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Original Article

In vitro Antioxidant Activities and Comparison of Different Fractions of the Ethanolic Extract of *Ficus racemosa* Fruits

Sadia Afrin Eva[1], Mirza Alimullah[1], Md. Junaeid Rahman[1], Asif Ul Haque Shuvo[1], Shamima Sultana[1], Asma Akter[1], Aka Barua Joya[1], Tahasin Akther[1], Khondoker Shahin Ahmed[2], Hemayet Hossain[2], Md Ashraful Alam[1], Nusrat Subhan[1*]

[1] *Department of Pharmaceutical Sciences, North South University, Dhaka, Bangladesh*
[2] *BCSIR Laboratories, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh*

***Correspondence E-mail:** nusrat.subhan@northsouth.edu

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Abstract: Oxidative stress, a major contributor to chronic diseases like diabetes and cardiovascular disorders, demands safer and more effective solutions than synthetic antioxidants. Natural alternatives, such as *Ficus racemosa*, have gained attention for their therapeutic potential. This study investigates the antioxidant and enzyme-inhibitory properties of *Ficus racemosa* extracts through comprehensive *in vitro* analyses. Ethanolic extracts and fractions were assessed using DPPH radical scavenging, nitric oxide (NO) scavenging, and ferric reducing power assays. High-performance liquid chromatography (HPLC) identified bioactive polyphenols, including gallic acid, quercetin, and caffeic acid, while molecular docking demonstrated strong binding affinities to oxidative stress-related enzymes. The ethyl acetate fraction exhibited the highest antioxidant activity, with an IC_{50} value of 0.194µg/mL for DPPH scavenging and 12.49µg/mL for NO scavenging. For the DPPH and the NO scavenging methods, IC_{50} value for other fractions are as followed, the ethyl acetate-methanol fraction (1.095 µg/mL, 13.25 µg/mL), the n-hexane-ethyl acetate fraction (1.521 µg/mL, 23.95 µg/mL), the crude extract (1.748 µg/mL, 16.18 µg/mL), ascorbic acid (2.024 µg/mL), the methanol fraction (2.411 µg/mL, 23.60 µg/mL), and the n-hexane fraction (2.738 µg/mL, 24.79 µg/mL). Docking results revealed quercetin as the most potent compound, with a binding affinity of –9.5 kcal/mol. These findings validate the traditional use of *Ficus racemosa* in folk medicine and highlight it's potential as a natural, safe, and effective alternative to synthetic antioxidants, paving the way for their application in functional foods and therapeutic formulations.

Keywords: antioxidant; *Ficus racemosa;* dumur; molecular docking; oxidative stress

1. Introduction

The widespread impact of oxidative stress on human health has become a growing concern. It disrupts the delicate balance of biological systems, tipping the scale between reactive molecules and the body's defense

mechanisms. The modern lifestyle, with its processed diets, environmental pollution, and high levels of stress, fuels an excess production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1]. While these molecules play essential roles in immune defense and cellular signaling [2], their uncontrolled accumulation disrupts biological stability. Lipids, proteins, and DNA become primary targets [3], leading to irreversible damage that accelerates aging and contributes to chronic diseases such as diabetes [4, 5], cardiovascular disorders, and cancer [6]. Left unchecked, oxidative stress weakens cellular integrity, paving the way for disease progression.

To counteract this, the body relies on antioxidants- compounds that neutralize reactive molecules and restore balance [7]. They are classified as endogenous, produced by the body (e.g., SOD, catalase, and glutathione peroxidase), or exogenous, obtained from diet and supplements [8]. While endogenous antioxidants provide a primary defense, excessive ROS and RNS can overwhelm their capacity, necessitating support from dietary antioxidants. Recent studies have increasingly focused on functional food ingredients and their potential health benefits [9, 10, 11]. The precision of *in vitro* assays offers unparalleled insights into their antioxidant potential, enabling researchers to dissect mechanisms at a molecular level. Techniques such as the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay, ferric reducing power assay, and nitric oxide (NO) scavenging assay form the cornerstone of antioxidant evaluation [12, 13]. These assays illuminate the multi-faceted roles of antioxidants, from radical neutralization to electron donation, providing a window into their functional dynamics. High-performance liquid chromatography (HPLC) adds depth to this analysis by identifying and quantifying the bioactive compounds responsible for these effects.

Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been extensively utilized for their free radical-scavenging abilities; however, multiple studies have raised concerns about their possible carcinogenicity and adverse metabolic effects [14, 15]. Toxicological studies have indicated that BHT and BHA, when administered at high doses (typically 0.1–0.5% in food), can induce liver toxicity, promote tumorigenesis, and disrupt endocrine signaling pathways [15]. These limitations highlight the urgent need for natural, biocompatible alternatives that not only combat oxidative stress effectively but also provide additional health benefits. Plant-derived antioxidants, including polyphenols, flavonoids, carotenoids, and vitamins, have emerged as powerful and safer alternatives [16]. These bioactive molecules not only exhibit superior antioxidant activity but also contribute to a range of additional health benefits [17]. Polyphenols, a diverse class of compounds found abundantly in fruits, vegetables, herbs, and medicinal plants, possess significant free radical-scavenging properties [16]. For instance, polyphenolic compounds such as resveratrol and quercetin (6) demonstrate DPPH radical scavenging activity with IC_{50} values ranging from 5–25μM [18]. In addition to scavenging ROS and RNS, polyphenols modulate key cellular signaling pathways, including NF-kB [19], MAPK [20], and SIRT1 [21], which regulate inflammation, apoptosis, and oxidative stress responses. These effects are critical in preventing the onset and progression of chronic diseases, including cardiovascular diseases, cancer, and neurodegenerative disorders [22, 23]. Flavonoids, a subclass of polyphenols, further enhance the antioxidant defense system by chelating metal ions, such as iron and copper, and inhibiting the formation of ROS. The flavonoid quercetin, for example, has been shown to chelate metal ions with a binding constant of 3.8×10^5 M^{-1}, thereby preventing Fenton-type reactions that generate highly reactive hydroxyl radicals [24]. Additionally, flavonoids exert anti-inflammatory, anti-cancer, and neuroprotective effects, making them valuable candidates in therapeutic strategies [25, 26]. Other notable dietary antioxidants include vitamins C and E, beta-carotene, and minerals such as selenium and zinc, which play essential roles in maintaining oxidative balance and supporting overall health [27].

Ficus racemosa, commonly known as “dumur” in certain regions, are a member of the *Moraceae* family and are widely recognized for their nutritional and therapeutic properties. They are rich in bioactive compounds such as phenolics, flavonoids, anthocyanins, and dietary fibers, contributing to their pharmacological effects [28, 29]. Traditionally, *Ficus racemosa* have been used in folk medicine for their antioxidant, anti-inflammatory, and antimicrobial properties [29]. It also helps to regulate blood pressure and blood cholesterol due to the presence of potassium, omega-6, and omega-3 fatty acids [30, 31]. *Ficus racemosa* is rich in trace and macro elements, along with a diverse array of biologically active phytochemicals, such as anthocyanins, carotenoids, flavonoids, polyphenols, phenolic acids, triterpenoids, glycosides, polysaccharides, reducing agents, and vitamins C, K, and

E [27]. Its active components play a vital role in combating oxidative stress and improving metabolic health through metal ion chelation, metal reduction, and free radical scavenging [32]. Phytochemical analyses of *Ficus racemosa* have also revealed the presence of ceramides such as zeaxanthin, lutein, *a*-carotene, lycopene, *β*-carotene, and cryptoxanthin [33]; also cerebrosides, steroids, pentacyclic triterpenes, flavonoids, and phenolic compounds [34, 35].

Some scientific studies confirm the antioxidant potential of *Ficus racemosa*. Methanolic extracts of *Ficus racemosa* show strong DPPH radical scavenging activity, demonstrating their ability to neutralize free radicals [36, 37]. Similarly, ferric reducing power assays highlight their electron-donating potential, while NO scavenging studies reveal their effectiveness in reducing reactive nitrogen species [37]. These findings validate the use of *Ficus racemosa* in traditional medicine for managing conditions related to oxidative stress. However, few reports have been published on studies isolating the bioactive from *Ficus racemosa* and focusing on their individual antioxidant effects and contents. This study focuses on the antioxidant potential of *Ficus racemosa* extracts and their fractions, aiming to explore their role in mitigating oxidative stress and their application in therapeutic formulations.

