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Research Article

Phytochemical Characterization, In-Vitro Antioxidant Activity, and Molecular Docking of Quercetin, Rutin and Apigenin

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Abstract

This study investigates the phytochemical characterization, *in-vitro* antioxidant activity, and molecular docking of natural flavonoids like Quercetin, Rutin, and Apigenin. FTIR analysis showed that each component possesses hydroxyl and carbonyl groups through the identification of specific functional groups that confirmed their structural nature. The results from phytochemical screening confirmed the presence of tannins and phenolics but also confirmed both flavonoids and flavanol glycosides and these findings were validated by fluorescence analysis. The total phenolic content analysis showed Quercetin possessed the greatest chemical content while Apigenin and Rutin followed with lower contents.

The strongest antioxidant performance detected in the in-vitro assays using DPPH and nitric oxide radical scavenging (NORSA) and reducing power tests showed Quercetin to have IC50 values of 9.64 µg/ml and 6.71 µg/ml respectively. The antioxidant functions of both Rutin and Apigenin proved to be robust in the assessments.

The binding ability of these flavonoids with Inducible Nitric Oxide Synthase (INOS) and xanthine oxidase proteins underwent molecular docking assessment to determine their binding affinity levels. The multiple hydroxyl groups in Quercetin enabled strong binding with proteins while Rutin and Apigenin followed behind.

Keywords: Quercetin, Rutin, Apigenin, Antioxidant Activity, Molecular Docking

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1. INTRODUCTION

Natural antioxidants have emerged as therapeutic agents because of the rising number of diseases connected to oxidative stress. The wide range of plant-derived polyphenolic compounds known as flavonoids continue to gain interest because of their potential to fight oxidative damage. Three active flavonoid compounds emerge as Quercetin and Rutin together with Apigenin because these substances possess both robust antioxidant functionality and several therapeutic possibilities including cardioprotective functions along with anti-inflammatory and neuroprotective effects. [1–3]

The damage from oxidative stress mainly results from Reactive Oxygen Species (ROS) which drive the evolution of chronic diseases including cancer cardiovascular issues and neurodegenerative conditions. [4,5] The protective function of natural antioxidants contributes to eliminating dangerous species that sustain harmful oxidative damage to cells. Experimental studies demonstrate that flavonoids become promising candidates for fighting oxidative stress because they possess electron-donation properties and stabilization functions for free radicals. [6,7]

Determining phytochemical contents together with measuring bioactive components' amounts helps experts evaluate the medicinal properties of natural substances. The compounds Quercetin Rutin and Apigenin contain high concentrations of flavonoid while showing various biological activities. The characterization of these flavonoids using FTIR spectroscopy together with quantitative phytochemical analysis improves their clinical effectiveness for therapeutic applications. [8]

The modern threat analysis can be complemented by molecular docking studies which provide fundamental insights about the molecular relationships between bioactive compounds and biological targets. The in-silico method provides predictions about how natural antioxidants function and targets specific stress-related enzymes through computational analyses. [9]

This research investigates Quercetin, Rutin, and Apigenin identification by FTIR and preliminary phytochemical screening and measures their secondary compound content alongside assessing their standard in vitro

antioxidant activity. A docking method evaluated the binding capacities and protein interactions of these target molecules toward oxidative stress regulatory proteins. This study's results will establish complete knowledge of antioxidant properties and structural features for these flavonoids which challenges future pharmacological examination.

2. MATERIAL AND METHOD

2.1 Material

All reagents were of analytical grade. Quercetin (≥ 95%), Rutin (≥ 99%), and Apigenin (≥ 98%) were purchased from Sigma-Aldrich and TCI Chemicals. Silymarin (≥ 98%) and Gentamicin sulfate (pharmaceutical grade) were obtained from Sigma-Aldrich and Himedia Laboratories, respectively. Potassium bromide (IR grade), Folin-Ciocalteu reagent, aluminium chloride (≥ 99%), ethanol (≥ 99.9%), and methanol (HPLC grade, ≥ 99.8%) were sourced from Merck and Loba Chemie. All chemicals were used following standard protocols to ensure reliability and reproducibility.

2.2 Identification of Quercetin, Rutin, and Apigenin Using FTIR Spectroscopy

To verify the structural authenticity of Quercetin, Rutin, and Apigenin as well as to analyze their functional groups researchers employed FTIR spectroscopy. FTIR spectroscopy uses molecular vibrations to detect infrared light absorptions at specific frequencies which create unique spectral signatures of each chemical compound. [10]

The flavonoid samples were mixed with potassium bromide (KBr) until they formed a properly prepared fine powder. The combination underwent compression into transparent pellets utilizing hydraulic press equipment. The measurement of FTIR spectra occurred over a wavelength scale between 4000–400 cm^{-1} by using an FTIR spectrometer manufactured by PerkinElmer. Analysis of functional group-specific peaks found in the compounds validated both the identity and purity through reference spectra comparison. [11]

2.3 Preliminary Screening of Flavonoid Samples

2.3.1 Qualitative Investigation

The qualitative investigation confirmed that the methanolic Apigenin, Quercetin and Rutin extracts contained essential phytochemical groups including flavonoids and tannins along with phenolics and flavanol glycosides. The initial screening method served critically to confirm phytochemical contents because basic and advanced characteristics of phytochemicals become undetectable in methanolic extracts. The standard testing procedure revealed the existence of specific phytochemical groups by generating characteristic colour indicators. The reaction between aluminum chloride and flavonoids protected their chemical structure which caused yellow color formation. [12,13] Two main reactions confirmed phytochemical components: Tannins caused a greenish-black color change with ferric chloride whereas the Folin-Ciocalteu reagent produced blue colorations as a sign of phenolic compounds. The identification of flavanol glycosides depended on their reaction with sodium nitroprusside which yielded a reddish color while adding sodium hydroxide. These tests produced positive findings which proved Apigenin, Quercetin, and Rutin contain substantial phytochemical content for quantitative and biological research progression. [14,15]

2.3.2 Fluorescence Analysis of Powdered Plant Material

A fluorescence analysis was performed on powdered Apigenin, Quercetin, and Rutin samples to detect their phytoconstituent components including flavonoids together with phenolic compounds. The analysts placed powdered samples onto clean watch glasses before reacting these samples with NaOH water solution and HCl and H_2SO_4 and HNO_3 and CH_3COOH and picric acid and $FeCl_3$. Each treated sample received analysis by visible light and by ultraviolet light that operated at 254 nm and 365 nm wavelengths. [16]

