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LC–MS/MS-Based Phytochemical Profiling, In Vitro Antioxidant and Anti-Inflammatory Activities, and Molecular Docking Studies of Hydroethanolic Extract from *Foeniculum vulgare* Leaves of Western Algeria

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Abstract

Compounds derived from natural sources remain a cornerstone for the development of prophylactic and therapeutic strategies in human healthcare. In this study, we aimed to systematically explore the phytochemical composition and antioxidant and anti-inflammatory activities of *Foeniculum vulgare(F.vulgare)* leaf hydroalcoholic extract (HEE) using in vitro and in silico approaches. The extract, obtained by maceration at room temperature, exhibited considerable levels of total polyphenols (23.80 ± 0.19 mg GAE/g) and flavonoids (17.62 ± 0.99 mg QE/g). LC-MS/MS profiling revealed a diverse array of bioactive molecules, including chlorogenic acid, ferulic acid, salicylic acid, rutin, trans-cinnamic acid, gentisic acid, vanillin, quercetin, morin, and caffeic acid, with chlorogenic acid and trans-ferulic acid as the major constituents. HEE displayed pronounced, dose-dependent antioxidant activity, with IC_{50} values of 15.39 ± 0.96 µg/mL (DPPH) and 19.36 ± 0.16 µg/mL (H_2O_2). A marked anti-inflammatory effect was also observed, reaching 71.53% inhibition of bovine serum albumin (BSA) denaturation at 500 µg/mL, with an IC_{50} of 111.53 ± 0.26 µg/mL. Molecular docking studies demonstrated that phenolic compounds, particularly rutin and quercetin, possess strong binding affinities toward multiple inflammatory targets, suggesting a synergistic, multi-target mechanism rather than the action of a single dominant compound. To the best of our knowledge, this is the first study to investigate the bioactive potential of *Foeniculum vulgare* leaves in Algeria and North Africa, underscoring their value as a natural source of bioactive compounds with promising applications in managing oxidative stress and inflammation, and in the development of plant-based products for pharmaceutical, agricultural, and food sectors.

Keywords : *Foeniculum vulgare*; hydroalcoholic extract; phenolic compounds; LC-MS/MS; antioxidant; anti-inflammatory; molecular docking.

Introduction

Since ancient times, humans have relied on medicinal plants not only for nourishment but also for treating a wide range of diseases due to their numerous health benefits **(Al Qaisi et al. 2024)**. Today, more than 70% of the global population still depends on medicinal plants for primary healthcare (**Barrahi et al. 2020)**. Moreover, the World Health Organization has emphasized the integration of medicinal plants into formal healthcare systems **(Vitale et al. 2022)**. Recent studies have attributed the health and nutritional benefits of these plants to their diverse array of bioactive compounds including polyphenols, flavonoids, alkaloids, procyanidins, essential minerals, and vitamins which contribute to dietary balance and possess significant biological properties such as antioxidant, antimicrobial, anticancer, and anti-inflammatory activities **(Si-larbi et al. 2025)**. *Foeniculum vulgare,* commonly known as fennel, is a resilient plant native to the Mediterranean region and a member of the Apiaceae family. Recognized for its distinctive aroma and flavor, fennel is widely used in culinary practices especially in Portugal and has long been employed in traditional medicine for treating a variety of ailments **(Singh et al. 2020**). In modern contexts, fennel is also utilized in the production of cosmetic products (**Aider et al., 2024**). Nutritionally, *Foeniculum vulgare* is rich in plant-based proteins, fibers, and carbohydrates **(Aider et al. 2024**), and it harbors considerable levels of bioactive compounds including polyphenols and volatile constituents that are responsible for its antioxidant, anti-inflammatory, antispasmodic, antibacterial, and antithrombotic effects. These compounds also play a role in cancer and cardiovascular disease prevention, respiratory and digestive relief, and even support lactation in breastfeeding mothers (**Barakat et al. 2022**). Furthermore, fennel demonstrates hepatoprotective, chemopreventive, hypoglycemic, and estrogenic properties (**Malin et al. 2022**). It also exhibits antiseptic, carminative, analgesic, and diuretic effects, underscoring its value in managing digestive disorders (**Sharma et al. 2024**). The essential oil extracted from fennel exhibits additional biological activities, including antioxidant effects, inhibition of fungal and bacterial growth, and both anti-inflammatory and analgesic actions. Traditionally, it has been used to treat respiratory conditions and colic in children (**Birdane et al. 2007**). Meanwhile, fennel seeds are highly valued in the pharmaceutical industry for their rich content of essential minerals (e.g., calcium, phosphorus, iron), vitamins (thiamine, riboflavin, niacin), proteins, and carbohydrates. Oils extracted from the seeds contain compounds such as anethole, known for its anticancer, anti-inflammatory, and hepatoprotective effects (**Belabdelli et al. 2020**). Particularly noteworthy are the leaves of *Foeniculum vulgare*, which are abundant in phenolic compounds and vitamins, and are considered the richest part of the plant in terms of fatty acid content. They also contain potent antioxidants including glycosylated compounds, iridoids, and chlorophyll along with aromatic essential oils (**Aider et al. 2024**). Traditionally, fresh fennel leaves have been used in treating glandular tuberculosis and kidney disorders **(Noreen et al. 2024**). Despite

the well-documented nutritional and therapeutic value of fennel's various parts, research on its phytochemical composition, nutritional profile, and biological activities remains insufficient, particularly regarding the leaves. In Algeria and much of North Africa, fennel leaves are often discarded as waste. Therefore, the objective of this study is to evaluate the levels of phenolic and flavonoid compounds in the hydroethanolic extract (HEE) of *Foeniculum vulgare* leaves using spectrophotometric methods, to characterize its main bioactive constituents via LC–MS/MS analysis, and to assess its antioxidant and anti-inflammatory activities in vitro. In addition, molecular docking was performed to predict the binding interactions of the identified phenolic compounds with key pro-inflammatory targets, providing insights into their potential mechanisms of action.

Materials and Methods

Plant materials

Cultivated *F. vulgare* leaves were harvested in March 2024 from Sira fields, Mostaganem Province, Western Algeria (35°46'01.1"N 0°11'18.3"E), and botanically authenticated at Oran 1 University. Immediately after collection, the leaves were carefully cleaned, sliced, and air-dried at room temperature (25 °C) for several days until complete dehydration. The dried material was then ground into a fine powder, achieving an average particle size of approximately 500 µm, and stored in airtight containers at room temperature. Following slight modifications to Chaoua et al., 2019's protocol, 20 g of the leaf powder was macerated in 200 mL of 75% ethanol under magnetic stirring for 5 hours. The mixture was filtered twice, and the combined filtrates were concentrated at ≤45 °C using rotary evaporation. The final HEE was stored at 4 °C for subsequent analysis.

Yield of HEE

The yield of the HEE was calculated as the mass of the extracted (g) relative to 100 g of dry plant material in accordance with the following equation (**Mir et al. 2025**):

$$\text{Yield (\%)} = \text{Dry plant material mass (g)}/\text{Amount of extracted HEE (g)} \times 100$$

Quantification of different phenolic classes

Total Phenol Content

Phenol content was estimated using the Folin-Ciocalteu reagent method, following the procedure described by (**Benahmed et al. 2021**) with minor modifications. Gallic acid was used as the standard. Total phenol content (TPC) was expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dry weight of plant material.

Total Flavonoid Content:

Flavonoid content in fennel extracts was determined using the aluminum chloride ($AlCl_3$) method according to t the procedure reported by(**Benahmed et al. 2021**). Quercetin was used to construct the calibration curve, and the results were expressed as milligrams of quercetin equivalents (mg QE) per gram of dry weight of plant material.

LC-MS/MS analysis of Foeniculum vulgare leaves

Sample preparation and LC-MS/MS analysis were conducted following the procedure described by Erlenner et al. (2023) and other established protocols in the literature

Sample Preparation:

Approximately 50 mg of each extract was transferred into a 2 mL Eppendorf tube. Samples were dissolved by adding 1 mL of a mixed solvent (acetonitrile:methanol:water, 1:1:1, v/v/v) and vortexed until complete dissolution. Any insoluble residues were further treated using an ultrasonic bath to ensure full solubilization. Extraction was then performed by adding 0.8 mL of hexane to the solution, followed by centrifugation at 7000 rpm for 5 minutes.

