinement) in preclinical screening have

increased interest in computational molecular docking, cell-free microplate enzyme kinetic assays, and biophysical protein denaturation assays. These computational models provide reliable, repeatable pharmacological data while removing the economic and moral constraints associated with using animals (Shringi 2026).

This work aimed to develop a thorough *in vitro* and *in vivo* pharmacological profile of the crude hydroethanolic extract of *Nigella sativa* folium. We evaluated its capacity to scavenge free radicals, interact with target macromolecular receptors *in silico*, inhibit certain inflammatory and neuromodulatory enzymes (5-LOX, COX-2, AChE, and BChE), and prevent heat protein denaturation.

2. MATERIAL AND METHOD

2.1. Extraction & Plant Material

Fresh *Nigella sativa* leaves were taken from grown plants, examined for botanical authenticity, and then cleaned with distilled water. An electric herbal mill was used to grind the leaves into a coarse powder (mesh size 40) after they were shade-dried for 12 days at room temperature (25 ± 2 °C) until their weight remained constant. Using the cold maceration method, 500 g of dried powder was extracted for 72 hours at room temperature with sporadic shaking in 70% analytical grade ethanol (1:10 w/v, 5.0 L). Whatman Grade No. 1 filter paper was used after the mixture had been filtered through two layers of muslin fabric. To prevent the heat degradation of the active ingredients, the filtrate was concentrated on a rotating vacuum evaporator (Büchi, Switzerland) at 38 °C under low pressure. Per cent yield (%) is calculated by dividing the dry weight of the crude extract (g) by the initial plant material (g) and multiplying by 100. For further use, the dried extract was stored at -20 °C in sealed amber glass vials.

2.2. Typical Chemicals and Reagents

Acetylthiocholine iodide (ATChI), S-butylthiocholine iodide (BTChI), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), zileuton, donepezil hydrochloride, aspirin, gallic acid, and quercetin were all obtained commercially. We bought human recombinant COX-2 inhibitor screening assay kits from Cayman Chemical (Ann Arbor, MI, USA). A 96-well microplate reader (BioTek Instruments, USA) was used for the spectrophotometric measurements.

2.3. Standardisation of Phytochemicals

2.3.1. TPC, or total phenolic content

The TPC was measured using the Folin-Ciocalteu microplate technique (Sari *et al.* 2023). Briefly, 20 µL of crude extract (1.0 mg/mL) or standard gallic acid solutions (12.5-200 µg/mL) were mixed with 100 µL of 10% Folin-Ciocalteu reagent in a 96-well plate. 80 µL of 7.5% (w/v) Na₂CO₃ was added after five minutes. The optical density at 765 nm was measured following a 90-minute dark incubation period at 25 °C. Milligrams of GAE/g of dried extract (mg GAE/g) were used to express TPC.

2.3.2. Total Flavonoid Content (TFC)

TFC was measured using the aluminium chloride colourimetric test (Chang *et al.*, 2002). The crude extract (25 µL) or regular quercetin solutions (6.25-100 µg/mL) were mixed with 100 µL distilled water, followed by 7.5 µL 5% NaNO₂. Six minutes later, 15 µL of 10% AlCl₃ was added. 50 µL of 1 M NaOH and 52.5 µL of distilled water were added after five minutes. At 510 nm, the absorbance was determined. The mg of quercetin equivalents per gram of dried extract (mg QE/g) was used to calculate the TFC.

2.4. Protein Protection *In Vitro* Anti-Inflammatory Tests

2.4.1. Bovine Serum Albumin (BSA) Protein Assay

The purpose of this assay was to quantify the denaturation and denaturation resistance of the Bovine Serum Albumin (BSA) protein. With a few

minor adjustments for use in microplates, the anti-denaturation assay was performed as outlined by Mizushima and Kobayashi (1968). 0.05 mL of NS and 0.45 mL of 0.5% (w/v) aqueous BSA made up the test reaction (0.5 mL). The final concentrations of Cr or standard aspirin/diclofenac are 25, 50, 100, 200, and 400 µg/mL. 1 N HCl was added to bring the pH down to 6.3. The mixtures were kept at 37 °C for 20 minutes, and then heated to 57 °C for 30 minutes in a water bath to thermally denature the mixtures. After cooling to room temperature, 200 µL of each solution was poured in a 96-well plate. The turbidity was then measured at 660 nm. Control wells containing a vehicle devoid of extract were used to establish 100% denaturation.