The laboratory analysis recorded different shining patterns for each test substance through its corresponding solution. Flavonoids were present in samples when they displayed bluish or greenish fluorescence under UV light examination. At the same time, a brownish-green colour appeared with $FeCl_3$ solution confirmed phenolic compounds. The researchers documented their findings under normal light and UV fluorescent light to gain significant information about the sample's phytochemical makeup. [17,18]

2.4 Quantitative Determination of Secondary Phytocomponents

2.4.1 Quantification of Total Phenolic Content (TPC)

The total phenolic content (TPC) of Apigenin Quercetin and Rutin was determined through the Folin-Ciocalteu technique at the wavelength of 765 nm. The presence of phenolic hydroxyl groups reduces phosphomolybdic and phosphotungstic acid complexes into blue chromophores that produce an absorbance at 765 nm wavelength. The intensity of light absorption correlates directly to phenolic substance levels in the solution. [19,20]

A standard solution of Gallic acid was designated. Research scientists prepared standard solutions of gallic acid at different concentrations from 0.2 ml to 1.0 ml to create a calibration curve. The prepared extract required 30 µl of solution which was mixed with Folin-Ciocalteu reagent together with sodium carbonate solution for each sample. The measurement occurred at 765 nm wavelength after the incubation time of 40 minutes at room temperature. The researchers stated their findings as milligrams of gallic acid equivalents (mg GAE) per gram of analyzed material. [21,22]

2.4.2 Quantification of Total Flavonoid Content (TFC)

The method measured Total Flavonoid content through an absorption-based colorimetric method that generated a flavonoid-aluminum chloride complex which produced an absorbance peak at 510 nm. Through this method,

researchers evaluate flavonoid quantities using the pink-coloured complex strength measurement. [23,24]

The analysis used quercetin as its standard compound. The laboratory created a calibration curve by using quercetin solutions spread between 10–100 µg/ml concentrations. The mixture of sample solution (0.5 ml) and sodium nitrite, aluminium chloride and sodium hydroxide solutions took place. And after consuming 15 minutes at room temperature the researchers measured the absorbance parameters at 510 nm. Results of TFC appeared as milligrams of quercetin equivalents per gram of sample material. [25]

2.4 Assessment of *In-Vitro* Antioxidant Activity of Flavonoid Samples

2.4.1 DPPH Radical Scavenging Activity

The DPPH (1,1-Diphenyl-2-picrylhydrazine) radical scavenging experiment was used to assess flavonoids' antioxidant potential. [26].This method assesses antioxidants' ability to neutralize free radicals by observing a colour change from deep violet to pale yellow when the DPPH radical is reduced. At 517 nm, the reaction was observed spectrophotometrically; a lower absorbance suggested a higher level of radical scavenging activity. Ascorbic acid served as the reference standard, while stock solutions of apigenin, quercetin, and rutin were prepared by dissolving them in ethanol. [27,28] To preserve pH stability, equal amounts of the flavonoid or standard solutions were combined with a 0.3 mM DPPH solution in ethanol and 0.1 M acetate buffer. For half an hour, the reaction mixtures were incubated at a low temperature in the dark. The IC50 values, which show the concentration needed to block 50% of DPPH radicals, were computed for each flavonoid after absorbance was measured at 517 nm. Of the flavonoids that were evaluated, quercetin showed the highest antioxidant activity, followed by rutin and apigenin. [29,30]

2.4.2 Nitric Oxide Radical Scavenging Activity

The nitric oxide radical scavenging activity was determined using sodium nitroprusside as the source of nitric oxide radicals. Flavonoid solutions of different concentrations were prepared and incubated with sodium nitroprusside in phosphate-buffered saline (pH 7.4) at 25°C for 150 minutes. [31,32] After incubation, the Griess reagent (sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride) was added to detect nitric oxide radicals, resulting in the formation of a pink chromophore. The intensity of the colour was measured at 546 nm. Ascorbic acid was used as the reference standard, and a dose-dependent increase in nitric oxide scavenging activity was observed. Quercetin showed the highest scavenging potential, followed by Rutin and Apigenin. [33,34]

2.4.3 Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity was evaluated using the Fenton reaction, which generates hydroxyl radicals by the interaction of hydrogen peroxide with ferrous ions. The degradation of 2-deoxyribose was used as a marker for hydroxyl radical activity, with malondialdehyde (MDA) formation indicating the extent of radical generation. [35,36] The reaction mixture containing the flavonoid samples, hydrogen peroxide, and ferrous sulfate was incubated, followed by the addition of thiobarbituric acid (TBA). The resulting pink chromophore was measured at 532 nm using a spectrophotometer. Ascorbic acid served as the standard. The percentage inhibition of hydroxyl radicals by flavonoids was calculated, with Quercetin showing the highest activity, followed by Rutin and Apigenin. [37,38]

2.4.4 Reducing Power Assay

The reducing power assay assessed the electron-donating ability of the flavonoids by measuring their capacity to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The flavonoid samples were prepared in phosphate buffer and mixed with potassium ferricyanide [39,40]. After incubation at 50°C, trichloroacetic acid was added to stop the reaction. The supernatant was mixed with distilled water and ferric chloride, resulting in the formation of a green-coloured complex measured at 700 nm. The reducing power was directly proportional to the absorbance, with higher absorbance indicating stronger antioxidant potential. Quercetin exhibited the highest reducing power, comparable to ascorbic acid, while Rutin and Apigenin showed moderate reducing power. [41]

2.5 Molecular Docking Study

Molecular docking was conducted to evaluate the antioxidant and nephroprotective potential of selected flavonoids and phenolic compounds by assessing their binding affinity with key renal injury biomarkers—NGAL, KIM-1, serine/threonine kinases, and NF-κB. Ligands such as quercetin, rutin, apigenin, kaempferol, caffeic acid, gallic acid, and ferulic acid were chosen for their known antioxidant effects, with quercetin and rutin serving as reference compounds. Protein structures were retrieved from the RCSB PDB database and prepared by optimizing their 3D conformations for accurate docking simulations. [42,43] Molecular docking was performed using virtual screening software, and the binding energies (kcal/mol) were calculated to predict interaction strength. Hydrogen bonding, Van der Waals forces, and other interactions were analyzed in 2D and 3D formats. Stronger binding (more negative binding energy) indicated higher antioxidant and nephroprotective potential. The results highlighted the ability of these phytoconstituents to interact with renal injury biomarkers, suggesting their therapeutic potential in mitigating nephrotoxicity. [44]

