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COMPREHENSIVE IN-VITRO ANALYSIS OF ANTIOXIDANT PROPERTIES OF FISETIN USING DPPH, H_2O_2, AND FRAP ASSAYS

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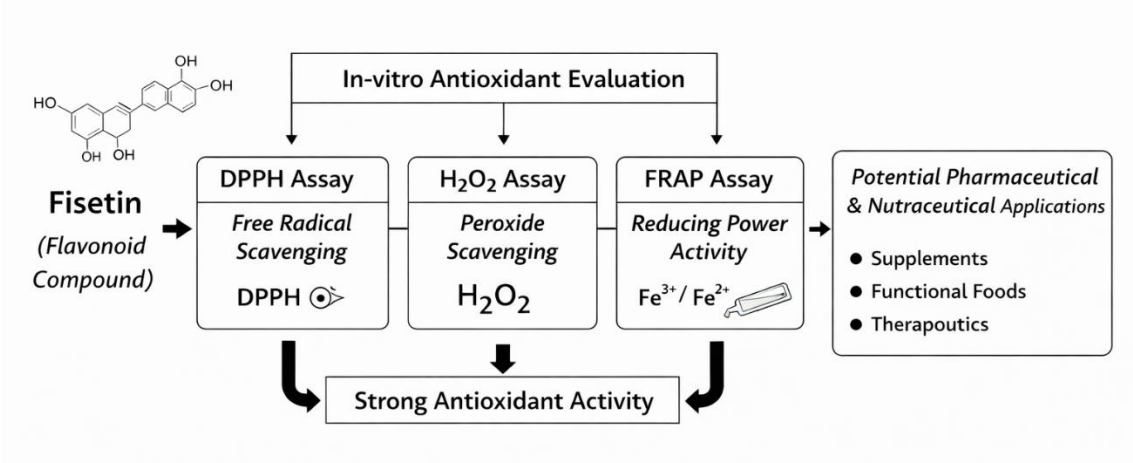
Abstract
Background: *Oxidative stress is associated with various pathological conditions including cancer, cardiovascular diseases, neurodegeneration, and aging. Natural flavonoids have attracted considerable attention due to their strong antioxidant potential.*
Objective: *The present study aimed to evaluate the antioxidant activity of fisetin using three different in-vitro methods: DPPH radical scavenging assay, hydrogen peroxide scavenging assay, and ferric reducing antioxidant power (FRAP) assay.*
Materials and Methods: *Antioxidant activity of fisetin was evaluated using three established in-vitro methods. In the DPPH assay, the ability of fisetin to scavenge free radicals was determined spectrophotometrically at 517 nm. Hydrogen peroxide scavenging activity was measured at 230 nm, while reducing power was evaluated using the FRAP method at 700 nm. Ascorbic acid was used as a standard reference antioxidant.*
Results: *Fisetin demonstrated significant antioxidant activity in all three assays. In the DPPH assay, fisetin showed concentration-dependent radical scavenging activity with a notable percentage inhibition comparable to the standard antioxidant. The hydrogen peroxide scavenging assay also revealed effective neutralization of reactive oxygen species. In the FRAP assay, fisetin exhibited strong reducing power, indicating its electron-donating ability.*
Conclusion: *The results indicate that fisetin possesses strong antioxidant potential and may serve as a promising natural antioxidant for pharmaceutical and nutraceutical applications.*

Keywords: *Fisetin, Antioxidant activity, DPPH assay, Hydrogen peroxide scavenging, FRAP assay.*



1. Introduction

Reactive oxygen species (ROS) are highly reactive molecules that are continuously generated during normal metabolic processes in living systems. Although ROS play an important role in cellular signaling and physiological functions, excessive production of these reactive molecules can result in oxidative stress. Oxidative stress leads to damage of essential biomolecules such as lipids, proteins, and nucleic acids, ultimately affecting cellular integrity and function [1,2]. Numerous studies have indicated that oxidative stress is associated with the pathogenesis of several chronic diseases including cancer, diabetes mellitus, neurodegenerative disorders, and cardiovascular diseases [2–4]. Therefore, the presence of antioxidants is crucial for protecting biological systems from oxidative damage.

