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Evaluation of Antioxidant and Anti-Inflammatory Activities of Aqueous Extract of *Malva Sylvestris* Leaves in Association with Serum Albumin

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Abstract: *Malva sylvestris* L., commonly known as common mallow, is an herbaceous plant widely used in traditional Algerian medicine for its anti-inflammatory and antioxidant properties. The richness of its leaves and flowers in phenolic compounds is the source of these therapeutic virtues. In Algeria, various remedies based on this species are commonly used in the treatment of inflammation, joint pain, and digestive disorders. Serum albumin is the most abundant protein in blood plasma and plays a key role in regulating oncotic pressure. Its versatile nature also allows it to transport numerous molecules such as fatty acids, hormones, drugs, and metal ions, thus contributing to the proper functioning of essential physiological processes. This study aims to evaluate certain biological activities resulting from the association between serum albumin and the aqueous extract of *Malva sylvestris* L. leaves. The results obtained highlight a notable antioxidant activity of this association, with respective IC50 values of 3.825 ± 0.422 µg/ml, 296.13 ± 69.33 µg/ml, and 217.9 ± 31.5 µg/ml for the ability to scavenge DPPH, OH, and H_2O_2 radicals. The mixture exhibits a total antioxidant capacity with an IC50 of 310.5 ± 11.33 µg/ml and a ferric ion reducing power with an IC50 of 42.93 ± 46.38 µg/ml. The ferrous ion chelating ability is evaluated with an IC50 of 137.96 ± 30.07 µg/ml. Regarding the anti-inflammatory activity, no stabilizing effect on erythrocyte membranes against osmotic stress, HOCl-induced oxidative stress, and heat was observed with this association. These results clearly demonstrate that the combination of serum albumin and the aqueous extract of *Malva sylvestris* leaves significantly potentiates the antioxidant properties of the plant. This combination represents a promising source of bioactive molecules and constitutes an interesting therapeutic alternative for the treatment of pathologies related to oxidative stress.

Keywords: *Malva sylvestris*, BSA, Antioxidant , Anti-radical , Anti-inflammatory

Introduction

For centuries, medicinal plants have been a valuable source of bioactive molecules, offering numerous therapeutic benefits, particularly in the prevention of oxidative damage. The growing interest in these biomolecules, particularly polyphenols, is justified by their well-documented antioxidant and anti-inflammatory effects (Singh, 2015). At a time when synthetic antioxidants are raising concerns about their safety, natural antioxidants from plants, such as those from *Malva sylvestris*, are seen as a promising alternative (Velioglu et al., 1998).

Malva sylvestris, widely known for its medicinal properties, contains various polyphenols, such as flavonoids and tannins, which are widely studied for their ability to neutralize free radicals (Bhattacharyya et al., 2014). However, beyond the exploration of these antioxidant properties, the association of these compounds with

serum proteins such as HSA (Human Serum Albumin) is attracting increasing attention. HSA, a major plasma protein, plays a crucial role in the transport and stabilization of small bioactive molecules (Zhang et al., 2021). Its interaction with polyphenols, particularly those present in *Malva sylvestris*, could amplify their biological effects by stabilizing these compounds in physiological environments.

In this sense, this study focuses on assessing the antioxidant and anti-inflammatory effects *of Malva sylvestris* in interaction with BSA, with the aim of better understanding the impact of this interaction on the mechanisms of protection against oxidative stress.

Materials and Methods

Plant Collection

Malva Sylvestris leaves were collected in October 2022 from M'sila, Algeria. The plant was identified by Doctor Mahmoud Laribi, botanist at Mouloud Mammeri University of Tizi-Ouzou, department of vegetal biology, where a voucher specimen was deposited (FSBSA/MK/oct2722). The sample was dried and then ground to obtain a powder that was stored at room temperature and in the dark until extraction.

Extract Preparation

20g of powder are dissolved in 200ml of distilled water. After 24 hours of maceration at room temperature, the filtrate was lyophilized.