3. RESULT AND DISCUSSION

3.1 Identification of Apigenin, Quercetin, and Rutin Using FTIR Spectroscopy

The FTIR spectrum of Apigenin displayed characteristic absorption bands confirming its identity. A broad and intense peak at 3400 cm^{-1} corresponds to the stretching vibrations of hydroxyl groups (–OH), indicating the presence of phenolic hydroxyl groups typical of flavonoids. A significant peak at 1650 cm^{-1} represents the carbonyl group (C=O) stretching vibration of the flavone backbone, confirming the keto group. Additional peaks at 1500 cm^{-1} and 1450 cm^{-1} reflect aromatic ring

(C=C) stretching, while the 1300–1000 cm^{-1} region revealed ether bond (C–O) vibrations, consistent with Apigenin's known structure (Figure 1).

The FTIR spectrum of Quercetin showed similar characteristic peaks, aligning with its molecular structure. A strong broadband at 3400 cm^{-1} is indicative of the multiple phenolic hydroxyl groups in the molecule. The absorption peak at 1650 cm^{-1} corresponds to carbonyl (C=O) stretching in the flavonol ring. Peaks at 1500 cm^{-1} and 1450 cm^{-1} represent the aromatic (C=C) stretching, while the region between 1200–1000 cm^{-1} corresponds to ether linkages (C–O) and glycosidic bonds (C–O–C), confirming the structural integrity of Quercetin (Figure 2).

The FTIR spectrum of Rutin revealed a broad peak at 3400 cm^{-1}, reflecting multiple hydroxyl groups in its glycoside structure. A sharp peak at 1640 cm^{-1} was attributed to carbonyl (C=O) stretching in the flavonoid glycoside backbone. Peaks at 1500 cm^{-1} and 1450 cm^{-1} indicated aromatic ring (C=C) stretching, while strong bands between 1200–1000 cm^{-1} were due to glycosidic bonds (C–O–C), characteristic of Rutin's sugar moieties (Figure 3).

The FTIR spectra of Apigenin, Quercetin, and Rutin confirmed the presence of key functional groups, including hydroxyl groups, carbonyl groups, and glycosidic bonds, consistent with their chemical structures. These results validate the identity and purity of the flavonoids, ensuring their suitability for further pharmacological evaluations.

