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Comparative evaluation of anti-inflammatory and antioxidant properties of *Globularia alypum* L. extracts and gels from Northern Tunisia

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Abstract

Background *Globularia alypum* L. is frequently used in traditional medicine to treat skin diseases and abscesses; however, there is no scientific evidence indicating the main organ of this plant responsible for its biological activity.

Methods The present study aimed to assess the total phenolic and flavonoid contents, as well as the antioxidant properties and anti-inflammatory activity (AIA) of various parts (leaves, flowers, stems, and roots) of *Globularia alypum* L. collected from three regions in Tunisia. Additionally, hydrogels were formulated using aqueous leaf extracts to evaluate their rheological properties and the retention of bioactive compounds. Antioxidant capacity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, and AIA was determined using the enzyme lipoxygenase-5 (LOX-5).

Results High levels of total phenolic content (TPC) were detected in both the leaf and flower extracts, with 451.97 ± 11.24 and 421.95 ± 15.38 mg gallic acid equivalents per gram of dry material (mg GAE/g DM). The leaf extract exhibited a high total flavonoid content (TFC), with a concentration of 35.17 ± 0.82 mg catechin equivalents per gram of dry material (mg EQ/g DM) and showed the strongest antioxidant activity, with an inhibitory concentration of 50% (IC_{50}) was 0.149 mg/mL) and a significant LOX-5 inhibition (IC_{50} = 0.15 mg/mL). Hydrogels made with aqueous extracts from leaves retained 57% of polyphenols and maintained 58.6% of antioxidant activities. A rheological analysis of these hydrogels confirmed that the gel formulations are suitable for skincare applications, highlighting their potential for dermatological and anti-inflammatory treatments.

Conclusion This comparative study offers a scientific basis for the traditional use of this local plant's leaves, emphasising their potential as natural antioxidants and sources of bioactive compounds with anti-inflammatory properties.

Keywords *Globularia alypum L.*, Hydrogel, Phenolic content, Flavonoids, Antioxidant activity, Anti-inflammatory activity

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1 Introduction

Natural items have served as vital sources of drugs since ancient times, and a significant portion of modern pharmaceuticals has some connection to these natural sources. Recently, interest in obtaining biologically active chemicals from natural sources has grown noticeably.

There is a great deal of interest in plant polyphenols, as evidenced by the numerous papers devoted to various aspects of these substances [1]. Recently, their importance for the human diet and antibacterial action has been shown [2]. Many studies have shown that the antioxidant and antimicrobial properties of these chemicals [3] confer anti-inflammatory effects [4] to plant-based beverages.

Globularia alypum L. belongs to the Plantaginaceae family and is a shrub that thrives in dry environments and is typically found alongside cistus and rosemary [5]. This perennial plant grows well in rocky, arid, and overgrown areas, particularly favouring limestone. Often, these shrubs grow on cliffs or large, solitary boulders about 60 cm high. The evergreen leaves are oval, lanceolate, leathery, and broadened at the tip. They are described as spatulate because their base is tapered.

The ciliated bracts surround the dense flower heads, which are nearly 2 cm in diameter and are pale blue. The branch ends present a single spherical flower head [6]. Zrigua, as it is known locally, is one of the most often used traditional plant remedies in Tunisian pharmacopoeia. Its leaves have historically been used as a stomachic, purgative, laxative, cholagogue, and sudorific agent [7].

The biological activities of *Globularia alypum* L. can be attributed to its high concentration of various chemicals compounds as polyphenols, based on phytochemical analysis [8]. Moreover, their hypoglycemic leaves possess laxative, cholagogue, gastric, purgative, and sudorific properties, as well as stimulating insulin production [9].

The importance of oxidative stress in preventing chronic diseases, such as heart disease, cancer, diabetes, hypertension, and Alzheimer's disease, highlights the need to research the antioxidant properties of *Globularia alypum* L. leaves [10].

Several studies on the species Globularia have reported the presence of total polyphenols, flavonoids, and anthocyanins [11]. According to research on this species, extracts of *Globularia alypum* L. demonstrated antioxidant activity [12].

