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Phytochemical profiling and anti-inflammatory activity of *Atriplex halimus* L. leaf extracts

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Abstract

Climate change, particularly drought and soil salinity, severely affects agricultural productivity and biodiversity in arid regions. Halophytic plants such as *Atriplex halimus* L., naturally adapted to saline and oxidative stress, represent promising sources of bioactive compounds. This study investigated the phytochemical profile and in vitro anti-inflammatory activity of *Atriplex halimus* leaves collected from the M'sila region, central Algeria.

Preliminary phytochemical screening revealed the presence of major classes of secondary metabolites, including phenolic compounds (tannins, flavonoids, and coumarins), terpenoids (phytosterols, triterpenes, saponins, and iridoids), and alkaloids. A high foam index indicated a substantial saponin content and cardiotonic glycosides were not identified in the extract.

Two extracts were prepared by maceration: a hydroethanolic extract and an aqueous extract. Anti-inflammatory activity was evaluated using protein denaturation inhibition and erythrocyte membrane stabilization assays. Both extracts demonstrated measurable activity, with the hydroethanolic extract consistently showing higher efficacy than the aqueous extract.

These findings suggest that *Atriplex halimus* is a natural source of compounds with moderate anti-inflammatory potential, warranting further phytochemical and in vivo investigations.

Keywords: *Atriplex halimus*; Leaves; Aqueous extract; Phytochemical Screening; Anti-inflammatory activity

1. Introduction

Faced with the increasing threat of global warming, which manifests itself through drought and soil salinity, these phenomena worsen soil degradation, limit agricultural productivity, floristic biodiversity, and impact the living conditions of local populations[1]. The so-called halophytic plants that can grow in these extreme climatic conditions are a real alternative and an ace problem solution[2].

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Atriplex halimus L. is a halophytic plant that grows spontaneously in the Algerian steppe[3], this plant called *Gteff* in Arabic or *Armâs* in Berber and Tuareg[4], used in traditional medicine as a hypoglycemic agent and to treat female pathologies.

This steppe plant is characterized by a persistent foliage rich in nitrogenous matter and resistance to salinity, which makes it mainly a halophytic and forage plant, highly appreciated by livestock during the dry season[5].

As a halophytic plant, *Atriplex halimus* L. has a remarkable ability to adapt to salt and oxidative stress, thanks to a particularly effective antioxidant system. Halophytes are indeed recognized for their richness in phenolic compounds, whose biological activity sometimes exceeds that of synthetic antioxidants, often controversial for their potential toxicity. These halophytic plants represent practically an untapped reservoir of a new generation of foods and medicines[6].

The present study aims to identify the phytochemical constituents of *Atriplex halimus* L. leaves through preliminary screening and to evaluate their anti-inflammatory activity.

2. Materials and Methods

2.1. Plant material

Leaves of *Atriplex halimus* L. were collected from El Maarif locality near Chott El Hodna, in M'sila Province, located in central Algeria. Sampling was carried out during the flowering season (June–September 2021). The plant parts used in this study are the leaves. The leaves were dried out of direct sunlight, at room temperature in a dry and ventilated place. The dried plant parts are crushed and sieved. The water content is checked (less than 10%). The recovered powder is stored in a glass container at room temperature and protected from light.

2.2. Phytochemical Screening

Preliminary phytochemical screening of *Atriplex halimus* leaf extracts was performed using standard qualitative methods based on colorimetric and precipitation reactions to detect major classes of secondary metabolites. The results were recorded as present (+), absent (−), or weakly present (±).

Alkaloids were detected using Mayer's, Wagner's, and Dragendorff's reagents after acid extraction of the plant powder. The formation of characteristic precipitates indicated a positive reaction.

Phenolic compounds were identified using ferric chloride solution, which produces a blue-black coloration in the presence of phenols. Tannins were detected using Stiasny and Bate-Smith reactions, while flavonoids were identified using ammonium hydroxide, aluminum chloride, and cyanidin tests based on characteristic color changes.