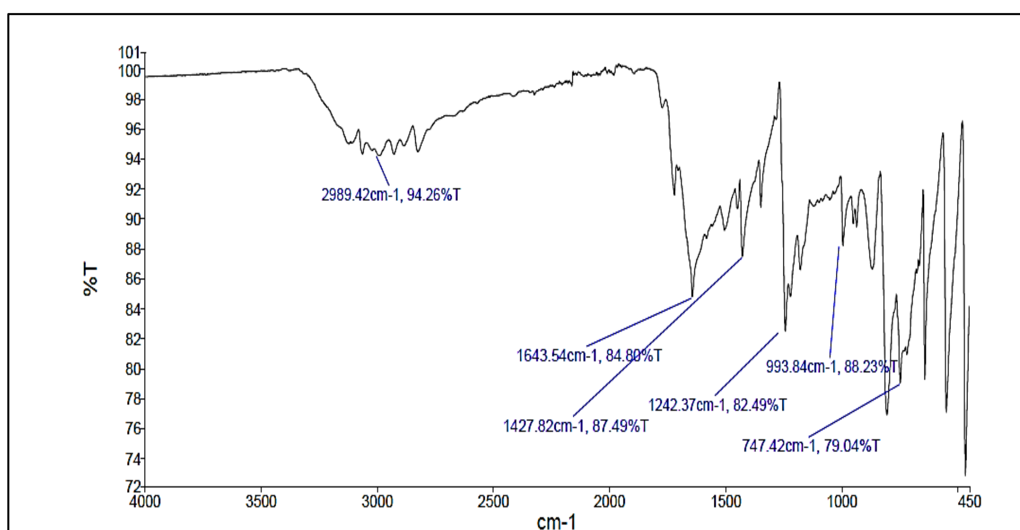

Figure 1: FTIR Spectrum of Apigenin

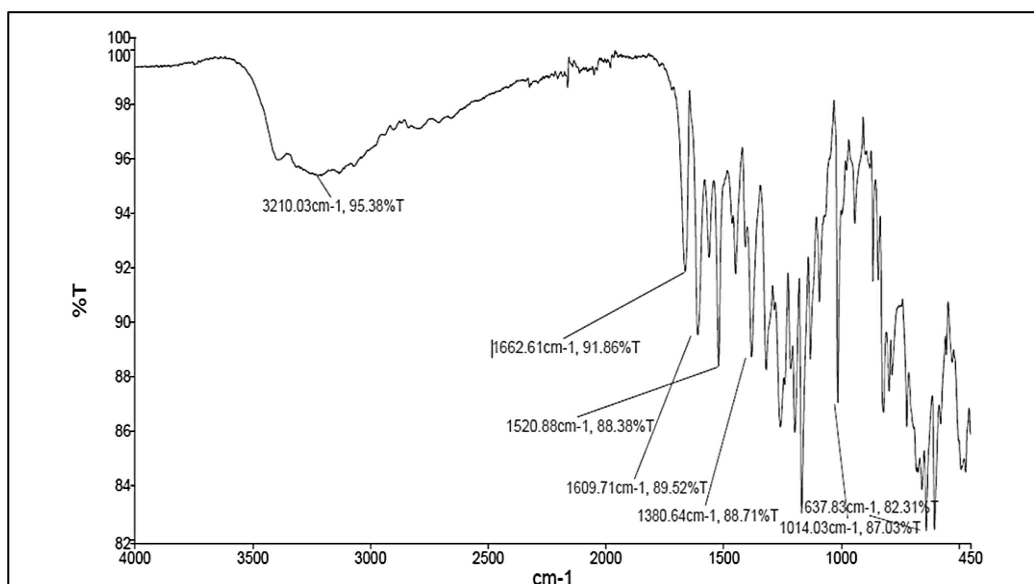

Figure 2: FTIR Spectrum of Quercetin

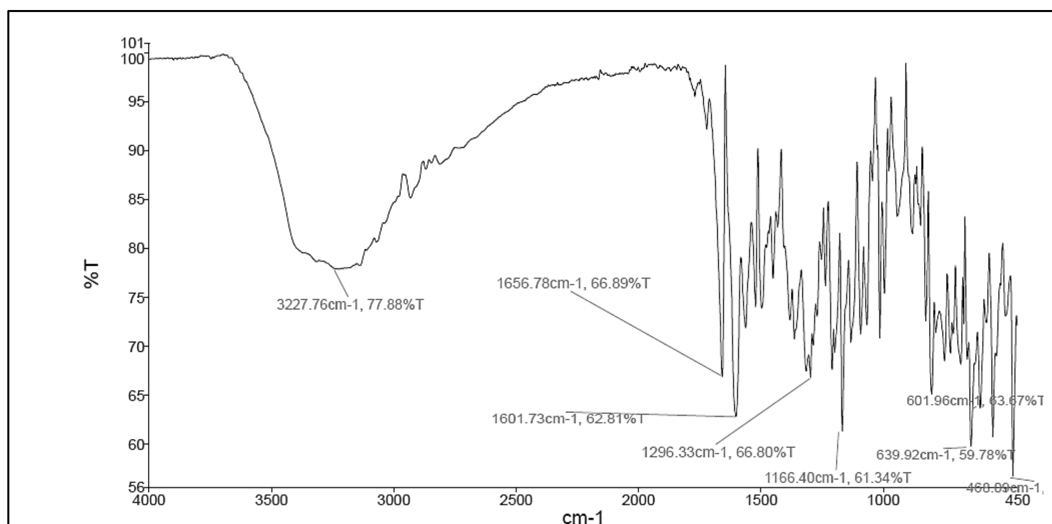

Figure 3: FTIR Spectrum of Rutin

3.2 Preliminary Screening of Flavonoid Samples

3.2.1 Qualitative Investigation

Qualitative phytochemical screening confirmed the presence of tannins, phenolic compounds, flavanol glycosides, and flavonoids in the flavonoid samples. The Ferric Chloride test showed a brown colour, indicating tannins, while a green colour confirmed gallotannins. The Lead Acetate test resulted in a white precipitate, verifying phenolic compounds, and the Gelatine test produced a white precipitate, further confirming phenolic content. The Magnesium and Hydrochloric Acid test for flavanol glycosides revealed a pink to scarlet colour, confirming their presence. These glycosides are indicative of antioxidant activity and potential therapeutic benefits.

Flavonoids were identified through several tests. The Shinoda test resulted in a reddish-pink colour, confirming flavonoid content. The Lead Acetate test produced a yellow precipitate, while the Alkaline Reagent test showed a deep yellow colour that disappeared upon acidification. The Sulfuric Acid test generated an orange-yellow colour, further confirming flavonoids. Furthermore, the Aqueous test developed a light-yellow colour, indicating good solubility and potential bioavailability. The results confirm the presence of key bioactive compounds, including flavonoids, phenolic compounds, and flavanol glycosides, which contribute to the antioxidant and therapeutic potential of the samples (Table 1).

Table 1: Qualitative Phytochemical Analysis of Flavonoid Samples

	Samples of quercetin						Samples of rutin		
Test Name									
Flavonoids test									
Shinoda	-	-	+	+		--	-	+	+
Lead acetate	-	-	+	+		-	-	+	+
Alkaline reagent test									
Sulphuric acid test									
Aqueous test									
Tannins and phenolic acid test									
FeCl3	-	-	+	+		-	-	+	+
Lead acetate	-	-	+	+		-	-	+	+
Gelatine	-	-	+	+		-	-	+	+
Flavanol glycosides test									
Flavanol glycosides	-	-	+	+		-	-	+	--
Terpenoid test									
Terpenes		- +	+	+		-	+	+	-
+ Present – Absent									

3.2.2 Fluorescence Analysis of Powdered Plant Material

The fluorescence analysis of quercetin and rutin samples under visible and ultraviolet (UV) light at 254 nm and 365 nm revealed distinct patterns indicating the presence of flavonoids, phenolics, and tannins. Both samples exhibited light green fluorescence under visible light, which darkened under UV exposure, confirming the presence of natural compounds.

Upon treatment with aqueous NaOH, the fluorescence shifted to greenish-blue under 365 nm UV light, while alkaline NaOH intensified the fluorescence, indicating phenolic groups. Acid treatments resulted in varied responses. The samples turned fluorescent green with 1 N HCl, suggesting flavonoid stability under acidic conditions. In contrast, treatment with 50% H_2SO_4 produced dark greenish-brown fluorescence, highlighting the presence of tannins with extensive hydroxylation.

Fluorescence intensified with 50% HNO_3, producing a blue-green colour under UV, typical of flavonoids and tannins. Picric acid treatment led to a characteristic yellowish-green fluorescence under UV light. Acetic acid showed a stable dark green fluorescence in both visible and UV light, suggesting tannin content. Ferric chloride treatment produced brownish-green fluorescence at 254 nm and greenish-blue at 365 nm, further indicating phenolic compounds. These distinct fluorescence patterns confirm the presence of key bioactive components, supporting their potential therapeutic applications and forming a basis for further quantitative analysis (Tables 2 and 3).

Table 2: Fluorescence characteristic analysis of *quercetin sample*

Sr. No.	Plant Sample + Reagents	UV light		Visible light
		254 nm (Short)	365 nm (Long)	
1.	Sample powder	Light green	Dark green	Light green
2.	Sample Powder + aq. NaOH	Light green	Greenish blue	Light green
3.	Sample Powder + alkaline NaOH	Dark green	Dark green	Dark green
4.	Sample Powder + 1 N HCL	Green	Fluorescent green	Green
5.	Sample Powder + 50 % H_2SO_4	Dark greenish brown	Green	Dark brown
6.	Sample Powder + 50 % HNO_3	Dark green	Blue-green	Dark green
7.	Sample Powder + picric acid (C6H3N3O6)	Greenish yellow	Yellowish green	Green
8.	Powder + CH_3COOH	Dark green	Black green	Dark Green
9.	Powder + $FeCl_3$	Brownish Green	Greenish blue	Light green
10.	Powder + HNO_3+ NH_3	Dark green	Dark green	Green

