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Assessment of the Antioxidant and Anti-inflammatory Properties of Aqueous Extract of *Rosa sempervirens* Leaves

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ABSTRACT

The antioxidant and anti-inflammatory properties of an aqueous natural extract obtained from *Rosa sempervirens* leaves were assessed. The ability of the extract to scavenge DPPH, •OH, and H_2O_2 radicals, chelate ferrous ions, reduce ferric ions, and protect β-carotene-linoleic acid in emulsion from peroxidation was investigated in vitro. Furthermore, the anti-inflammatory activity of the extract was evaluated by measuring the stability of the membrane of human red blood cells against different hypotonic concentrations of NaCl and heat, as well as by inhibiting the denaturation of albumin. A high total phenolic content (278.38± 11.07 mg GAE/g) and flavonoid content (34.22± 0.12 mg QE /g) were found in the extract. The extract exhibited significant scavenging activity of DPPH (IC50 6.201 ± 0.126 µg/ ml), •OH (IC50 = 894.57 ± 21.18 µg/ml), and H_2O_2 (IC50= 107±09.58 µg/ml) radicals, and good antioxidant activity by chelating ferrous ions (IC50 = 2499.086 ± 28.267µg/ml), reducing ferric ions (IC50=141.33±2.34 µg/ml), exhibiting total antioxidant capacity (IC50 465.65 ± 9.71 µg/ml), and protecting β-carotene-linoleic acid against peroxidation (I% = 90.05 ± 1.65% at 1000µg/ml). *R. sempervirens* displayed anti-inflammatory activity in aqueous extract by inhibiting heat-induced albumin denaturation and stabilizing the membrane of human red blood cells. It was suggested from the results that *R. sempervirens* aqueous extract could help prevent oxidative and inflammatory processes due to its good antioxidant and anti-inflammatory properties.

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Introduction

For centuries, medicinal plants have been the foundation of traditional medicine and a promising source of bioactive molecules that can be exploited for modern medicine (1). Recently, natural compounds have gained increased attention due to their potential as alternative antioxidants to synthetic ones (2). Exogenous chemicals and endogenous metabolic processes generate free radicals that can oxidize biomolecules, leading to tissue damage and cell death. To prevent this, the organism employs antioxidant molecules, including polyphenols, and various defense systems to keep free radicals at low concentrations (3). Polyphenols possess unique chemical structures that act as efficient electron donors or hydrogen atoms, making them effective at neutralizing free radicals and reactive oxygen species, resulting in their antioxidant and anti-inflammatory activities and other biological functions (4).

Molecules that can stabilize erythrocytes under stress conditions, such as osmotic and heat stress, may also play a role in maintaining the integrity of biological membranes and stabilizing lysosomes. *Rosa sempervirens* (*R. sempervirens*), commonly known as the evergreen rose, is a climbing perennial with prickly stems belonging to the family Rosaceae (5). This plant has been widely used in traditional medicine and has been scientifically proven to possess antioxidant, radical scavenging, and antimicrobial properties (6-8). Despite its wide use in conventional medicine, the antioxidant and anti-inflammatory capacities of *R. sempervirens* leaves are not well understood. Therefore, this study aimed to investigate these properties in the aqueous extract of *R. sempervirens* leaves.

In this study, we evaluated the antioxidant and anti-inflammatory activities of the aqueous extract of *R. sempervirens* leaves. Our results demonstrate that the extract can scavenge DPPH, •OH, and H_2O_2 radicals, chelate ferrous ions, reduce ferric ions, and protect β-carotene-linoleic acid in emulsion from peroxidation in vitro, indicating its potent antioxidant properties. Furthermore, the extract exhibited anti-inflammatory activity by stabilizing

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the membrane of human red blood cells against different hypotonic concentrations of NaCl and heat and inhibiting the denaturation of albumin. These findings suggest that the aqueous extract of *R. sempervirens* leaves possesses potential therapeutic benefits against oxidative stress and inflammation-related diseases.

Materials and Methods

Collection and Processing of R. Sempervirens Leaves for Extraction

In December 2021, a batch of *R. sempervirens* leaves was harvested from Tizi-Ouzou, Algeria, and identified by Dr. Mahmoud Laribi, a botanist from the Department of Vegetal Biology at Mouloud Mammeri University of Tizi-Ouzou. A voucher specimen (FSBSA/MK/2707) was deposited after identification. The leaves were then dried and, ground into a powder, stored at room temperature in the dark until the extraction process.

Extract preparation

Extracting the active compounds from the *R. sempervirens* leaves involved several steps. Initially, 20 grams of the dried and ground leaves were added to 200 milliliters of distilled water. The mixture was then allowed to stand for 24 hours at room temperature, a process known as maceration. During this time, the water acted as a solvent and extracted the desired compounds from the leaves.

After the maceration period, the mixture was filtered to remove any solid plant material, leaving a clear liquid known as the filtrate. This filtrate was then subjected to lyophilization, which involved freezing the juice and removing the water by sublimation under a vacuum. The resulting extract was a dry powder that contained the concentrated active leaves compounds.

The choice of solvent, extraction method, and other parameters can significantly impact the yield and quality of the extract obtained. In this case, using distilled water as a solvent and the maceration method allowed for extracting a significant amount of active compounds from the leaves. The lyophilization step also helped preserve the extract's stability and bioactivity.