The resulting supernatant was carefully collected and diluted at a ratio of 1:4 (1 part supernatant + 3 parts solvent mixture) to adjust the sample concentration for LC-MS/MS analysis. Prior to injection, all samples were filtered through a 0.25 µm membrane filter to remove any particulate matter.

LC-MS/MS Analysis:

Analysis was performed on an Agilent 6460 Triple Quad LC-MS system equipped with a Poroshell 120 EC-C18 column (50 mm × 4.6 mm I.D., 2.7 µm particle size). The injection volume was 4.0 µL, with a flow rate of 0.4 mL/min, and the column temperature was maintained at 30 °C. The total analysis time was 40 minutes.

Mobile Phases and Gradient:

The mobile phases consisted of Phase A (water containing 0.1% formic acid and 5 mM ammonium formate) and Phase B (acetonitrile containing 0.1% formic acid). A gradient elution was applied, starting at 75% A / 25% B, gradually changing to 25% A / 75% B at 15 min, reaching 0% A / 100% B at 16–20 min, and returning to 85% A / 15% B at 22 min until the end of the run at 40 min.

Antioxidant activity

DPPH free radical-scavenging activity

The DPPH radical decolorization method outlined in reference (**Benahmed et al. 2021**) was adopted to assess antioxidant activity, with minor modifications. Briefly, in 50 mL test tubes, each diluted concentration of the extract was mixed with 5 mL of a 0.004% DPPH solution prepared in methanol. The mixture were incubated at room temperature for 30 minutes in the dark. The absorbance of both the extract samples and the control was measured at 517 nm using a spectrophotometer. The percentage of free radical inhibition was calculated using the following equation:

$$I\%_{DPPH} = \left[\frac{Abs_0 - Abs_1}{Abs_0}\right] \times 100$$

Abs_0 = the absorbance of the blank

Abs_1 = the absorbance of the extract.

The IC_{50} value, defined as the concentration required to achieve 50% inhibition, was calculated from the dose–response curve obtained by plotting the percentage of inhibition (or absorbance) against the logarithm of extract concentrations. The data were analyzed using nonlinear regression in Microsoft Excel. All measurements were performed in triplicate, and the results are expressed as mean ± SD.

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Hydrogen Peroxide Scavenging Assay

Antioxidant activity was evaluated by measuring the extract's ability to scavenge hydrogen peroxide (H_2O_2), based on the method reported in (**Surana et al. 2022**), with some necessary modifications. A 10 mM H_2O_2 solution was prepared in a phosphate buffer (pH 7.4). The reaction mixture consisted of 10 mM of the prepared H_2O_2 solution and varying concentrations of the extract. Ascorbic acid was used as a reference standard. Absorbance was measured at 240 nm. The percentage of H_2O_2 scavenging was calculated using the following equation:

$$I\%_{\mathrm{H2O2}} = \left[\frac{Abs_0 - Abs_1}{Abs_0}\right] \times 100$$

Abs_0 = the absorbance of the blank

Abs_1 = the absorbance of the extract

The IC_{50} value, defined as the concentration required to achieve 50% inhibition, was calculated from the dose–response curve obtained by plotting the percentage of inhibition (or absorbance) against the logarithm of extract concentrations. The data were analyzed using nonlinear regression in Microsoft Excel. All measurements were performed in triplicate, and the results are expressed as mean ± SD.

Anti-inflammatory activity

The anti-inflammatory activity of the extracts was evaluated using the heat-induced albumin denaturation method, following the protocol reported in (**Bailey-Shaw et al. 2017**), with necessary modifications. Briefly, 1 mL of each diluted concentration of the extract was mixed with 1 mL of 1% bovine serum albumin (BSA) in separate test tubes. Each assay was performed in three biological replicates. The mixture were first incubated at 37 °C for 20 minutes, followed by a second incubation at 70 °C for 5 minutes. The samples were then cooled to room temperature, and absorbance was measured using a spectrophotometer. Phosphate-buffered saline (PBS) was used as the control, and diclofenac was used as the reference standard. The percentage inhibition of albumin denaturation by the extract was calculated using the following equation:

$$I\% = \left[\frac{Abs_0 - Abs_1}{Abs_0}\right] \times 100$$

Abs_0 = the absorbance of the blank Abs_1 = the absorbance of the extract.

IC_{50} values were determined through linear interpolation of the inhibition–concentration curves, with all assays conducted in triplicate to ensure precision and reproducibility.

In Silico study

Ligand Preparation

The 3D structures of the 10 Compound library of flavonoids identified from the plant extract through LCMSMS analysis and the reference drug diclofenac were downloaded in structure data file (sdf) format from pubchem (https://pubchem.ncbi.nlm.nih.gov/) (**Kim et al. 2019**) . The LigPrep interface of the 2017-V2 version of Schrodinger was used to prepare the compounds at pH 7 (±2) and the OPLS3 force field using Epik. Desalt and

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generate tautomers were also selected on the LigPrep interface, and the stereoisomer computation was left to retain specific chiralities (vary other chiral centers) and to generate at most 1 per ligand (**Omoboyowa et al. 2025** ; **Balogun et al. 2021**).

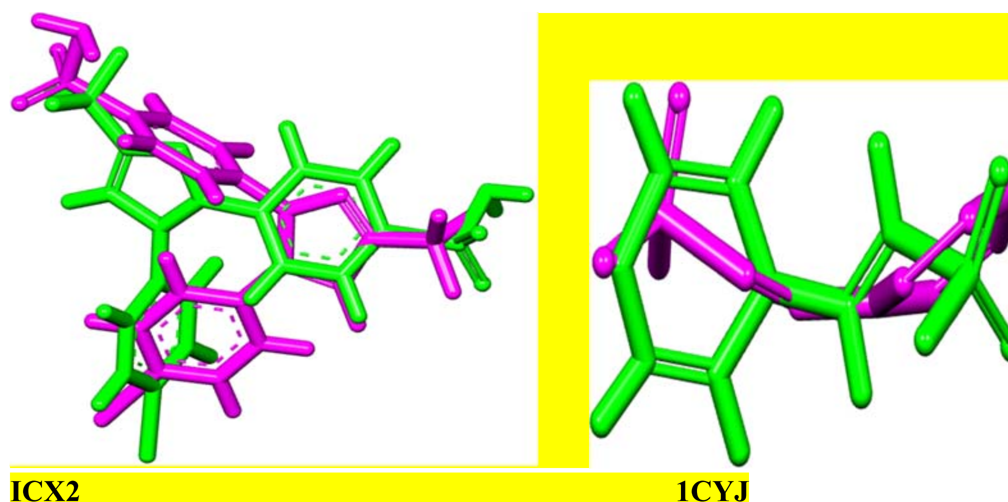
Protein Preparation

The crystallographic structures of The target proteins, COX-2, IL1β, NFKβ, PLA2 and TNFα with PDB ID: 1CX2, ILOB, 8TQD, 1CJY AND 5MU8 respectively for Homo sapiens were retrieved from protein database repository (www.rcsb.org) (**Berman et al. 2000**), the monomeric (chain A) protein was prepared using the protein preparation wizard of Schrodinger suit (2017 v2). The receptor grid generation tool of the software was used to generate a grid coordinate (1CX2: x = 24.42, y = 21.81, and z = 16.26; ILOB: x = 7.54, y = 6.03, and z = 7.31; 8TQD: x = 19.98, y = 10.26, and z = -7.91; 1CJY: x = 36.46, y = -1.31, and z = 82.70 and 5MU8: x = 31.19, y = 11.18, and z = 17.90) at the binding domain generated by sitemap of the maestro Schrodinger (**Rondeau et al. 2015**).

Molecular Docking Analysis

The prepared compounds were subjected to virtual screening within the binding sites of the selected targets, using grid coordinates generated for each receptor. Binding affinities were evaluated through an extra-precision (XP) docking protocol to carry out site-directed docking of the compounds. The resulting protein–ligand complexes were then analyzed, and their two-dimensional interaction profiles were visualized using Discovery Studio 2020 (**Omoboyowa et al. 2023**).

The co-crystallized ligand was re-docked into the catalytic site of the proteins to confirm the accuracy of the screening and docking scores (**Omoboyowa et al. 2021**). The Root means square deviation (RMSD) of the re-docked co-crystallized ligands from its original geometry were 1.897 Å, 2.075 Å, 2.008 and 5.213 Å for 1CX2, 1CYJ, 5MU8 and 8TQD, respectively. Fig. 1 shows the binding mode of the re-docked (pink) and the co-crystallized ligands (green) within the binding pocket of the target for the validation of the docking procedure.



