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Evaluation of the in-vitro anti-inflammatory and hemolytic activity of aqueous extract of *Silene Vulgaris (Moench) Garcke* in Algeria.

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Abstract

Silene vulgaris (Moench) Garcke is a popular edible plant that is consumed by a significant population in many countries. The present research focused on determining the in-vitro anti-inflammatory ability and hemolytic activity of aqueous extracts derived from the soil and aerial parts of *S. vulgaris*. The study involved many plant parts, including roots, leaves, stems, and flowers. The extraction method adopted consisted of decoction and maceration, with distilled water acting as the solvent for extraction. Bovine Serum Protein Denaturation was used as a method to evaluate the anti-inflammatory activity of *S.vulgaris*. Moreover, the evaluation of the hemolytic effect of aqueous extracts from *S.vulgaris* parts was prepared in vitro on an erythrocyte suspension of human blood incubated in phosphate buffer solution (PBS). The results of in-vitro anti-inflammatory activity showed that the macerated aqueous extract (Mac-H_2O) and decocted aqueous extract (Dec-H_2O) of leaves (at concentrations of 1 mg/ml) exhibited a considerably greater level of inhibition (88.31% and 66.88%, respectively) compared to the other parts. Additionally, an evaluation of hemolytic potential was conducted on human erythrocytes, revealing that the root extracts (Mac-H_2O and Dec-H_2O) display high activity against erythrocytes, leading to hemolysis (82.97 ± 3.56% and 86, 62 ± 1.44%, respectively). In contrast, the aerial parts showed very weak hemolytic activity. These results indicate that *S.vulgaris* has considerable antioxidant properties, particularly the leaves, which can make it a good nominee for subsequent investigations.

Keywords: *Silene vulgaris* (*Moench) Garcke,* Antiinflammatory activity, Decoction, Hemolytic activity, Maceration.

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1. Introduction

For thousands of years, people have used naturally growing plants as medicinal cures for a wide range of ailments and pains due to their high antioxidant content, such as polyphenols, flavonoids, and tannins, which may provide protection against a number of diseases, such as Alzheimer's, inflammation, and cancer (1). Plants are also a valuable resource for the pharmaceutical industry

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and a rich source of antimicrobial agents (2). *S.vulgaris* is an herbaceous perennial plant with flowers that belongs to the Caryophyllaceae family (3). It commonly grows in weedy, semi-arid, and open-dry areas, reaching a height of up to 60 cm (4). It is often referred to as bladder campion, which is the most widely recognized English common name, derived from the plant's distinctive inflated calyx (10-13 mm) that resembles a bladder (5). Although *S. vulgaris* is native to Europe, it has spread to other parts of the world, including the Mediterranean region and sections of North America (6, 7). Its adaptability to different soil types and environments has facilitated its spread beyond its native area (8). Due to its widespread distribution and various cultural interactions, it has accumulated a multitude of vernacular (common) names across different regions and languages, in Algerian west; it is called “Tighighet” and “Tighecht”. These names often reflect the plant's physical characteristics and uses.

Historically, people have used *S. vulgaris* for a variety of purposes. In Italy, people commonly eat the young leaves and stems of S. vulgaris raw in salads as an antianemic (9), sedative, anti-inflammatory, and antitoxic agent (10, 11). Also, in Corsica, *S. vulgaris* is commonly used in soup along with other kinds of natural herbs (12). In Spain, *S. vulgaris* is commonly referred to as 'bladder campion' and is known to contain high levels of biologically active substances such as lutein and β-carotene (13; 14), which possess antioxidant effects (15, 16). Moreover, people commonly use the plant's roots, rich in saponins, as a soap substitute in washing sheep’s wool and clothes for their detergent properties [17].

Therefore, there is not much scientific proof to back up its traditional applications for treating ailments, including inflammation. Moreover, research on in-vitro hemolytic activity is emerging as a new field in drug discovery. Since a large population consumes this plant, the primary aim of our research is to determine the hemolytic activity and anti-inflammatory properties of aqueous extracts derived from *S. vulgaris* using different parts of the plant.

2 Material and Methods

2.1 Extraction

After collecting *S.vulgaris* parts (roots, leaves, stems, and flowers) from Ain Dehab province, Tiaret city (located in the Algerian west in a semi-arid climate)(Figure 1), all the parts were cleaned and dried away from sunlight, then crushed to get fin powder and stored until further use. A voucher specimen was deposited at the herbarium of the laboratory under the code number EHB-SV05-07. In addition, the plant was molecularly identified, and the sequence was uploaded to the Genbank database (accession number: PP316332.1).