2. Materials and Methods

2.1 Plant Material Collection

As a plant sample, the ripe fruits of *Ficus racemosa* were collected from Tangail, Bangladesh. An experienced taxonomist then recognized the sample and identified it (Accession Number of DACB87264) at the Mirpur National Herbarium.

2.2 Extraction of plant materials

The plant material was washed, air-dried, and powdered. The powdered material was subjected to maceration in ethanol (70% v/v) for 7 days at room temperature with occasional stirring. The extract was filtered and concentrated using a rotary evaporator under reduced pressure to yield a crude ethanolic extract.

2.3 Different fraction preparation

2.3.1 Column Chromatography

Preparation of reagent:

The crude extract was fractionated via column chromatography on silica gel, employing a gradient solvent system (hexane, ethyl acetate, and methanol). Initially, 1 gram of crude extract was processed through the column. Subsequently, a total of 8 grams of crude extract was fractionated in successive runs using the solvent gradient system. This process yielded 13 sub-fractions, which were later grouped into 5 major fractions based on their thin-layer chromatography (TLC) profiles and stored at 4°C for further analysis.

Solvent gradient system:

The gradient solvent ratio to be applied in column chromatography is summarized in the following table.

Table 1: Gradient solvent system to be used in the column chromatography

Solvent system	Ratio	Volume	Fraction No.
Hexane	100%	60 ml	1
Hexane: Ethyl acetate	10:1	60 ml	2
Hexane: Ethyl acetate	5:1	60 ml	3
Hexane: Ethyl acetate	1:1	60 ml	4

Solvent System	Ratio	Volume	Fraction No.
Hexane: Ethyl acetate	1:5	60 ml	5
Hexane: Ethyl acetate	1:10	60 ml	6
Ethyl acetate	100%	60 ml	7
Ethyl acetate: Methanol	10:1	60 ml	8
Ethyl acetate: Methanol	5:1	60 ml	9
Ethyl acetate: Methanol	1:1	60 ml	10
Ethyl acetate: Methanol	1:5	60 ml	11
Ethyl acetate: Methanol	1:10	60 ml	12
Ethyl acetate: Methanol	100%	60 ml	13

2.4 Quantitative and Qualitative Analysis through HPLC

2.4.1 Chemicals

Gallic acid, 3,4-dihydroxybenzoic acid, catechin-hydrate, catechol, (-) epicatechin, caffeic acid, vanillic acid, syringic acid, rutin hydrate, p-coumaric acid, trans-ferulic acid, rosmarinic acid, myricetin, quercetin, trans-cinnamic acid, and kaempferol were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC), methanol (HPLC), acetic acid (HPLC), and ethanol were obtained from Merck (Darmstadt, Germany). α-amylase, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and naphthyl ethylenediamine dihydrochloride were obtained from Sigma-Aldrich Chemical Co. (USA). Starch soluble (extra pure) was obtained from J.T. Baker Inc., Phillipsburg, USA. Other chemicals and reagents used were of analytical grade.

2.4.2 RP-HPLC-DAD Analysis

2.4.2.1 Preparation of standard solutions

Sixteen phenolic compounds were dissolved in methanol to produce stock standard solutions. The stock solution concentrations ranged from (4.0-50 µg/ml). Each stock solution's appropriate volumes were mixed, then diluted serially to prepare the working standard solutions. All solutions were stored under refrigeration for further analysis.

Methanol stock solutions containing Gallic acid (20 µg/ml), 3,4-Dihydroxybenzoic acid (15 µg/ml), Catechin (1) hydrate (50 µg/ml), Catechol, (-) Epicatechin (2), rosmarinic acid (30 µg/ml each), Caffeic acid, vanillic acid, Syringic acid, rutin (4) hydrate, p-coumaric acid, trans-ferulic acid, quercetin (6) (10 µg/ml each), myricetin (5), kaempferol (8 µg/ml each), and trans-cinnamic acid (7) (4 µg/ml) were prepared and diluted to appropriate concentrations for the construction of calibration curves. The calibration curves were constructed by plotting the peak areas under the curve versus the amount of the analytes.

2.4.2.2 Chromatographic protocol development

Ethanolic extract of *Ficus racemosa* fruits was thawed, filtered through 0.22 µm membrane filters, and then separated by RP-HPLC-DAD analysis as described by Ahmed et al. (2021) [38] with some modifications to obtain chromatograms. Detection and quantification of selected polyphenolic compounds were performed on a Shimadzu (LC-20A, Japan) equipped with a binary solvent delivery pump (LC-20AT), an auto sampler (SIL-20A HT), column oven (CTO-20A), a photodiode array detector (SPD-M20A) and Phenomenex Luna C18 column (4.6 x 250 mm, 5µm) at ambient temperature. The RP-HPLC-DAD machine was controlled by the LC Lab Solution software. The mobile phase comprises A (1% acetic acid in acetonitrile) and B (1% acetic acid in water), and the flow rate was set at 0.5 mL/min. The gradient program was set as follows: 0-20 min, eluent A was kept at 5-25%; 21-30 min, eluent A was increased from 25% to 40%; then in the next 5 min, eluent A was

increased to 60%; but next 5 min (36-40 min), eluent A decreased to 60-30%; this down fall was continued for next 5 min, and eluent A was reached from 30 to 5% and the final 5 min eluent A was kept at 5%. The sample injection volume was 20µL. The UV detector was set at 270 nm and applied to validate the method and analysis. The mobile phase was filtered through a 0.45 µm Nylon 6, 6 membrane filter (India) and degassed under vacuum.

2.5 In Vitro Antioxidant Assays

2.5.1 DPPH Radical Scavenging Assay

In HPLC grade methanol, a DPPH solution (0.004% w/v) was prepared [39]. To make the stock solution (500 µg/mL), each of the five fractions (A, B, C, D & E) and the *Ficus racemosa* crude extract was combined individually with Milli-Q water. 1mL extracts were taken to the test tubes of serially diluted different concentrations (12.5 µg/mL to 500 µg/mL), and then a freshly made 1mL DPPH solution (0.004% w/v) was added. So, the total volume was 2 mL, and after 10 minutes of incubation in a dark place, the absorbance was measured at 515 nm using a spectrophotometer (HACH 4000 DU UV-visible spectrophotometer). As a reference standard, ascorbic acid was dissolved in Milli-Q water to create a stock solution with the same strength (500 mg/mL). The percent scavenging of the DPPH free radical activity was measured by using the following equation:

% of inhibition = [(absorbance of the control – absorbance of the test sample) / absorbance of the control] X 100

Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. All the tests were performed in triplicate.

2.5.2 Nitric Oxide (NO) Radical Scavenging Assay

For the NO radical scavenging assay, a previously described method was used [40]. At a physiological pH, sodium nitroprusside solution produced nitric oxide (NO) radicals. In phosphate buffer, 1 ml of sodium nitroprusside (10 mM) was combined with 1 ml of ethanolic extract and fractions of various concentrations (12.5 - 150 µg/ml) (pH 7.4). 150 minutes were spent incubating the mixture at 25°C. Griess' reagent (1% sulfanilamide, 2% o-phosphoric acid, and 0.1% naphthyl ethylene diamine dihydrochloride) was added to 1ml of the incubated solution. The absorbance was measured at 546 nm, and the % inhibition was measured by using the following equation:

% of inhibition = [(absorbance of the control – absorbance of the test sample) / absorbance of the control] X 100

2.5.3 Ferric Reducing Power Assay

The reducing power of the fractions was evaluated by mixing 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%) with 1 mL of each fraction. The mixture was incubated at 50°C for 20 minutes, followed by the addition of 2.5 mL of trichloroacetic acid (10%). After centrifugation at 3000 rpm for 10 minutes, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%). The absorbance was measured at 700 nm.

2.6 Molecular Docking

2.6.1 Receptor Preparation

The proteins/receptors, Human milk xanthine Alpha amylase (PDB ID: 1PPI), iNOS (PDB ID: 4NOS), NADPH oxidase (PDB ID: 5VOH), in PDB format, were downloaded from the Protein Data Bank. (https://www.rcsb.org/). By using PyMol, Water molecules and original ligands were deleted [41, 42]. AutoDock tools 1.5.7 were used to prepare the protein; Addition of polar hydrogen and Kollman charge. The receptors/proteins were saved in PDBQT format [43, 44].