ICX2 **1CYJ**

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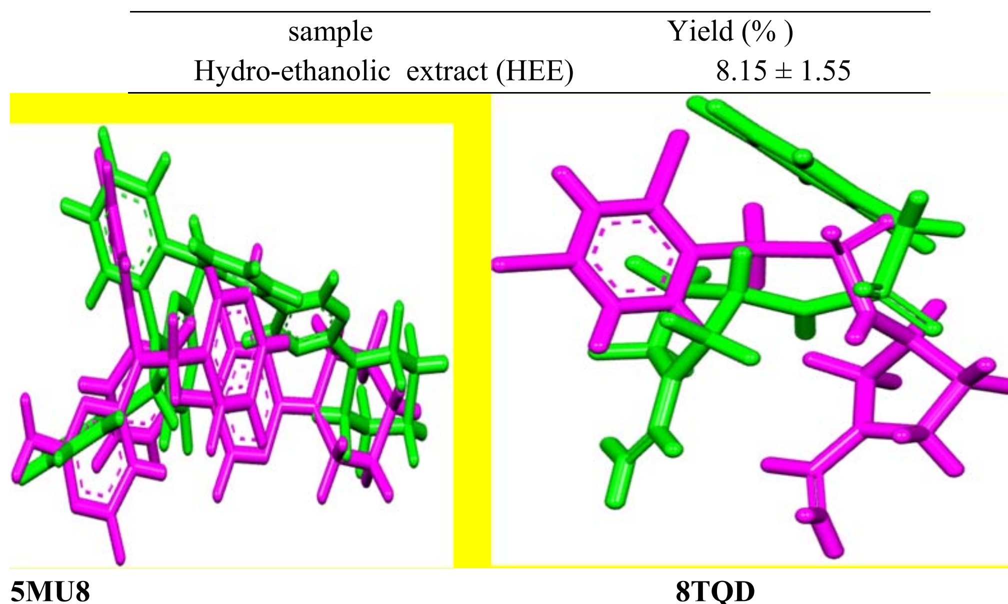
sample	Yield (%)
Hydro-ethanolic extract (HEE)	8.15 ± 1.55



5MU8 8TQD

Fig. 1: The binding mode of the re-docked (pink) and the co-crystallized ligands (green) within the binding site of the targets.

Statistical Analysis

Statistical Analysis

All data are expressed as mean ± standard deviation (SD) from three independent replicates (n = 3). Statistical analyses were performed using GraphPad Prism version 8.00. Student's t-test was used for pairwise comparisons when appropriate, while one-way ANOVA followed by Tukey's post-hoc test was applied to evaluate differences among multiple groups. Two-way ANOVA was performed to assess the effects of extract concentration and treatment type (HEE vs. DCF) on heat-induced BSA denaturation. When a significant interaction or main effects were detected, Bonferroni's post-hoc test was applied for multiple comparisons. Statistical significance was set at $p < 0.05$. Pearson's correlation coefficients (two-tailed) were calculated to assess relationships between phytochemical contents and biological activities.

Results and discussion

Extraction Yield of HEE

In this study, the extraction rate of *F. vulgare* leaves was evaluated using hydro-maceration method with hydro-ethanol solvent. HEE showed a yield of 8.15 ± 1.55 %, as shown in Table 1

Table1. Yield and density of hydro-ethanol extract (HEE) obtained by hydro-maceration method with hydro-ethanol solvent of *F. vulgare* leaves.

Phytochemical Composition Assessment

The total phenolic content (TPC) measured in the hydroethanolic extract (HEE) of *F. vulgare* leaves in this study indicate that the HEE of *F. vulgare* leaves contains 23.80 ± 0.19 mg GAE/g extract of total polyphenols and 17.62 ± 0.99 mg QE/g extract of total flavonoids, was lower than the values reported by [Singh, 2013] for ethanolic (44.11 ± 0.50 mg GAE/g dry weight) and hexane extracts (37.11 ± 1.73 mg GAE/g DW) of the same plant material. However, the TPC observed herein was comparable to that reported for the aqueous extract (25.75 ± 1.73 mg GAE/g DW), and exceeded the concentration obtained using methanol (17.81 ± 0.49 mg GAE/g DW). Furthermore, the TPC value in the current HEE of *F. vulgare* leaves was lower than those reported for *F. vulgare* seeds in studies by (**Moumen et al. 2025**), which recorded concentrations of 551.45 ± 0.010, 38.2 ± 2.3, and 27.09 mg GAE/g DW, respectively. Nonetheless, the result obtained surpassed the value reported by (**Kalleli et al. 2019**) for Tunisian *F. vulgare* seeds (15.69 ± 3.39 mg GAE/g DW). In terms of flavonoid content, the concentration measured in the HEE of *F. vulgare* leaves was slightly lower than the 19.71 mg QE/g reported by (**Moumen et al. 2025**) for a seed decoction. Nevertheless, it surpassed flavonoid levels in aqueous (14.78 mg/g) and ethanolic extracts (5.08 mg/g), as well as those in Tunisian seeds reported by (**Khammassi et al. 2022**) and (**Kalleli et al. 2019**) (16.42 ± 0.20 mg QE/g and 12.65 ± 0.88 mg QE/g, respectively). These findings imply that *F. vulgare* leaves may serve as a superior source of flavonoids compared to seeds, highlighting their relevance as a natural reservoir of bioactive compounds. Discrepancies in flavonoid and polyphenol content across studies are likely due to several variables affecting phytochemical extraction, including extraction technique, solvent characteristics, duration, temperature, solvent-to-material ratio, and intrinsic plant matrix composition (**Putra et al. 2024**). Additional influencing factors include climatic conditions, genetic background, plant maturity, and harvest timing (**Bentahar et al. 2025**). Collectively, these results confirm that *Foeniculum vulgare* leaves represent a valuable source of polyphenols and flavonoids, with notable variability compared to other plant parts and extraction methods. Although the hydroethanolic extraction yielded promising bioactive compound levels, the results underline the potential for further optimization. Future research should focus on applying advanced extraction technologies to better preserve and enrich the phytochemical profile of *F. vulgare* leaf extracts.

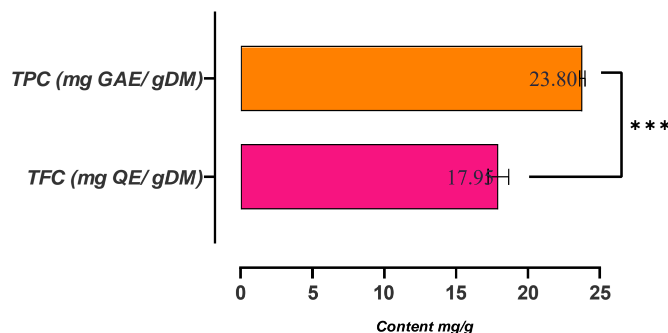


Figure 1. Total bioactive compound content in the hydroethanolic extract (HEE) of *F. vulgare* Mill. Error bars indicate standard deviation. *** denotes a statistically significant difference at $p < 0.001$.

LC-MS/MS Analysis

LC-MS/MS profiling (Table 2) of the HEE revealed a complex array of phenolic acids and flavonoids, consistent with the phytochemical richness characteristic of Apiaceae species. Chlorogenic acid emerged as the predominant constituent (95.73 µg/g extract), aligning with reports for Moroccan fennel seeds (**Moumen et al. 2025**) and Tunisian *Pimpinella anisum* (**Rebey et al. 2019**). This caffeoylquinic derivative demonstrates significant bioactivities through Nrf2-mediated oxidative stress modulation and NF-κB pathway regulation (**Liang and Kitts, 2015**), with recent studies confirming hepatoprotective efficacy against NAFLD in rodent models (**Alamri et al. 2023**). Key phenolic acids included trans-ferulic acid (18.32 µg/g extract), recognized for multi-target effects including free radical scavenging and pro-inflammatory mediator inhibition (**Bhuia et al. 2024**); salicylic acid (15.97 µg/g extract), which regulates inflammation through COX-2 suppression and iron chelation (**Hussein et al. 2020**); and gentisic acid (12.09 µg/g extract), demonstrating nephroprotective activity via renin-angiotensin system modulation (**Altinoz and Ozpinar 2019**). Trans-cinnamic acid (12.34 µg/g extract) is an organic compound displaying a wide range of biological activities and high antioxidant effectiveness (**Sova 2012**). The flavonoid fraction featured rutin (15.53 µg/g extract), a glycosylated flavonol with documented cardioprotective and neuroprotective activities through PPAR-γ activation (**Choi et al., 2021**); quercetin (2.54 µg/g extract), enhancing wound repair via ERK/NF-κB pathway regulation (**Azeem et al. 2023**); morin (2.01 µg/g extract), showing enhanced neuroprotection through AChE inhibition in cerebral ischemia models (**Alla et al. 2024**); and vanillin (3.31 µg/g extract), suppressing IBD inflammation via MAPK/NF-κB signaling inhibition (**Kafali et al. 2023**). Minor constituents like caffeic acid (1.01µg/g extract), contributed synergistically to vasodilation and antioxidant effects (**Silva and Lopes; 2020**).