The protection % was calculated as:

% protein protection = $[1 - (\text{sample} - \text{sample blank}) / (\text{control} - \text{control blank})] \times 100$

2.4.2. Egg Albumin Denaturation Assay-Buffer

Fresh hen egg albumin (0.2 mL) was combined with 2.8 mL of phosphate-buffered saline (pH 6.4) and 2.0 mL of either ordinary Diclofenac (25-400 µg/mL) or various concentrations of NSLE. The mixtures were heated to 70 °C for five minutes after being placed at 37 °C for fifteen minutes. After cooling, absorbance at 660 nm was measured, and the above formula was used to calculate the percentage of protection.

2.5. Enzyme Inhibition Assays by Microplate Technique

2.5.1. 5-Lipoxygenase (5-LOX) Assay

The enzymatic conversion of linoleic acid to 13-hydroperoxyoctadecadienoic acid was used to measure the 5-LOX inhibitory action (Baylac and Racine 2003). 10 µL of NSLE and sodium phosphate buffer 0.1 M (pH 8.0) were utilised for the reaction mixture. 20 µL of 5-LOX enzyme solution (0.05 mg/mL) and either regular zileuton or Cr (10-200 µg/mL). Following a pre-incubation period of 10 minutes at 25 °C, 10 µL of the linoleic acid substrate (1.25 mM in ethanol) was added to initiate the enzymatic reaction. Using a UV-transparent 96-well microplate reader set at 234 nm, the absorbance increase was continuously monitored for ten minutes.

2.5.2. Cyclooxygenase-2 (COX-2) Assay

In accordance with the manufacturer's instructions, the commercial colourimetric screening kit was used to assess the human recombinant system's capacity to suppress COX-2. In summary, assay buffer, heme and human recombinant COX-2 enzyme were pre-incubated with test quantities of NSLE (10-200 µg/mL) or celecoxib was added for 15 minutes at 37 °C. Arachidonic acid (substrate) was added to start the reaction. The production of prostaglandin was colourimetrically followed by oxidising N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) at 590 nm.

2.5.3 Assays for Cholinesterase (AChE and BChE):

Ellman's approach, which was developed for use in 96-well plates, was one way to measure cholinesterase inhibition (Ellman *et al.* 1961). AChE Assay: Each well was pre-incubated for 15 minutes at 25 °C with 10 µL of AChE (0.1 U/mL), 20 µL of NSLE (12.5–200 µg/mL) or donepezil, and 100 µL of 0.1 M phosphate buffer (pH 8.0). Next, 10 µL each of 0.01 M DTNB and 0.075 M acetylthiocholine iodide (ATChI) were added. For ten minutes, a yellow 5-thio-2-nitrobenzoate anion production curve was observed at 412 nm as a function of time. Equine serum BChE (0.1 U/mL) and S-butyrylthiocholine iodide (BTChI) were used as substrates in the BChE Assay, which was conducted under the same conditions.

2.6. Antioxidant Tests

2.6.1. Radical Scavenging Assay for DPPH

Serial dilutions of NSLE in 50 µL aliquots or normal ascorbic acid solutions (5–100 µg/mL) were combined with 150 µL of ethanolic DPPH solution at 0.1 mM. The absorbance was then measured at 517 nm following a 30-minute dark incubation period at 25°C.

2.6.2. Radical Decolourisation Assay for ABTS

To create the ABTS radical cation (ABTS•+), 2.45 mM potassium persulfate was added to a 7 mM ABTS solution and left in the dark for 16 hours. The original absorbance value at 734 nm was 0.70

± 0.02 after the solution was thinned down with ethanol. After six minutes, the absorbance at 734 nm was measured after adding 20µL of extract to 180µL of ABTS working reagent.

2.6.3. The Ferric Reducing Antioxidant Power (FRAP) Assay

This assay was used to gauge antioxidant reducing power. The three solutions, 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (dissolved in 40 mM HCl) and 20 mM FeCl₃.6H₂O were mixed in a 10:1:1 (v/v/v) ratio to create the working FRAP reagent. After mixing 180 µL of FRAP reagent with 20 µL of extract in each well, the mixture was incubated at 37 °C for 10 minutes. Results were expressed in terms of µmol Fe (II)/g dry extract. Absorbances were measured at 593 nm and compared with a FeSO₄ calibration curve.