Table 3: Fluorescence characteristic analysis of raw powder of *rutin sample*

Sr. No.	Plant Sample + Reagents	UV light		Visible light
		Short 254 nm	Long 365 nm	
1.	Sample powder	Light green	Green	Light green
2.	Sample Powder + aq. NaOH	Light green	Blueish Green	Light green
3.	Sample Powder + alkaline NaOH	Pale green	Pale green	Green
4.	Sample Powder + 1 N HCL	Dark green	Blue-green	Dark brown
5.	Sample Powder + 50 % H_2SO_4	Dark greenish brown	Green	Dark brown
6.	Sample Powder + 50 % HNO_3	Black green	Blue-green	Green
7.	Sample Powder + picric acid (C6H3N3O6)	Yellow-green	Yellow-green	Green
8.	Powder + CH_3COOH	Dark green	Dark green	Dark Green
9.	Powder + $FeCl_3$	Brownish Green	Greenish blue	Light green
10.	Powder + HNO_3 + NH_3	Dark green	Dark green	Green

3.2.3 Quantitative Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The total phenolic content (TPC) and total flavonoid content (TFC) of Apigenin, Quercetin, and Rutin samples were quantified, revealing significant amounts of bioactive compounds.

For Apigenin, TPC ranged from 8.21 ± 0.27 mg GAE/g to 16.74 ± 0.55 mg GAE/g, while TFC values varied from 9.12 ± 0.19 mg QE/g to 14.63 ± 0.41 mg QE/g, with the highest content observed. In Quercetin, TPC values ranged from 9.39 ± 0.42 mg GAE/g to 21.87 ± 0.84 mg GAE/g, and TFC values from 11.04 ± 0.15 mg QE/g to 18.78 ± 0.57 mg QE/g. For Rutin, TPC ranged from 7.59 ± 0.16 mg GAE/g to 17.38 ± 0.37 mg GAE/g, and TFC values from 7.05 ± 0.26 mg QE/g to 15.88 ± 0.19 mg QE/g, with Sample showing the highest levels (Figure 4).

Overall, Quercetin exhibited the highest phenolic and flavonoid content, followed by Apigenin and Rutin. These findings highlight the antioxidant potential of these flavonoids, reinforcing their therapeutic relevance in oxidative stress management and related health benefits.

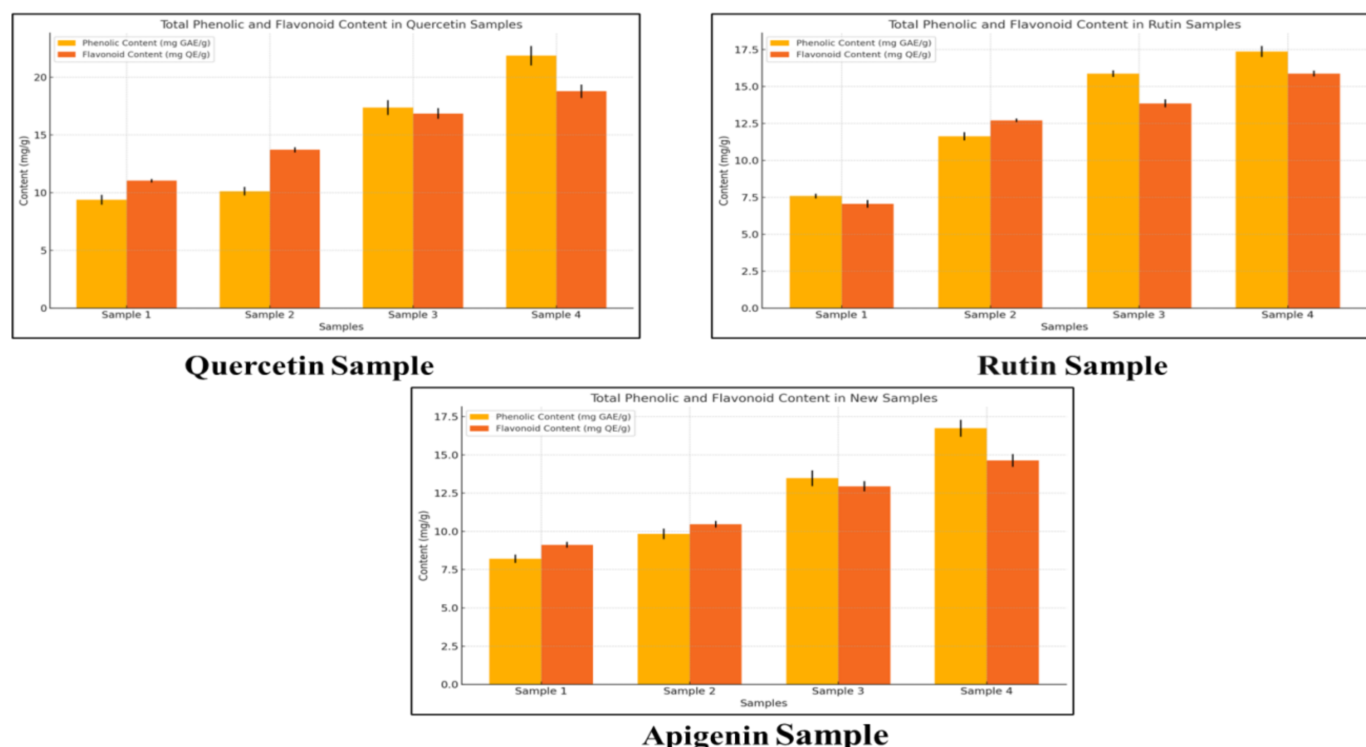

Figure 4: Total Phenolic (TPC) and Flavonoid Content (TFC) in Quercetin, *Rutin, and* Apigenin Sample

3. 3 Assessment of *In-Vitro* Antioxidant Activity of Flavonoid Samples

3.3.1 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity assay evaluated the antioxidant potential of Quercetin, Rutin, and Apigenin, with ascorbic acid as the reference standard. The assay quantified the capacity of these compounds to neutralize DPPH radicals by measuring the decrease in absorbance at 517 nm. The percentage of radical scavenging activity (RSA) and IC50 values were calculated to assess the antioxidant potency.

Quercetin showed the highest antioxidant activity, achieving 87.71% inhibition at 100 µg/ml with an IC50 value of 9.64 µg/ml, closely matching ascorbic acid (IC50 = 8.91 µg/ml). Rutin exhibited moderate activity, with 44.21% inhibition at 100 µg/ml and an IC50 value of 9.168 µg/ml, whereas Apigenin demonstrated comparatively weaker antioxidant activity with 44.21% inhibition at 100 µg/ml and an IC50 value of 9.168 µg/ml (Figure 5).