The hydroethanolic extract of *Globularia alypum* L. shows potential as a source of antioxidants, indicating that this plant could be responsibly utilised in food technology, processing, and medicinal applications. Over the past decade, there has been a notable increase in interest and use of natural cosmetic products, especially cosmetic gels for wound care [11]. Numerous studies have highlighted the therapeutic potential of herbal hydrogels, particularly their ability to reduce inflammation, protect mucosal tissues, and promote wound healing. Owing to their hydrophilic matrix, these formulations are especially suitable for delivering water-soluble bioactive compounds such as polyphenols and flavonoids [11, 12].

Although several studies have described the properties of *Globularia alypum* L., no work has compared its different plant parts or examined how extraction solvent and formulation influence its biological potential. In this study, we first evaluated the four organs and identified the leaves as the richest and most biologically active. Based on this finding, we then compared leaf aqueous extracts, hydroethanolic extracts, and a leaf-based hydrogel to determine which formulation provides the greatest functional value and whether the gel preserves the properties of its source extract. Since the traditional anti-inflammatory use of *Globularia alypum* L had never been validated through enzyme-based assays, we also investigated the anti-collagenase activity of both the extracts and the hydrogel. This integrated strategy, combining organ comparison, solvent comparison, and formulation assessment, fills a clear gap in the literature and offers the first comprehensive evaluation of *Globularia alypum* L. leaves for potential natural skincare applications.

2 Materials and methods

2.1 Plant material

Samples of *Globularia alypum* L. were collected from three different altitudes in the governorate of Kef (North–West Tunisia). The geographical coordinates of our sites are shown in Table 1.

The species *Globularia alypum* L (Fig. 1) was collected in February 2021 from three locations in the Kef region. The various parts of the plant—leaves, flowers, stems, and roots, were separated and dried in a well-ventilated area, away from direct light. Once dried, they were crushed and stored in glass bottles in a dark place. The identification of the plant material was carried out by Dr. Ridha El Mokni, a botanist in the Laboratory of Botany, Cryptogamy, and Plant Biology at the Faculty of Pharmacy of Monastir, Tunisia, where some voucher herbarium specimens—referred to as PLANTAG/Glob.aly., 10101/2021; 10201/2021; 10301/2021—are preserved in the personal

Table 1 Geographical coordinates of the three study sites

Provenance	Site	Elevation (m)	Latitude	Longitude
Neber	Jbal Twila	415	36°19.38′ N	008°45.43′ E
Seres	Jbal Maiza	650	36°04.706′ N	008°54.970′ E
Kalaat Snan	Ain Snan	1 048	35° 45. 4″ N	008° 23′ 14″ E

Fig. 1 *Globularia alypum* L

herbarium of Dr. Ridha El Mokni, housed at the Faculty of Pharmacy of Monastir (Monastir University).

2.2 Polyphenol extraction

2.2.1 Hydro-ethanolic and aqueous extracts

The four parts of fresh plants—leaves, flowers, stems, and roots—were air-dried in the shade at room temperature. After drying, the plant parts were ground into a fine powder. A 0.25 g sample of the powdered plant material from each part was mixed with 5 mL of 70% ethanol. This mixture was stirred for 3 h on a stirring plate in a water bath set at 50 °C, as described by Djeridane et al. Lenovo [13]. The tubes were centrifuged at 4500 rpm for 15 min at 25 °C. The supernatant was collected, and the same procedure was repeated by adding 5 mL of solvent to the pellet. The resulting solutions were then filtered using Whatman paper, resulting in a solution of unknown concentration.

To prepare an aqueous extract, ten grams of plant material were combined with 100 mL of distilled water. This mixture was then subjected to ultrasonic treatment in a water bath at 50 °C for 30 min. Following this, the solution was centrifuged at 4500 rpm for 15 min, then filtered through Whatman paper and processed in a manner similar to the hydro-ethanolic extracts to ensure consistency in preparation.