Coumarins were detected under UV light (360 nm) after alkaline treatment. Anthocyanins were identified based on pH-dependent color changes, whereas anthraquinones were detected using the Borntrager and modified Borntrager tests.

Phytosterols and triterpenes were identified using Liebermann–Burchard and Salkowski reactions. Saponins were evaluated using the foam index method. Cardiotonic glycosides were detected using Keller–Killiani, Baljet, and Kedde reactions. Mucilages were identified based on their precipitation in ethanol.

All tests were conducted according to standard phytochemical procedures reported in the literature.

2.3. Preparation of extract

Two extracts were obtained by maceration, a hydroethanolic extract and an aqueous extract with 1 :10 ratio , to evaluate and compare their biological activities.

2.4. In Vitro Anti-Inflammatory Activity

The anti-inflammatory activity of *Atriplex halimus* L. leaf extracts was evaluated using two in vitro assays: inhibition of protein denaturation and erythrocyte membrane stabilization. Diclofenac sodium was used as the reference anti-inflammatory drug in both assays. All experiments were performed in triplicate.

2.4.1. Inhibition of Protein Denaturation

The inhibition of protein denaturation was assessed according to the method described by Adekola et al. (2022), with slight modifications. This assay is based on the ability of plant extracts to prevent heat-induced denaturation of albumin[7].

Egg albumin solution was prepared by diluting fresh egg white with distilled water to a final volume of 200 mL, followed by filtration. Different concentrations of the extracts (0.1–5 mg/mL) were prepared in distilled water.

For the assay, 2 mL of extract solution was mixed with 200 µL of egg albumin and 2.8 mL of phosphate-buffered saline (PBS, pH 6.4). The mixture was incubated at 37 ± 2°C for 15 min, then heated at 70°C for 5 min to induce protein denaturation. After cooling to room temperature, absorbance was measured at 540 nm using a UV–visible spectrophotometer.

Distilled water served as the negative control, while diclofenac sodium (10–100 µg/mL) was used as the positive control.

The percentage inhibition of protein denaturation was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

The IC_{50} value, defined as the concentration required to inhibit 50% of protein denaturation, was determined from the dose–response curve using linear regression analysis.

2.4.2. Erythrocyte Membrane Stabilization Assay

Membrane stabilization activity was evaluated following the method of Gupta et al. (2021), with minor modifications. This assay measures the ability of extracts to protect human erythrocytes against heat-induced hemolysis[8].

Phosphate-buffered saline (PBS, pH 6.4) was prepared by dissolving Na_2HPO_4 (1.79 g), KH_2PO_4 (1.36 g), and NaCl (7.02 g) in distilled water and adjusting the volume to 1000 mL.

Fresh human blood was collected from a healthy volunteer who had not taken anti-inflammatory drugs for at least two weeks prior to sampling. Blood samples were collected in EDTA tubes and centrifuged at 3000 rpm for 10 min. The erythrocyte pellet was washed three times with isotonic saline solution and resuspended in PBS to obtain a 3% (v/v) erythrocyte suspension.

Extract solutions (0.1–5 mg/mL) were prepared in distilled water. Diclofenac sodium (10–100 µg/mL) was prepared in PBS as a positive control.

For the assay, 1 mL of erythrocyte suspension was mixed with 1 mL of extract solution. The negative control consisted of erythrocyte suspension mixed with PBS, and the positive control consisted of erythrocyte suspension mixed with diclofenac solution.

The mixtures were incubated at 54°C for 30 min to induce hemolysis, followed by centrifugation at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 540 nm.

The percentage inhibition of hemolysis was calculated as:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

The IC_{50} value was determined from the dose–response curve and corresponds to the concentration required to inhibit 50% of hemolysis.