2.6.2 Ligand preparation

The 3D structure of Ligands Caffeic acid (PubChem ID: 689043), Gallic acid (PubChem ID: 370), and Quercetin (PubChem ID: 5280343) were downloaded from PubChem as SDF format. (https://pubchem.ncbi.nlm.nih.gov/). PDB format is required for using AutoDock tools; therefore, using PyMol, SDF files were converted into PDB format. By using AutoDock tools 1.5.7, the ligands were prepared for docking and saved as a PDBQT file [43, 44].

2.6.3 Grid preparation

The grid menu of AutoDock tools was used to calculate the AutoGrid parameters [43]. The proteins were uploaded in the PDBQT format, and from the grid menu, the grid box was selected, and a box appeared. The default centers and the default dimensions of X, Y, and X coordinate were taken. The grid file was saved in the GPF format [43, 44].

2.6.4 Docking

AutoDock Vina is a complete computational docking method based on a quick conformational search and a basic scoring system [45]. The default techniques in AutoDock and AutoDock Vina have been extensively utilized for applications like virtual screening since they are quite efficient for typical drug-like ligands [46]. AutoDock Vina was run using the command prompt [45], and the docked file was saved in the PDBQT format [44].

2.6.5 Visualization

For visualizing the 2D and 3D structure of docked protein and ligand, Biovia Discovery Studio client 2021 is used [47].

3. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.1 RP-HPLC-DAD Analysis

HPLC, the most remarkable chromatographic technique, is a powerful and widely used technique that is used to analyze plant extracts because this technique can systematically profile the composition of samples, and it focuses on the identification and consistency assessment of the components. Ethanolic extract of *Ficus racemosa* fruits and selected standards for HPLC analysis were carried out. HPLC analysis provides accurate quantitative precision and accuracy to allow the identification of the chemicals in the selected ethanolic extract. The chromatogram revealed that *Ficus racemosa* fruit extract depicts the presence of phenolic acid, phenolics, such as flavonoids. It was observed that the extract contains catechin hydrate (10.12±0.22 mg/100 g dry extract), epicatechin (92.41±0.32 mg/100 g dry extract), caffeic acid (6.22±0.25 mg/100 g dry extract), rutin hydrate (8.24±0.03 mg/100 g dry extract), myricetin (132.69±0.63 mg/100 g dry extract), quercetin (22.81±0.37 mg/100 g dry extract), and trans-cinnamic acid (1.85±0.21 mg/100 g dry extract), which is shown in **Figure 1** and **Table 2** respectively.

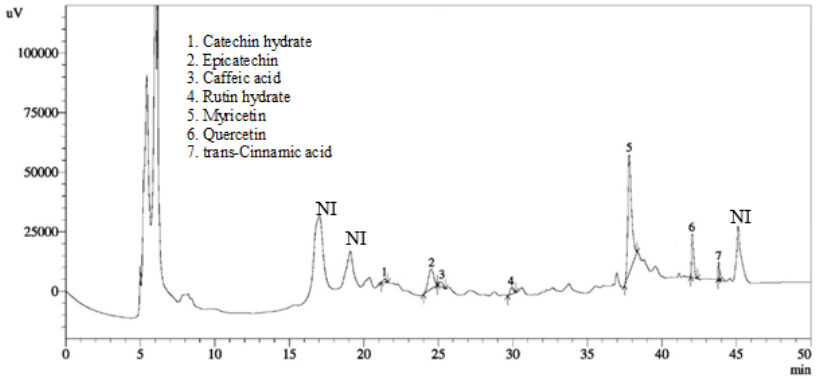

Figure 1. HPLC Chromatographic profiles of the ethanolic extract of *Ficus racemosa*. The peak labelled NI was not identified. The phenolic compound found in the *Ficus racemosa* extract was calculated from the corresponding standard curve and was presented as the mean±SD as shown in **Table 2.**

Table 2: Identification and quantification of phenolic compounds in *Ficus racemosa*.

Peak no.	Name of phenolic compounds	Retention time (min)	Concentration (mg/100 g dry extract)
1.	Catechin hydrate	21.37	10.12±0.22
2.	(-) Epicatechin	24.52	92.41±0.32
3.	Caffeic acid	25.15	6.22±0.25
4.	Rutin hydrate	29.91	8.24±0.03
5.	Myricetin	37.8	132.69±0.63
6.	Quercetin	42.03	22.81±0.37
7.	trans-Cinnamic acid	43.8	1.85±0.21

3.2 DPPH Radical Scavenging Activity

The DPPH radical contains an odd electron responsible for the visible deep purple color. Antioxidant compounds can donate an electron, and DPPH accepts this electron. When DPPH accepts an electron, then decolorizes the purple color, which can be quantitatively measured from the changes in absorbance. The antioxidant activity of the extract and its fractions is shown in **Figure 2**. Ascorbic acid was also used as a standard.

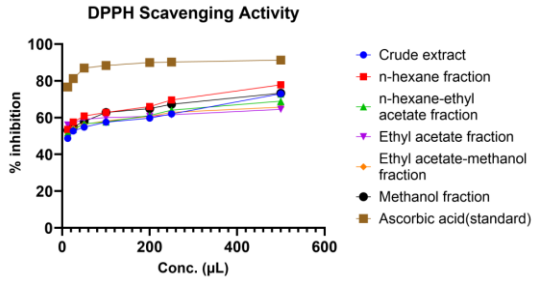

Figure 2: Dose-response curve of DPPH scavenging activity of the crude ethanol extract of *Ficus racemosa* fruit and its five different fractions.

3.3 NO Scavenging Activity:

The dose-response curve of nitric oxide (NO) scavenging activity of the crude ethanol extract of *Ficus racemosa* fruit and its five different fractions are given in the **Figure 3**. It represents the ethyl acetate fraction as the most effective in mitigating nitrosative stress, with an IC_{50} of 12.49 µg/mL found in **Table 2**.

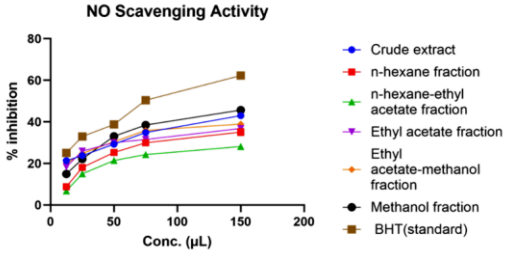

Figure 3: Dose-response curve of NO scavenging activity of the crude ethanol extract of *Ficus racemosa* fruit and its five different fractions.

3.4 Ferric Reducing Power Activity

The dose-response curve of reducing power of the crude ethanol extract of *Ficus racemosa* fruit and its five different fractions are shown in the **Figure 4**. It established a concentration-dependent increase in activity, with the ethyl acetate fraction exhibiting the highest reducing potential.

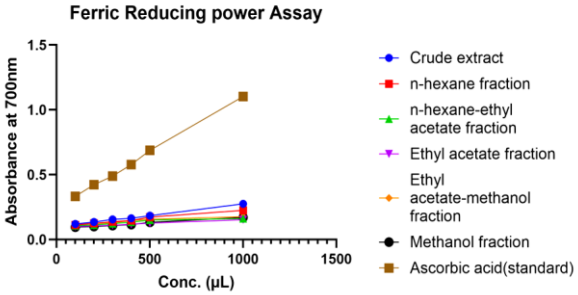

Figure 4: Dose-response curve of reducing power of the crude ethanol extract of *Ficus racemosa* fruit and its five different fractions.

Table 3: IC_{50} values of *Ficus racemosa* extract and its five fractions in different antioxidant assays such as DPPH and NO Scavenging Method.

Sample	**DPPH Scavenging Method (µg/mL)**	**NO Scavenging Method (µg/mL)**
Ascorbic Acid	2.024	-
BHT	-	21.36
Crude Extract	1.748	16.18
n-hexane Fraction	2.738	24.79
n-hexane - Ethyl acetate Fraction	1.521	23.95
Ethyl acetate Fraction	0.1940	12.49

Sample	DPPH Scavenging Method (µg/mL)	NO Scavenging Method (µg/mL)
Ethyl acetate - Methanol Fraction	1.095	13.25
Methanol Fraction	2.411	23.60

Values are expressed as IC_{50} values of triplicate analysis. Ascorbic Acid is used as a DPPH standard, and BHT is used as a NO Scavenging.