The differences observed in the chemical composition of the leaves compared to previous studies on fennel seeds can be attributed to organ-specific metabolic specialization, whereby leaves may synthesize or accumulate certain compounds at higher or lower levels than seeds. Additionally, environmental and ecological variations, such as climatic conditions and soil characteristics, as well as responses to stress-induced metabolites, may contribute to these discrepancies. In summary, these differences reflect the natural variability among plant organs and across diverse environmental contexts.

Table 2: LC-MS/MS analysis data of detected metabolites from the hydro-ethanolic extract (HEE) **of *Foeniculum vulgare Mill*. Leaves.**

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Nº	Compound name	Retention time	HEE (µg/g)	Prec Ion	Prod Ion	Dwell	Frag (V)	CE (V)	Polarity
1	Chlorogenic acid	2.54	95.73	353	191	9	110	12	Negative
2	Trans-Ferulic acid	5.85	18.32	195.1	176.9	9	75	10	Positive
3	Salicylic acid	7.25	15.97	137	93.1	38	85	18	Negative
4	Rutin	2.95	15.53	609.1	300.2	9	185	40	Negative
5	Trans-cinnamic acid	10.81	12.34	149.1	131.1	18	90	6	Positive
6	Gentisic acid	3.37	12.09	152.8	108.9	9	100	13	Negative
7	Vanillin	4.97	3.31	152.9	124.9	9	65	13	Positive
8	Quercetin	11.63	2.54	300.8	179	18	130	20	Negative
9	Morin	11.64	2.01	302.9	136.8	18	145	32	Positive
10	Caffeic acid	3.22	1.01	178.9	135	9	80	16	Negative

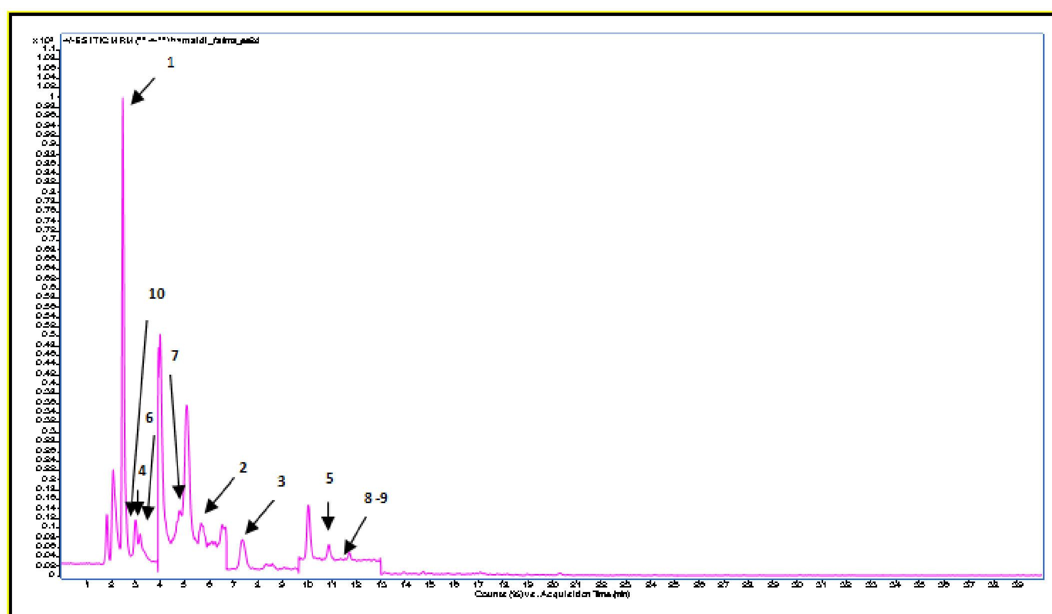

Figure2. LC-MS/MS Chromatogram of Phenolic Compounds in Hydro-Ethanolic Extract of *Foeniculum vul*gare Mill. Leaves 1; Chlorogenic acid, 2 ; Trans-Ferulic acid, 3 ; Salicylic acid, 4 ; Rutin, 5 ; Trans-cinnamic acid,6; Gentisic acid,7; Vanillin,8 ; Quercetin,9; Morin, and 10; Caffeic acid

Antioxidant Activity

Growing safety concerns regarding synthetic antioxidants like BHA and BHT have intensified interest in natural alternatives. Phenolic-rich plants represent valuable sources of such antioxidants, effectively neutralizing free radicals and mitigating reactive oxygen species (ROS), thereby reducing oxidative stress for applications in health, nutrition, and cosmetics (**Tlili et al. 2019**). All antioxidant assays revealed concentration-dependent enhancement of free radical scavenging capacity in the extract. In DPPH assays, an IC_{50} of 15.39 ± 0.96 µg/mL was observed, with 65% inhibition at 200 µg/mL. For H_2O_2 scavenging, the IC_{50} was 19.36 ± 0.16 µg/mL, reaching 80% inhibition at identical concentrations. The HEE of *F. vulgare* leaves exhibited potent antioxidant activity comparable to ascorbic acid and BHA standards (Figures 3-4). Spectrophotometric methods (DPPH and H_2O_2 assays) were employed to evaluate antioxidant capacity, leveraging their sensitivity and reliability. The DPPH method assesses hydrogen/electron donation to stabilize DPPH• radicals, while the H_2O_2 assay quantifies neutralization of non-radical ROS involved in cellular damage. This dual approach provides comprehensive insight into antioxidant mechanisms. The HEE's robust activity aligns with its high phenolic/flavonoid content. Although reference standards showed marginally greater efficacy, the correlation supports polyphenols as primary contributors to antioxidant effects. Comparatively, our results demonstrate superior activity: Tunisian *F. vulgare* seed extracts exhibited higher IC_{50} values (27.17 ± 2.82 and 23.66 mg/mL) **(Kalleli et al. 2019; Khammassi et al. 2022**). Notably, Egyptian *F. vulgare* essential oil displayed low efficacy (IC_{50} = 15.33 mg/mL) **(Shahat et al. 2011).**

In H_2O_2 scavenging, the extract outperformed literature values. Previous studies reported $IC_{50} \approx 100$ µg/mL for *F. vulgare* leaf essential oil (**Chatterjee et al. 2012**) and 63.5 ± 4.46% inhibition at 5 mg/mL in edible parts (**Romojaro et al. 2013**) significantly lower than our findings. This antioxidant potency is attributable to abundant polyphenolic/flavonoid compounds (**Tița, et al. 2025**), corroborated by strong positive Pearson correlations between TPC/TFC and activity metrics (Table 3). LC-MS confirmed key antioxidant phenolics (e.g., chlorogenic acid), and potential synergistic interactions among constituents may further enhance efficacy (**Belahcene et al. 2023**).