The antioxidant activity of the flavonoids was dose-dependent, with Quercetin consistently outperforming Rutin and Apigenin, indicating its strong electron-donating ability due to the presence of multiple hydroxyl groups.

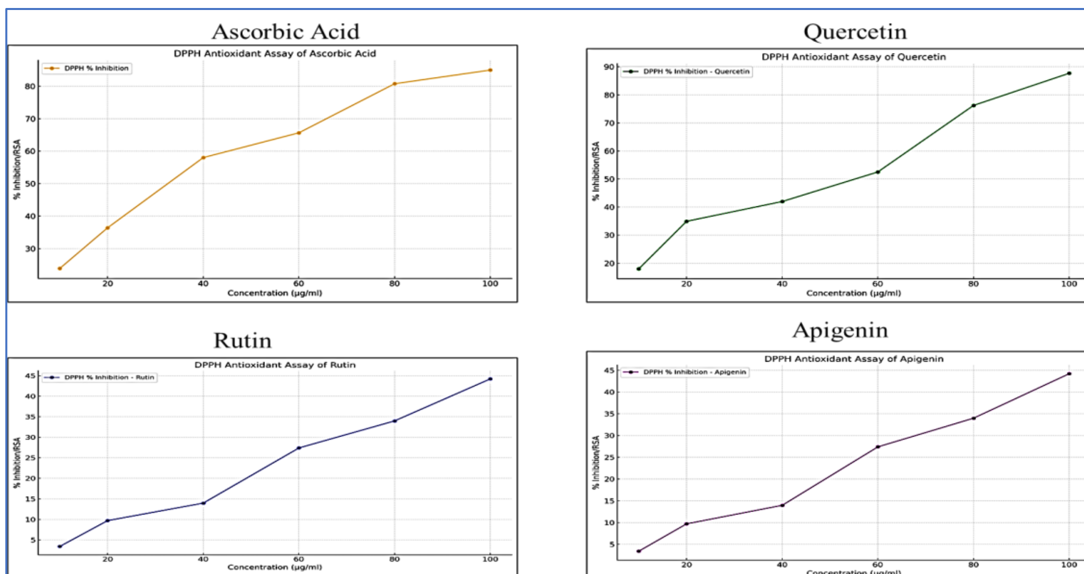

Figure 5: DPPH Antioxidant Assay Curves for Ascorbic Acid, Quercetin, Rutin, and Apigenin, showing % Radical Scavenging Activity (RSA) at various concentrations (µg/ml).

3.3.2 Nitric Oxide Radical Scavenging Activity (NORSA) of ascorbic acid sample

Nitric oxide (NO) plays a crucial role in physiological processes; however, its overproduction contributes to oxidative stress, inflammation, and tissue damage. The nitric oxide radical scavenging activity (NORSA) assay was employed to evaluate the antioxidant potential of Quercetin, Rutin, and Apigenin, with ascorbic acid serving as the standard.

Quercetin demonstrated the strongest nitric oxide scavenging activity, achieving 71.11% inhibition at 100 µg/ml with an IC50 value of 6.71 µg/ml. This superior performance can be attributed to the presence of multiple hydroxyl groups, enhancing its electron-donating capacity and radical stabilization potential. Rutin showed substantial activity, with 80.35% inhibition at 100 µg/ml and an IC50 value of 1.43 µg/ml. Despite its glycosidic structure, which may reduce radical scavenging efficiency compared to Quercetin, Rutin's activity remains significant. Apigenin exhibited the lowest nitric oxide scavenging activity among the tested flavonoids, with 97.31% inhibition at 100 µg/ml and an IC50 value of 2.43 µg/ml. Although less potent due to its fewer hydroxyl groups, Apigenin still demonstrated considerable antioxidant capacity. Ascorbic acid provided a benchmark with 76.31% inhibition at 100 µg/ml and an IC50 value of 9.12 µg/ml, confirming its well-documented role as a potent antioxidant.

The results indicate that all tested flavonoids possess notable nitric oxide radical scavenging activity, with Quercetin emerging as the most effective, closely followed by Rutin and Apigenin. This dose-dependent response underscores the therapeutic potential of these natural antioxidants in mitigating nitric oxide-induced oxidative stress and preventing related pathologies.

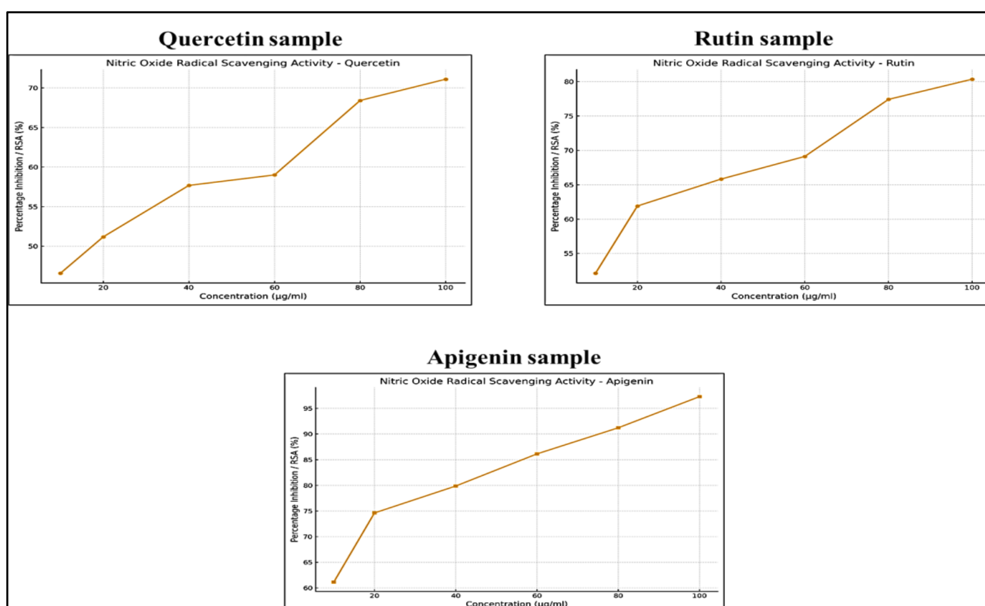

Figure 6: Nitric Oxide Radical Scavenging Activity (NORSA) of Quercetin, Rutin, Apigenin sample

3.2.3 Reducing Power Assay (RPA)

The reducing power assay was used to assess the electron-donating capacity of the flavonoids by measuring the reduction of Fe^{3+} to Fe^{2+}. This activity correlates with the antioxidant potential of the compounds, as higher reducing power reflects a greater ability to neutralize free radicals.