3. Results and discussion

3.1. Preliminary Phytochemical Screening

Preliminary phytochemical screening of *Atriplex halimus* L. leaves collected from M'sila revealed a broad spectrum of secondary metabolites, as summarized in Table 1. The three principal classes of plant secondary metabolites, namely phenolic compounds, terpenoids, and alkaloids, were represented in the analyzed extract.

Phenolic constituents were prominently detected, including condensed and hydrolysable tannins, flavonoids, and coumarins (figure 1 and 2). Positive reactions with ferric chloride confirmed the presence of phenolic compounds, while aluminum chloride and ammonium hydroxide tests suggested flavonol-type flavonoids. In contrast, anthocyanins and anthraquinones were not detected under the applied experimental conditions. The occurrence of flavonoids and tannins is consistent with previous studies conducted in various Algerian regions such as Biskra[9], Mascara and Ouargla[10,11]. However, the absence of anthocyanins, reported in some investigations, may reflect geographical, seasonal, or methodological differences.

Alkaloids were positively identified using Mayer's, Wagner's, and Dragendorff's reagents (figure 3), in agreement with several published reports[10–12]. Nonetheless, other studies have described negative findings for alkaloids in *Atriplex halimus*[9], suggesting that environmental factors, plant physiological state, and extraction parameters may influence their detectability.

Within the terpenoid class, phytosterols, triterpenes, and saponins exhibited strong positive reactions (figure 4). The foam index of 250 indicates a substantial saponin content (figure 5), exceeding values reported for samples from other regions, such as Biskra[9]. Iridoids were also detected. Cardiotonic glycosides were not clearly detected; moreover, their identification through colorimetric reactions remains method-dependent and may generate false-positive results.

Overall, discrepancies between studies on *Atriplex halimus* L. may be attributed to variations in geographical origin, climatic conditions, harvesting period, genetic background, and extraction protocols, all of which are known to influence phytochemical profiles.

Table 1 Qualitative phytochemical screening of *Atriplex halimus* L. leaves**.**

Phytochemical Class	Test / Reagent	Result
Alkaloids	Meyer	++
	Dragendorff	++
	Wagner	++
Phenolic compounds	$FeCl_3$	+++
Condensed tannins	Stiasny	+++
	Bath Smith	+++
Hydrolysable tannins	$FeCl_3$	+
Coumarins	NH_4OH	++
Flavonoids	NH_4OH	++
	$AlCl_3$	++
	Cyanidine	++
Anthocyanins	HCl/NaOH	−
Anthraquinones	Borntrager (without hydrolysis)	−
	Borntrager (with hydrolysis)	−
Iridoids	HCl	+
Phytosterols	Libermann-Burchard	+++

Triterpenes	Salkowski	+++
Saponins	Foam index	+++
Cardiotonic glycosides	Keller-Kiliani	+
	Baljet	–
	Kedde	–
Les mucilages	Ethanol precipitation	++

Results are expressed as follows: +++ strong presence; ++ moderate presence; + weak presence; – absence; ± doubtful presence.