2.3 Formulation of hydrogels

The natural gel was formulated using the fusion method [14], 15. For a 50 g gel, the ingredients were as follows: 1% xanthan gum (gelling agent) (Sigma-Aldrich, St. Louis, MO, USA), 0.6% Cosgard (Benzyl alcohol, Dehydroacetic acid as a preservative) (Azelis, Antwerp, Belgium), 5% aqueous extract from *Globularia alypum* L. leaves and the remainder distilled water. The detailed composition of the gels is shown in Table 2:

The ingredients were weighed using a precision balance, and the gel was created by carefully mixing the components with a whisk until a homogeneous mixture was achieved. The formulations were stored in opaque plastic containers with lids to prevent evaporation. Quality control analyses were carried out according to the Cosmetic Products Stability Guide [16].

2.4 Evaluation of the yield

Oven-dried the flasks at 55 °C and weighed them empty before placing them in a rotary evaporator to remove the ethanol (at 40 °C and 80 mbar) and water (at 50 °C and 80 mbar).

2.5 Total phenolic content

Extracts from the samples and gels were used in quantities of 100 µL of each solution mixed with 500 µL of Folin-Ciocalteu (Sigma-Aldrich, St. Louis, MO, USA), reagent diluted 10 times in distilled water. After 2 min, 2 mL of 20% Na_2CO_3 (20 g/100 mL) was added, which favours an alkaline medium to trigger the oxidation–reduction reaction. The mixtures were placed in the dark for 30 min, and then the absorbance was measured at wavelengths of 750–760 nm using a blank solution that contained all the reagents except for the gallic acid [17]. All measurements were performed in triplicate.

The total phenolic content in the extracts was determined using a calibration curve with gallic acid (97.5–102.5%, Sigma-Aldrich, St. Louis, MO,USA)., expressed in milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g of DM) [18].

Table 2 The gels compositions

Gel code	Aqueous extract source	Xanthan Gum (%)	Cosgard (%)	Distilled water
G1	Leaves from Neber	1	0.6	q.s
G2	Leaves from Seres	1	0.6	q.s
G3	Leaves from Kalaat Snan	1	0.6	q.s
GT	Control (Distilled Water)	1	0.6	q.s

q.s: sufficient quantity (quantum satis)

2.6 Total flavonoid content

Flavonoids have a free hydroxyl group (OH) at position 5, which is likely to give, with the CO group, a colored complex with aluminum chloride (Sigma-Aldrich, St. Louis, MO, USA). Flavonoids form a yellowish complex through the chelation of metals such as iron and aluminum. This demonstrates that the metal (Al) loses two electrons to bond with two oxygen atoms in the phenolic molecule, which acts as an electron donor [18].

The TFC in the samples (extracts and gels) was quantified using a spectrophotometric assay. A mixture of 1 mL of 2% aluminum chloride and 1 mL of the extract was incubated in the dark for 15 min. The absorbance at 430 nm was measured against a blank prepared in the same way but the extract was replaced with 70% ethanolic solvent. All measurements were performed in triplicate in a microplate reader (BioTek Synergy 2, Winooski, VT, USA).

The TFC in the extracts was determined using a calibration curve with quercetin (≥ 95%, Sigma-Aldrich, St. Louis, MO, USA), expressed in milligrams of quercetin equivalents per gram of dry matter (mg EQ/g of DM) [17].

2.7 Biological activities

2.7.1 DPPH assay

DPPH (Sigma-Aldrich, St. Louis, MO, USA) is a stable purple free radical in solution. It exhibits a characteristic absorbance in the range of 512–517 nm. This colour disappears quickly when DPPH is reduced to diphenyl picryl hydrazine by a compound with antiradical properties, thus causing discoloration. The colour intensity was proportional to the capacity of the antioxidants present in the medium to donate protons [13]. 1 mL of a methanolic DPPH solution (8%) was added to 1 mL of the samples. For the hydro-ethanolic extracts, we used different dilutions (1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.1 mg/mL, 0.05 mg/mL, 0.025 mg/mL, 0.0125 mg/mL and 0.01 mg/mL).