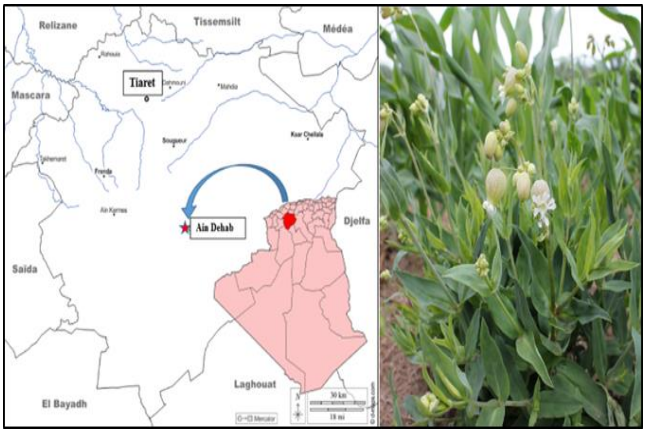

Figure 1. Geographic map of the collecting area of *S. vulgaris.*

The process of preparing plant extracts involves the adoption of decoction and maceration-filtration procedures. The aqueous decocted extract was prepared by combining 10 grams of powdery plant parts with 100 ml of distilled water. The raw materials were cooked in the reflux assembly for 30 minutes until they reached boiling point, after which they were filtered and concentrated until they turned

dehydrated. The macerated extract was placed in the rotator at a speed of 200 rpm, using the same proportion. After an entire day of 24 hours, the mixture was subjected to filtration and subsequent evaporation until it reached a state of total dryness. After calculating the yield, it was kept at 4 °C out of direct sunlight for later use (18).

2.2. Anti-inflammatory activity

Bovine Serum Protein Denaturation Method

Preparation of Reagents

Phosphate buffer solution (PBS): dissolve 0.2 grams of potassium chloride (KCl), 1.44 grams of disodium hydrogen phosphate (Na_2HPO_4), and 8 grams of sodium chloride (NaCl) and 0.24 grams of potassium dihydrogen phosphate (KH_2PO_4) in 800 ml of distilled water. The pH of the solution was adjusted to 6.3 by adding 1N hydrochloric acid (HCl), and the volume was adjusted to 1L using distilled water (19).

Bovine serum albumin (BSA) 0.5%: in 100 ml of PBS, dissolve 500 mg of BSA.

Method: The test solution consists of 0.45 ml of a 0.5% BSA solution and 0.05 ml of the sample solution with varying doses (0.5, 1, 2, and 4 mg/ml). The test control solution contains 0.45 ml of 0.5% BSA solution and 0.05 ml of distilled water. A product control solution is prepared by combining 0.45 ml of distilled water with 0.05 ml of the sample solution in different doses. The standard solution is composed of 0.45 ml of 0.5% BSA solution and 0.05 ml of diclofenac sodium in varying doses (0.125, 0.25, 0.5, and 1 mg/ml).

Procedure: 0.05 ml of test samples/ the standard was combined with 0.45 ml (0.5% w/V BSA). The specimens were placed under incubation at a temperature of 37°C for 20 minutes in a water bath. The temperature was elevated to continually keep the samples at a constant 75°C for a duration of 3 minutes inside the same water bath. Following the cooling process, introduce 2.5 ml of phosphate buffer into the specimens. The absorbance was measured at a wavelength of 255nm against a blank containing PBS. The test control signifies total denaturation of the protein. A comparison was made between the outcomes and the used standard. The calculation for determining the percentage inhibition of protein denaturation is as follows:

Percentage inhibition = [{(optical density of test solution – optical density of product control) / optical density of test control} - 1] × 100 (20).

2.3 Hemolytic activity

2.3.1. Preparation of the erythrocyte suspension

A healthy donor provided fresh blood, which was collected in a heparinized tube and subjected to centrifugation at 1500 rpm for a period of 5 minutes. After the elimination of the plasma, the pellet underwent three steps of washing using a PBS solution (pH= 7, 2 - 7, 4) and was centrifuged each time at 1500 rpm; the pellet was thus diluted with PBS to get an erythrocyte suspension (1–3%).

2.3.2. Preparation of extracts

The different extracts of *S.vulgaris* are diluted in PBS to obtain various doses (0.125, 0.25, 0.5, and 1 mg/ml).

2.3.4. Hemolysis test

Place in each hemolysis 0.5 ml of the prepared erythrocyte suspension with 0.5 ml extract at varying doses (0.125, 0.25, 0.5, and 1 mg/ml). Incubate the tubes in a water bath at 37°C for 30 min. Every 15 minutes, 1000 µl samples are taken and added to 1000 µl of PBS. The tubes are gently mixed and subjected to centrifugation at 1500 rpm for 10 minutes, after which the reaction is stopped with a cold bath.