2.7. In Silico molecular docking analysis was performed on the molecule

Ligand Preparation: The NCBI PubChem provided the 3D structures of thymoquinone (CID: 3989), quercetin (CID: 5280343), rutin (CID: 5280805), and kaempferol (CID: 5280863). These structures were then energy-minimised using Avogadro v1.2's MMFF94 force field and converted to PDBQT format using AutoDockTools v1.5.7.

Human 5-lipoxygenase (PDB: 6N2W), human cyclooxygenase-2 with inhibitor (PDB: 5KIR), and human acetylcholinesterase (PDB: 4EY7) crystal structures were obtained from the RCSB Protein Data Bank. Heteroatoms and water molecules were removed, Gasteiger charges were added, and missing loops were repaired.

Docking Execution: A grid box measuring 50 × 50 × 50 Å at a spacing of 0.375 Å was used to dock around the co-crystallised inhibitor binding pockets. For computations, AutoDock Vina v1.2.0 was utilised (exhaustiveness = 32). The various conformations were arranged according to binding free energy (kcal/mol), and BIOVIA Discovery Studio Visualizer was used to construct the 2D and 3D interaction diagrams.

2.8. Statistical Analysis

There were three separate experiments ($n = 9$) with triplicate assays. To find the median inhibitory concentration (IC_{50}), GraphPad Prism version 9.0 was used to perform non-linear regression of log inhibitor vs normalised response (varying slope). The differences between extract concentrations and the positive standards were compared using one-way ANOVA and Tukey's multiple comparisons test ($p < 0.05$ was employed as a criterion of significance).

3. Results

3.1. Extraction Yield and Standardisation of Phytochemicals

An extraction yield of 12.84% (w/w) was obtained by cold extraction of 500 g of dry *Nigella sativa* leaf powder, which produced 64.2 g of dry crude extract. High quantities of phenolic and flavonoid scaffolds in the foliar matrix were verified by colourimetric tests (Table 1).

Table 1: *Nigella sativa* folium extract's physicochemical yield and quantitative phytochemical profile

Parameter	Value	Reference Standard / Method
Extraction Yield (% w/w)	12.84 ± 0.38	Cold maceration (70% EtOH)
Total Phenolics (TPC)	71.28 ± 2.14 mg GAE/g	Gallic acid ($R^2 = 0.9984$)
Total Flavonoids (TFC)	36.85 ± 1.62 mg QE/g	Quercetin ($R^2 = 0.9979$)
FRAP Reducing Value	482.5 ± 12.4 μ mol Fe(II)/g	FeSO ₄ standard curve ($R^2 = 0.9991$)

The values are the mean $\pm$ SEM of three separate experiments conducted in triplicate.

3.2. Anti-Inflammatory Activity in Vitro (Denaturation of Proteins)

In a concentration-dependent manner (25–400 μ g/mL), NSLE prevented heat-induced denaturation of both egg albumin and bovine serum albumin (Table 2). NSLE protected against BSA denaturation at $78.45 \pm 2.30\%$ at 400

μ g/mL, with an IC_{50} of 114.6 ± 4.2 μ g/mL (standard aspirin: 78.2 ± 2.8 μ g/mL). In the model for egg albumin, diclofenac sodium, which acted as the standard, produced % inhibition 64.5 ± 2.1 μ g/mL and yielded an IC_{50} of 128.4 ± 4.8 μ g/mL. Our extract produced comparable inhibition at higher doses (Table 2).

Table 2. Inhibition of thermal protein denaturation by NSLE compared with standard reference drugs.

Treatment	Conc. (μ g/mL)	BSA Inhibition (%)	Egg Albumin Inhibition (%)	IC_{50} BSA (μ g/mL)
NSLE	25	$18.4 \pm 1.2^{**}$	$14.2 \pm 1.1^{**}$	114.6 ± 4.2
	50	$32.6 \pm 1.8^{***}$	$26.8 \pm 1.5^{***}$	
	100	$47.2 \pm 2.1^{***}$	$42.1 \pm 1.9^{***}$	
	200	$64.8 \pm 2.4^{***}$	$59.4 \pm 2.2^{***}$	
	400	$78.5 \pm 2.3^{***}$	$72.6 \pm 2.6^{***}$	
Untreated Control	—	4.3 ± 2.2	3.1 ± 1.9	—
Aspirin	100	$61.2 \pm 2.1^{***}$	—	78.2 ± 2.8
Diclofenac Na	100	—	$69.4 \pm 2.4^{***}$	64.5 ± 2.1