Determination of total phenolic content

The Folin-Ciocalteu spectrophotometric method was utilized in determining the concentration of phenolic compounds in the *R. sempervirens* extract (9). To prepare the reaction mixture, 200 µl of the extract (40 µg/ml) was combined with 1 ml of diluted Folin-Ciocalteu reagent and 800 µl of sodium carbonate solution (75 mg/ml). The mixture was then incubated at room temperature for 45 minutes, and the absorbance was measured at 760 nm against a blank. A calibration curve was generated for the standard solution of gallic acid using a similar procedure. The number of phenolic compounds in the extract was expressed as milligrams of gallic acid equivalents per gram.

The Folin-Ciocalteu spectrophotometric method widely estimates plant extracts' total phenolic content (9). The results obtained using this method demonstrate that the *R. sempervirens* extract contains many phenolic compounds. Phenolic compounds have been shown to possess antioxidant, anti-inflammatory, and other beneficial properties for human health (4). Therefore, the high concentration of phenolic compounds in the *R. sempervirens* extract may contribute to its potential therapeutic benefits. The Folin-Ciocalteu method is a simple and reliable method for determining the phenolic content in plant extracts. Its use can facilitate the characterization and standardization of plant extracts for pharmaceutical and nutraceutical purposes.

Determination of total flavonoid content

The aluminum chloride spectrophotometric assay was employed to determine the concentration of flavonoids in the *R. sempervirens* leaf extract, following the method described in previous studies (10,11). Initially, 1 ml of the plant extract was mixed with an equal volume of a methanolic solution containing 2% aluminum trichloride. The mixture was then incubated for 10 minutes at room temperature, allowing for the formation of a stable complex between the flavonoids and the aluminum ion.

Following incubation, the absorbance of the reaction mixture was measured at a wavelength of 430 nm against a methanol blank using a spectrophotometer. The absorbance value was used to calculate the flavonoid content of the extract. A standard curve of quercetin was generated to convert the absorbance value to flavonoid concentration, expressed as mg of quercetin equivalents (QE) per gram of extract.

The aluminum chloride spectrophotometric assay allowed for the accurate and reliable quantification of flavonoids in the *R. sempervirens* leaf extract. The method is commonly used to analyze flavonoids in plant extracts due to its sensitivity and specificity. The standard curve generated using quercetin allowed for the conversion of absorbance values to flavonoid concentration, providing a more accurate measure of the flavonoid content of the extract.

Determination of DPPH radicals scavenging activity

Using the method described by (12,13), the free radical scavenging potential of the R. sempervirens extract was assessed by employing the stable free radical DPPH test. To carry out the test, 3.75 ml of the extract was mixed with 250 µl of 0.8 mM DPPH in ethanol, and the resulting solution was incubated in triplicate for 30 minutes in the dark. Subsequently, the absorbance of the solution was measured at 517 nm against a blank ethanol solution. L-Ascorbic acid was used as a reference standard to determine the scavenging activity. The scavenging activity percent (SA %) was calculated using the equation: $SA\% = (Ac - As)/Ac \times 100$, where Ac represents the absorbance of the control and As represents the absorbance of the sample. The results indicated the extract's ability to neutralize free radicals and potentially provide protection against oxidative stress.

β-Carotene Bleaching Assay

The antioxidant activity of the *R. sempervirens* leaf extract and the level of BHT were measured using the method outlined by (14,15). A mixture of 1 ml of HPLC-grade chloroform, 0.5 mg of β-carotene, 25 µl of linoleic acid, and 200 mg of Tween 40 was prepared in a 50 ml round-bottom flask. The mixture was evaporated entirely using a vacuum evaporator at 40°C for 10 minutes. The resulting mixture was then diluted in 100 ml of distilled water saturated with oxygen. Next, 350 µl of ethanolic stock extract solution (with concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg/ml) was added to 2.5 ml of the emulsion. The same procedure was repeated for the positive control BHT. The

mixtures were then measured for their absorbance at 470 nm immediately after their preparation (t=0 min) and after incubation for 120 minutes (t=120 min) against the blank. The percentage of inhibition (I %) was calculated using the following equation: I% = [(AA(120) – AC(120))/(AC(0) – AC(120))] × 100, where AA(120) is the absorbance of the antioxidant at t=120 min, AC(120) is the absorbance of the control at t=120 min, and AC(0) is the absorbance of the control at t=0 min.

Hydroxyl radical scavenging assay

The extract's ability to scavenge hydroxyl radicals was measured following the method outlined by (16). Firstly, various concentrations of the extract (500 µL) were mixed with FeSO4 (1.5 mM, 1 ml), hydrogen peroxide (6 mM, 0.7 ml), and sodium salicylate (20 mM, 0.3 ml) to obtain a final reaction solution of 3 ml. The reaction mixture was incubated at 37°C for 1 hour, and the resulting color was measured at 560 nm against a blank. L-Ascorbic acid was used as a reference standard.