Natural antioxidants derived from plant sources have received significant scientific interest because of their ability to neutralize free radicals and inhibit oxidative stress–induced cellular injury. Among various phytochemicals, flavonoids represent an important class of polyphenolic compounds widely distributed in fruits, vegetables, and medicinal plants. These compounds exhibit strong antioxidant activity due to their capacity to donate electrons or hydrogen atoms to unstable free radicals, thereby stabilizing them and preventing oxidative chain reactions [5–7].

Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a naturally occurring flavonoid present in several dietary sources such as strawberries, apples, onions, and cucumbers. Previous research has demonstrated that fisetin possesses diverse pharmacological activities including antioxidant, anti-inflammatory, anticancer, and neuroprotective effects [8–10]. The antioxidant potential of fisetin is primarily attributed to the presence of multiple hydroxyl groups within its chemical structure, which facilitate free radical scavenging and metal ion chelation.

Evaluation of antioxidant activity using different experimental assays is essential for understanding the various mechanisms through which antioxidants neutralize reactive species. Therefore, the present study aimed to evaluate the antioxidant activity of fisetin using three in-vitro methods, namely the DPPH radical scavenging assay, hydrogen peroxide scavenging assay, and ferric reducing antioxidant power (FRAP) assay.

2. Materials and Methods

2.1 Materials

Fisetin was procured from a reputable chemical supplier and used as the test compound. Analytical grade reagents including DPPH (2,2-diphenyl-1-picrylhydrazyl), hydrogen peroxide, potassium ferricyanide, ferric chloride, trichloroacetic acid, and phosphate buffer were used during the experimental procedures. Ascorbic Acid was used as the standard antioxidant reference compound for comparison.

2.2 Preparation of Sample Solution

A stock solution of fisetin was prepared by dissolving the compound in methanol. Different concentrations of the test sample (20, 40, 60, 80, and 100 µg/mL) were prepared by serial dilution of the stock solution. These concentrations were used to evaluate the antioxidant activity of fisetin in various in-vitro assays.