The mixture was vigorously shaken, and then the tubes were incubated at room temperature in the dark for 30 min. The blank was represented by ethanol. The maximum absorption wavelength was previously determined. All readings were taken at 517 nm in the microplate reader.

According to [19], the antioxidant activity was estimated according to the following equation:

$$DPPH\,(\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \quad (1)$$

where is the DPPH radical percentage inhibition; A_{blank} and A_{sample} are the absorbance values of the control and of the test sample extract, respectively. It was possible to determine the sample concentration of the antioxidant activity that provided 50% inhibition of DPPH present in the test solution, referred to as IC_{50}. The lower IC50 indicate the higher antiradical activity. All tests were carried out in triplicate, and IC_{50} values were reported as means ± SD of the triplicate measurements. However, for the aqueous extracts and the gels, we used a solution of Trolox (2-carboxylic acid-6-hydroxy-2,5,7,8-tetramethylchroman) (98%, Sigma-Aldrich, St. Louis, MO, USA) as the reference antioxidant to create the calibration curve and perform the sensitivity analysis of the method applied [20], 21.

2.7.2 Anti-inflammatory activity (AIA)

2.7.2.1 5-lipoxygenase activity The extract's LOX-5 (Sigma-Aldrich, St. Louis, MO,USA) activity was assessed using the methodology of Baylac and Racineand [21] and Nikhila et al. [22], using linoleic acid as the substrate. As a result of a series of dilutions of a stock solution with a concentration of with a concentration of 100 mg/ml (10, 5, 2.5, 1, 0.5, 0.25, 0.125, 0.1, 0.05 and 0.01 mg/mL), we generated hydroethanolic extracts (70%) of the various *Globularia alypum* L. organs (with triplicates for each organ) via the preliminary method described by Touaibia and Chaouch [23].

Next, 10 µL of LOX-5 was incubated with 800 µL of 0.1 M borate buffer solution (pH = 9.3) for 15 min at 25 °C. The reaction was started after adding 10 µL of the substrate (linoleic acid). The absorbance was then measured at 234 nm, in the microplate reader. Quercetin was used as a standard inhibitor at the same concentration as the extracts. The percentage of LOX-5 inhibition was calculated as follows:

$$I(\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \quad (2)$$

where I (%) is the inhibition percentage of the LOX-5, A_{blank} is the absorbance of the reaction without the extract and A_{Sample} is the absorbance of the reaction with the extract. The extract concentration providing 50% inhibition (IC50) was determined by plotting the inhibition percentage against the concentrations of the extract solution.

2.7.2.2 Collagenase activity The collagenase activity assay is used to evaluate the enzymatic activity of collagenase (Sigma-Aldrich, St. Louis, MO, USA) and to investigate the potential inhibition of aqueous leaf extracts and the corresponding hydrogels of *Globularia alypum* L. at a concentration of 250 µg/mL. Collagenase is dissolved in a 50 mM tricine buffer containing 10 mM $CaCl_2$ and 400 mM NaCl, adjusted to pH 7.5, to obtain an enzymatic activity concentration of 0.8 U/mL, fol-

lowing the supplier's recommendations. The substrate FALGPA (N-[3-(2-Furyl) acryloyl]-Leu-Gly-Pro-Ala) (Sigma-Aldrich, St. Louis, MO, USA), which is specific to collagenase, is also prepared in the same buffer at a concentration of 2 mM.

In the 96-well microplate reader, each well receives 25 µL of buffer, 25 µL of water or a solution containing the test inhibitor, and 25 µL of the collagenase solution. The mixtures are pre-incubated for 15 min at room temperature to allow initial interaction between the enzyme and the inhibitor. Subsequently, 50 µL of the FALGPA substrate solution is added to each well. Absorbance is measured at 340 nm immediately after substrate addition and then every two minutes for a total duration of 20 min [24]

The slope obtained from the linear regression of the recorded absorbance values, which corresponds to the reaction velocity (V), determines the enzymatic activity. A reference inhibitor, such as EDTA was 250 µg/mL (reference concentration), is also tested to validate the effectiveness of the studied compounds. Additionally, different substrate concentrations, ranging from 0.5 to 2.5 mM, can be tested to analyse the inhibition mechanism and its effect on collagenase activity.

