



IN VITRO ANTIOXIDANT ACTIVITY ASSESSMENT OF CAPPARIS ZEYLANICA FLOWERS

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ABSTRACT

This study evaluates the *in vitro* antioxidant activity of Capparis zeylanica flowers, emphasizing the plant's potential as a natural source of antioxidants that can mitigate oxidative stress. Oxidative stress, driven by free radicals, is associated with chronic conditions such as cardiovascular disease, cancer, and neurodegenerative disorders. As natural antioxidants are sought to replace synthetic options with potential toxicity concerns, Capparis zeylanica offers a promising alternative, particularly due to its bioactive compounds, including phenols, flavonoids, and tannins. Fresh Capparis zeylanica flowers were extracted using a hydroalcoholic solvent, and the resulting extract was assessed through DPPH radical scavenging, ferric reducing antioxidant power (FRAP), and total phenolic content assays. The DPPH assay demonstrated moderate radical scavenging activity, while the FRAP assay revealed the extract's reducing power, though less effective compared to standard ascorbic acid. Total phenolic content analysis confirmed the presence of significant phenolic compounds, which are key contributors to antioxidant efficacy. However, the extract's antioxidant potency was lower than the synthetic standard, likely due to lower concentrations of phenolic compounds. The findings affirm Capparis zeylanica flowers as a moderate yet promising antioxidant source, suitable for applications in nutraceuticals and functional foods aimed at reducing oxidative stress. Given its natural origin and diverse bioactive profile, further investigations could enhance the efficacy of the extract through optimized extraction methods or isolation of specific compounds. This study supports the potential of Capparis zeylanica in antioxidant-rich supplements and underscores the need for additional research to fully understand and maximize its therapeutic applications in health preservation and disease prevention.

Key words: Capparis Zeylanica, Antioxidant Activity, Phytochemicals, Oxidative Stress.

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INTRODUCTION

The *in vitro* antioxidant activity assessment of Capparis zeylanica flowers [1], like many phytochemical studies, investigates the plant's potential in combating oxidative stress—a fundamental biological process associated with various chronic diseases [2], including cardiovascular disorders [3], diabetes [4], cancer [5], and neurodegenerative conditions [6].

Antioxidants are critical in neutralizing free radicals [7], which are reactive molecules with unpaired electrons that can cause cellular damage if not adequately balanced by antioxidants [8].

This imbalance leads to oxidative stress, impacting cellular components like DNA [9], proteins, and lipids [10]. Capparis zeylanica, a species within the Capparidaceae family, is noted for its traditional medicinal uses across various cultures [11]. Its flowers, particularly, are reputed for their bioactive compounds, including flavonoids [12], phenolic acids, and tannins [13]. These compounds are known for their antioxidant properties, where flavonoids act as hydrogen donors and phenolic compounds have reducing capabilities [14]. *In vitro* studies commonly apply assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging, ferric reducing

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power, and total phenolic content determination to measure the antioxidant activity of plant extracts [15]. These methods evaluate the radical scavenging ability, the reduction of ferric ions, and the quantification of phenolic compounds, respectively [16].

In vitro methods offer a controlled environment to analyze the specific antioxidant properties of extracts, isolating variables that could otherwise affect the results in *in vivo* studies [17]. By comparing the antioxidant activities of the plant extract with known standards like ascorbic acid (Vitamin C) [18], researchers can infer the potency of the natural antioxidants present in *Capparis zeylanica*. The presence of high levels of phenolic compounds is typically indicative of substantial antioxidant potential, given their efficiency in stabilizing and neutralizing free radicals. The growing interest in plant-derived antioxidants stems from the adverse effects associated with synthetic antioxidants [19], such as carcinogenicity in some cases. *Capparis zeylanica*'s antioxidant profile underscores its potential as a natural alternative for developing antioxidant-rich supplements or therapeutic agents [20]. Consequently, further investigations, including identifying the specific compounds responsible for the observed activity and understanding their mechanisms, could advance its application in pharmaceuticals and functional foods [21].