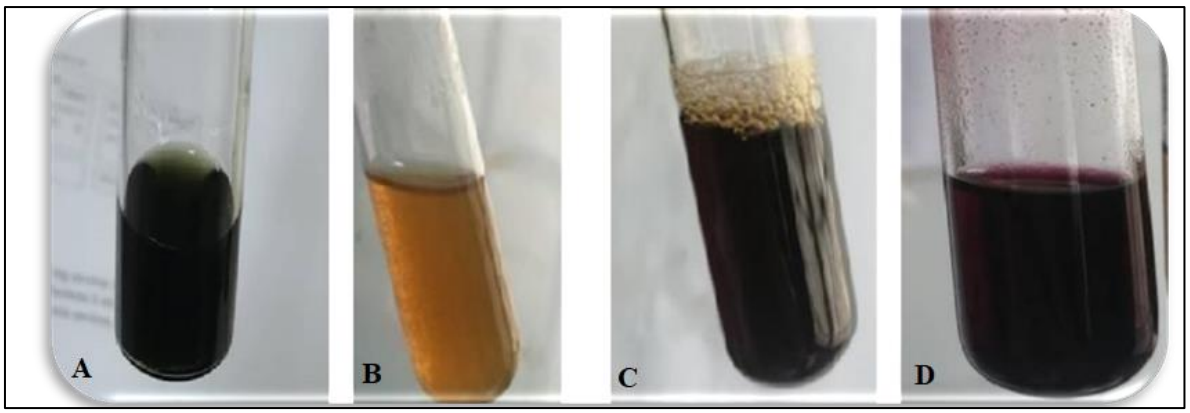

Figure 1 Qualitative identification tests for polyphenols and tannins in the extract of *Atriplex halimus* L. (A) Ferric chloride test for total phenolics; (B–C) Stiasny test for condensed tannins; (D) Bate–Smith test for condensed tannins

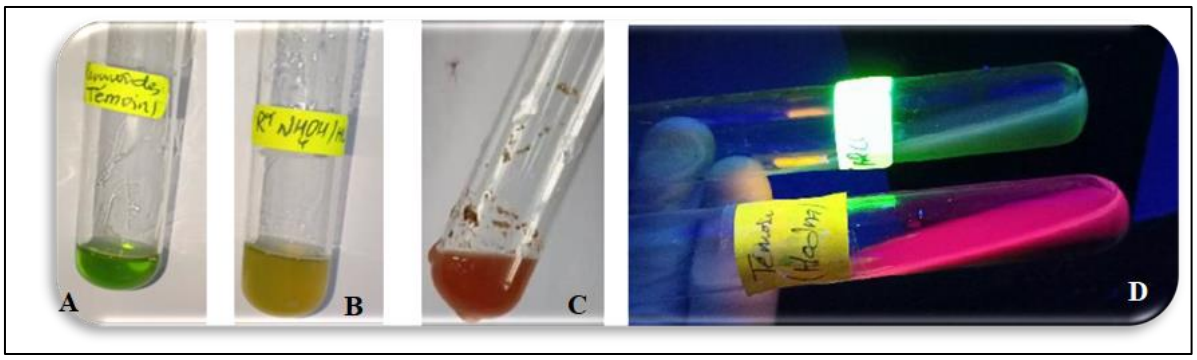

Figure 2 Qualitative identification tests for flavonoids in the extract of *Atriplex halimus* L. (A) Negative control; (B) ammonium hydroxide test; (C) cyanidin test; (D) aluminum chloride test

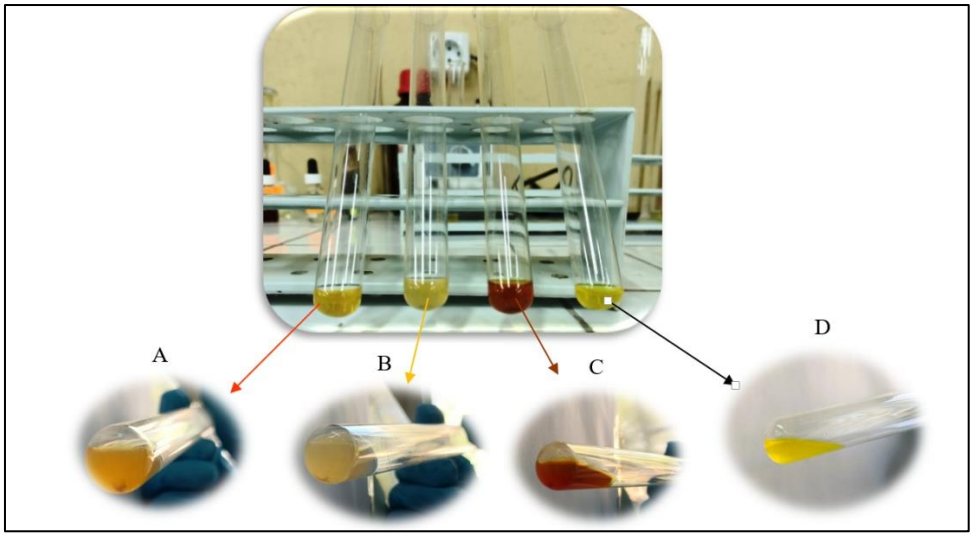

Figure 3 Qualitative identification tests for alkaloids in extract of *Atriplex halimus* L.
(A) Orange precipitate with Dragendorff's reagent; (B) white precipitate with Mayer's reagent; (C) brown precipitate with Wagner's reagent; (D) negative control