The following formula is used to determine the percentage of AIA (collagenase activity inhibition):

$$Inhibition(\%) = \left(\frac{V_c - V_a}{V_c}\right) \times 100 \quad (3)$$

V_c is the negative control's reaction velocity (without inhibitor), and V_a is the reaction velocity in the presence of the tested compound (gel).

2.8 Rheological test

The Anton Paar MCR-92 rheometer (Modular Compact Rheometer Series, Anton Paar GmbH, Austria) operates based on the rotational rheometric principle, allowing the measurement of rheological properties under shear stress. It features a cone-plate geometry (CP50-1, 50 mm diameter, 1° angle), where each gel is placed between a fixed plate and a rotating cone. As the cone rotates at a controlled speed, it applies shear stress to the sample, allowing the measurement of viscosity (η), shear rate ($\gamma^{\cdot}$), and shear stress (τ). The rheometer can analyse how these parameters evolve with temperature, offering a thorough characterisation of the rheological behaviour of the fluid or gel. To start, the sample is homogenized and positioned on the lower plate of the rheometer. The cone is then lowered to the calibrated gap to ensure a uniform distribution of the sample [15]. Two types of measurements are conducted:

1. Viscosity as a function of shear rate, applying a γ sweep (e.g., 0.1 to 1000 s^{-1}) and recording viscosity (η);
2. Viscosity as a function of temperature, programming a thermal ramp (e.g., 20–80 °C) to observe η variations. The results help determine whether the material exhibits Newtonian or non-Newtonian behavior and assess its thermal stability.

2.9 Statistical analysis

SAS software version 9.4 was used to perform statistical analyses (SAS Institute, Cary, NC, USA). The significance of the differences between the extracts of the four plant sections and the three research sites, as well as between aqueous extracts and hydrogels, was evaluated using an analysis of variance (ANOVA) and the Tukey HSD test.

Values are expressed as mean ± SD ($n=3$). Different letters (a, b, c) indicate significant differences between organs ($p \leq 0.05$), while asterisks (*) indicate significant differences between provenances.

3 Results

3.1 Extract yield (EY)

A comparison of the various parts (flowers, leaves, stems, and roots) of *Globularia alypum* L. at three sites (Neber, Seres, and Kalaat Snan) showed significant differences ($P<0.01$) in extract yields among the plant parts (Table 3). Leaves and flowers exhibited the highest yields (EY) compared to stems and roots. Specifically, leaves had the highest EY, with percentages of 43.65 ± 4.40%, 43.71 ± 0.82%, and 40.32 ± 0.22% at Neber, Seres, and Kalaat Snan, respectively. Flowers followed, with values of 28.79 ± 0.32%, 27.27 ± 1.95%, and 28.42 ± 0.31% at the same sites Snan.

The results shown in Table 3 reveal notable differences in extraction yield (EY) among various plant parts. Specifically, the aqueous extraction of leaves produced 43.16 ± 0.85% for Neber, 37.46 ± 0.62% for Seres, and 45.09 ± 0.56% for Kalaat Snan. These results indicate that the choice of solvent does not significantly influence the extraction yield of polyphenols from the leaves.

3.2 Total polyphenol content (TPC)

The TPC was analysed in the leaves, flowers, stems, and roots of our species from the three provenances (Fig. 2). The leaves have greater TPC values than the other organs.

The TPC of the flower extracts significantly ($P<0.05$) differed between provenances, with the highest values observed in Kalaat Snan flower extracts (421.95 ± 15.38 mg GAE/g DM), followed by Neber and Seres.