Objective

The purpose of this study is to investigate the antioxidant activity of *Capparis zeylanica* flowers for their potential ability to counteract oxidative stress. As synthetic antioxidants have become more harmful, the potential health benefits of natural antioxidants derived from plants have become increasingly evident. The number of phenolic acids, flavonoids, and tannins in *Capparis zeylanica* gives the plant its antioxidant properties. In this investigation, the antioxidant activity of the flower extract of *Capparis zeylanica* will be evaluated *in vitro* by means of radical scavenging by DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric-reducing antioxidant activity, and phenolic analysis. Reactive oxygen species are reduced and free radicals are neutralized by aspartate acid, for example. These plants' ability to protect cellular components against oxidative damage *in vitro* could provide insight into their potential to prevent cancer, cardiovascular disorders, and neurological diseases in the future. *Capparis zeylanica*'s phytochemical profile is being investigated as part of the study to encourage further exploration of its therapeutic potential. As a result of these findings, nutraceuticals, functional foods, and therapeutic agents containing plant-based antioxidants could also be developed for health preservation and disease prevention, as well as for the development of *Capparis zeylanica* as a natural antioxidant.

MATERIALS AND METHODS

The *in vitro* antioxidant activity of *Capparis zeylanica* flowers involved several key steps. Fresh flowers were collected and authenticated based on botanical standards, followed by preparation for extraction. The plant material was air-dried in shade to prevent degradation of sensitive compounds, then ground to a fine powder using a mechanical grinder [22]. This powdered material was subjected to extraction with a hydroalcoholic solvent mixture through a Soxhlet apparatus, a standard method for efficient phytochemical extraction [23]. The extraction was conducted over a controlled period at a temperature not exceeding the boiling point of the solvent to maintain the stability of bioactive compounds. After extraction, the mixture was filtered using Whatman filter paper, and the filtrate was concentrated under reduced pressure with a rotary evaporator, yielding a dark brown solid residue, which was then stored under refrigeration until further use [24].

The extract was analyzed using multiple assays in order to determine its antioxidant activity. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was first used to measure the ability to scavenge free radicals. We prepared DPPH solutions in methanol and added plant extract at varying concentrations [25-27]. As a result of 30 minutes of DPPH reaction time, spectrophotometric measurements at 517 nm were taken to determine DPPH reduction. A standard antioxidant, ascorbic acid, was used to estimate the antioxidant capacity of the extract [28]. Extracts with lower absorbance exhibited higher scavenging activity [29]. The FRAP assay was then used to determine the extract's reducing power. The reaction was induced after the extract was mixed with potassium ferricyanide and trichloroacetic acid was added [30]. Increased absorbance indicates a greater reduction power as Fe^{3+} transforms to Fe^{2+} at 700 nm. Last but not least, the Folin-Ciocalteu reagent was used to estimate the total phenolic content [31]. In the presence of the reagent and extract, a blue color was observed at 765 nm as a result of the reaction. Further elucidation of antioxidant potential was achieved by expressing total phenolic content as gallic acid equivalents. The present study assesses the *in vitro* antioxidant activity of *Capparis zeylanica* flower extract and concludes that it is a valuable source of natural antioxidants [32].

RESULTS

The results of the *in vitro* antioxidant activity and phytochemical assessments of *Capparis zeylanica* flowers demonstrate the potential antioxidant properties of the hydroalcoholic extracts. Phytochemical Screening (Table 1): The phytochemical analysis of *Capparis zeylanica* flowers revealed the presence of multiple secondary metabolites, including alkaloids, tannins, flavonoids, saponins, triterpenoids, steroids, glycosides,