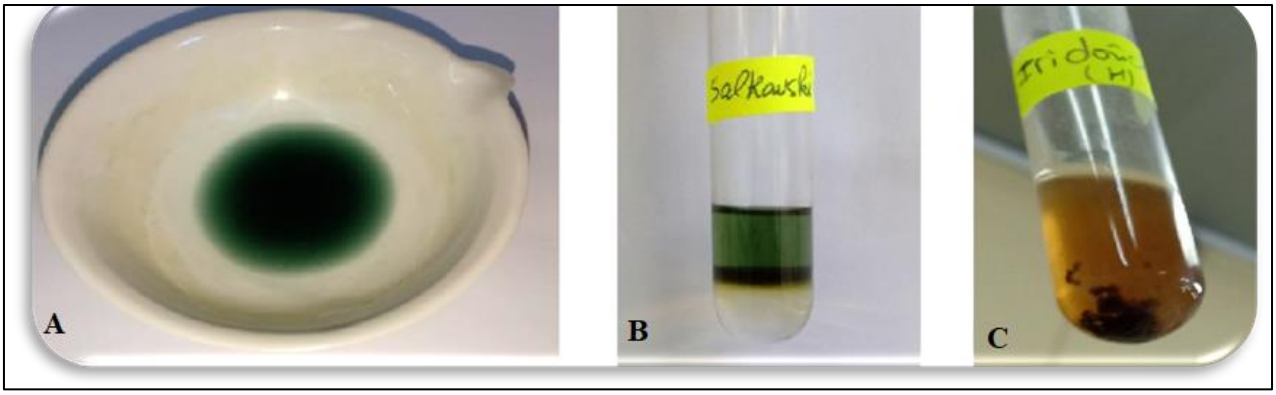

Figure 4 Qualitative identification tests for terpenoid compounds in the extract of *Atriplex halimus* L.
(A) Liebermann–Burchard test for phytosterols; (B) Salkowski test for triterpenes; (C) HCl test for iridoids

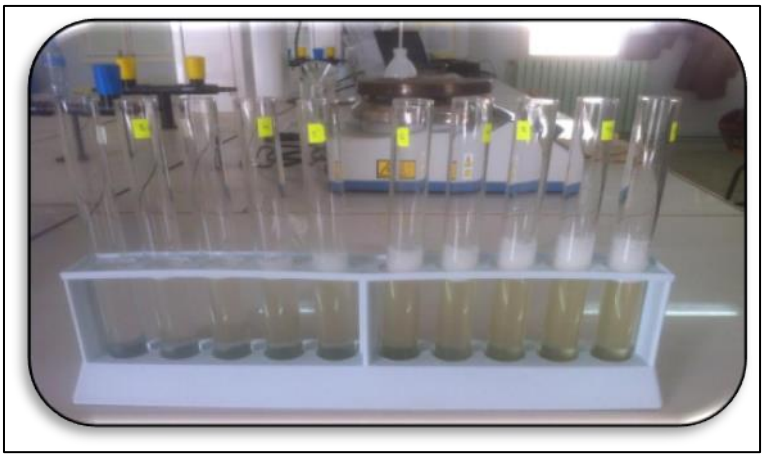

Figure 5 Determination of the Foam Index of the decoction of *Atriplex halimus* L

3.2. *In Vitro* Anti-Inflammatory Activity

The anti-inflammatory activity of *Atriplex halimus* L. leaf extracts was evaluated using two *in vitro* assays: inhibition of protein denaturation and membrane stabilization against heat-induced hemolysis. The results were expressed as IC_{50} values (mg/mL), calculated from linear regression curves. Diclofenac sodium was used as the reference drug (table 2).