Table 3 Extract yield (EY) with standard deviation (SD) of *Globularia alypum* L. (leaves, flowers, stems, and roots) in the studied regions (Neber, Seres and Kalaat Snan)

Region of Tunisia		% of extract yield in different parts of *Globularia alypum* L. ± SD			
	Aqueous extract	Hydro-ethanolic extracts			
	Leaves	Leaves	Flowers	Stems	Roots
Neber	$43.16^{a} \pm 0.85$	$43.65^{a} \pm 4.40$	$28.79^{a} \pm 0.32$	$13.08^{a} \pm 0.61$	$9.84^{a} \pm 1.14$
Seres	$37.46^{b} \pm 0.62$	$43.71^{a} \pm 0.82$	$27.27^{a} \pm 1.95$	$11.07^{b} \pm 0.20$	$6.61^{b} \pm 0.87$
Kalaat Snan	$45.09^{a} \pm 0.56$	$40.32^{a} \pm 0.22$	$28.42^{a} \pm 0.31$	$11.14^{a} \pm 0.66$	$6.69^{b} \pm 0.36$

n = 3, different letters within the same column indicate significant differences (one-way ANOVA and Tukey test), with $p < 0.05$, except for the stems and roots columns where $p < 0.01$

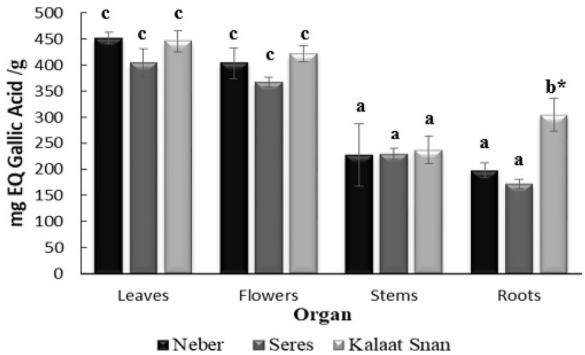

Fig. 2 The TPC (mg GAE/g DM) of hydro-ethanolic extracts of four *Globularia alypum* L. organs of in three locations of the Kef region (Neber, Seres and Kalaat Snan)

According to Fig. 2, the total phenolic content (TPC) in the stems ranged from 228.76 ± 10.69 to 236.94 ± 27.32 mg GAE/g DM. The TPC in the roots varied considerably among different provenances, with the highest values observed in Kalaat Snan at 304.02 ± 31.48 mg GAE/g DM, followed by Neber and Seres. These findings indicate a significant difference ($P < 0.01$) in TPC among the four plant parts (flowers, leaves, stems, and roots) across the three provenances (Neber, Seres, and Kalaat Snan). Generally, the flowers and leaves demonstrated relatively high polyphenol contents.

Table 4 compares the TPC of aqueous and hydro-ethanolic extracts from the leaves of *Globularia alypum* L. across three provenances: Neber, Seres, and Kalaat Snan. The results indicate that hydro-ethanolic extracts have significantly higher polyphenol content compared to aqueous extracts. For instance, the TPC value for the hydro-ethanolic extracts of *Globularia alypum* L. leaves from Neber was 451.97 ± 11.24 mg GAE/g DM, while the aqueous extracts showed a TPC of 95.29 ± 8.22 mg GAE/g DM. Hydro-ethanol exhibits approximately 4 to 5 times greater extraction efficiency than aqueous solvents. This is mainly because of its ability to solubilise a wider range of phenolic compounds, especially less polar ones constituents.

Hydrogels formulated from aqueous leaf extracts contain only 5% of the extract, which results in a substantial reduction in polyphenol content. The TPC values for the hydrogels are lower than those in aqueous extracts but remain significant, constituting about 5% of the original quantity. For instance, the Kalaat Snan hydrogel retains approximately 57% of the polyphenols present in the corresponding aqueous extract.

Additionally, the TPC of the aqueous and hydro-ethanolic extracts of *Globularia alypum* L. leaves was compared across three provenances: Neber, Seres, and Kalaat Snan. The results are presented in Table 4.