Hydrogen peroxide radical scavenging activity

The ability of the water extract of *R. sempervirens* to scavenge hydrogen peroxide was determined using the method outlined by (17). A 40 mM hydrogen peroxide solution was prepared in phosphate buffer with a pH of 7.4. A mixture of 3.4 ml extract in distilled water added 0.6 ml of the hydrogen peroxide solution. After incubation for 10 minutes, the absorbance of hydrogen peroxide was measured at 230 nm against a blank solution of phosphate buffer without hydrogen peroxide. The scavenged H_2O_2% was calculated using the equation: Scavenged H_2O_2% =1-As/Ac x100, where Ac represents the absorbance of the control (without extract) and As represents the absorbance in the presence of the extract. The experiment was carried out in triplicate.

Ferrous ion chelating activity

The inhibition of the iron (II)–ferrozine complex formation was used to evaluate the chelating activity of the extract, as per the method described in (18,19). The ferrozine complex was formed by mixing 100 µl of 0.6 mM $FeCl_2$ with 100 µl of 5 mM ferrozine solution, and the absorbance was measured at 562 nm to establish the control. To test the extract's chelating ability, 500 µl of various extract concentrations or EDTA concentrations (positive control) were added to 100 µl of 0.6 mM $FeCl_2$. The mixture was made up to a final volume of 1.5 ml with methanol. The solution was incubated for 5 min at room temperature, and then 100 µl of 5 mM ferrozine solution was added. After shaking well, the absorbance was measured at 562 nm, and the percentage of chelation was calculated using the formula: Chelation % = (A sample/A control) x 100.

Ferric-reducing power assay

The reducing power was determined using the method described by (20, 21). First, 1.25 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1.25 ml of potassium ferricyanide (1%) were mixed with different extract concentrations. The mixture was incubated at 50°C for 20 min. After incubation, the reaction mixture was added with 1.25 ml of trichloroacetic acid (10%) and centrifuged at 3000 rpm for 10 min. The supernatant was added with 0.5 ml of freshly prepared $FeCl_3$ (0.1%). The absorbance at 700 nm was measured. The standard ascorbic acid was used at various concentrations.

Total antioxidant capacity

The phosphomolybdenum assay was employed to estimate the total antioxidant capacity of the extract. A reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was mixed with the extract, and the resulting mixture was incubated at 95°C for 90 min. After cooling the solution to room temperature, the absorbance was measured at 695 nm. Ascorbic acid was used as the standard (22, 23).

Antihemolytic activity

Red blood cell suspension

Venipuncture was used to draw blood from healthy volunteers, which was subsequently centrifuged at 2000 rpm for 10 minutes at 4°C. Red blood cells (RBCs) were separated from plasma and washed three times in a phosphate-buffered saline solution (0.9 % NaCl).

Hypotonic solution-induced hemolysis

We assessed the extract's membrane stabilizing ability using the hypotonic solution-induced hemolysis technique, as reported in (24). The sample was a 40 µl dilution of a stock suspension of washed erythrocytes (RBCs) in hypotonic solution (0.1, 0.3, 0.5, 0.7, 0.9 % NaCl) in sodium phosphate buffer saline (pH 7.4) with various quantities of the aqueous extract. After 30 minutes of slow stirring at 37 degrees Celsius, the mixture was centrifuged at 2000 revolutions per minute for 10 minutes before its absorbance at 540 nanometers was determined. This equation was used to determine what fraction of hemolysis was prevented: percentage of Hemolysis Inhibition = (OD1 - OD2) / OD1 X 100 where OD1 is the optical density of pure hypotonic-buffered saline solution (the control) and OD2 is the optical density of the test sample in the same solution.

Heat-induced hemolysis

According to the protocol described in (25), 2% RBCs suspension was combined with 1 milliliter of extract or aspirin dissolved in isotonic phosphate buffer saline (pH 7.4) and incubated in a water bath at 56 degrees Celsius for 30 minutes. The absorbance of the supernatants was measured at 560 nm after the tubes were cooled under running tap water and centrifuged at 2000 rpm for 10 minutes. % Protection = 100 - (OD sample / OD Control X 100), where OD sample and OD Control are optical densities measured before and after exposure to heat, respectively.

Oxidant induced hemolysis

This experiment involved adding various amounts of extract to 1 ml of 5% RBC solution in PBS (pH 7.4) and incubating the mixture for 15 minutes at 37°C. After 10 minutes of centrifugation at 4°C and 2000 rpm, the supernatant was thrown away. After incubation, the packed RBCs were resuspended in 0.5 mM HOCl in PBS. To determine the degree of resistance to HOCl-induced hemolysis, the absorbance was monitored at 540 nm. References (26, 27) detailed this technique. % Protection = 100 - (OD sample/OD control) X 100 was used to derive the level of safety.

Inhibition of albumin denaturation

This assay measures the extract's ability to prevent protein denaturation. Here is how it's supposed to go down:

- Phosphate-buffered saline with 0.2% (w/v) egg albumin should be prepared as a stock solution (pH 6.4).
- Mix 50 µl with 5 ml of the stock solution to prepare a standard or extract.
- Warm the test tubes to 72 degrees Celsius for 5 minutes before cooling them.
- Take a reading at 660 nm to see how absorbent the solutions are.

By contrasting the absorbance of the test samples with that of the control, one may calculate the degree to which protein denaturation has been hindered (containing only the stock solution of egg albumin). Inhibition of protein denaturation increases as sample absorbance decreases (28).