3.5 Molecular docking of caffeic acid, gallic acid, and quercetin present in Ficus racemosa fruit extract.

Docking studies revealed strong binding affinities of gallic acid, quercetin, and caffeic acid with key oxidative stress-related enzymes. Quercetin exhibited the highest binding affinity with iNOS (−9.5 kcal/mol), followed by NADPH oxidase (−8.4 kcal/mol). Hydrogen bonds and hydrophobic interactions were the primary forces stabilizing these complexes. These results provide a molecular basis for the antioxidant efficacy observed *in vitro* and suggest potential therapeutic applications for *Ficus racemosa* bioactive in oxidative stress-related diseases.

Compounds from *Ficus racemosa*, such as Gallic acid, Caffeic acid, and Quercetin, strongly bind with Alpha amylase, iNOS, and NADPH oxidase. (**Figure: 4, 5, 6**) Their binding affinity is shown in **Table 3**. Ligands form several types of bonds, such as hydrogen bonds, hydrophobic bonds, and salt bridges with proteins/receptors. (**Table 2, 3, and 4)**

Table 4: Binding properties of caffeic acid, gallic acid, and quercetin present in *Ficus racemosa* fruit extract.

Ligand	Receptor	Affinity (Kcal/mol)	rmsd
Caffeic acid	Alpha amylase (1ppi)	-7.3	0.000
	iNOS (4nos)	-6.3	
	NADPH ox (5voh)	-6.8	
Gallic acid	Alpha amylase (1ppi)	-5.8	0.000
	iNOS (4nos)	-5.7	
	NADPH ox (5voh)	-5.6	
Quercetin	Alpha amylase (1ppi)	-8.9	0.000
	iNOS (4nos)	-9.5	
	NADPH ox (5voh)	-8.4	

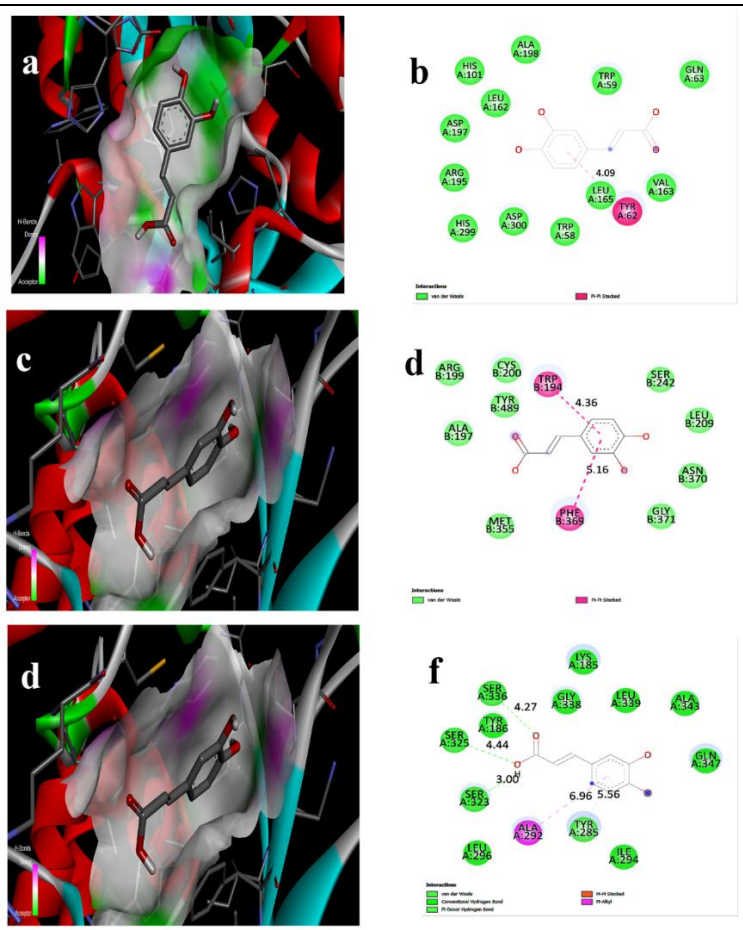

Figure 4: Best rank imposes of caffeic acid and alpha amylase 3D (a), 2D (b); caffeic acid and iNOS 3D (c), 2D (d); caffeic acid and NADPH ox 3D (e), 2D (f).

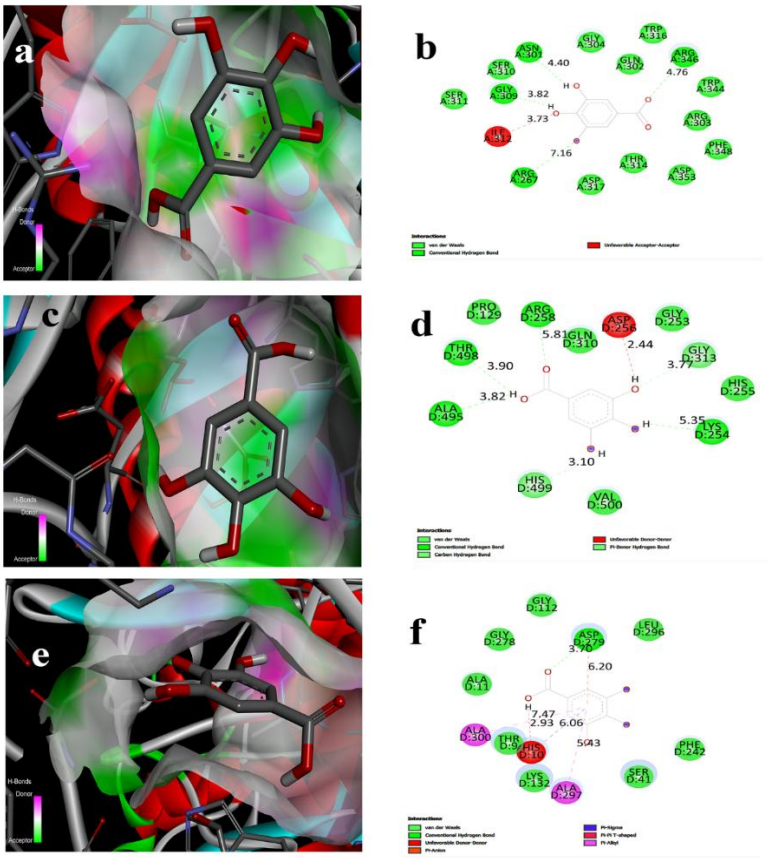

Figure 5: Best rank imposes of gallic acid and alpha amylase 3D (a), 2D (b); gallic acid and iNOS 3D (c), 2D (d); gallic acid and NADPH ox 3D (e), 2D (f).

3.6 Ficus racemosa extract component caffeic acid interaction with protein target 1ppi

The caffeic acid interaction with protein target 1ppi (the complex of a pancreatic alpha-amylase with a carbohydrate inhibitor refined to 2.2-A resolution). This table summarizes key non-covalent interactions stabilizing the binding of caffeic acid to the protein 1ppi. It is divided into two sub-tables:
Hydrogen bonds significantly enhance ligand-protein specificity and affinity through directional electrostatic interactions. **Table 4.1** catalogs hydrogen bonds formed between caffeic acid and the protein 1ppi. Metrics include distances (H-A: hydrogen-acceptor; D-A: donor-acceptor), donor angle geometry, and roles of protein donor groups/side chains. Notably, GLN63A and ARG195A act as key donors, with strong angles (153°–174°) and short H-A distances (2.22–2.89 Å), indicating robust interactions critical for ligand anchoring. Together, these interactions elucidate structural mechanisms driving ligand-protein affinity and specificity.

Table 4.1: Hydrogen bonds interaction data between caffeic acid and the target protein 1ppi

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	63A	GLN	2.22	3.13	153.01	Y	Y	521 [Nam]	3922 [O3]
2	195A	ARG	2.89	3.87	174.11	Y	Y	1526 [Ng+]	3924 [O3]

AA: Amino acid residue; Distance H-A: Distance between hydrogen and acceptor atom; Distance D-A: Distance between donor and acceptor atom; Donor Angle: Angle between donor, acceptor, and hydrogen atom; Protein donor?- Does protein provide the donor group?; Side chain: Is the hydrogen bond formed with the amino acid side chain?; Donor Atom: ID of donor atom; Acceptor Atom: ID of acceptor atom.