The values are the mean $\pm$ SEM of three separate experiments conducted in triplicate. *** $p < 0.001$, ** $p < 0.01$

3.3. Multiple-Target Inhibition Profiles of Enzymes

All four examined enzymes (5-LOX, COX-2, AChE, and BChE) were inhibited in a concentration-dependent manner by Ns.Cr (Table 3). NSLE inhibited human recombinant COX-2 with an IC_{50} of 68.34 ± 2.45 $\mu\text{g/mL}$ (celecoxib:

0.85 ± 0.04 $\mu\text{g/mL}$) and 5-LOX with an IC_{50} of 42.18 ± 1.95 $\mu\text{g/mL}$ (zileuton standard: 12.45 ± 0.82 $\mu\text{g/mL}$). NSLE showed greater selectivity for AChE ($IC_{50} = 84.12 \pm 3.10$ $\mu\text{g/mL}$) over BChE ($IC_{50} = 108.50 \pm 4.20$ $\mu\text{g/mL}$) in cholinesterase tests (Table 3).

Table 3: NSLE and reference inhibitors' inhibitory potencies (IC_{50}) against specific enzymes.

Target Enzyme	NSLE IC_{50} ($\mu\text{g/mL}$)	Standard Inhibitor	Standard IC_{50} ($\mu\text{g/mL}$)
5-Lipoxygenase (5-LOX)	42.18 ± 1.95	Zileuton	12.45 ± 0.82
Cyclooxygenase-2 (COX-2)	68.34 ± 2.45	Celecoxib	0.85 ± 0.04
Acetylcholinesterase (AChE)	84.12 ± 3.10	Donepezil	5.21 ± 0.34
Butyrylcholinesterase (BChE)	108.50 ± 4.20	Donepezil	16.40 ± 0.75

Mean $\pm$ SEM (n = 9) is represented by the values. Every non-linear regression curve had an R^2 of at least 0.985.

3.4. Capacity for Radical Scavenging

In cell-free spectrophotometric tests, NSLE showed potent radical scavenging action. In the test, the DPPH radical was quenched by the extract with an IC_{50} of 38.64 ± 1.42 $\mu\text{g/mL}$ (ascorbic acid standard: 8.45 ± 0.35 $\mu\text{g/mL}$). The extract yielded an IC_{50} of 29.15 ± 1.12 $\mu\text{g/mL}$ in the ABTS assay (ascorbic acid: 5.80 ± 0.22 $\mu\text{g/mL}$).

3.5. Molecular Docking and Target Interactions in Silico

In the catalytic sites of 5-LOX, COX-2, and AChE, molecular docking assessed the binding positions and interaction energies of the primary foliar constituents (Table 4).

Table 4: Free energy for in silico binding (kcal/mol) and anticipated molecular interactions

Ligand	5-LOX Binding	Key Residues (5-LOX)	COX-2 Binding	Key Residues (COX-2)	AChE Binding	Key Residues (AChE)
Rutin	-9.1	His367, His372, Leu607	-9.4	Arg120, Tyr355, Ser530	-9.6	Trp286, Tyr124, Asp74
Quercetin	-8.4	His372, Val604, Leu368	-8.7	Val349, Tyr385, Phe518	-8.9	Trp86, Gly121, Ser203
Kaempferol	-8.0	Gln363, His367, Leu414	-8.2	Val116, Arg513, Phe518	-8.5	Trp286, Tyr337, Phe338

Thymoquinone	−6.4	Leu368, Ile406, Phe555	−6.7	Tyr355, Leu352, Val523	−6.9	Trp86, Tyr337, Phe338
Standard	−8.2 (Zileuton)	His372, Leu607, Ile406	−9.8 (Celecoxib)	Arg120, Ser530, Val523	−11.2 (Donepezil)	Trp86, Trp286, Phe338

Among all the targets, rutin and quercetin showed the strongest binding affinities. Rutin established coordination bonds with catalytic Fe^{2+} -coordinating residues (His367, His372) in 5-LOX (6N2W). Quercetin formed hydrogen bonds with Tyr385 and Ser530 in COX-2 (5KIR) after anchoring inside the cyclooxygenase channel. Quercetin directly attached to Ser203 of the catalytic triad in AChE (4EY7) and π -stacked with Trp86 at the choline-binding site.