Results

Phenolic Content

Studies have demonstrated that polyphenols have a range of biological properties, with antioxidant activity being the most widely studied because of their significant contribution to human health. In the case of *R. sempervirens*, the aqueous extract was analyzed for its total phenolic content, calculated as Gallic Acid Equivalent (GAE) using the equation y = 0.006x + 0.027 with an R2 value of 0.990. The results indicate that the extract contains 278.38±11.07 mg GAE/g of extract.

The findings suggest that the aqueous extract of *R. sempervirens* is a rich source of polyphenols, mainly phenolic compounds. These compounds have been shown to possess several biological activities, including antioxidant, anti-inflammatory, and anti-carcinogenic properties. The use of GAE as a measure of total phenolic content is a common practice in analytical chemistry. The high R2 value indicates that the calculation method was accurate and reliable. The error range of ±11.07 mg GAE/g also suggests that the analysis was precise and repeatable.

Overall, the results suggest that the aqueous extract of *R. sempervirens* could be a potential source of phenolic compounds, which may benefit human health. Further research could explore the potential therapeutic properties of the extract and its applications in the medicine and food industries.

Flavonoid content

After constructing a calibration curve with the equation y = 0.022x + 0.182 and an R2 value of 0.994, the collected data suggest that the aqueous extract has a considerable quantity of flavonoids, with a concentration of 34.22 ± 0.12 mg QE/g.

This result implies that the aqueous extract has a high flavonoid content, a group of naturally occurring compounds with potential health benefits. The calibration curve and high R2 value indicate that the measurement method used to determine the flavonoid concentration was precise and accurate. The error range of ±0.12 mg QE/g means a low degree of variability, making the results highly reliable.

Overall, the findings suggest that the aqueous extract could be a potential source of flavonoids, which may benefit human health. Further research could explore the potential therapeutic properties of the extract and its applications in the medicine and food industries.

DPPH scavenging activity

The experiment aimed to evaluate the ability of the aqueous extract to scavenge DPPH radicals and compare it with that of ascorbic acid, a known antioxidant. Radical scavenging activity was measured using the IC50 value, representing the concentration of antioxidants required to scavenge 50% DPPH radicals. The results indicated that the IC50 value for ascorbic acid (2.359 ± 0.091 µg/ml) was lower than that of the aqueous extract (6.201 ± 0.126 µg/ml). Figure 1 presents the inhibition % data.

The findings suggest that while the aqueous extract of the sample did have DPPH radical scavenging activity, it was not as potent as ascorbic acid, a well-known and powerful antioxidant. The comparison of IC50 values helps in understanding the relative antioxidant strength of the aqueous extract. The presented data suggest that a higher extract concentration is required to scavenge 50% of DPPH radicals than ascorbic acid.

Overall, the results suggest that the aqueous extract of the sample has some antioxidant activity, but it is less potent than ascorbic acid. Further research could investigate the specific antioxidant compounds present in the extract and their potential health benefits.

β-Carotene bleaching

The β-Carotene bleaching inhibition assay is a crucial parameter in assessing the antioxidant activity of an extract as it measures the extract's ability to prevent lipid peroxidation. The results of this assay are presented in Figure 2 as % inhibition values. The study found that the

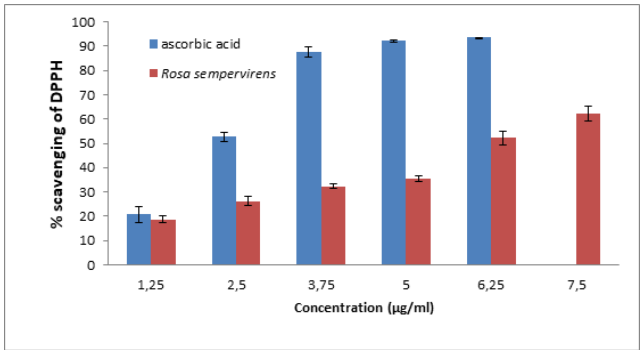

Figure 1. DPPH radical scavenging activity of ascorbic acid and aqueous extract of *Rosa sempervirens*.

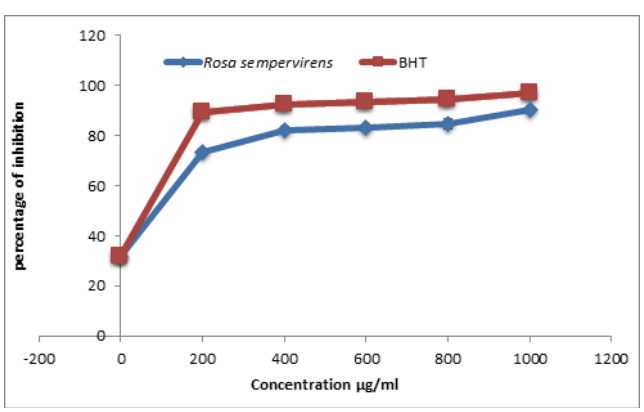

Figure 2. β-Carotene Bleaching inhibition by BHT and aqueous extract of *Rosa sempervirens*.

observed antioxidant activity increased with increasing extract concentration. At a concentration of 1000 µg/ml, the results for the aqueous extract of *R. sempervirens* were comparable to the positive control, BHT. The % inhibition for the extract and BHT were 90.05 ± 1.65% and 96.88 ± 0.34%, respectively.