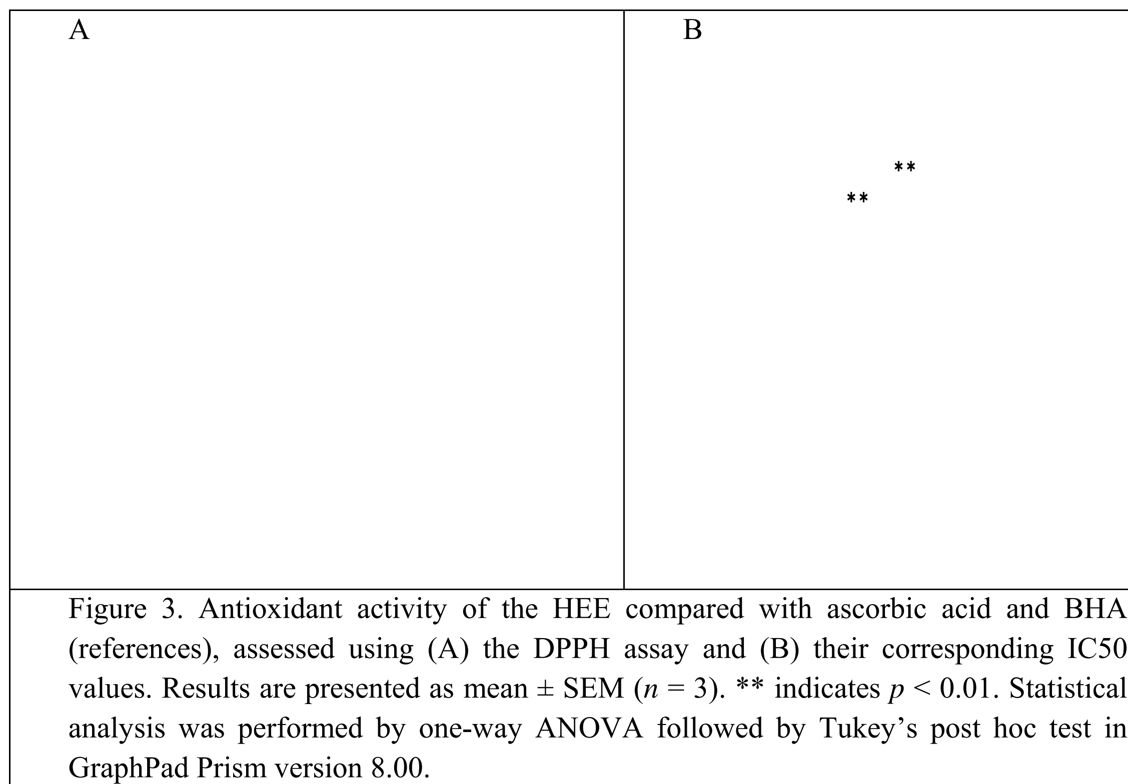

Figure 3. Antioxidant activity of the HEE compared with ascorbic acid and BHA (references), assessed using (A) the DPPH assay and (B) their corresponding IC50 values. Results are presented as mean ± SEM (n = 3). ** indicates $p < 0.01$. Statistical analysis was performed by one-way ANOVA followed by Tukey's post hoc test in GraphPad Prism version 8.00.

Anti-inflammatory Activity

Plant-derived compounds are increasingly explored as safer and more accessible alternatives to synthetic anti-inflammatory drugs, which are often associated with adverse effects. Within this framework, the anti-inflammatory potential of the hydroethanolic extract (HEE) from *F. vulgare* leaves was assessed through its inhibition of heat-induced bovine serum albumin (BSA) denaturation, a well-established and reliable *in vitro* model. Protein denaturation is a pivotal mechanism in the pathogenesis of inflammation, and its inhibition represents a promising therapeutic strategy.

The HEE exhibited a significant concentration-dependent inhibition of BSA denaturation: 40.12% at 62.5 µg/mL, 58.63% at 125 µg/mL, and 71.53% at 500 µg/mL (Figure 5). The calculated IC_{50} value was 111.53 ± 0.26 µg/mL. In comparison, the reference drug diclofenac showed near-complete inhibition (99%) at 500 µg/mL with a significantly lower IC_{50} of 16.82 ± 0.14 µg/mL, confirming its superior potency. This anti-denaturation activity strongly aligns with the extract's high polyphenol and flavonoid content, as quantified in Table 3, and reflects the well-documented nexus between oxidative stress and inflammation (**Al Qaisi et al. 2024**). The observed effect is likely attributable to the identified phenolic and flavonoid constituents, as revealed by LC-MS/MS analysis. The literature indicates that such compounds can interact with amino acid-rich domains on proteins, stabilizing their native conformation and preventing denaturation, thereby preserving biological

function (**Barrahi et al. 2020**). While less potent than diclofenac, the HEE outperformed *F. vulgare* seed extracts reported in the literature, which showed ≤38% inhibition at a higher concentration of 250 µg/mL (**Ekor. 2014**). Its efficacy was slightly lower than that of the essential oil from leaves of the same plant (IC_{50} = 95.9 ± 2.4 µg/mL (**Vitale et al. 2022**), but was comparable to *F. vulgare* fruit extracts, recently reported to show IC_{50} values of 105–120 µg/mL in similar assays (**Goudjil et al. 2024**).

In conclusion, the HEE of *Foeniculum vulgare* leaves demonstrates noteworthy *in vitro* anti-inflammatory activity, mediated likely by its polyphenolic constituents. This finding supports the traditional use of fennel and warrants further investigation to isolate the active principles and evaluate their efficacy in *in vivo* models. LC-MS/MS analysis suggests the bioactivity stems from identified phenolic and flavonoid compounds. Contemporary studies indicate these phytoconstituents may stabilize protein conformations by binding to amino acid-rich domains, thereby preventing denaturation (**Goudjil et al. 2024**) These findings underscore the relevance of plant extracts as potentially safer alternatives to synthetic anti-inflammatories in managing chronic inflammatory diseases (**Singh et al.**

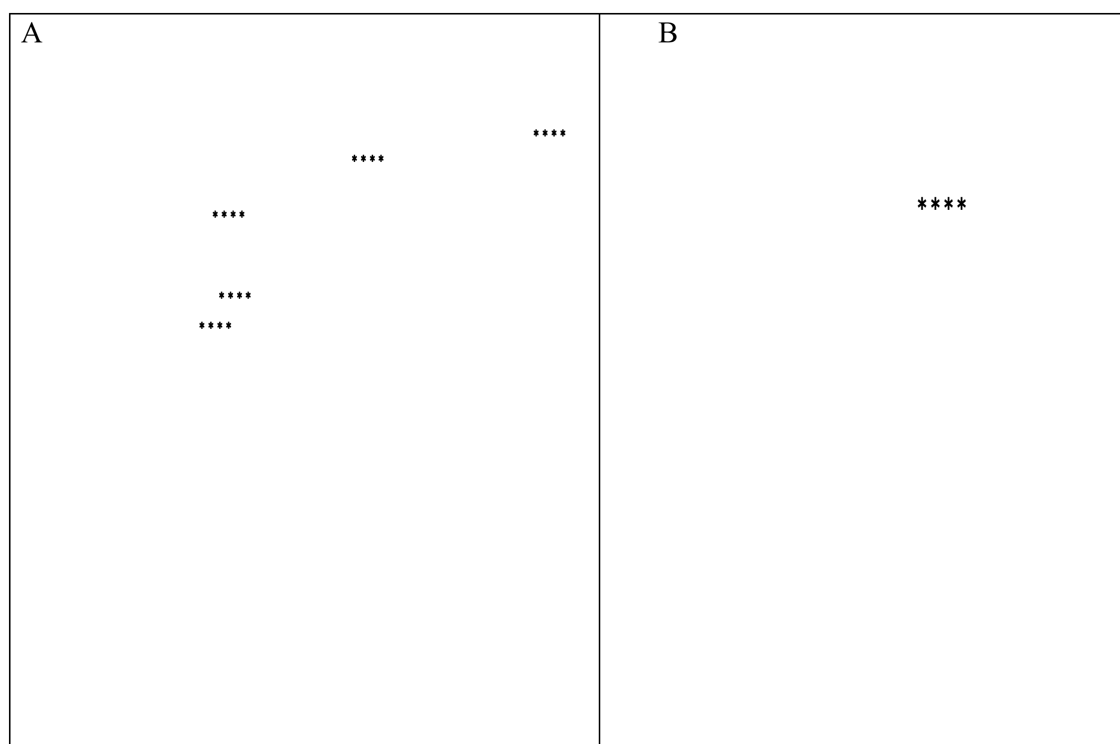

Figure 4. Antioxidant activity of the HEE compared with ascorbic acid (reference) determined by (A) the H_2O_2 assay and (B) their IC50 values. Data are expressed as mean ± SD of three independent replicates (n = 3). *ns* = no significant difference *p*=0,1843 ; **** indicates significance at $p < 0.0001$. Error bars represent standard deviation. Statistical analysis was performed using Student's *t*-test in GraphPad Prism version 8.00.