Hydrophobic interactions are critical non-covalent forces that stabilize ligand-protein binding by excluding water from non-polar interfaces. **Table 4.2** details key hydrophobic contacts between the ligand and residues in the target protein 1ppi. Entries include the residue index, amino acid type (AA), distance (Å) between interacting carbon atoms, and identifiers for the ligand and protein atoms involved. Distances ≤4.0 Å highlight residues like TYR62A and LEU165A that contribute to binding stability through van der Waals forces and desolvation effects.

Table 4.2: Hydrophobic Interactions between ligand caffeic acid and protein target 1ppi

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	58A	TRP	3.78	3915	466
2	62A	TYR	3.45	3914	506
3	62A	TYR	3.66	3915	507
4	62A	TYR	3.83	3917	508
5	63A	GLN	3.91	3920	519
6	165A	LEU	3.94	3920	1286

AA: Amino acid residue; Distance: Distance between interactions' carbon atoms; Ligand Atom: ID of ligand carbon atom; Protein Atom: ID of protein carbon atom.

3.7 Ficus racemosa extract component caffeic acid interaction with protein target 4nos

The protein target human inducible nitric oxide synthase with inhibitor (4nos) is used for the interaction with Caffeic Acid as the ligand. The results have been given below in **Table 4.3** and **Table 4.4.**

Through directed electrostatic interactions, hydrogen bonds significantly improve the specificity and affinity of ligands for proteins. Here in **Table 4.3**, it shows the hydrogen bond between ligand caffeic acid and protein target 4nos. Here ASN370B act as key donor, with a strong angle of 160.43° and short H-A distance of 2.82 Å, demonstrating a strong interaction that is essential for ligand anchoring.

Table 4.3: Hydrogen bonds interaction data between caffeic acid and the target protein 4nos

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	370B	ASN	2.82	3.75	160.43	No	No	13684 [O3]	5740 [O2]

AA: Amino acid residue; Distance H-A: Distance between hydrogen and acceptor atom; Distance D-A: Distance between donor and acceptor atom; Donor Angle: Angle between donor, acceptor, and hydrogen atom; Protein donor?- Does protein provide the donor group?; Side chain: Is the hydrogen bond formed with the amino acid side chain?; Donor Atom: ID of donor atom; Acceptor Atom: ID of acceptor atom.

Since hydrophobic interactions keep water out of non-polar surfaces, they are essential non-covalent forces that maintain ligand-protein binding. In **Table 4.4**, the hydrophobic interactions between the ligand and residues in the target protein 4nos has been described. Residues like PHE369B and TRP194B, which support binding stability through van der Waals forces and desolvation effects, are highlighted at distances ≤4.0 Å.

Table 4.4: Hydrophobic Interactions between ligand caffeic acid and protein target 4nos

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	194B	TRP	3.87	13686	4310
2	194B	TRP	3.62	13689	4304
3	194B	TRP	3.69	13690	4306
4	369B	PHE	3.55	13692	5734
5	369B	PHE	3.91	13690	5730
6	489B	TYR	3.83	13692	6712
7	489B	TYR	3.58	13689	6714

AA: Amino acid residue; Distance: Distance between interactions carbon atoms; Ligand Atom: ID of ligand carbon atom; Protein Atom: ID of protein carbon atom.

3.8 Ficus racemosa extract component caffeic acid interaction with protein target 5voh

The caffeic acid interaction with protein target 5voh (Crystal structure of engineered water-forming NADPH oxidase (TPNOX) bound to NADPH). This table describes critical non-covalent interactions that stabilize the binding of caffeic acid to the protein 5voh. It's separated into two sub-tables.

In **Table 4.5**, hydrogen bonds between the ligand caffeic acid and protein target 5voh has been shown. Here, residue- GLN347A has shown strong donor angle of 162.45° and short H-A distance of 4.05 Å, demonstrating a strong interaction that is essential for ligand anchoring.

Table 4.5: Hydrogen Bonds interaction data between caffeic acid and the protein target 5voh

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	323A	SER	2.21	3.02	139.59	-	-	13719 [O.co2]	2462 [O3]
2	336A	SER	2.40	2.88	109.88	-	-	2558 [O3]	13718 [O.co2]
3	347A	GLN	3.10	4.05	162.45	-	-	2633 [Nam]	13721 [O3]

AA: Amino acid residue; Distance H-A: Distance between hydrogen and acceptor atom; Distance D-A: Distance between donor and acceptor atom; Donor Angle: Angle between donor, acceptor, and hydrogen atom; Protein donor?- Does protein provide the donor group?; Side chain: Is the hydrogen bond formed with the amino acid side chain?; Donor Atom: ID of donor atom; Acceptor Atom: ID of acceptor atom.

In **Table 4.6**, the hydrophobic interactions between the ligand and residues in the target protein 4nos have been described. Residues like TRY285A, which support binding stability through van der Waals forces and desolvation effects, are highlighted at distances ≤4.0 Å.

Table 4.6: Hydrophobic Interactions between ligand caffeic acid and protein target 5voh

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	285A	TYR	3.62	13713	2170
2	285A	TYR	3.91	13709	2171
3	294A	ILE	3.96	13713	2245

AA: Amino acid residue; Distance: Distance between interactions' carbon atoms; Ligand Atom: ID of ligand carbon atom; Protein Atom: ID of protein carbon atom.

3.9 Ficus racemosa extract component gallic acid interaction with protein target 1ppi

Molecular interactions between gallic acid and human pancreatic α-amylase (PDB: 1PPI). The 1PPI structure (resolved at 2.2 Å) depicts the enzyme co-crystallized with a carbohydrate inhibitor, providing a proven active site for docking analysis.

Hydrogen bonds increase ligand affinity and specificity via directional interactions. GLY309A and ARG346A serve as the leading hydrogen donors to gallic acid in key bond interactions. Near-ideal geometry, characterized by bond angles of 150°–152° and H-A distances ranging from 1.86 Å to 2.93 Å, suggests significant stability. This demonstrating geometrically complementary bonding that inhibits starch substrate access through competitive mechanisms.

Table 5.1: Hydrogen Bonds interaction data between ligand gallic acid and target protein 1ppi

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	267A	ARG	2.76	3.23	110.26	y	y	2095 [Ng+]	3918 [O3]
2	301A	ASN	2.37	3.05	126.61	x	x	3924 [O3]	2373 [O2]
3	304A	GLY	3.53	3.98	110.17	y	x	2398 [Nam]	3924 [O3]
4	309A	GLY	1.86	2.75	151.80	x	x	3910 [O2]	2428 [O2]
5	346A	ARG	2.93	3.81	149.62	y	y	2737 [Ng+]	3921 [O3]

AA: Amino acid residue; Distance H-A: Distance between hydrogen and acceptor atom; Distance D-A: Distance between donor and acceptor atom; Donor Angle: Angle between donor, acceptor, and hydrogen atom; Protein donor?- Does protein provide the donor group?; Side chain: Is the hydrogen bond formed with the amino acid side chain?; Donor Atom: ID of donor atom; Acceptor Atom: ID of acceptor atom.

3.10 Ficus racemosa extract component gallic acid interaction with protein target 4nos

The interactions at the molecular level between gallic acid and human inducible nitric oxide synthase (iNOS) (PDB: 4NOS). The 4NOS structure, co-crystallized with a selective inhibitor, offers a validated binding site for evaluating the interactions of gallic acid, highlighting key residues within the catalytic domain.

Hydrogen bonds stabilizing gallic acid within the inhibitor-binding site of human inducible nitric oxide synthase (PDB: 4NOS). Donor residues ASP256D and VAL500D establish essential hydrogen bonds with gallic acid, demonstrating near-linear geometry (bond angles: 162°–165°) and short H-A distances (2.03–3.42 Å). The interactions indicate significant electrostatic complementarity in the catalytic domain, implying competitive displacement of the native inhibitor.

Table 5.2: Hydrogen Bonds interaction data between ligand gallic acid and protein target 4nos

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	256D	ASP	2.03	3.00	165.21	y	x	11651 [Nam]	13692 [O3]
2	256D	ASP	3.04	3.75	131.35	x	y	13692 [O3]	11658 [O.co2]
3	313D	GLY	2.36	3.00	122.78	x	x	13684 [O3]	12116 [O2]
4	495D	ALA	2.64	3.15	112.75	x	x	13696 [O.co2]	13611 [O2]
5	498D	THR	2.57	3.07	112.69	y	y	13642 [O3]	13695 [O.co2]
6	499D	HIS	2.39	3.21	140.90	x	y	13698 [O3]	13650 [N2]
7	500D	VAL	2.46	3.42	162.49	y	x	13653 [Nam]	13698 [O3]

AA: Amino acid residue; Distance H-A: Distance between hydrogen and acceptor atom; Distance D-A: Distance between donor and acceptor atom; Donor Angle: Angle between donor, acceptor, and hydrogen atom; Protein donor?- Does protein provide the donor group?; Side chain: Is the hydrogen bond formed with the amino acid side chain?; Donor Atom: ID of donor atom; Acceptor Atom: ID of acceptor atom.