The findings suggest that the aqueous extract of *R. sempervirens* has a high potential for antioxidant activity, as demonstrated by the comparable results to the positive control, BHT. The increased inhibition percentage values at a concentration of 1000 µg/ml suggest that the extract can prevent lipid peroxidation, which could protect against oxidative stress-related diseases. Figure 2 provides a visual representation of the dose-dependent antioxidant activity of the extract.

Overall, the study demonstrates that the aqueous extract of *R. sempervirens* has the potential as an antioxidant. Further research could investigate the specific antioxidant compounds present in the extract and their potential health benefits.

Hydroxyl radical scavenging

The study evaluated the ability of the aqueous extract of *R. sempervirens* to scavenge hydroxyl radicals by measuring its ability to compete with salicylic acid. The findings, presented in Figure 3, indicate that the hydroxyl radical scavenging activity of the extract increased with increasing extract concentration. The study also found that ascorbic acid had a more effective scavenging ability than the aqueous extract, with an IC50 value of 758.83 ± 7.40 µg/ml compared to the extract's IC50 value of 894.57 ± 21.18 µg/ml.

The results suggest that the aqueous extract has the potential to scavenge hydroxyl radicals, which are reactive oxygen species that can cause cellular damage and lead to oxidative stress-related diseases. The maximum scavenging activity was observed at 2 mg/ml, with ascorbic acid demonstrating a higher scavenging ability than the extract. Figure 3 visually represents the extract's dose-dependent hydroxyl radical scavenging activity.

In conclusion, the study demonstrates that the aqueous extract of *R. sempervirens* has the potential to scavenge hydroxyl radicals, albeit less effectively than ascorbic acid. Further research could investigate the specific antioxidant compounds present in the extract and their potential health benefits.

Hydrogen peroxide radical scavenging activity

The study evaluated the ability of the aqueous extract to scavenge hydrogen peroxide and compared it to ascorbic acid as a reference compound. The findings, presented in Figure 4, indicate that the scavenging activity of the extract was concentration-dependent and increased with increasing extract concentration. However, the effective concentration required for scavenging activity significantly differed between the extract and ascorbic acid. The extract showed 88.35% scavenging activity at 500µg, whereas ascorbic acid showed 99.4% scavenging activity at 90µg.

The study also found that the IC50 value for hydrogen peroxide scavenging was 107 ± 9.58 µg/ml for the extract, indicating a less effective scavenging potential than that of ascorbic acid, whose IC50 value was 49.19 ± 2.70 µg/ml. These findings suggest that while the aqueous extract of *R. sempervirens* has the potential to scavenge hydrogen peroxide, its effectiveness is lower than that of ascorbic acid.

Overall, the results of this study suggest that the aqueous extract has potent antioxidant activity, although its effectiveness may be lower than that of ascorbic acid. Further research could investigate the specific antioxidant compounds present in the extract and their potential health benefits.

Ferrous ion chelating activity

The chelating activity of *R. sempervirens aqueous* extract was investigated as a possible secondary antioxidant mechanism, specifically its ability to chelate pro-oxidant metals like iron. The assay utilized the formation of a red-colored complex between Ferrozine and Fe^{2+}, which is disrupted by the presence of a chelating agent. The extent of color reduction indicates the degree of chelation by the extract. Results depicted in Figure 5 demonstrated that both the extract and EDTA exhibited concentration-dependent metal chelating effects. However, EDTA showed higher activity compared to the extract in this assay, with an

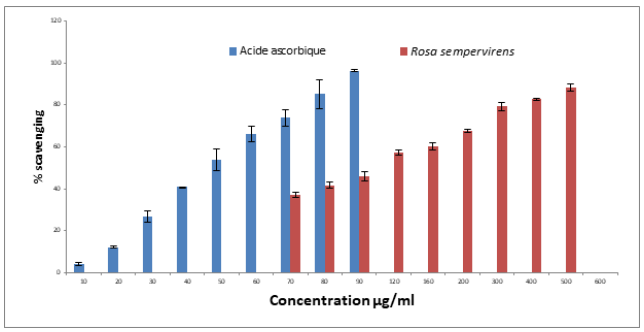

Figure 4. Hydrogen peroxide radical scavenging activity of ascorbic acid and aqueous extract of *Rosa sempervirens*.

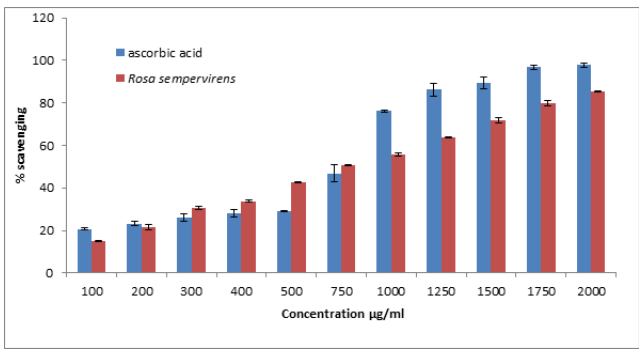

Figure 3. Hydroxyl radical scavenging activity of ascorbic acid and aqueous extract of *Rosa sempervirens*.