In the protein denaturation assay, both extracts exhibited inhibitory activity. The hydroethanolic extract (HEO) showed an IC_{50} value of 1.51 mg/mL, slightly lower than that of the aqueous extract (1.75 mg/mL), indicating comparatively stronger activity. However, both extracts were markedly less potent than diclofenac sodium (IC_{50} = 0.103 mg/mL).

A similar trend was observed in the membrane stabilization assay. The hydroethanolic extract demonstrated an IC_{50} value of 8.01 mg/mL, compared to 9.13 mg/mL for the aqueous extract, confirming its slightly higher protective effect against erythrocyte hemolysis. Nevertheless, diclofenac sodium exhibited significantly greater activity (IC_{50} = 0.248 mg/mL), indicating that the plant extracts display moderate anti-inflammatory potency under the tested conditions.

These findings are consistent with previous reports, including those of Tadj et al. (2024), who described superior anti-inflammatory activity for ethanolic extracts of *Atriplex halimus* L. compared with aqueous preparations [13]. However, direct numerical comparison between studies should be interpreted cautiously due to methodological variations in extraction procedures, assay conditions, and reference standards.

The relatively higher activity of the hydroethanolic extract may be related to the enhanced extraction of polar and semi-polar bioactive compounds in the ethanol–water solvent system. Phenolic constituents, particularly flavonoids, are known to contribute to membrane stabilization and modulation of inflammatory processes, which may partially explain the observed activity.

Overall, the hydroethanolic extract demonstrated measurable *in vitro* anti-inflammatory effects, although its potency remains substantially lower than that of the synthetic reference drug.

Table 2 *In Vitro* Anti-Inflammatory Activity of *Atriplex halimus* L. leaf extracts.

Assay	Hydroethanolic Extract	Aqueous Extract	Diclofenac Sodium
Protein Denaturation (IC_{50}, mg/mL)	1.51	1.75	0.103
Anti-hemolytic Activity (IC_{50}, mg/mL)	8.01	9.13	0.248

4. Conclusion

The present study demonstrated that *Atriplex halimus* L. leaves collected from the M'sila region are characterized by a diverse phytochemical profile. Preliminary screening revealed the presence of major classes of secondary metabolites, including phenolic compounds (tannins, flavonoids, and coumarins), terpenoids (phytosterols, triterpenes, saponins, and iridoids), and alkaloids. The relatively high foam index further confirmed the richness of the extract in saponins, suggesting a notable presence of bioactive constituents.

The *in vitro* anti-inflammatory assessment demonstrated that both hydroethanolic and aqueous extracts of *Atriplex halimus* L. exhibit measurable activity in protein denaturation inhibition and membrane stabilization assays. In both models, the hydroethanolic extract consistently showed slightly greater effectiveness than the aqueous extract.

These findings suggest that *Atriplex halimus* L. constitutes a potential natural source of bioactive compounds with moderate anti-inflammatory properties. Further investigations, including detailed phytochemical characterization and *in vivo* studies, are warranted to clarify the mechanisms responsible for these effects and to better evaluate its therapeutic potential.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

I declare and all co-authors that we participated in the design, execution and analysis of the document and that I approve the final version. In addition, there is no conflict of interest in connection with this document, and the material described is not in the process of being published nor is it intended for publication elsewhere.

Statement of Ethical Approval

The experimental procedures involving human blood samples were conducted in accordance with institutional guidelines and ethical standards for research involving human participants. Blood collection was performed under strict hygienic conditions and used exclusively for in vitro laboratory analysis.

Statement of Informed Consent

Written informed consent was obtained from the healthy volunteer prior to blood collection.

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