anthraquinones, reducing sugars, carbohydrates, gum, mucilage, proteins, and amino acids. The presence of these compounds is significant, as they are known for their antioxidant properties. For instance, flavonoids and tannins can contribute to free radical scavenging activities, which are beneficial in counteracting oxidative stress. However, coumarin and starch were absent, which may impact specific pharmacological properties. Hydrogen Peroxide Scavenging Activity (Tables 2 and 3): The hydrogen peroxide scavenging activity of *Capparis zeylanica* flower extracts at various concentrations indicated a dose-dependent increase in inhibition percentage. For the standard antioxidant (ascorbic acid), EC₅₀ was achieved at 6.2 µg/ml, while the flower extract did not reach 50% inhibition within the tested range, suggesting a moderate antioxidant activity. The highest inhibition observed for the extract was 42.22% at 60 µg/ml, showing its potential, though not as effective as the standard. Ferric Reducing Power (Tables 4 and 5): The ferric reducing power assay, which measures the reduction of ferric ions by antioxidants, showed increasing absorbance with rising concentrations. The standard had higher absorbance values at all concentrations, peaking at 0.471 for 100 µg/ml, while the flower extract's maximum absorbance was 0.225 at the

same concentration. These results indicate that the *Capparis zeylanica* extract possesses moderate reducing power, although it is less effective than the standard antioxidant.

Total Phenolic Content (Tables 6 and 7): The total phenolic content determination, a measure of phenolic compounds which are strong antioxidants, showed that the *Capparis zeylanica* extract had lower absorbance values compared to the standard gallic acid. The absorbance for the flower extract at the highest tested concentration (250 µg/ml) was 0.377, while the standard at 200 µg/ml showed a significantly higher absorbance of 1.181. This result suggests that while *Capparis zeylanica* flowers contain phenolic compounds, the quantity is lower than that of the gallic acid standard, potentially impacting its overall antioxidant efficacy. The results indicate that the hydroalcoholic extract of *Capparis zeylanica* flowers exhibits moderate antioxidant activity, with positive results in free radical scavenging and reducing power assays. However, its effectiveness is relatively lower than standard antioxidants, which may be attributed to its lower phenolic content. These findings support further investigation into optimizing extraction methods or exploring synergistic effects with other natural antioxidants for potential therapeutic applications.

Table 1. Screening of *Capparis zeylanica* flowers for phytochemicals

S.No.	Secondary Metabolite	Flowers (Hydroalcoholic extract of <i>Capparis zeylanica</i>)
1	Alkaloids	+
2	Tanins	+
3	Flavonoids	+
4	Saponins	+
5	Triterpenoids	+
6	Steroids	+
7	Glycosides	+
8	Coumarin	-
9	Anthraquinones	+
10	Reducing Sugars	+
11	Carbohydrates	+
12	Gum & Mucilage	+
13	Starch	-
14	Proteins	+
15	Amino acids	+

Table 2. Hydroxyperoxide concentration (#g/ml) is used to calculate inhibition as percentages

Concentration (µg/ml)	% Inhibition	EC ₅₀
5	43.40±0.58	
10	51.73±0.21	
15	61.62±0.32	6.2 µg/ml
25	77.56±0.31	
50	97.78±0.27	
60		

Table 3. Using a hydrogen peroxide scavenging model, this figure shows the percentage inhibition of *Capparis zeylanica* Flowers at various concentrations (g/ml)

Concentration ($\mu\text{g/ml}$)	% Inhibition	EC50
10	15.27 \pm 0.20	
15	17.80 \pm 0.24	
25	26.28 \pm 0.34	-
50	40.52 \pm 0.27	
60	42.22 \pm 0.34	

Table 4. This graph illustrates the absorbance of the standard at different concentrations (g/ml) for the ferric reducing power determination model

Concentration ($\mu\text{g/ml}$)	Absorbance
10	0.080 \pm 0.001
25	0.121 \pm 0.002
50	0.220 \pm 0.001
75	0.341 \pm 0.003
100	0.460 \pm 0.001

Table 5. This graph shows the absorption of *Capparis zeylanica* flowers at various concentrations (g/ml) in ferric reducing power determination model

Concentration ($\mu\text{g/ml}$)	Absorbance
10	0.031 \pm 0.001
25	0.078 \pm 0.002
50	0.120 \pm 0.001
75	0.160 \pm 0.001
100	0.225 \pm 0.001

Table 6. The model can be used to determine the total phenolic content by varying the concentration (g/ml) of gallic acid

Concentration ($\mu\text{g/ml}$)	Absorbance
25	0.181 \pm 0.011
50	0.384 \pm 0.010
75	0.612 \pm 0.008
100	0.780 \pm 0.014
200	1.181 \pm 0.010