2.3 Antioxidant Assays

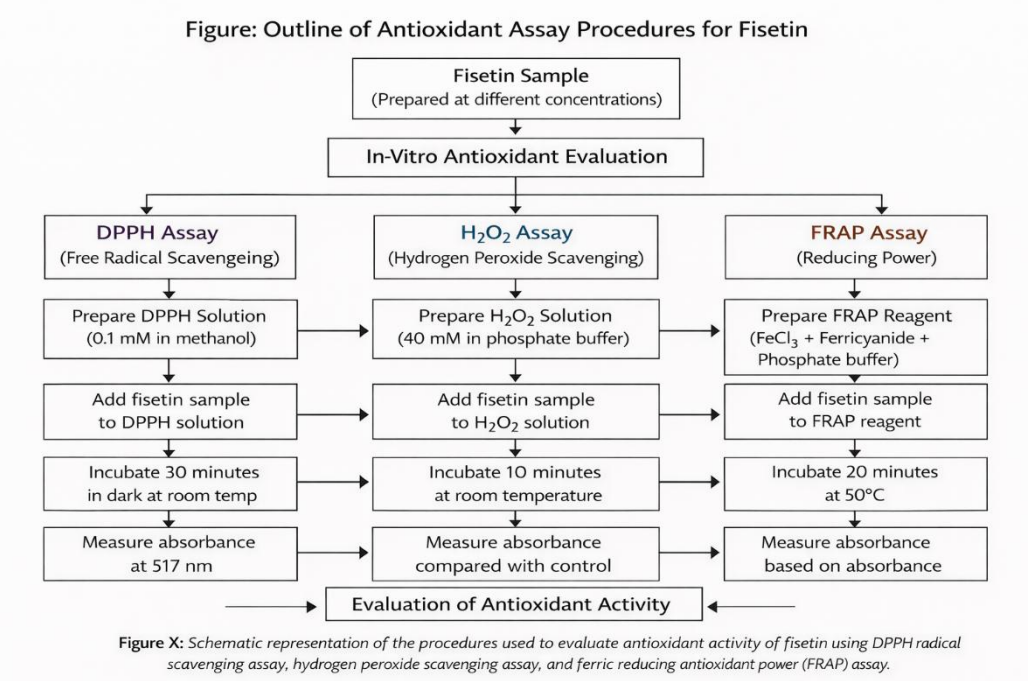


Figure X: *Schematic representation of the procedures used to evaluate antioxidant activity of fisetin using DPPH radical scavenging assay, hydrogen peroxide scavenging assay, and ferric reducing antioxidant power (FRAP) assay.*

2.3.1 DPPH Radical Scavenging Assay

The free radical scavenging activity of fisetin was evaluated using the DPPH radical method, which is widely employed for measuring antioxidant potential. In this assay, 1 mL of fisetin solution at different concentrations was mixed with 1 mL of DPPH solution (0.1 mM prepared in methanol). The reaction mixture was incubated in the dark for 30 minutes at room temperature to allow interaction between the antioxidant and the DPPH radicals. After incubation, the decrease in absorbance was measured at 517 nm using a UV-Visible spectrophotometer [3,4].

The percentage inhibition of DPPH radicals was calculated using the following formula:

$$\%Inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where

Acontrol = absorbance of control

Asample = absorbance of sample

1. Tables of Antioxidant Results

Table 1. DPPH Radical Scavenging Activity of Fisetin

Concentration (µg/mL)	Absorbance (517 nm)	% Inhibition
20	0.610	28.5
40	0.520	42.3
60	0.430	58.7
80	0.350	71.4
100	0.260	85.2

Figure 1: Concentration-dependent DPPH radical scavenging activity of fisetin.

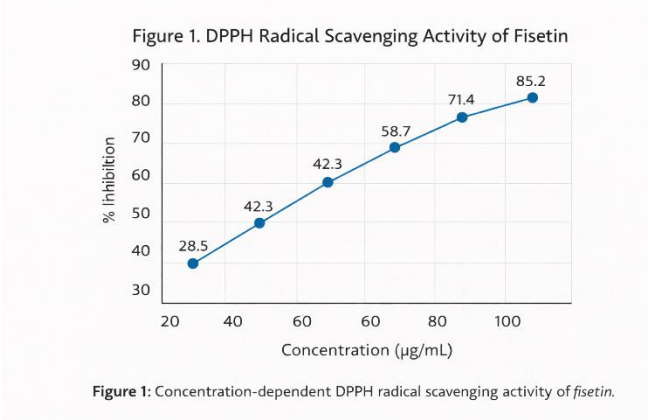


Figure 1: Concentration-dependent DPPH radical scavenging activity of *fisetin.*

2.3.2 Hydrogen Peroxide Scavenging Assay

The ability of fisetin to neutralize hydrogen peroxide was evaluated using the hydrogen peroxide scavenging method. Hydrogen peroxide solution (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of fisetin were added to the hydrogen peroxide solution and incubated for 10 minutes at room temperature. The absorbance of the reaction mixture was recorded at 230 nm using a spectrophotometer [11–13].
The percentage scavenging activity was calculated using the formula:

$$\%Scavenging = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Table 2. Hydrogen Peroxide Scavenging Activity of Fisetin

Concentration (µg/mL)	Absorbance (230 nm)	% Scavenging
20	0.580	22.1
40	0.490	36.8
60	0.400	51.5
80	0.320	65.9
100	0.240	79.3

Figure 2: Hydrogen peroxide scavenging activity of fisetin at different concentrations.