Determination Of DPPH Radicals Scavenging Activity

The free radical scavenging activity of the extract was measured using the stable free radical DPPH test according to the method described by (Sharma & Bhat, 2009; Santos et al., 2010). 250 µl of 0.8 mM DPPH in ethanol was mixed with 3.65 ml of extract and 100µl of BSA. The reaction was carried out in triplicate and the absorbance was measured at 517nm after 30 minutes in dark. L-Ascorbic acid was used as reference standard.

Hydroxyl Radical Scavenging Assay

Scavenging activity of hydroxyl radical of the extract was measured according to the method of (Rajamanikandan et al., 2011) Three millilitres of the final reaction solution consisted of aliquots (500 µl) of various concentrations of the extract,100µl of BSA, 1ml FeSO4 (1.5 mM), 0.7 ml hydrogen peroxide (6 mM) and 0.3 ml sodium salicylate (20 mM). The reaction mixture was incubated for 1 h at 37°C. L-Ascorbic acid was used as the standard. The colour development was measured at 560 nm against a blank.

Hydrogen Peroxide Radical Scavenging Activity

The scavenging ability of water extract of *Malva Sylvestris* on hydrogen peroxide was determined according to the method of (Serteser et al., 2009). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). BSA (100µl) and extract in distilled water (3.4 ml) was added to a hydrogen peroxide solution (0.6 ml, 40mM). Absorbance of hydrogen peroxide at 230 nm was measured 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide.

Ferrous Ion Chelating Activity

Ferrous ion chelating activity was determined by inhibition of the formation of iron(II)–ferrozine complex, following the method of (Dinis et al., 1994; Nabavi et al., 2012). Briefly, 100 µl of 0.6 mM $FeCl_2$ was added to 500µl of different concentrations of the extract mixed with 100µl of BSA or EDTA (positive control). The reaction mixture was adjusted to a final volume of 1.5ml with methanol, and then 100µl of 5 mM ferrozine solution were added. The mixture was shaken vigorously and left to stand at room temperature for 5 min. Absorbance was determined at 562nm.

Ferric Reducing Power Assay

Reducing power was determined by the method described by (Oyaizu, 1986; Hazra et al., 2008). Different concentrations of extract and BAS were mixed with 1.25 ml of 0.2 M, pH 6.6 sodium phosphate buffer and 1.25 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. After incubation, the reaction mixture was acidified with 1.25 ml of trichloroacetic acid (10%) and centrifuged at 3000 rpm for 10 min. Finally, 0.5 ml of freshly prepared FeCl3 (0.1%) was added to this solution, and the absorbance was measured at 700nm. Aascorbic acid at various concentrations was used as standard.

Total Antioxidant Capacity

Total antioxidant capacity was estimated by phosphomolybdenum assay (Prieto et al., 1999; Rao et al., 2010) The tubes containing extract and BSA and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95°C for 90 min.Then the solution was cooled to room temperature and absorbance was read at 695 nm. Ascorbic acid was used as standard.

Antihemolytic Activity

Red Blood Cell Suspension

Blood was obtained by venipuncture from healthy volunteers collected in heparinized tubes and centrifuged at 2 000 r/min for 10 min at 4 °C. After removing the plasma, red blood cells (RBCs) were washed for three successive times using phosphate buffer saline (PBS) (0.9% NaCl). The study protocol was performed according to the Helsinki declaration and approved by Scientific Committee of the Faculty of Biology (CSFB). Informed written consent was obtained from Hospital Department of Hematology (University Hospital Nedir Mohamed of Tizi-Ouzou).

Hypotonic Solution Induced Hemolysis

Membrane stabilizing activity of extract and BSA was assessed using hypotonic solution induced hemolysis, and the method was described by de Freitas et al. (de Freitas et al., 2008). In hypotonic solution, the test sample consisted of washed stock erythrocyte (RBC) suspension (40 mL) with 1 mL of hypotonic solution (0.1%, 0.3% and 0.7% NaCl) in sodium PBS (pH 7.4) containing either of the different concentrations of BSA. The mixture was incubated for 30 min at 37 °C under gentle stirring, centrifuged for 10 min at 2 000 r/min and the absorbance of the supernate was measured at 540 nm.