Table 7. The scatter plot represents the absorbance at various concentrations (g/ml) used to determine the content of total phenols in *Capparis zeylanica* flowers

Concentration ($\mu\text{g/ml}$)	Absorbance
25	0.060 \pm 0.008
50	0.119 \pm 0.012
75	0.127 \pm 0.007
100	0.191 \pm 0.010
200	0.270 \pm 0.011
250	0.377 \pm 0.007

DISCUSSION

The *in vitro* antioxidant activity assessment of *Capparis zeylanica* flowers offers a valuable insight into the plant's bioactive compounds and their potential applications. This study's results highlight that the hydroalcoholic extract contains essential phytochemicals, such as phenols, flavonoids, tannins, and glycosides,

known for their antioxidant properties. These compounds may play a crucial role in the observed antioxidant activity, aligning with findings from similar medicinal plants that are rich in polyphenols and flavonoids. Phenolic compounds, specifically, are noted for their capacity to donate hydrogen atoms, making them effective in neutralizing free radicals. The results from the

DPPH assay indicated moderate free radical scavenging activity for *Capparis zeylanica* flowers. Although the extract did not reach 50% inhibition at tested concentrations, it still demonstrated measurable activity. This moderate activity might be attributed to the relatively lower concentration of total phenols compared to synthetic antioxidants like ascorbic acid. However, natural antioxidants are often advantageous for their lower toxicity and additional bioactive benefits. Studies on similar plants, such as *Cassia fistula*, have shown significant antioxidant properties in their hydroalcoholic extracts, supporting the relevance of using such plant sources as natural antioxidants. The ferric reducing antioxidant power (FRAP) assay results further validated the antioxidant potential of the extract, though it was less potent than the standard at all concentrations. This observation may reflect the specific composition of *Capparis zeylanica*, where antioxidant activity could be enhanced through optimized extraction processes or combining extracts from different parts of the plant. Reducing power correlates well with the presence of phenolic content, suggesting that higher phenolic compounds could improve the antioxidant strength of *Capparis zeylanica*. In while *Capparis zeylanica* flowers exhibit promising antioxidant potential, further studies focusing on enhancing extraction techniques or isolating specific compounds could maximize their efficacy. This research underscores the plant's potential as a natural source of antioxidants, suitable for developing supplements or functional foods aimed at mitigating oxidative stress-related conditions. However, additional research on dosage optimization and potential synergistic effects with other natural compounds would provide a more comprehensive understanding of its therapeutic value.

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CONCLUSION

The *in vitro* antioxidant activity assessment of *Capparis zeylanica* flowers demonstrates the plant's potential as a natural antioxidant source, owing to its diverse phytochemical composition. This study identified key bioactive compounds, including phenols, flavonoids, tannins, and glycosides, which are recognized for their antioxidant properties. Through DPPH radical scavenging and ferric reducing power (FRAP) assays, the extract showed moderate antioxidant activity, affirming the role of these compounds in neutralizing free radicals and reducing oxidative stress. Despite the extract's lower potency compared to synthetic antioxidants like ascorbic acid, the natural origin of these antioxidants offers several advantages, such as reduced toxicity and additional health benefits from other bioactive compounds. The moderate efficacy observed could potentially be enhanced through optimized extraction methods or concentration adjustments, which may isolate and enrich the active compounds. The presence of phenolic compounds, specifically, suggests that *Capparis zeylanica* could play a significant role in free radical scavenging and offer protective benefits against oxidative damage, a factor linked to numerous chronic diseases. These findings contribute to a growing body of research supporting the use of plant-based antioxidants as alternatives to synthetic options. The study also highlights the need for further research to maximize the therapeutic potential of *Capparis zeylanica*. Investigating the synergy between this extract and other antioxidants, understanding dose-response effects, and conducting *in vivo* studies could provide additional insights into its applications in functional foods, nutraceuticals, or pharmaceuticals. Thus, *Capparis zeylanica* flowers hold promise as a naturally derived, accessible antioxidant resource, with implications for health preservation and chronic disease prevention through dietary and medicinal applications.

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