Quercetin exhibited the strongest reducing power among the tested flavonoids. At 100 µg/ml, it showed an absorbance of 1.73 ± 0.42 at 700 nm, with 51.20% inhibition and an IC50 of 6.71 µg/ml. The dose-dependent increase in absorbance highlights its efficient electron-donating capacity, confirming its robust antioxidant activity. Rutin demonstrated moderate reducing power compared to Quercetin, with an absorbance of 1.88 ± 0.039 at 100 µg/ml and 45.83% inhibition. The IC50 value for Rutin was 8.51 µg/ml, indicating slightly lower electron-donating ability. The results suggest that Rutin retains significant antioxidant properties, though less potent than Quercetin due to its glycosidic structure. Apigenin exhibited the lowest reducing power, with an absorbance of 1.89 ± 0.039 at 100 µg/ml and 45.83% inhibition, like Rutin. Its IC50 value was 8.51 µg/ml. The lower reducing power of Apigenin is attributed to its structural features, such as fewer hydroxyl groups, reducing its capacity to donate electrons.

The reducing power assay confirmed that Quercetin has the highest antioxidant potential, followed by Rutin and Apigenin (Table 4). The structural differences among these flavonoids, including the number and position of hydroxyl groups, significantly influence their electron-donating ability. The dose-dependent increase in absorbance for all samples reinforces their antioxidant efficacy and therapeutic relevance in oxidative stress management.

Table 4: Reducing power assay of Quercetin, Rutin, Apigenin

Sr. No.	Conc. (µg/ml)	Absorbance at 700 nm	% Inhibition/ RSA	IC50 (µg/ml)
Quercetin				
1	10	0.79±0.041	25.01	6.709018 (Quercetin)
2	20	0.86±0.32	27.16049	16.70902 (Quercetin)
3	40	1.13±0.045	35.92233	36.70902 (Quercetin)
4	60	1.46±0.033	44.02985	56.70902 (Quercetin)
5	80	1.59±0.044	46.62162	76.70902 (Quercetin)
6	100	1.73±0.42	51.20482	96.70902 (Quercetin)
Rutin				
1	10	0.66±0.043	17.10	8.51 (Rutin)
2	20	0.74±0.047	19.04	18.51 (Rutin)
3	40	1.02±0.052	26.47	38.51 (Rutin)
4	60	1.43±0.056	30.08	58.51 (Rutin)
5	80	1.69±0.049	39.45	78.51 (Rutin)
6	100	1.88±0.039	45.83	98.51 (Rutin)
		Apigenin		
1	10	0.96±0.043	17.10	8.51 (Apigenin)
2	20	0.84±0.047	19.04	18.51 (Apigenin)
3	40	1.01±0.052	26.47	38.51 (Apigenin)
4	60	1.33±0.056	30.08	58.51 (Apigenin)
5	80	1.59±0.049	39.45	78.51 (Apigenin)
6	100	1.89±0.039	45.83	98.51 (Apigenin)

3.4 Molecular Docking Study

3.4.1 Binding Affinity Analysis

The molecular docking study revealed strong interactions of quercetin, rutin, and apigenin with key renal injury biomarkers NF-κB, KIM-1, and NGAL. The binding affinity scores indicated potential renoprotective effects through the modulation of inflammation and oxidative stress pathways. Quercetin exhibited the highest binding affinity for KIM-1 (-8.4 kcal/mol) and NGAL (-8.5 kcal/mol), suggesting its strong potential to mitigate oxidative stress and stabilize these protein targets. The interaction with NF-κB (-7.8 kcal/mol) was characterized by multiple hydrogen bonds, enhancing molecular stability.

3.4.2 Rutin and Apigenin Interactions

Rutin demonstrated a significant binding affinity with NF-κB (-8.3 kcal/mol) and NGAL (-8.1 kcal/mol), supporting its role in reducing inflammation and oxidative damage. Although the binding affinity with KIM-1 was moderate (-3.1 kcal/mol), rutin's overall interaction profile indicates antioxidant potential. Apigenin showed notable binding with NGAL (-8.3 kcal/mol), stabilized by hydrogen bonding and Van der Waals forces, while interactions with NF-κB (-7.6 kcal/mol) and KIM-1 (-7.9 kcal/mol) reinforce its anti-inflammatory and renoprotective activity.

3.4.3 Visualization of Docking

The molecular interactions of quercetin, rutin, and apigenin with the target proteins NF-κB, KIM-1, and NGAL were visualized through 2D and 3D docking images, providing insights into the nature of their binding interactions. These visual representations highlight the molecular-level interactions that contribute to the antioxidant and anti-inflammatory properties of the flavonoids. The docking visualization of quercetin with NF-κB (Figure 5) reveals strong binding interactions, primarily facilitated by hydrogen bonding and hydrophobic contacts, which stabilize the quercetin-protein complex. Rutin's interactions with KIM-1 and NGAL (Figure 6) indicate stabilizing effects within the protein-binding pockets, suggesting its potential to attenuate oxidative damage. Similarly, apigenin's binding with NGAL (Figure 7) demonstrates significant hydrogen bonding and Van der Waals forces, enhancing the stability of the complex and contributing to its antioxidant activity.

The molecular docking results confirm that quercetin, rutin, and apigenin exhibit strong binding affinities and stable interactions with key renal injury biomarkers. These interactions align with their proposed antioxidant and anti-inflammatory effects, supporting their role in mitigating renal damage and promoting cellular homeostasis. Table 5 summarizes the binding affinity scores for the flavonoids with the respective target proteins, further reinforcing the molecular evidence for their renoprotective potential.