The blank (negative control) is prepared using the same experimental steps. It comprises 1 ml of

erythrocyte suspension and 1 ml of PBS. Under the same conditions and experimental procedures, we prepared the positive control tube of total hemolysis, which contains 0.5 ml of the erythrocyte suspension and 0.5 ml of Triton X-100. Each tube's absorbance reading is recorded with a UV-visible spectrophotometer at 540 nm.

The extracts had colors that were detected with the spectrophotometer; this could influence the real absorbance of the hemolytic activity, so in the same conditions, a positive control was prepared of the extracts, took 1000 ul of each concentration, and mixed with 1000 ul of PBS tampon. The same blank was used to read the absorbance, and then we subtracted it from the absorbance of the extracts with erythrocyte suspension.

The rate of hemolysis is calculated as a percentage relative to the hemolysis total after 30 minutes of incubation, according to the following formula:

Hemolysis rate (%) = (Abs (extract) - Abs (negative control)-Abs (positive control) / Abs (total hemolysis) × 100 (21, 22, 23).

3. Result

3.1. Anti-inflammatory activity

The denaturation of the egg albumin method was applied to assess the anti-inflammatory properties of the studied plant. Dec-H_2O and Mac-H_2O extracts, particularly the leaves, exhibited the maximum inhibition rate at a dose of 4 mg/ml (Figures 2 and 3).

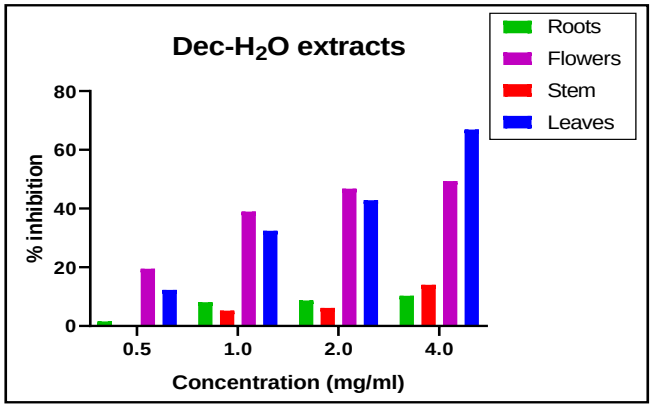

Figure 2. Inhibition percentage of the anti-inflammatory activity of aqueous decocted extracts of *S.vulgaris* parts.

The Mac-H_2O leaf extract exhibited a considerably greater level of inhibition (88.31%) compared to the Dec-H_2O leaf extract (66.88%).

Also, the Mac-H_2O stem extracts exhibited a good inhibition rate even at low concentrations (42.85% at 1 mg/ml). In addition, as the concentration went up, the inhibition rate for Dec-H_2O extracts, Mac-H_2O extract, and the standard diclofenac sodium (Figure 4) steadily increased.

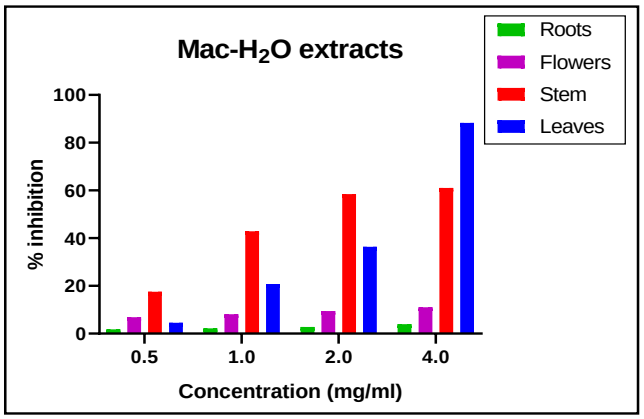

Figure 3. Inhibition percentage of the anti-inflammatory activity of aqueous macerated extracts of *S.vulgaris* parts.

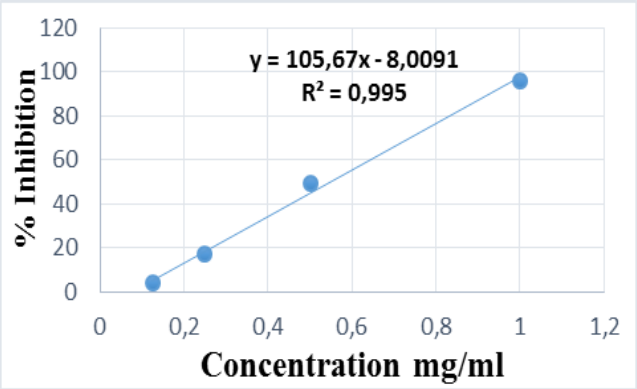

Figure 4. Inhibition percentage of the anti-inflammatory activity of diclofenac sodium (standard).