Table 5.3 presents the recognized salt bridges detected within the protein-ligand complex, mentioning the interacting residues, their distances, and the nature of the ligand groups present. This table also assumes that the protein residue aiding in the interaction carries a positive charge, concurrent with enumerating the specific ligand atoms participating in the salt bridge formation.

Table 5.3: *Ficus racemosa* extract ligand with protein target salt bridge

Index	Residue	AA	Distance	Protein positive?	Ligand Group	Ligand Atoms
1	258D	ARG	4.23	Yes	Carboxylate	13696, 13695

3.11 Ficus racemosa extract component gallic acid interaction with protein target 5voh

The gallic acid interaction with protein target 5voh (Crystal structure of engineered water-forming NADPH oxidase (TPNOX) bound to NADPH). This table describes critical non-covalent interactions that stabilize the binding of gallic acid to the protein 5voh.

High-affinity hydrogen bonds within the iNOS-gallic acid complex (PDB: 4NOS). Residues ALA11D and ASP279D establish geometrically optimized hydrogen bonds with gallic acid, demonstrating near-linear donor angles (168°–173°) and notably short H-A distances (2.01–2.04 Å). The interactions illustrate electrostatic complementarity essential for competitive inhibition in the heme-containing catalytic domain.

Table 5.4: Hydrogen Bonds interaction data between ligand gallic acid and target protein 5voh

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	10D	HIS	2.28	3.12	142.69	Y	X	10340 [Nam]	13719 [O.co2]
2	11D	ALA	2.04	3.02	172.85	Y	X	10350 [Nam]	13719 [O.co2]
3	41D	SER	2.67	3.22	116.50	Y	Y	10591 [O3]	13707 [O3]
4	41D	SER	2.47	3.22	134.25	X	Y	13707 [O3]	10591 [O3]
5	132D	LYS	2.88	3.25	102.27	Y	Y	11278 [N3+]	13721 [O3]
6	279D	ASP	2.01	2.98	168.48	Y	X	12398 [Nam]	13718 [O.co2]

AA: Amino acid residue; Distance H-A: Distance between hydrogen and acceptor atom; Distance D-A: Distance between donor and acceptor atom; Donor Angle: Angle between donor, acceptor and hydrogen atom; Protein donor?- Does protein provide the donor group?; Side chain: Is the hydrogen bond formed with the amino acid side chain?; Donor Atom: ID of donor atom; Acceptor Atom: ID of acceptor atom.

3.12 Ficus racemosa extract component quercetin interaction with protein target 1ppi

Quercetin interaction with protein target 1ppi (the complex of a pancreatic alpha-amylase with a carbohydrate inhibitor refined to 2.2-A resolution).

Hydrogen bonds facilitate the binding of quercetin to pancreatic α-amylase (PDB: 1PPI). Donor residues GLU233A and ARG195A establish geometrically optimized hydrogen bonds with quercetin, demonstrating near-linear angles (152°–155°) and short H-A distances (2.12–2.23 Å). The interactions stabilize the flavonoid core within the catalytic pocket.

Table 6.1: Hydrogen Bonds interaction data between ligand quercetin and target protein 1ppi

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	63A	GLN	3.47	4.01	117.97	X	Y	3935 [O3]	522 [O2]
2	195A	ARG	2.12	3.03	151.52	Y	Y	1525 [Ng+]	3929 [O3]
3	195A	ARG	2.25	3.12	147.03	Y	Y	1526 [Ng+]	3929 [O3]
4	233A	GLU	2.23	3.14	155.29	X	Y	3927 [O3]	1836 [O3]
5	305A	HIS	2.22	2.98	132.93	Y	Y	2409 [Nar]	3931 [O3]

AA: Amino acid residue; Distance H-A: Distance between hydrogen and acceptor atom; Distance D-A: Distance between donor and acceptor atom; Donor Angle: Angle between donor, acceptor, and hydrogen atom; Protein donor?- Does protein provide the donor group?; Side chain: Is the hydrogen bond formed with the amino acid side chain?; Donor Atom: ID of donor atom; Acceptor Atom: ID of acceptor atom.

Hydrophobic interactions stabilize quercetin within the substrate-binding site of pancreatic α-amylase (PDB: 1PPI). Hydrophobic interactions improve ligand binding by promoting desolvation-induced entropy

increases, thereby removing water from nonpolar surfaces. Key residues TYR62A (3.8 Å) and LEU165A (3.6 Å) establish significant van der Waals interactions with the flavonoid rings of quercetin, thereby competitively obstructing the catalytic triad from starch substrates.

Table 6.2: Hydrophobic Interactions between ligand quercetin and target protein 1ppi

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	59A	TRP	3.94	3920	477
2	62A	TYR	3.64	3925	510
3	163A	VAL	3.99	3918	1275
4	165A	LEU	3.45	3918	1286

AA: Amino acid residue; Distance: Distance between interactions' carbon atoms; Ligand Atom: ID of ligand carbon atom; Protein Atom: ID of protein carbon atom.

Table 6.3 briefly states the salt bridge interactions discovered within the analyzed protein-ligand complex, referring to the residue information, interatomic distances, and the charge status of the interacting protein residues. It also denotes the ligand groups involved and lists the ligand atom IDs that participate in the formation of these salt bridges.

Table 6.3: *Ficus racemosa* extract ligand with protein target salt bridge

Index	Residue	AA	Distance	Protein positive?	Ligand Group	Ligand Atoms
1	305A	HIS	5.10	Yes	Carboxylate	3910, 3911

3.13 Ficus racemosa extract component quercetin interaction with protein target 4nos

The protein target 4nos (human inducible nitric oxide synthase with inhibitor) is used for the interaction with Quercetin as the ligand. The results have been given below in **Table 6.4** and **Table 6.5**

Hydrogen bonds facilitating the interaction of quercetin with human inducible nitric oxide synthase (PDB: 4NOS). Key bonds include ALA197C (H-A: 2.98 Å), ARG199C (H-A: 2.50 Å), and SER242C (H-A: 3.53 Å), with donor angles ranging from 115° to 125°, suggesting geometrically constrained interactions. The minimal H-A distance for ARG199C (2.50 Å) indicates significant electrostatic complementarity in proximity to the heme cofactor, effectively displacing the native inhibitor.

Table 6.4: Hydrogen Bonds interaction data between ligand quercetin and target protein 4nos

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	197C	ALA	2.98	3.63	125.68	x	x	13701 [O3]	7756 [O2]
2	199C	ARG	2.50	3.16	124.17	y	x	7765 [Nam]	13701 [O3]
3	242C	SER	3.53	4.05	115.47	x	y	13707 [O3]	8127 [O3]

AA: Amino acid residue; Distance H-A: Distance between hydrogen and acceptor atom; Distance D-A: Distance between donor and acceptor atom; Donor Angle: Angle between donor, acceptor, and hydrogen atom; Protein donor?- Does protein provide the donor group?; Side chain: Is the hydrogen bond formed with the amino acid side chain?; Donor Atom: ID of donor atom; Acceptor Atom: ID of acceptor atom.

Hydrophobic interactions that stabilize quercetin within the catalytic domain of human inducible nitric oxide synthase (iNOS) (PDB: 4NOS). Critical contacts include TRP194C (3.50–3.58 Å), LEU209C (3.52 Å), PHE369C (3.64–3.79 Å), and TYR489C (3.30 Å), with distances of ≤4.0 Å signifying high-affinity van der Waals interactions. The interactions competitively displace the native inhibitor by occluding the heme cofactor and limiting substrate access to the active site.