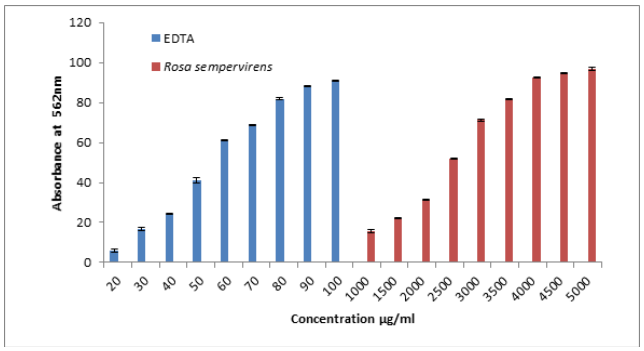

Figure 5. Ferrous ion chelating activity of EDTA and aqueous extract of *Rosa sempervirens*.

IC50 value of 57.21 ± 0.44 µg/ml compared to 2499.086 ± 28.267 µg/ml for the extract. This indicates that EDTA had a more substantial chelating effect on Fe2+ ions than the extract. Chelating agents are essential in antioxidant activity as they neutralize free radicals produced by pro-oxidant metals. Thus, the ability of aqueous extract to chelate iron suggests that it may have potential as a secondary antioxidant mechanism in addition to its primary antioxidant activity.

Ferric reducing power

It was observed (Figure 6) that the reducing power of Rosa sempervirens aqueous extract increased as the concentration increased, indicating a concentration-dependent activity. However, the extract exhibited lower reducing power than ascorbic acid, as evidenced by its IC50 value of 141.33±2.34 µg/ml compared to ascorbic acid's IC50 value of 56.72±2.79 µg/ml. These findings suggest that while the extract has considerable reducing power, its activity is not as potent as that of ascorbic acid.

Total antioxidant capacity

In this study, the test used to evaluate the antioxidant activity of *R. sempervirens* aqueous extract, and ascorbic acid was based on the reduction of Mo (VI) to Mo (V) at acidic pH by the extract, resulting in the formation of a green phosphate/Mo (V) complex. The extract and ascorbic acid concentrations were tested at 100 to 500 µg/ml, and both showed a dose-dependent antioxidant activity (Figure 7).

However, the IC50 value for the antioxidant capacity of ascorbic acid (292 ± 7.54 µg/ml) was found to be higher than that of the extract (465.65 ± 9.71 µg/ml). These results suggest that the extract has antioxidant potential and could act as a natural source of antioxidants, but its activity is not as intense as that of ascorbic acid.

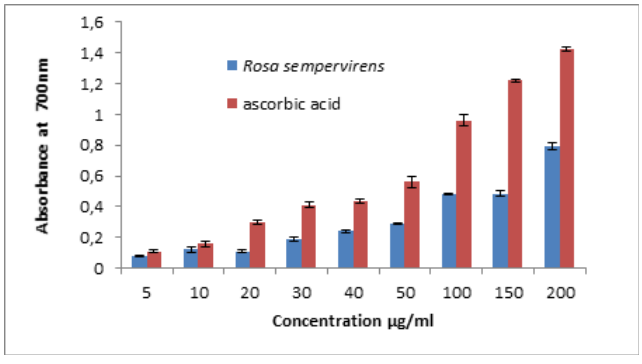

Figure 6. Ferric reducing power of ascorbic acid and aqueous extract of *Rosa sempervirens*.

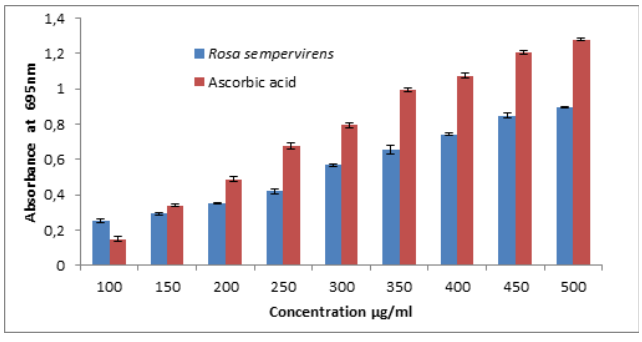

Figure 7. Total antioxidant capacity of ascorbic acid and aqueous extract of *Rosa sempervirens*.

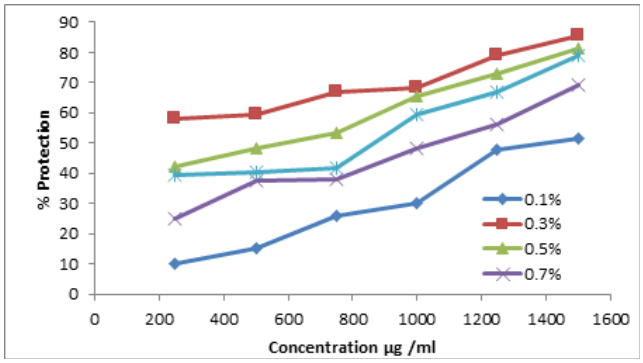

Figure 8. Effect of *Rosa sempervirens* aqueous extract on hypotonicity-induced hemolysis.