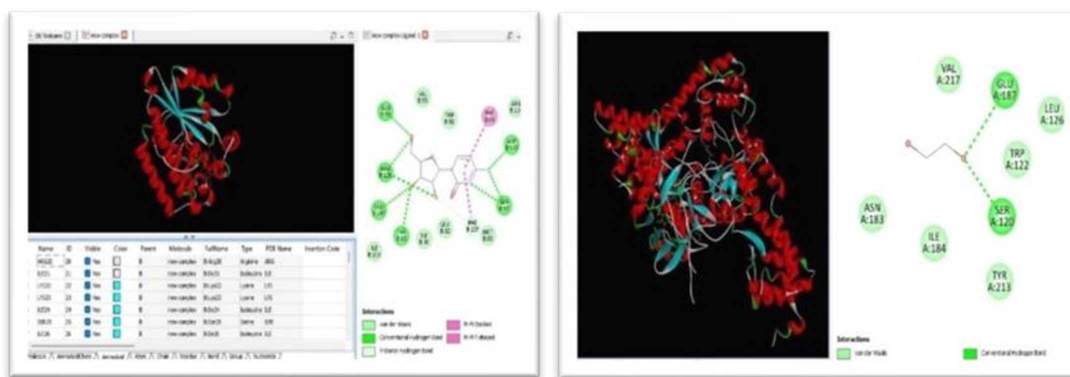

Figure 7: 3D and 2D docking interactions of quercetin with NF-κB, illustrating strong binding facilitated by hydrogen bonds and hydrophobic interactions.

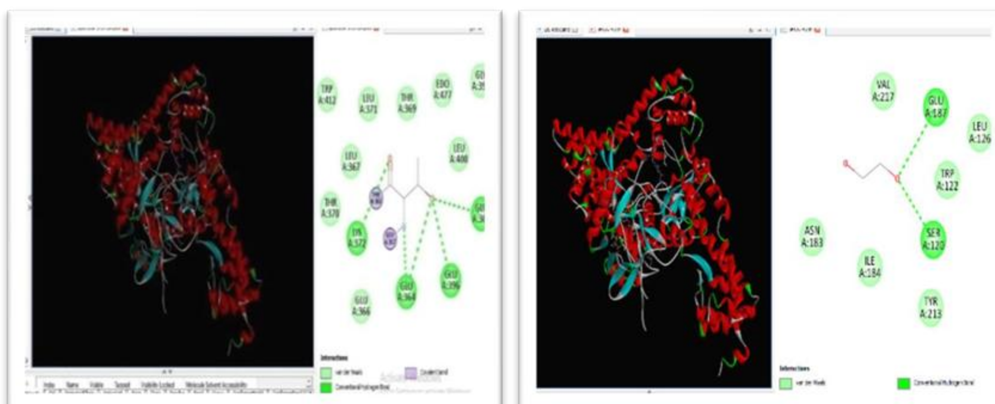

Figure 8: 3D and 2D docking interactions of rutin with KIM-1/NGAL molecules, highlighting the flavonoid's stabilizing interactions with protein pockets associated with inflammation.

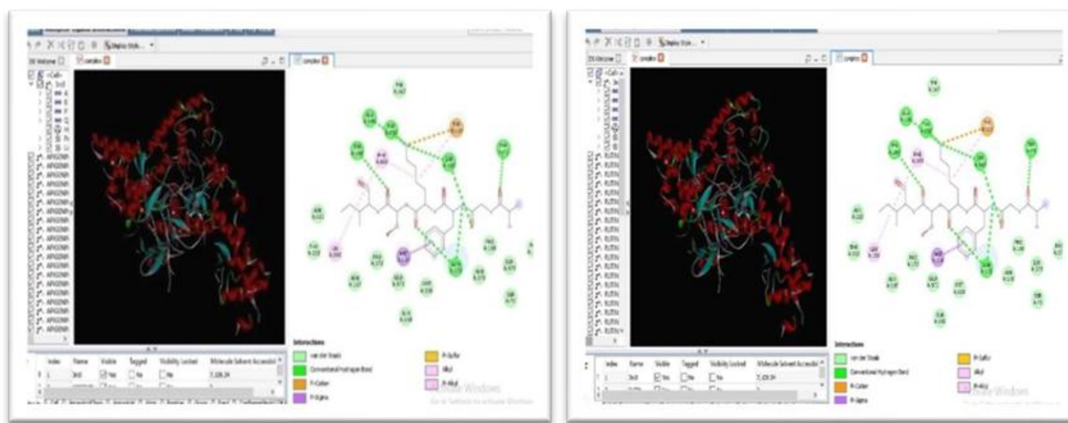

Figure 9: 3D and 2D docking interactions of apigenin with NGAL, showing significant bonding interactions, which may contribute to its antioxidant effects.

Table 5: Binding affinity score of ligands and target proteins/enzymes.

Sr. No.	Ligand Name	PubChem ID	Target protein molecules/ enzymes Binding affinity kcal/mol			
			NF-κB	KIM-1	NGAL	Serine kinase
1.	Quercetin	5280343	-7.8	-8.4	-8.5	--
2.	Rutin	5280805	-8.3	-3.1	-8.1	--
3.	Apigenin	5280443	-7.6	-7.9	-8.3	--

CONCLUSION

In conclusion, this study provides strong evidence for the therapeutic potential of quercetin, rutin, and apigenin. The phytochemical characterization confirmed their purity and structural identity, ensuring the reliability of subsequent analyses. The in vitro antioxidant assays demonstrated that these flavonoids possess remarkable free radical scavenging activity, particularly against DPPH, hydroxyl, and nitric oxide radicals, highlighting their ability to combat oxidative stress effectively.

The molecular docking studies indicate revealing significant binding interactions with key biomarkers associated with renal injury, such as NF-κB, KIM-1, and NGAL. Among the tested flavonoids, quercetin exhibited the highest binding affinity, suggesting a stronger potential to modulate oxidative stress and inflammatory responses. Rutin and apigenin also demonstrated stable interactions, supporting their antioxidant and renoprotective roles at the molecular level.

These findings indicate that quercetin, rutin, and apigenin are promising candidates for managing oxidative stress-related diseases. In future *in-vivo* studies and clinical trials will be crucial in translating these findings into therapeutic applications, paving the way for natural flavonoids as potential alternatives in oxidative stress and inflammation management.

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Abbreviations

NF-κB (Nuclear Factor Kappa B), **KIM-1** (Kidney Injury Molecule-1), **NGAL** (Neutrophil Gelatinase-Associated Lipocalin), **ROS** (Reactive Oxygen Species), **DPPH** (2,2-Diphenyl-1-Picrylhydrazyl), **ABTS** (2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid), **FRAP** (Ferric Reducing Antioxidant Power), **NO** (Nitric Oxide), **IC50** (Half-Maximal Inhibitory Concentration).

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