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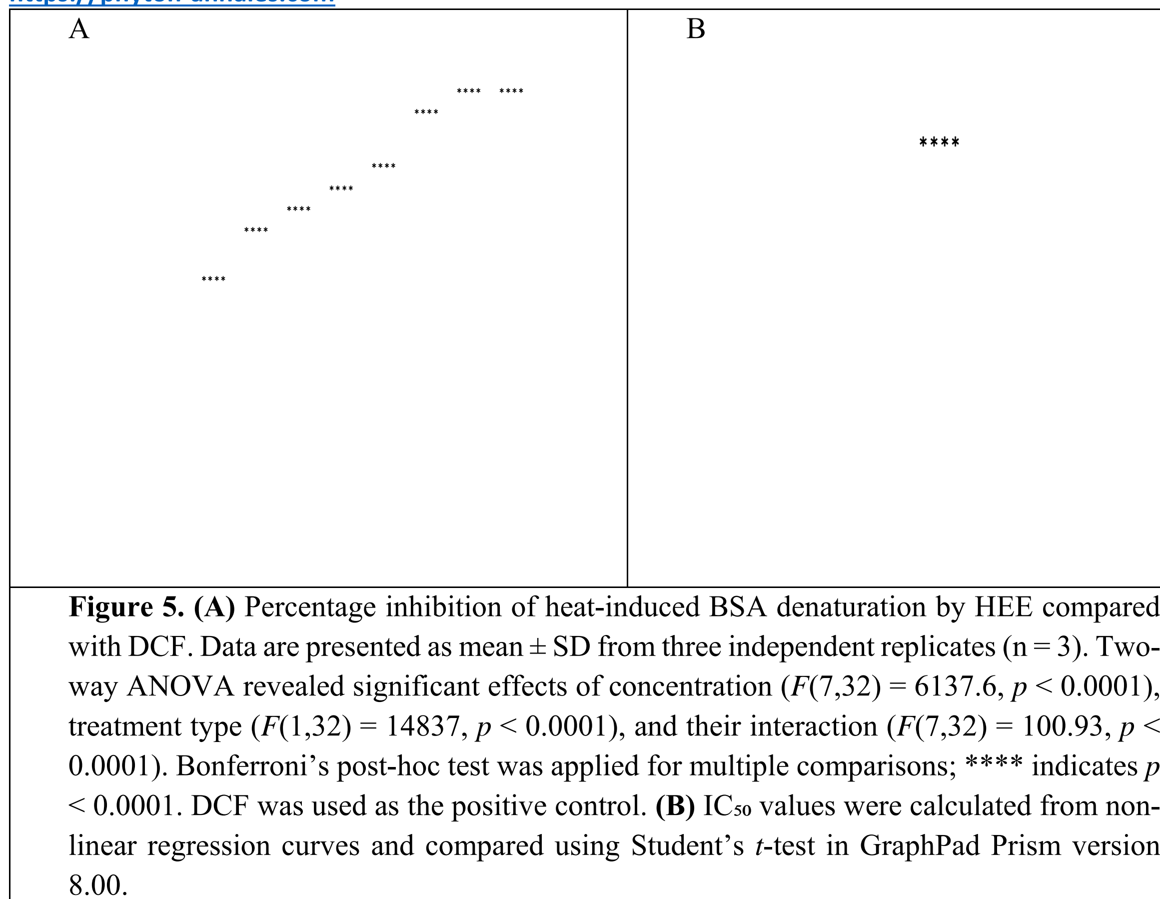

Figure 5. (A) Percentage inhibition of heat-induced BSA denaturation by HEE compared with DCF. Data are presented as mean ± SD from three independent replicates (n = 3). Two-way ANOVA revealed significant effects of concentration ($F(7,32) = 6137.6$, $p < 0.0001$), treatment type ($F(1,32) = 14837$, $p < 0.0001$), and their interaction ($F(7,32) = 100.93$, $p < 0.0001$). Bonferroni's post-hoc test was applied for multiple comparisons; **** indicates $p < 0.0001$. DCF was used as the positive control. **(B)** IC_{50} values were calculated from non-linear regression curves and compared using Student's *t*-test in GraphPad Prism version 8.00.

Table 3. Pearson's correlation values of TPC, and TFC contents and antioxidant (DPPH and H_2O_2) and anti-inflammatory activity of *Foeniculum vulgare Mill* HEE

IC_{50} TEST	Total bioactive component		Antioxidant activity		Anti inflamatory activity
	TPC	TFC	DPPH	H_2O_2	
TPC	1				
TFC	0.1767^{ns}	1			
DPPH	0.8349***	0.0002^{ns}	1		
H_2O_2	0.6561***	0.7614**	0,2519^{ns}	1	
Anti –inflamatory activity	0.4435**	0.9155***	0,0932ns	0,9543***	1

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In Silico study

Building on the encouraging in vitro anti-inflammatory results, a selection of phenolic compounds identified in the hydroalcoholic extract (HEE) of *F. vulgare* was subjected to molecular docking studies. The objective was to examine their potential interactions with five central proteins involved in inflammatory signaling: COX-2 (PDB ID: 1CX2), IL-1β (1LOB), NF-κB (8TQD), PLA2 (1CJY), and TNF-α (5MU8). These targets were chosen due to their well-established roles in driving inflammatory processes. For example, COX-2 is a key enzyme in prostaglandin synthesis, while IL-1β and TNF-α, are potent cytokines that orchestrate both acute and chronic inflammatory responses. NF-κB, a pivotal transcription factor, regulates the expression of numerous genes implicated in inflammation. PLA2, meanwhile, contributes to eicosanoid production by initiating the release of arachidonic acid via phospholipid hydrolysis. This computational approach aimed to characterize the binding behavior of these natural compounds and provide molecular insights that could help explain their experimentally observed anti-inflammatory effects. The non-steroidal anti-inflammatory drug diclofenac was used as a reference compound. Docking simulations were carried out using the Schrödinger suite (XP precision mode), with binding affinities expressed in kcal/mol more negative values indicating stronger interactions. An overview of docking scores is summarized in Table 4.

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Table 4 Binding Affinities of Phytochemicals and Reference Drugs to Pro-Inflammatory Targets : Molecular Docking Insights

Compounds	Pubchem ID	Docking Score (kcal/mol)				
		COX-2 (1CX2)	IL1β (1LOB)	NFKβ (8TQD)	PLA2 (1CJY)	TNFα (5MU8)
Chlorogenic acid	1794427	-5.958	-5.921	-4.308	-6.033	-5.832
Ferulic acid	445858	-6.774	-3.390	-1.917	-3.917	-3.560
Salicylic acid	338	-5.468	-3.683	-2.954	-3.882	-3.313
Rutin	5280805	0.000	-7.244	-5.231	-10.351	-10.278
Trans-cinnamic acid	444539	-5.258	-1.545	-1.750	-2.607	-2.256
Gentisic acid	3469	-5.630	-3.826	-3.760	-4.420	-3.699
Vanillin	1183	-6.555	-4.865	-2.296	-3.768	-3.710
Quercetin	5280343	-8.793	-5.040	-3.479	-6.018	-5.094
Morin	5281670	-7.663	-3.324	-3.338	-7.267	-4.647
Caffeic acid	689043	-7.275	-4.138	-1.588	-4.814	-4.532
Diclofenac	3033	-7.977	-2.102	-2.808	-2.426	-3.242

Out of the ten phenolic compounds analyzed, five (rutin, quercetin, chlorogenic acid, morin, and ferulic acid) showed significant binding affinities (≤ −5 kcal/mol) with at least one target, a value generally considered indicative of biologically relevant interactions. Rutin stood out, demonstrating favorable binding across all five targets, highlighting its broad potential as a multi-target anti-inflammatory agent.

Target-Based Binding Affinity Insights

Among all tested compounds, quercetin showed the strongest interaction with COX-2 (−8.793 kcal/mol), surpassing that of diclofenac (−7.977 kcal/mol), suggesting its strong potential to inhibit prostaglandin production. Additional compounds such as morin, caffeic acid, ferulic acid, chlorogenic acid, and salicylic acid also displayed meaningful interactions with COX-2, indicating possible additive or synergistic anti-inflammatory effects. For IL-1β, rutin emerged as the most potent binder (−7.244 kcal/mol), followed by chlorogenic acid and quercetin, indicating its capacity to modulate cytokine activity. Notably, rutin was the only compound to exhibit a significant binding score for NF-κB (−5.231 kcal/mol), suggesting it may disrupt key transcriptional regulatory mechanisms, even if direct inhibition of transcription factors is typically uncommon. In the case of PLA2, four compounds rutin, morin, chlorogenic acid, and quercetin showed strong affinities, with rutin again ranking highest (−10.351 kcal/mol), underscoring its potential to inhibit the release of phospholipid-derived inflammatory mediators. Rutin also displayed the strongest interaction with TNF-α (−10.278 kcal/mol), further emphasizing its potential role as a broad-spectrum modulator of inflammation.