Heat Induced Hemolysis

Different concentrations of the extract mixed with BSA (mg/mL) or aspirin dissolved in isotonic PBS (pH 7.4) was mixed with 1 mL of 2% RBCs suspension. The reaction mixture was incubated in a water bath at 56 °C for 30 min. After incubation, the tubes were cooled under running tap water, then centrifuged at 2 000 r/min for 10 min and the absorbance of the supernatants was estimated at 560 nm (Sakat et al., 2010).

Oxidant Induced Hemolysis

One milliliter of RBC suspension (5%) in PBS (pH 7.4) was incubated for 15 mn at 37°C with 1 ml of the extract mixed with BSA at different concentrations. After preincubation, the mixture was centrifuged at 2 000 r/min for 10 min at 4°C, the supernatant was removed and packed RBCs were resuspended with 0.5 mmol/L HOCl in PBS. After this, the incubation was performed as previously described. The absorbance was determined at 540 nm (Suwalsky et al., 2007; Chandler et al., 2013).

Results and Discussion

The results obtained in this study for both the antioxidant and the anti-inflammatory activity *of Malva sylvestris* aqueous extract alone and in combination with BSA are summarized in the following tables (Tables 1 and 2).

Table 1. IC 50 recorded for antioxidant activity

Test	**IC50 standard**	**IC50 *Malva sylvestris***	**IC50 *M sylvestris* + BSA**
DPPH radicals scavenging activity	2.359 ± 0.091 µg/ml	7.81 ± 0.402 µg/ml	3.825 ± 0.422 µg/ml
Hydroxyl radical scavenging assay	758.83 ± 7.40 µg/ml	971.28 ± 27.12 µg/ml	296.13 ± 69.33 µg/ml
Hydrogen peroxide radical scavenging activity	259.95 ± 9.33 µg/ml	431.06 ± 11.72 µg/ml	296.13 ± 69.33 µg/ml
Ferrous ion chelating activity	57.21± 0.44 µg/ml	74.631 ± 1.19µg/ml	137.96 ± 30.07 µg/ml
Ferric reducing power assay	88.17±1.39 µg/ml	46.7 ± 0.85 µg/ml	42.93 ± 46.38 µg/ml
Total antioxidant capacity	292 ± 7.54 µg/ml	348.357 ± 6.03 µg/ml	310.5 ± 11.33 µg/ml

Table 2. Percentage of protection recorded for erythrocyte membrane stabilization

Test	**Protection % *Malva sylvestris***	**Protection % *M sylvestris* + BSA**
Hypotonic solution induced hemolysis	0.3%NaCl:79.57% 0.7%NaCl: 60.3%	0.3% Na Cl:80.3% 0.7% Na Cl:60.7%
Heat induced hemolysis	90%	89.1%
Oxidant induced hemolysis	63.10%.	65.8%

DPPH Scavenging Activity

Table 1 presents the percentage inhibition of DPPH radical scavenging activity by the samples tested. The IC50 value, representing the concentration required to scavenge 50% of DPPH radicals, was used to compare the antioxidant potential of different samples. The IC50 of ascorbic acid was 2.359 ± 0.091 µg/ml, significantly lower than that of the aqueous extract of *Malva sylvestris* (IC50 = 7.81 ± 0.402 µg/ml). However, when combined with BSA, the antioxidant activity of the extract improved notably, with an IC50 value of 3.825 ± 0.422 µg/ml, indicating an enhanced scavenging capacity.

Hydroxyl Radical Scavenging

The ability of *Malva sylvestris* aqueous extract, alone and in combination with BSA, to scavenge hydroxyl radicals was assessed using salicylic acid as a reference compound. As shown in Table 1, scavenging activity increased with increasing concentration. Ascorbic acid (IC50 = 758.83 ± 7.40 µg/ml) showed the most effective scavenging ability, while the extract alone (IC50 = 971.28 ± 27.12 µg/ml) had a lower capacity. Interestingly, the combination of the extract with BSA significantly improved hydroxyl radical scavenging, achieving an IC50 of 296.13 ± 69.33 µg/ml, which is considerably lower than that of the extract alone.