4. DISCUSSION

One effective approach to sustainable drug development is the use of bioactive molecules made from leftover plant biomass. This study demonstrated the anti-inflammatory, anti-acne, anti-skin cancer, anti-allergic, and anti-asthmatic activities of *Nigella sativa* leaves (NSLE). Strong multi-target anti-inflammatory, antioxidant, and enzyme-inhibitory actions have been observed in *in vitro* systems. These elements are known to produce inflammatory diseases and arthritic symptoms, as well as denature proteins in biological tissue (Foo and Roofeh 2024). Heat destroys the tertiary structure of proteins, exposing cryptic antigenic determinants and triggering localised inflammatory reactions (Fouani *et al.* 2022). By binding to the hydrophobic surface cavities, the anti-inflammatory drugs stop heat denaturation and maintain serum albumin's natural fold (Wu *et al.* 2024).

Excessive production of reactive oxygen species (ROS) leads to lipid peroxidation and increased expression of inflammatory genes (Liu *et al.* 2023). NSLE's showed strong capacity to scavenge radicals. In addition to their enzymatic inhibition, the foliar extracts have a significant ferric-reduction potential (482.5 $\mu\text{mol Fe(II)}/\text{g}$) and an IC_{50} of 38.64 $\mu\text{g}/\text{mL}$ for DPPH and 29.15 $\mu\text{g}/\text{mL}$ for ABTS, indicating that they can also stop free-

radical chain reactions. Ultimately, it was determined that *Nigella sativa* leaves contain functional concentrations of polyphenols and flavonoids that may be used to neutralise free radicals, protect biological proteins, and inhibit key enzymes involved in inflammation (5-LOX, COX-2) and neuromodulatory processes (AChE). NSLE demonstrated concentration-dependent protective action against egg albumin ($\text{IC}_{50}=128.4 \mu\text{g}/\text{mL}$) and heat-induced denaturation of BSA ($\text{IC}_{50}=114.6 \mu\text{g}/\text{mL}$). The abundance of polyphenols (71.28 mg GAE/g) and flavonoids (36.85 mg QE/g) in the leaves is linked to this membrane- and protein-stabilising effect. By forming several hydrogen bonds with the polypeptide chains in non-covalent complexes, the polyphenolic metabolites prevent heat denaturation of polypeptide chains (Huynh *et al.* 2026). The production of pro-inflammatory prostaglandins and leukotrienes via the COX-2 and 5-LOX pathways is a key mechanism of chronic inflammation, oedema, and mucosal illness. Selective COX-2 inhibition may lead to a shift in arachidonic acid toward the 5-LOX pathway and increase the generation of bronchoconstrictive leukotrienes; nevertheless, dual 5-LOX/COX-2 inhibition is preferred (Agrawal 2025). For 5-LOX and COX-2, NSLE exhibited dual inhibitory action with IC_{50} values of 42.18 $\mu\text{g}/\text{mL}$ and 68.34 $\mu\text{g}/\text{mL}$, respectively. Our *in silico* docking results also supported this functional inhibition. The foliar flavonoid rutin directly bonded to the iron-coordinating residues His367 and His372 with a binding energy of $-9.1 \text{ kcal}/\text{mol}$ for 5-LOX. Similarly, quercetin was found to have binding capacity with Tyr385 and Ser530, which prevents arachidonic acid entry, while remaining in the COX-2 enzyme's catalytic channel ($-8.7 \text{ kcal}/\text{mol}$).

Studies have shown that dual inhibitors of butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) are applicable in cholinergic anti-inflammatory signalling and neurodegenerative illnesses (Cacabelos *et al.* 2024). In contrast to BChE ($IC_{50} = 108.50 \mu\text{g/mL}$), NSLE exhibited strong anticholinesterase action that was selective for AChE ($IC_{50} = 84.12 \mu\text{g/mL}$). Like the therapeutic inhibitor donepezil, quercetin and rutin both docked in the PAS and CAS of hAChE (binding energies: -8.9 and -9.6 kcal/mol, respectively), forming π - π stacking interactions with Trp86 and Trp286.

5. CONCLUSION

Significant in vitro multi-target anti-inflammatory, antioxidant, and enzyme-inhibitory effects are shown by *Nigella sativa* folium hydroethanolic extract (Ns.Cr). Without using experimental animal models, it quenches free radicals, inhibits 5-LOX, COX-2, and AChE, and protects against heat protein denaturation. Strong binding of foliar flavonoids (rutin and quercetin) to catalytic residues is responsible for these activities, according to molecular docking. The valuation of *N. sativa* vegetative leaves as a readily available source of bioactive phytopharmaceuticals is supported by these findings.

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