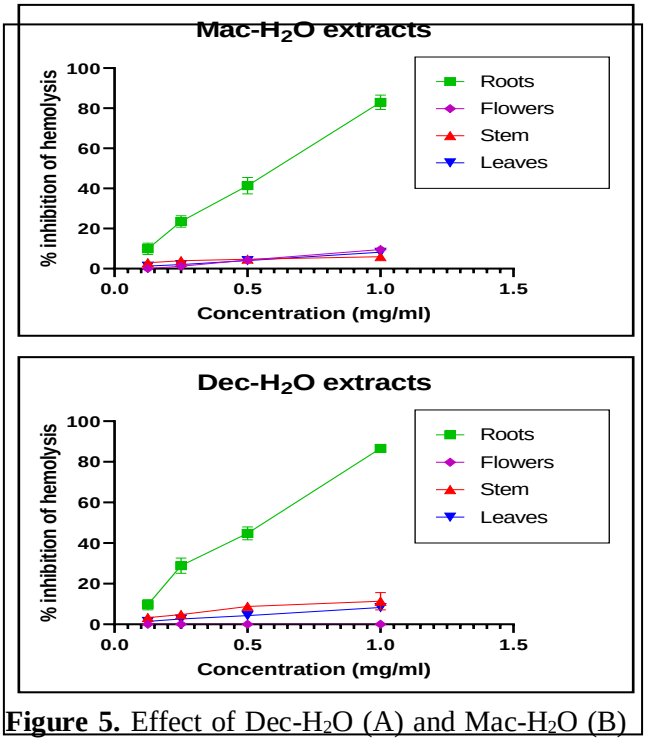

Figure 5. Effect of Dec-H_2O (A) and Mac-H_2O (B) extracts of S.*vulgaris* parts (Roots, flowers, Stem, Leaves) on inhibition of hemolysis. Data are presented as the means ± standard deviations of three replicate determinations.

3.2 Hemolytic activity

The present study aimed to evaluate the hemolytic activity of aqueous extracts derived from various parts of *S.vulgaris* against normal human erythrocytes. The extracts had a slight to strong hemolytic impact on human erythrocytes. The plant's hemolytic activity is determined as the percentage of inhibition of hemolysis and then represented as the mean ± standard deviation of three duplicates.

The outcomes in (Figure 5) show that for both types of aqueous extract, the hemolytic activity of *S. vulgaris* aerial parts was remarkably low (at doses of mg/mL). Conversely, at a concentration of 1 mg/ml, the root extracts prepared with Dec-H_2O and Mac-H_2O showed considerable hemolytic activity (86, 62± 1.44%, and 82.97± 3.56%, respectively).

4. Discussion

This study showed that the aerial parts of *S.vulgaris* vulgaris, particularly the leaves, exhibit an anti-inflammatory effect, which can be related to the presence of some particular bioactive chemicals such as flavonoids, phenolic acids, tannins, terpenoids, and saponins (24, 25). The findings of the research (26) indicated that the anti-inflammatory activity is attributed to flavonoids and terpenoids. In the same context, triterpenes, particularly saponins, have been found to possess anti-inflammatory properties (27). Prior research has already claimed that flavonoids also have anti-inflammatory properties (28). Hence, the anti-inflammatory effect of S.vulgaris could perhaps be attributed to the synergistic action of its secondary metabolites, including flavonoids, saponins, tannins, and terpenoids. It is well known that *S. vulgaris* roots contain an elevated level of saponins that have

detergent qualities (29). According to a study done on the impact of saponin on erythrocytes (30), it was possible to demonstrate that human erythrocyte exposure to saponin stimulates Ca2+ entry, which in turn causes cell membrane scrambling and ultimately leads to the suicidal death of human erythrocytes, and this effect is paralleled by hemolysis. Nevertheless, it is essential to acknowledge that although traditional applications and first findings indicate the potential efficiency of S. vulgaris as an anti-inflammatory agent, further studies need to be carried out to completely explain its biological mechanism of action, toxicity profile, therapeutic efficacy, and safety of consuming this edible plant.

5. Conclusion

The aqueous extracts of plants used in this investigation exhibited encouraging selectivity searches, with a particular focus on the leaves. These extracts revealed an anti-inflammatory impact in vitro, rendering them an interesting alternative for further investigation. The present study offers evidence from scientists about the impact of saponin-rich plants on human blood cells. Furthermore, we demonstrated the hemolytic capacity of many parts of *S. vulgaris* for the very first time, indicating that specific parts, such as the leaves and stem, may be acceptable for consumption, whereas the roots can damage human blood cells. Our findings have the potential to enhance the safe ethnopharmacological application of herbal remedies, hence fostering further investigation into their bioactive components' efficacy against inflammatory diseases and the underlying processes regulating their biological activity**.**

6. Conflicts of interest

“There are no conflicts to declare”.

7. Funding

“None”

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