Hydrogen Peroxide Radical Scavenging Activity

The scavenging activity of hydrogen peroxide for the samples was also evaluated, with results showing significant variation. Ascorbic acid (IC50 = 259.95 ± 9.33 µg/ml) exhibited the strongest activity, while *Malva sylvestris* alone had a higher IC50 (431.06 ± 11.72 µg/ml), indicating less scavenging potential. However, the combination with BSA resulted in a marked improvement in activity, with an IC50 of 296.13 ± 69.33 µg/ml, suggesting the potential of BSA to enhance the extract's antioxidant effects.

Ferrous Ion Chelating Activity

The ferrous ion chelating activity of the *Malva sylvestris* extract and its combination with BSA was measured by evaluating the reduction in color intensity of the Fe2+-Ferrozine complex. EDTA (IC50 = 57.21± 0.44 µg/ml) demonstrated the highest activity. The extract alone showed a lower chelating effect (IC50 = 74.631 ±

1.19 µg/ml), while the combination with BSA displayed a significantly higher IC50 of 137.96 ± 30.07 µg/ml, indicating that the combination is less effective at chelating iron than the extract alone.

Ferric Reducing Power Assay

The ferric reducing power of the samples was evaluated, and it was observed that the extract's reducing power was concentration-dependent. The IC50 of *Malva sylvestris* extract alone (46.7 ± 0.85 µg/ml) was higher than that of ascorbic acid (88.17 ± 1.39 µg/ml), indicating strong reducing capacity. When combined with BSA, the IC50 slightly improved to 42.93 ± 46.38 µg/ml, suggesting that the combination maintains the reducing capacity.

Total Antioxidant Capacity

The total antioxidant capacity was determined using the reduction of Mo (VI) to Mo (V). Ascorbic acid (IC50 = 292 ± 7.54 µg/ml) displayed stronger antioxidant capacity compared to the extract alone (IC50 = 348.357 ± 6.03 µg/ml). However, the combination of the extract with BSA showed a moderate improvement, with an IC50 of 310.5 ± 11.33 µg/ml, further confirming the synergistic effect of BSA on the antioxidant potential of the extract.

Heat-Induced Hemolysis

As illustrated in Table 2, both the native *Malva sylvestris* extract and the combination with BSA provided significant protection against heat-induced erythrocyte hemolysis. While aspirin offered maximum protection (62.97 ± 2.1%), both the extract alone and the combination with BSA achieved similar levels of protection, around 90%, with the combination slightly lower at 89.1%.

Hypotonic Solution-Induced Hemolysis

Table 2 demonstrates the protective effects of the samples against hypotonic solution-induced hemolysis. The extract alone provided 79.57% protection at 0.3% NaCl, while the combination with BSA slightly increased this protection to 80%. At 0.7% NaCl, the extract offered 60.3% protection, with a similar result for the combination at 60.7%.

Oxidant-Induced Hemolysis

In the presence of oxidative stress, as shown in Table 2, the *Malva sylvestris* extract alone exhibited 63.10% protection against hemolysis, whereas the combination with BSA increased this protection to 65.8%, further indicating the enhanced bioactivity of the extract when combined with BSA. This study investigates the impact of the combination of *Malva sylvestris* aqueous extract and bovine serum albumin (BSA) on antioxidant and anti-inflammatory activities. The results highlight the potential impact of this combination on the antioxidant and anti-inflammatory activity of the extract studied. The combination of *Malva sylvestris* with BSA demonstrated a remarkable synergy in the inhibition of free radicals, notably DPPH● and OH●. This improvement in free radical scavenging capacity can be attributed to several factors:

The polyphenols present in *Malva sylvestris* probably interact with the thiol residues (Cys-34) of BSA. These residues play a crucial role in the scavenging of reactive oxygen species (ROS), thereby increasing the overall efficiency of radical scavenging (Colombo et al., 2012). BSA can act as a redox buffer, stabilizing polyphenols in biological environments and extending their half-life under physiological conditions (Zhang et al., 2021). This stabilization could explain the observed increase in antioxidant activity. The hydroxyl radical, considered the most reactive of the free radicals, is particularly well neutralized by this combination. This is significant given its ability to interact with intracellular targets such as DNA, causing significant damage (Lushchak, 2014).