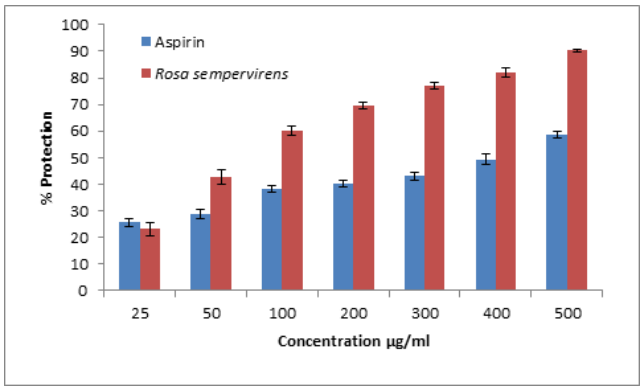

Figure 9. Effect of *Rosa sempervirens* aqueous extract on heat-induced hemolysis.

Membrane stabilizing activity

The ability of the extract to protect erythrocyte membranes against lysis was demonstrated by the results from Figures 8 and 9. The hypotonic solution-induced hemolysis test showed that the extract inhibited RBC hemolysis at concentrations ranging from 0.250 to 1.500 mg/ml. The inhibitory effect on RBC hemolysis at 0.1%, 0.3%, 0.5%, 0.7%, and 0.9% NaCl concentrations were 51.46 ± 1.63%, 85.66 ± 0.86%, 81.32 ± 1.34%, 69 ± 1.68%, and 79.05 ± 1.44%, respectively. These results suggest that the extract can potentially prevent the breakdown of erythrocyte membranes.

Similarly, in the heat-induced hemolysis test, the extract inhibited the lysis of erythrocyte membranes at concentrations ranging from 0.025 to 0.5 mg/ml. The inhibition rates ranged from 42.64 ± 2.83% to 90.29 ± 0.5%. In comparison, acetylsalicylic acid (aspirin) demonstrated inhibition rates ranging from 25.65 ± 1.57% to 58.49 ± 1.23%. These findings indicate that the extract can potentially protect erythrocyte membranes against different types of lysis and may have implications for preventing or treating conditions characterized by erythrocyte membrane breakdown.

Oxidant induced hemolysis

A dose-dependent protective effect against HOCl-induced hemolysis was observed in the extract, as shown in Figure 10. The extract exhibited a gradual decrease in hemolysis ratio with increasing concentrations, with a protective effect observed even at a concentration of 1mg/ml (83.1±1.29%). These results suggest that the extract poten-

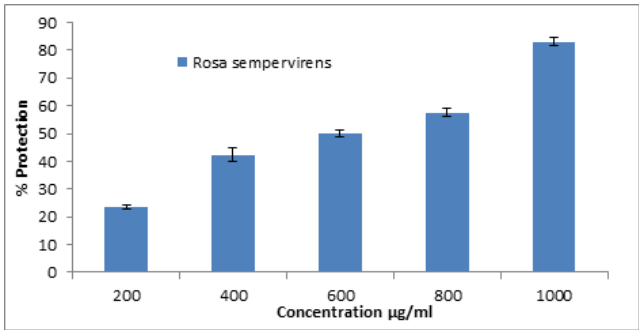

Figure 10. Effect of *Rosa sempervirens* aqueous extract on HOCl-induced hemolysis.

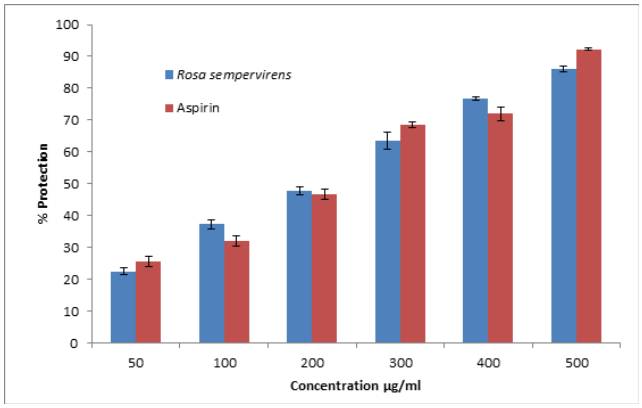

Figure 11. TEffect of *Rosa sempervirens* aqueous extract on albumin denaturation.

tially protects against hemolysis induced by HOCl.

Inhibition of albumin denaturation

Remarkable effectiveness in inhibiting thermally induced albumin denaturation was observed in the extract at different concentrations in this study. Protein denaturation is a crucial factor in inflammation, so plant extracts that can inhibit denaturation are often tested for their anti-inflammatory activity. Figure 11 shows that the extract demonstrated a maximum inhibition of 86.05 ± 0.8% at 500 µg/ml, while aspirin, the anti-inflammatory standard, showed 92.23 ± 0.32% inhibition at 500µg/ml. These results indicate that the extract has the potential to be used as an anti-inflammatory agent due to its ability to inhibit protein denaturation.

Discussion

The need for mechanisms to detect and detoxify reactive oxygen species, which are harmful byproducts of the oxygenated environment, is discussed in the passage. Exogenous molecules with antioxidant power, such as polyphenols, are being explored for their potential to prevent various pathological changes in cells by protecting the oxidation of their main constituents, including proteins, lipids, carbohydrates, and DNA.