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Molecular Interaction Profiles

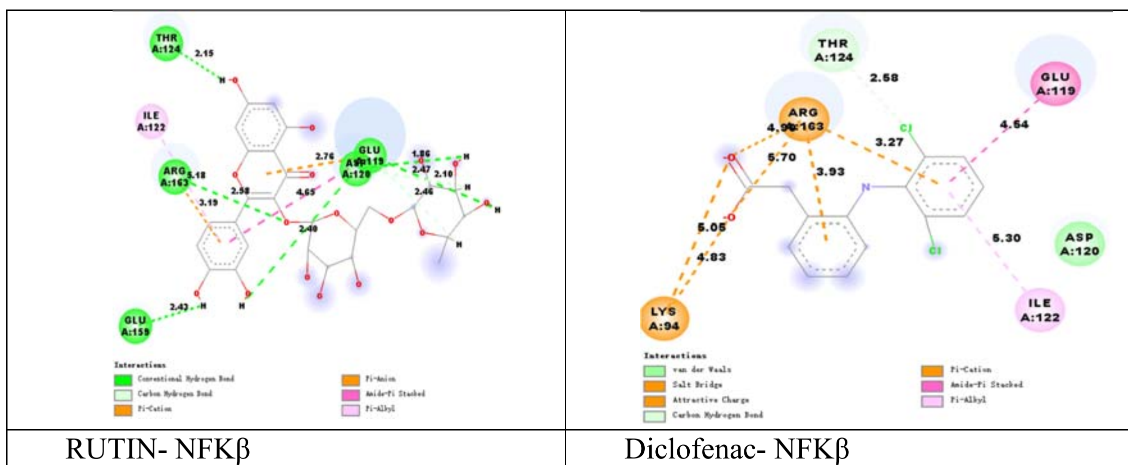
The interaction diagrams for the top-scoring compounds, along with diclofenac, are presented in Figure 6 (a–e). These visualizations highlight the involvement of key functional groups such as hydroxyl, carboxyl, and aromatic rings in stabilizing the ligand–protein complexes. These moieties contribute to various non-covalent interactions, including hydrogen bonds, π–π stacking, and hydrophobic contacts, which collectively enhance the binding stability, specificity, and overall affinity of the compounds for their targets.

Morin -1CX2	Quercetin -1CX2
Diclofenac -1CX2	Caffeic acid -1CX2

Figure 6a. Predicted 2D binding interactions of selected phenolic compounds with the active site of COX-2.

Chlorogenic acid- IL1β	Rutin- IL1β
Quercetin- IL1β	Diclofenac- IL1β

Figure 6b. Predicted 2D binding interactions of selected phenolic compounds with the active site of IL1β (1LOB).



RUTIN- NFKβ	Diclofenac- NFKβ

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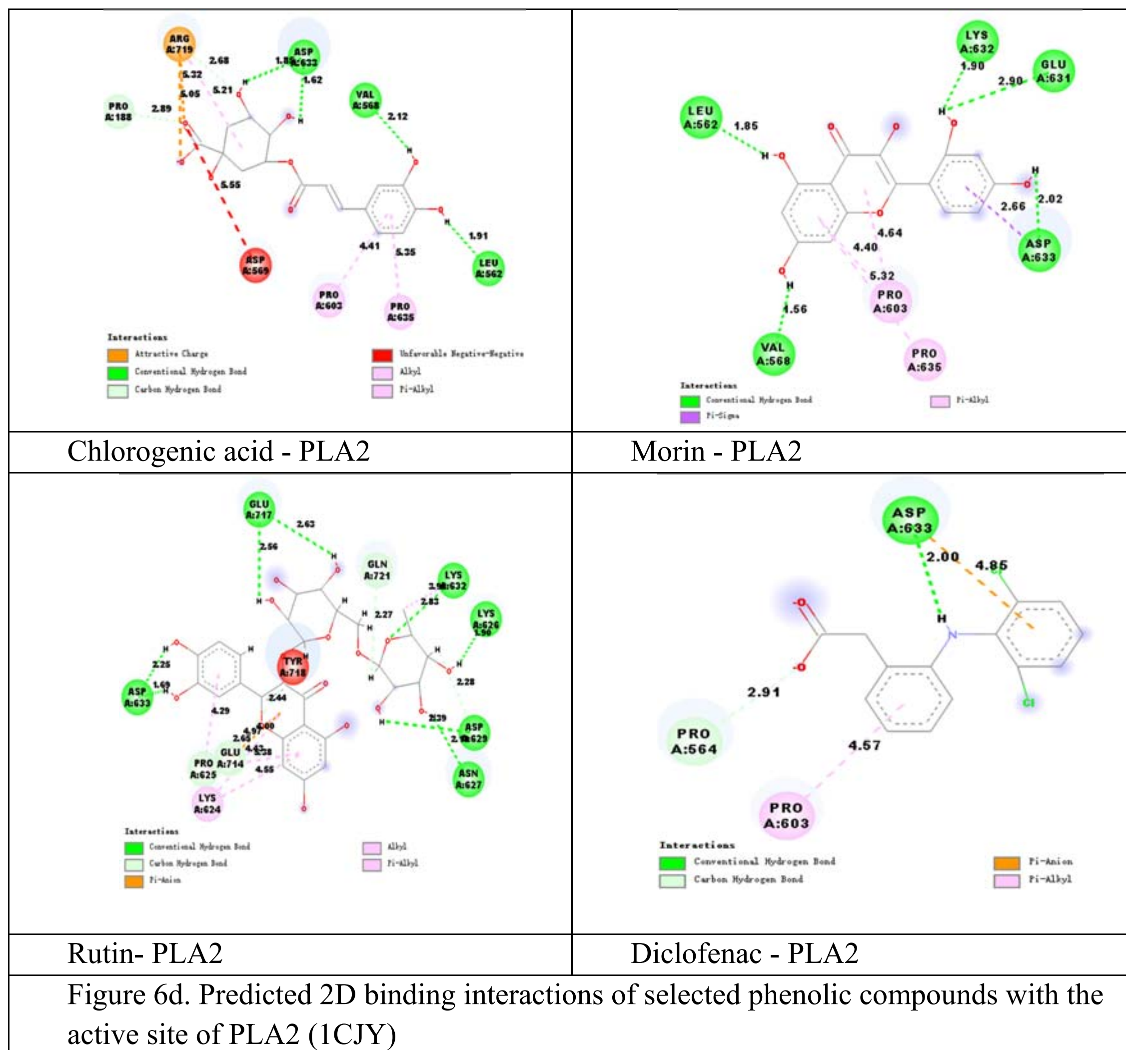
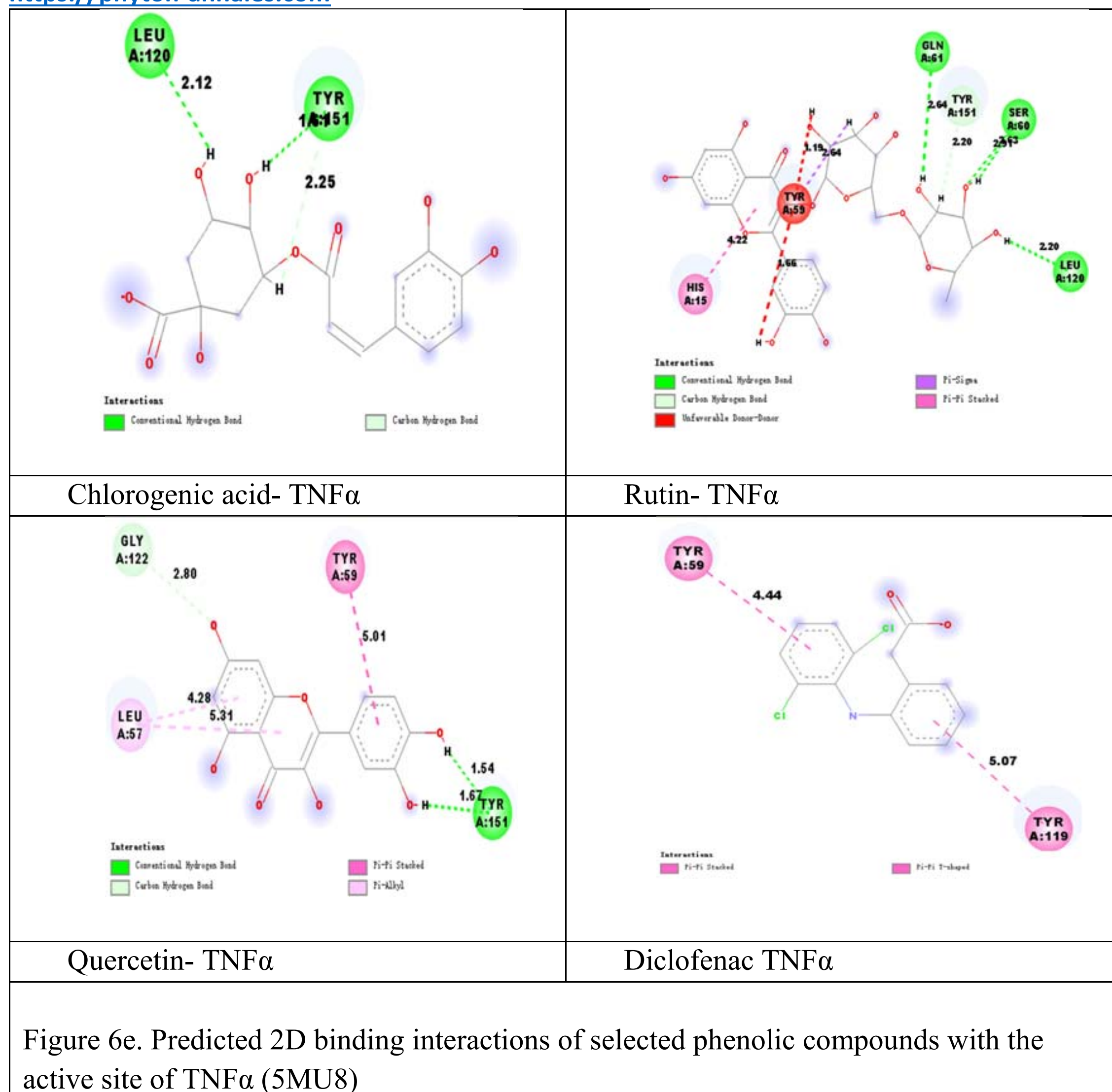
Figure 6c. Predicted 2D binding interactions of selected phenolic compounds with the active site of NFKβ (8TQD)