In contrast to the synergistic effect observed for free radical scavenging, the combination with BSA showed a negative impact on metal ion chelation capacity and iron reduction. This phenomenon can be explained by:
BSA, as a carrier protein, has metal-binding sites that can compete with *Malva sylvestris* polyphenols for the chelation of metal ions, particularly Fe2+ (Sugio et al., 1999).

This competition may limit the availability of metal ions to be sequestered by the extract's polyphenols, thus reducing the overall efficiency of iron chelation and reduction. This result highlights the importance of considering the relative concentration of proteins and polyphenols in optimizing the overall antioxidant effect. The combination of *Malva sylvestris* with BSA showed no significant improvement in the protection of erythrocyte membranes against oxidative stress, with a percentage protection of 65.8% versus 63.10% for the extract alone. The integrity of erythrocyte membranes is often used as a model for assessing the stabilization of lysosomal membranes, essential for preventing the release of pro-inflammatory enzymes (Omale & Okafor, 2008). By analogy with BSA, HSA, by stabilizing these membranes, could play a crucial role in inhibiting the release of lysosomal enzymes, thus offering better protection against inflammatory processes (Govindappa et al., 2011).

The stabilizing action of HSA on cell membranes, combined with the intrinsic anti-inflammatory properties of *Malva sylvestris*, suggests a synergistic anti-inflammatory effect that is not very effective *in vitro* but has potential *in vivo*. The complexes formed between BSA and polyphenols of *Malva sylvestris* could modulate the inflammatory response by inhibiting lipid peroxidation and stabilizing lysosomal membranes (Adefegha & Oboh, 2011).

The study did not reveal any significant synergistic or antagonistic effects for H_2O_2 scavenging and for the protection of erythrocyte cells against osmotic and thermal stress. However, these results remain relevant:
Although hydrogen peroxide is not highly reactive on its own, it can be toxic to cells by giving rise to the hydroxyl radical (Moualek et al., 2020). The elimination of H_2O_2 therefore remains crucial to cellular antioxidant defense. The fact that the combination with BSA does not diminish these capabilities of *Malva sylvestris* extract is positive, indicating that the combination maintains the extract's efficacy in these specific aspects of cellular protection.

Conclusion

This preliminary study highlights the promising potential of the combination of *Malva sylvestris* aqueous extract and BSA. The results show a significant improvement in certain antioxidant activities, notably free radical scavenging and cell membrane protection, although a moderate impact was observed on metal chelation and iron reduction capacities.

The synergy observed in membrane stabilization and anti-inflammatory activity underlines the protective role of this combination against oxidative damage and inflammatory processes. The interactions between *Malva sylvestris* polyphenols and BSA appear to enhance the bioactivity of the plant extracts, making this combination promising for future therapeutic applications, particularly in the fight against oxidative stress and inflammation.
However, the complexity of the interactions observed, with variable effects depending on the tests carried out, highlights the need for further research. It would be pertinent to explore in greater detail the molecular mechanisms underlying these interactions, to optimize the relative concentrations of extract and BSA, and to conduct *in vivo* studies to confirm these beneficial effects under physiological conditions.

In conclusion, this study opens up interesting prospects for the combined use of *Malva sylvestris* and BSA in the development of new antioxidant and anti-inflammatory strategies, while underlining the importance of a nuanced approach to the interpretation of interactions between natural compounds and proteins.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM Journal belongs to the authors.

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