Table 6.5: Hydrophobic Interactions between ligand quercetin and target protein 4nos

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	194C	TRP	3.50	13694	7727
2	194C	TRP	3.58	13693	7725
3	209C	LEU	3.52	13694	7856
4	369C	PHE	3.79	13692	9153
5	369C	PHE	3.64	13700	9155
6	489C	TYR	3.30	13692	10135

AA: Amino acid residue; Distance: Distance between interactions' carbon atoms; Ligand Atom: ID of ligand carbon atom; Protein Atom: ID of protein carbon atom. (1)

3.14 Ficus racemosa extract component quercetin interaction with protein target 5voh

The quercetin interaction with protein target 5voh (Crystal structure of engineered water-forming NADPH oxidase (TPNOX) bound to NADPH).

High-fidelity hydrogen bonds stabilize quercetin within the NADPH-binding site of engineered TPNOX (PDB: 5VOH). Significant interactions involve GLU161A (H-A: 2.17 Å; 169°; carbonyl acceptor) and GLY326A (H-A: 2.29 Å; 174°; backbone amide), demonstrating near-optimal geometry that competitively displaces the NADPH cofactor via electrostatic mimicry.

Table 6.6: Hydrogen Bonds interaction data between ligand quercetin and target protein 5voh

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	45A	ALA	2.72	3.16	107.98	y	x	327 [Nam]	13732 [O3]
2	131A	CYS	2.74	3.16	106.98	x	x	13730 [O3]	990 [O2]

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
3	161A	GLU	2.17	3.13	169.03	x	y	13724 [O3]	1220 [O.co2]
4	326A	GLY	2.29	3.27	174.37	y	x	2475 [Nam]	13726 [O3]
5	422B	PHE	2.40	3.24	144.73	x	x	13726 [O3]	6629 [O2]

AA: Amino acid residue; Distance H-A: Distance between hydrogen and acceptor atom; Distance D-A: Distance between donor and acceptor atom; Donor Angle: Angle between donor, acceptor, and hydrogen atom; Protein donor?- Does protein provide the donor group?; Side chain: Is the hydrogen bond formed with the amino acid side chain?; Donor Atom: ID of donor atom; Acceptor Atom: ID of acceptor atom.

Stabilization of quercetin through hydrophobic interactions within the NADPH-binding cleft of engineered TPNOX (PDB: 5VOH). Key residues ILE158A (3.69 Å), ILE44A (3.96 Å), and TYR157A (3.99 Å) establish complementary van der Waals interactions with the flavonoid scaffold of quercetin, working in conjunction with hydrogen bonds (**Table 6.6**) to obstruct cofactor access via competitive desolvation.

Table 6.7: Hydrophobic Interactions between ligand quercetin and target protein 5voh

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	44A	ILE	3.96	13716	323
2	157A	TYR	3.99	13719	1186
3	158A	ILE	3.89	13713	1201
4	158A	ILE	3.69	13717	1200

AA: Amino acid residue; Distance: Distance between interactions carbon atoms; Ligand Atom: ID of ligand carbon atom; Protein Atom: ID of protein carbon atom.

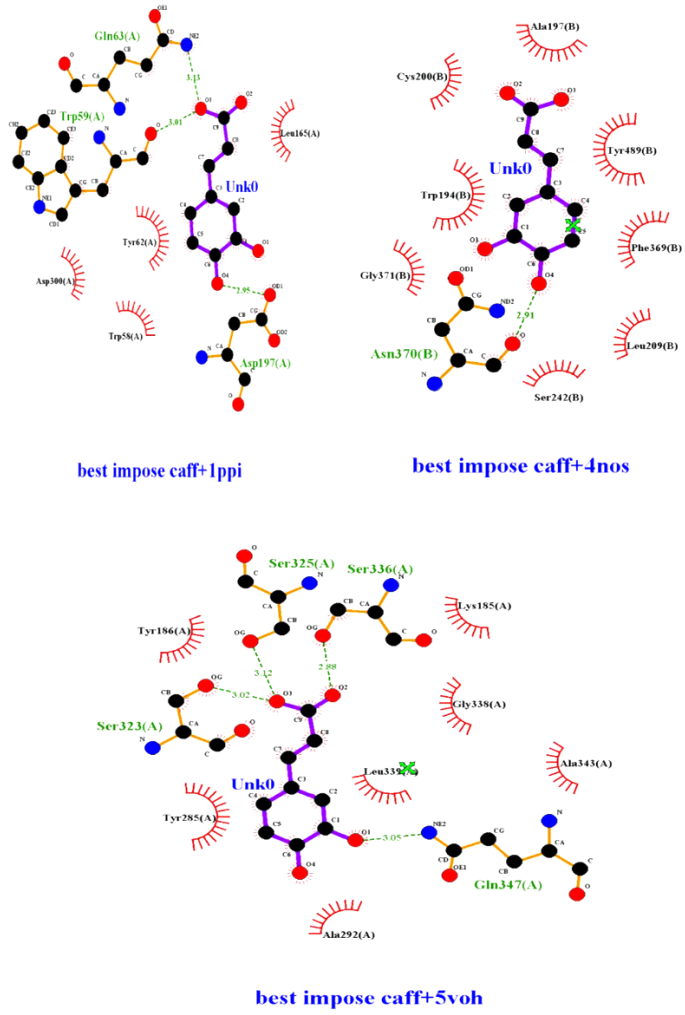

Figure 6: LigPlot demonstration of Caffeic acid and receptors.

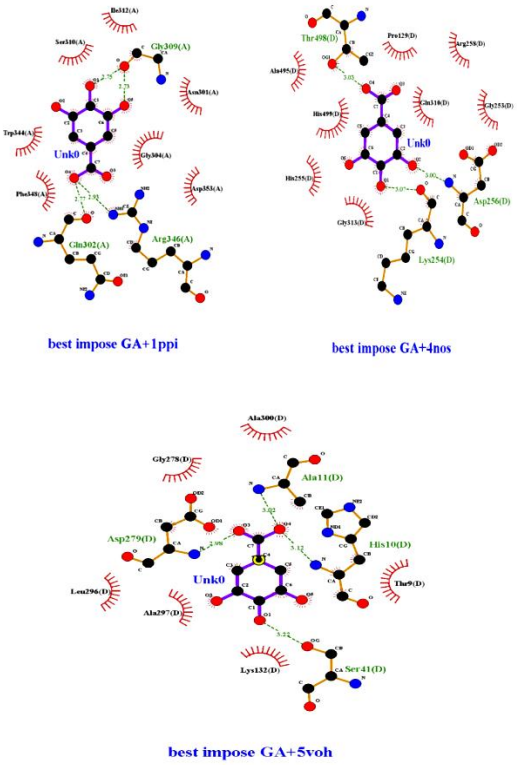

Figure 7: LigPlot demonstration of Gallic acid and receptors.

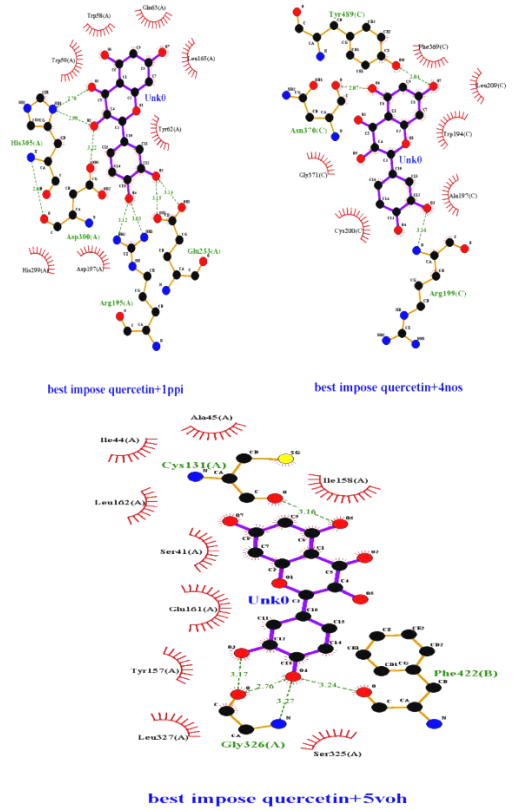

Figure 8: LigPlot demonstration of Quercetin and receptors.

4. Discussion:

The findings of this study highlight the importance of natural antioxidants, especially those derived from *Ficus racemosa*, in reducing oxidative stress. Phytochemicals such as phenolics and flavonoids, which are abundant in plant-based foods, are well-known for their ability to scavenge free radicals, neutralize reactive oxygen species (ROS), and prevent oxidative damage to biomolecules [48, 49, 50]. These bioactive compounds not only play a crucial role in the plant's defense mechanisms but also offer significant health benefits to humans when consumed.