The antioxidant activity of the aqueous extract of *R. sempervirens*, a plant species with a high concentration of polyphenols, is focused on in the study. The results show the exceptionally high antioxidant potential of the extract compared to other species described in the literature. For instance, the extract exhibited 86.05 ± 0.8% maximum inhibition of albumin denaturation at a concentration of 500 µg/ml, which was higher than that of aspirin, an anti-inflammatory standard, at the same concentration (92.23 ± 0.32%).

The extract also showed higher phenolic content than that reported by (30) and (31). Still, the flavonoid content was lower than that reported by (8) and higher than that reported by (32) for *Rosa canina.* The scavenging and antioxidant activity of the extract were monitored by several tests, which confirmed its high potential compared to other works. For instance, a lower IC50 value (i.e., higher scavenging activity) in the DPPH assay than the aqueous extract of *Rosa agrestis* and *Rosa sempervirens* reported in (34) and (29), respectively, was exhibited by the extract. However, it was found that the extract was less effective in scavenging hydroxyl radicals than that reported by (35).

The generation of hydroxyl radicals from superoxide anion and hydrogen peroxide, which are highly reactive free radicals and can cause significant damage by interacting with intracellular targets such as DNA, resulting in various free radical pathologies, can be led by the presence of metal ions in biological systems.

The removal of hydrogen peroxide (H_2O_2) is considered crucial for cellular systems or foods' antioxidant defense due to its potential toxicity and ability to generate a hydroxyl radical (36,37,38). Better scavenging activity was exhibited by the extract (88.35 ± 1.65%) compared to Rosa hibiscus (48.52 ± 3.03%) at 500 µg/ml [39]. Potent inhibition of lipid peroxidation and β-carotene bleaching was also observed at 1mg/ml (90.05 ± 1.65%) in comparison to *Rosa agrestis* (63.30 ± 2.27%) at 0.5mg/ml [34]. These findings suggest the extract's potential anti-cancer activity as lipid peroxidation is known to cause cancer. Additionally, the extract exhibited significant protective action against oxidative damage caused by iron (II) ions by demonstrating ferrous solid ion chelating activity (IC50=2499.086 ± 28.267 µg/ml) [40]. The extract's ferric-reducing power was also higher than *Rosa kordesii* water extract (IC50=261.67 µg/ml) (44). This indicates the extract's potential antioxidant activity and electron-donating capacity, which are closely related to reducing power (41,42,43). The extract also exhibited greater total antioxidant capacity than *Cakile maritima* (IC50=486.4 ± 2.27 µg/ml) (45), suggesting its potential as an antioxidant.

In the second part of the study, anti-inflammatory activity was observed as the extract stabilized RBCs membrane against hypotonic solution-induced hemolysis, heat-induced hemolysis, and oxidant-induced hemolysis, compared to *Clerodendrum paniculatum* Linn aqueous extract (34.7% at 1 mg/ml) (46), *Carica papaya* (38.8 ± 5% at 3mg/ml) (47), and *Rhus typhina* (61.06 ± 2.53% at 20 µg/ml) (48), respectively. The extract's protective effect against these forms of hemolysis supports its anti-inflammatory potential, which could be attributed to its ability to regulate calcium influx in erythrocytes (49). Furthermore, the extract's effect on erythrocyte stabilization could also apply to stabilizing the lysosomal membrane, which is similar to the erythrocyte membrane (50). At the same time, its anti-inflammatory activity could be explained by inhibiting lysosomal content release at the site of inflammation (51).

More excellent protection was observed for the *R. sempervirens* extract on albumin denaturation compared to *Pedalium murex* (70.43 ± 0.85% at 1000 µg/ml) (52). The denaturation of proteins is known to cause inflammation and rheumatoid arthritis, and the observed protection

against albumin denaturation confirms and contributes to the anti-inflammatory activity of the *R. sempervirens* extract.

Conclusion

In conclusion, the present study investigated the antioxidant and anti-inflammatory activities of *R. sempervirens* extract. The results demonstrated that the extract exhibited potent antioxidant activity, attributed to its ability to scavenge free radicals and chelate metal ions. The extract also showed anti-inflammatory activity by stabilizing the RBCs membrane against various forms of hemolysis and protecting proteins from denaturation. Furthermore, the extract exhibited potent inhibition of lipid peroxidation, suggesting its potential as an anti-cancer agent.

The findings of this study suggest that *R. sempervirens* extract could be a potential source of natural antioxidants and anti-inflammatory agents. Further investigations are needed to identify the active compounds responsible for the extract's activities and assess their bioavailability and safety in the pharmaceutical and food industries. Nonetheless, the study highlights the importance of exploring natural sources of antioxidants and anti-inflammatory agents to combat the growing incidence of oxidative stress-related diseases. The results obtained in this study could contribute to developing new therapeutic agents for the prevention and treatment of various diseases associated with oxidative stress and inflammation.

Declaration of patient consent

The authors acquired suitable consent forms from the patients, in which they provided permission to record their blood and clinical data in the journal. The patients were informed that their identities would remain anonymous and that their names or initials would not be disclosed.

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Conflict of interest

There are no conflicts of interest.

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