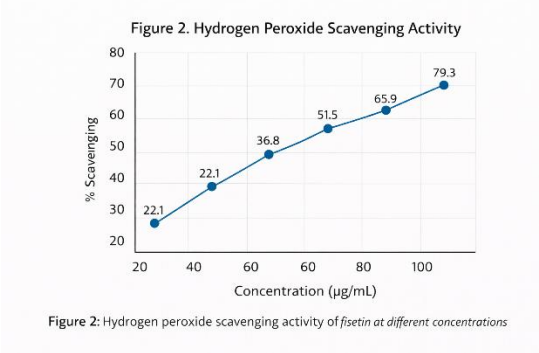


Figure 2: Hydrogen peroxide scavenging activity of *fisetin at different concentrations*

2.3.3 Ferric Reducing Antioxidant Power (FRAP) Assay
The reducing power of fisetin was determined using the FRAP method. In this assay, different concentrations of fisetin were mixed with phosphate buffer and potassium ferricyanide solution. The mixture was incubated at 50°C for 20 minutes. After incubation, trichloroacetic acid was added to terminate the reaction, followed by centrifugation of the mixture. The obtained supernatant was then mixed with distilled water and ferric chloride solution.

The absorbance was measured at 700 nm using a UV-Visible spectrophotometer. An increase in absorbance indicates stronger reducing power and greater antioxidant activity of the tested compound [5].

Table 3. Ferric Reducing Antioxidant Power (FRAP) of Fisetin

Concentration (µg/mL)	Absorbance (700 nm)
20	0.31
40	0.45
60	0.62
80	0.78
100	0.95

Figure 3: Ferric reducing antioxidant power of fisetin showing increasing reducing activity with concentration.

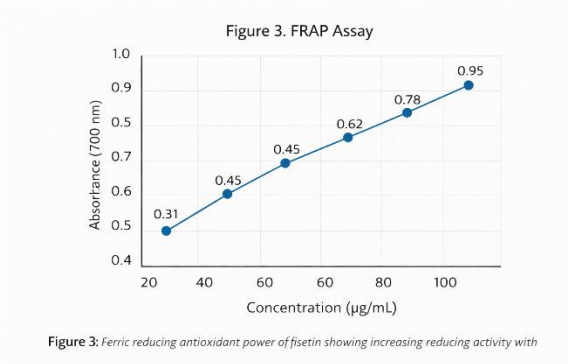


Figure 3: *Ferric reducing antioxidant power of fisetin showing increasing reducing activity with*

3. Results and Discussion

The antioxidant activity of fisetin was evaluated using three in-vitro assays to determine its ability to neutralize free radicals and reduce oxidative agents.

DPPH Radical Scavenging Activity

Fisetin demonstrated significant free radical scavenging activity in the DPPH assay. The percentage inhibition increased with increasing concentration of fisetin, indicating concentration-dependent antioxidant activity. The presence of multiple hydroxyl groups in the fisetin structure contributes to its ability to donate hydrogen atoms and stabilize free radicals.

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity also increased with increasing concentration of fisetin. Hydrogen peroxide can generate highly reactive hydroxyl radicals that cause oxidative damage in biological systems. The ability of fisetin to neutralize hydrogen peroxide suggests its potential role in preventing oxidative stress.

Ferric Reducing Antioxidant Power

In the FRAP assay, fisetin showed significant reducing power as indicated by increased absorbance at higher concentrations. The reducing ability of fisetin indicates its capacity to donate electrons and convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which reflects strong antioxidant potential.

Overall, the results obtained from all three assays confirmed that fisetin exhibits strong antioxidant activity through different mechanisms including free radical scavenging, hydrogen peroxide neutralization, and electron donation.

4. Conclusion

The present study evaluated the antioxidant activity of fisetin using three different in-vitro assays: DPPH radical scavenging assay, hydrogen peroxide scavenging assay, and FRAP assay. The results demonstrated that fisetin exhibits significant antioxidant activity in all tested methods. The antioxidant potential of fisetin may be attributed to the presence of hydroxyl groups capable of donating electrons or hydrogen atoms to neutralize free radicals. These findings suggest that fisetin could serve as a promising natural antioxidant for pharmaceutical, nutraceutical, and therapeutic applications.

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