Table 4 The TPC (mg GAE/g DM), of aqueous leaf extracts and the corresponding hydrogels *Globularia alypum* L. in three regions (Neber, Seres and Kalaat Snan)

	Leaves hydro-ethanolic extracts	Leaves aqueous extracts	Hydrogels of leaves aqueous extracts
Neber	$451.97^{a} \pm 11.24$	$95.29^{a} \pm 8.22$	$63.53^{a} \pm 3.22$
Seres	$403.56^{a} \pm 28.36$	$98.28^{a} \pm 7.10$	$51.57^{a} \pm 6.44$
Kalaat snan	$445.90^{a} \pm 19.83$	$116.08^{a} \pm 9.50$	$66.23^{a} \pm 9.35$

n = 3, different letters within the same column indicate significant differences (one-way ANOVA and Tukey test), with $p < 0.05$

3.3 Total flavonoid content (TFC)

In Table 5, the results for aqueous and hydroethanolic extracts (leaves, flowers, stems, and roots) and hydrogel leaves of *Globularia alypum* L. in the studied regions (Neber, Seres, and Kalaat Snan).

Quantitative analysis of TFC revealed highly significant differences ($P < 0.001$) among plant organs (leaves, flowers, stems, roots) in *Globularia alypum* L., across the three study regions (Neber, Seres, and Kalaat Snan), as shown in Table 5.

Table 5 demonstrates that among the leaves, Kalaat Snan contained the highest TFC at 35.17 ± 0.82 mg EQ/g DM, followed by Neber and Seres. For flowers, Neber also had the highest TFC, at 11.22 ± 0.41 mg EQ/g DM, exceeding those of Kalaat Snan and Seres. The stems showed the lowest TFC, ranging from 1.89 ± 0.12 to 5.73 ± 0.39 mg EQ/g DM. Similarly, the TFC in the root extract ranged from 1.95 ± 0.26 to 5.46 ± 0.25 mg EQ/g DM.

The TFC of aqueous and hydro-ethanolic leaf extracts of *Globularia alypum* L. was compared across three provenances (Neber, Seres, and Kalaat Snan). Aqueous extracts exhibited higher flavonoid contents than hydroethanolic extracts. For instance, TFC values for aqueous extracts were 34.32 ± 5.42 mg EQ/g DM for Neber, 34.08 ± 1.59 mg EQ/g DM for Seres, and 40.32 ± 3.89 mg EQ/g DM for Kalaat Snan, whereas the values for hydroethanolic extracts were 31.15 ± 1.43 mg EQ/g DM, 27.37 ± 0.97 mg EQ/g DM, and 35.17 ± 0.82 mg EQ/g DM, respectively. This difference may be due to the greater water solubility of some flavonoid compounds.

3.4 Biological activity

3.4.1 Antioxidant activity (AOA)

The AOA of the hydroethanolic extracts (70%) from various plant parts (leaves, flowers, stems, and roots) of *Globularia alypum* L. was evaluated using DPPH. Figure 3 shows that leaf extracts exhibited the highest AOA, with IC_{50} values of 0.149 ± 0.010 mg/mL for Kalaat Snan, 0.159 ± 0.004 mg/mL for Neber, and 0.178 ± 0.014 mg/mL for Seres, indicating that Kalaat Snan had the strongest AOA.

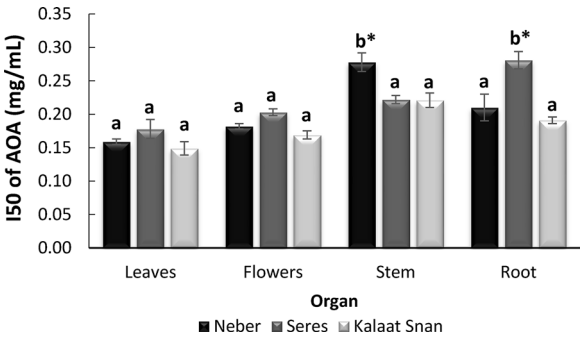

Fig. 3 IC_{50} of the AOA of the four organs of *Globularia alypum* L. (leaves, flowers, stems, and roots) in three regions (Neber, Seres and Kalaat Snan)

Neber and Kalaat Snan were important antioxidants, with IC50 values of 0.182 ± 0.004 mg/mL and 0.169 ± 0.006 mg/mL, respectively. Additionally, the stem and root extracts exhibited lower AOA compared to the leaf and flower extracts.