Chlorogenic acid - PLA2	Morin - PLA2
Rutin- PLA2	Diclofenac - PLA2

Figure 6d. Predicted 2D binding interactions of selected phenolic compounds with the active site of PLA2 (1CJY)



Chlorogenic acid- TNFα	Rutin- TNFα
Quercetin- TNFα	Diclofenac TNFα

Figure 6e. Predicted 2D binding interactions of selected phenolic compounds with the active site of TNFα (5MU8)

Phenolic compounds have emerged as promising alternatives to conventional anti-inflammatory drugs. Their biological activities span multiple stages of the inflammatory response, including the modulation of gene transcription and expression, inhibition of key inflammatory enzymes, and scavenging of reactive oxygen and nitrogen species **(Ribeiro et al. 2015**). Key mediators targeted by these compounds include interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), cyclooxygenase-2 (COX-2), phospholipase A2 (PLA2), and nuclear factor kappa B (NF-κB), all critically implicated in chronic inflammatory diseases (**Kany et al. 2019**). In the present study, molecular docking analysis elucidated the atomic-level interactions between selected phenolic compounds from *Foeniculum vulgare* extract and these key inflammatory targets (COX-2, PLA2, TNF-α, IL-1β, NF-κB), using a binding energy cut-off of −5 kcal/mol to define biologically relevant interactions. These results provide a structural basis for the compounds' anti-inflammatory potential, revealing stable binding conformations within the

active sites of their targets. Rutin emerged as the standout multi-target agent, exhibiting the highest binding affinities across all targets. Its particularly strong interactions with PLA2 (−10.351 kcal/mol) and TNF-α (−10.278 kcal/mol) are mechanistically explained by its rich interaction patterns: An extensive hydrogen bonding network with PLA2 residues (Glu717, Asp633, Lys632, Lys626, Asp629, Asn627) supported by carbon hydrogen bonds (Glu714, Pro625, Gln721) (Fig 6d), and stabilization within TNFα via hydrogen bonds (Gln61, Ser60, Leu120) and a carbon hydrogen bond (Tyr151) (Fig 6e). Rutin also engaged IL-1β through multiple hydrogen bonds (Glu64, Lys63, Glu37, Lys65, Ser21, Val41) and a carbon hydrogen bond (Gln38) (Fig 6b). Notably, rutin was the only compound showing meaningful affinity for NF-κB (−5.231 kcal/mol), involving key hydrogen bonds (Thr124, Arg163, Glu119, Asp120, Glu159) and carbon hydrogen bonds (Glu119, Asp120) (Fig 6c). This suggests a potential role in modulating pro-inflammatory gene expression, possibly by interfering with NF-κB's nuclear translocation or DNA-binding function, a mechanism observed for some polyphenols (**Forouzanfar et al. 2025**). Rutin's exceptional interaction capacity is structurally attributed to its polyhydroxylated flavonoid core and glycosylated moiety, facilitating these multiple stabilizing contacts (**Pentu et al. 2025**). Quercetin demonstrated significant potential, particularly as a COX-2 inhibitor (−8.793 kcal/mol), surpassing the reference drug diclofenac (−7.977 kcal/mol). The docking results shed light on this strong affinity, revealing a stable binding conformation facilitated by a conventional hydrogen bond with Leu352, carbon hydrogen bonds with Ala527, and crucial π–alkyl/π–π stacking interactions with hydrophobic residues (Val349, Val523, Leu531, Ser353) (Fig 6a). Quercetin also showed effective binding to PLA2 and cytokine targets. These findings align with its established role as a broad-spectrum modulator of inflammation-related enzymes and signaling molecules (**Triwardhani et al. 2023**). Furthermore, its observed binding profiles support reports that quercetin reduces pro-inflammatory cytokine expression (IL-1β, TNF-α, IL-6), likely via NF-κB pathway modulation (**Bastin et al. 2023**). Chlorogenic acid and morin also showed consistent binding to COX-2 and PLA2, with moderate interactions for IL-1β and TNF-α.Morin exhibited a notably strong affinity toward PLA2 (−7.267 kcal/mol), suggesting a significant inhibitory role in phospholipid metabolism, a crucial step in the inflammatory cascade (**Hong et al. 2025**). Overall, the detailed in silico binding profiles revealing specific hydrogen bonding, hydrophobic, and π-interactions provide strong mechanistic support for the extract's previously reported in vitro anti-inflammatory effects. The compounds with the highest docking scores (rutin, quercetin, morin) are known for their antioxidant and enzyme-inhibitory properties, consistent with biological assay results. The diversity and stability of the interactions observed (e.g., rutin's multi-target networks, quercetin's strong COX-2 binding, morin's PLA2 affinity) reinforce that the anti-inflammatory potential of *Foeniculum vulgare* stems not from a single compound, but from the synergistic, multi-target effects of its phenolic constituents.

Conclusion

Based on the results of this study clearly indicate that the hydroethanolic extract from *F. vulgare* leaves contains a notable amount of phytochemical compounds, including polyphenols and flavonoids such as chlorogenic acid and ferulic acid, as confirmed by LC-MS/MS analysis. These compounds are likely responsible for the antioxidant and anti-inflammatory activity demonstrated by the HEE from Foeniculum vulgare leaves, positioning it as a promising source for the development of anti-inflammatory agents and potential treatments for diseases related to oxidative stress in the therapeutic research. Furthermore, this study highlights and confirms the therapeutic and medicinal value of *Foeniculum vulgare* leaves through its promising in vitro results, thus paving the way for future research including in vivo studies. It also emphasizes the need to adopt the most appropriate experimental methods to obtain an accurate and comprehensive assessment of the therapeutic benefits of this plant species, with the aim of supporting its integration into evidence-based health interventions. In conclusion, the molecular docking results suggest that phenolic compounds from *Foeniculum vulgare* hydroalcoholic extract (HEE), particularly rutin and quercetin, are capable of modulating multiple inflammatory pathways at the molecular level. These findings support the traditional medicinal use of this plant and justify further in vivo investigations to explore the pharmacological relevance of its bioactive compounds.

Conflicts of Interest: The authors declare no conflict of interest.

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