The DPPH assay results demonstrated that the ethyl acetate fraction exhibited the highest free radical scavenging activity, with an IC_{50} value of 0.194 µg/mL. This value surpasses standard antioxidants such as ascorbic acid, which typically falls within the 0.5–20 µg/mL [48, 51]. Extracts with IC_{50} values below 50 µg/mL are considered potent antioxidants, and the ethyl acetate fraction's significantly lower IC_{50} value underscores its remarkable antioxidant efficacy. Hexane hardly carries phenolic compounds in extraction. However, n-hexane acetate fraction has shown greater antioxidant activity than the methanol fraction, which may be due to the presence of acetate solvent or other chemicals present in this selected fraction. The order of radical scavenging activity was as follows:

Ethyl acetate fraction > ethyl acetate-methanol fraction > n-hexane-ethyl acetate fraction > crude extract > methanol fraction > n-hexane fraction

The nitric oxide (NO) scavenging assay further validated these findings, highlighting the ethyl acetate fraction as the most effective in mitigating nitrosative stress, with an IC_{50} of 12.49 µg/mL**.** Given that strong NO scavengers like gallic acid and quercetin typically exhibit IC_{50} values in the range of 10–50 µg/mL [52, 53], the ethyl acetate fraction's activity is well within this range, indicating its strong nitrosative stress-reducing potential. The reduction in nitrite levels is critical, as excessive NO production leads to the formation of reactive peroxynitrite species, exacerbating cellular damage and inflammatory responses. The ranking of NO scavenging activity was as follows:

Ethyl acetate fraction > ethyl acetate-methanol fraction > crude extract > Methanol fraction > n-hexane-ethyl acetate fraction > hexane fraction.

The ferric-reducing power assay demonstrated a concentration-dependent increase in activity, with the ethyl acetate fraction displaying the highest reducing potential. This result aligns with previous studies linking ferric-reducing ability to polyphenolic content [54, 55]. Since phenolics and flavonoids are known to donate electrons to neutralize free radicals, the correlation between HPLC-detected polyphenolic content and reducing power further supports their antioxidant capacity.

The HPLC analysis of the ethanolic extract was carried out to identify the phenol and flavonoid molecules that have important antioxidant activity, and they are used to reduce the risk of cell damage and cell death [1]. Seven natural polyphenolic compounds, such as catechin hydrate (1), (-) epicatechin (2), caffeic acid (3), rutin (4) hydrate, myricetin (5), quercetin (6), and trans-cinnamic acid (7), were identified in the fruit extract of *Ficus racemosa* **Figure 9**. The antioxidant property of phenolic compounds is attributed to their ability to prevent the formation of reactive species, neutralizing (scavenging) free radicals, forming chelate complexes with pro-oxidizing metals, and also the number of hydroxyl groups in the molecule correlates positively with the antioxidant activity [2]. Catechin (1) (structurally an isomer of epicatechin) and epicatechin (2), flavanols, have strong antioxidant activity, which can donate one electron of the phenolic OH group, thus reducing free radicals, and are responsible for deactivating free radicals [3]. Phenolic acids are the most prominent and well-characterized phenolic compounds in plants [4]. Hydroxycinnamic acids such as caffeic acid (3) and trans-cinnamic acid (7) were found in the extract, which are structurally simple and widely distributed in plants. Normally, this kind of phenolic has antioxidant activity and interesting pharmacological properties, though their activity decreases due to their difficulty in penetrating cells because of their high polarity [5]. Other phenolic

compounds like rutin (4) hydrate, myricetin (5), and quercetin (6) have a long history of different pharmacological activities [6, 7, 8, 9]. Rutin or hydrated rutin, a flavanol, has demonstrated excellent antioxidant, anti-inflammatory, and anti-diabetic properties [10]. Myricetin (5) and quercetin (6) are representatives of flavonols subgroups, which have high prevalence in the plant kingdom, and are important components of a healthy diet [11]. Previous literatures explain that the greater the number of hydroxyl substituents present in the B ring Figure **9**; the compound can give the stronger the antioxidant properties [9]. Myricetin (5), which was identified in our sample, has the highest number of hydroxyl groups present, and it has evidence of antioxidant, anti-hyperglycemic, and renoprotective effects [12]. Quercetin (6), though it has one hydroxyl group absent in the B ring, also exhibits a similar kind of pharmacological effect like myricetin [13]. The HPLC and various antioxidant assay results indicate that phenolic and flavonoid compounds are key contributors to the antioxidant activity of the selected plant extract and its fraction. A significant positive correlation has been found in the crude extract of *Ficus racemosa*, and the ethyl acetate fraction due to the presence of phenolic compounds. Methanol fraction comes after ethyl acetate fraction, for this, the phenolic compound presence is at a minimum range, so as their antioxidant activity.

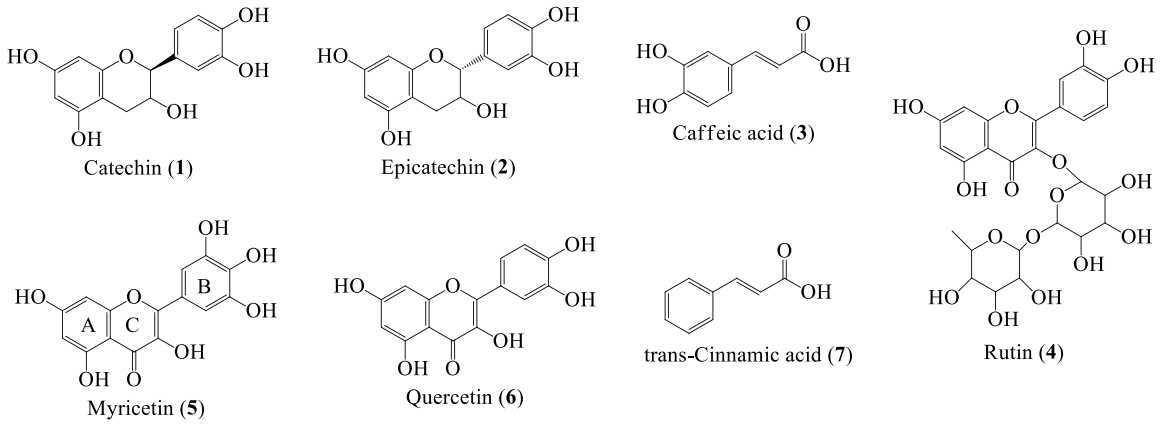

Figure 9: Phenolic compounds found in the ethanolic extract of *Ficus racemosa*.

Molecular docking studies revealed strong binding affinities of key bioactive compounds to oxidative stress-related enzymes. Quercetin (6) exhibited the highest binding affinity with inducible nitric oxide synthase (iNOS) at -9.5 kcal/mol, followed by caffeic acid (3) (-7.3 kcal/mol) and gallic acid (-5.8 kcal/mol). Given that docking scores below -6 kcal/mol indicate strong interactions [56, 57]. Quercetin's high affinity suggests a potential inhibitory role against iNOS, which is implicated in oxidative and inflammatory responses. These interactions, stabilized by hydrogen bonding and hydrophobic forces, suggest that the bioactive compounds in *Ficus racemosa* can inhibit enzyme activity, thereby reducing ROS and reactive nitrogen species (RNS) production, contributing to its therapeutic potential.

Overall, these findings provide strong evidence that *Ficus racemosa* exhibits significant antioxidant properties, with the ethyl acetate and methanol fractions showing the highest efficacy across different assays. The potent free radical scavenging, nitrosative stress mitigation, and enzyme inhibition activities highlight the potential application of these extracts in preventing oxidative stress-related diseases.

5. Conclusion:

The findings of this study reinforce the role *of Ficus racemosa* as a promising natural antioxidant source. Its rich composition of polyphenols, flavonoids, and other bioactive compounds contributes to significant free radical scavenging, nitrosative stress mitigation, and redox balance restoration. The strong antioxidant potential observed across various assays suggests its potential therapeutic application. These findings validate the traditional use of *Ficus racemosa* in folk medicine and position them as promising candidates for functional food development and therapeutic formulations. Given the concerns associated with synthetic antioxidants, the exploration of plant-derived alternatives like *Ficus racemosa* is crucial for developing safer and more effective

health-promoting interventions. The comparative analysis of antioxidant assays and docking results also suggests that *Ficus racemosa* extracts could serve as lead candidates for developing therapeutic agents targeting oxidative stress-related diseases.

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