Interestingly, Kalaat Snan root extracts show significantly higher AOA than stem extracts and Neber and Seres' roots. AOA varies considerably ($P < 0.001$) among the different organs of *Globularia alypum* L. Leaves and flowers display notably higher AOA than stems and roots.

In our investigation, the AOA values were significantly higher than those of Khlifi et al. [25] analysed in Algeria. Nouir et al. [7] reported an antioxidant potential with an IC50 value of 0.240 mg/mL, determined through β-carotene assays, which is comparable to our AOA in root extracts. Furthermore, our AOA values were substantially higher than those reported by Mohamed et al. [26] in Tunisia, which had IC50 values of 0.067 mg/mL and 0.016 mg/mL, respectively.

Aqueous leaf extracts demonstrate significant AOA (Fig. 4), with IC50 values of 180.80 ± 2.46 µg/mL for Neber, 168.62 ± 5.15 µg/mL for Seres, and

Table 5 TFC with standard deviation (SD) of aqueous and hydroethanolic extracts (leaves, flowers, stems, and roots) and Hydrogel leaves of *Globularia alypum* L. in the studied regions (Neber, Seres, and Kalaat Snan)

Provenance	TFC (mg EQ/g of DM) in gels and different parts of *Globularia alypum* L. ± SD					
	Hydro-ethanolic Aqueous extracts				Aqueous extract	
	Leaves	Flowers	Stem	Root	Leaves	Gels
Neber	$31.55^{a*} \pm 1.43$	$11.22^{a} \pm 0.41$	$3.07^{a} \pm 0.02$	$3.39^{a} \pm 0.11$	$34.32^{a*} \pm 5.42$	$22.2^{a*} \pm 2.19$
Seres	$27.37^{b} \pm 0.97$	$8.49^{b} \pm 0.26$	$5.73^{b} \pm 0.39$	$1.95^{b} \pm 0.26$	$34.08^{a*} \pm 1.59$	$20.28^{a*} \pm 6.09$
Kalaat Snan	$35.17^{c} \pm 0.82$	$10.28^{a} \pm 0.65$	$5.46^{c} \pm 0.25$	$1.89^{c} \pm 0.12$	$40.32^{a*} \pm 3.89$	$22.20^{a*} \pm 4.95$

n = 3, different letters within the same column indicate significant differences (one-way ANOVA and Tukey test), with $p < 0.01$, except for the, *, where $p < 0.05$.

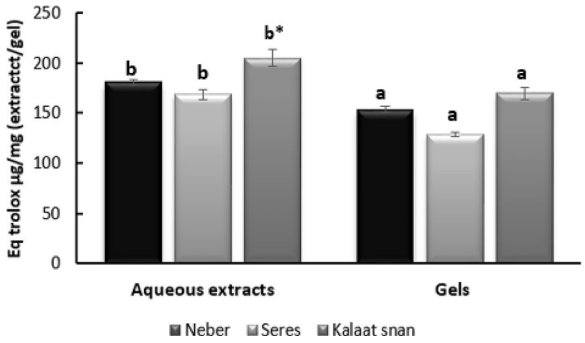

Fig. 4 AOA (DPPH) of aqueous leaf extracts and the corresponding hydrogels of *Globularia alypum* L. in three regions (Neber, Seres, and Kalaat Snan)

204.87 ± 8.45 µg/mL for Kalaat Snan. These results confirm that Kalaat Snan has the highest AOA, followed by Neber and Seres.

The results (Fig. 4) indicate that hydrogels retain significant AOA despite dilution (5%). The higher AOA is, according to Kalaat Snan, with 70.77% for the leave extract and 58.62% for the gel.

3.4.2 Anti-inflammatory activity (AIA)

3.4.2.1 Effect of hydro-ethanolic extracts on 5-lipoxygenase activity The results of the IC_{50} for the anti-inflammatory activity revealed that the different parts of *Globularia alypum* L. have a significant impact ($P < 0.001$) in the inhibitory effect of our hydroethanolic extract on the enzymatic activity of LOX-5, with values ranging from a maximum ($IC_{50} = 0.15 \pm 0.05$ mg/mL) to a minimum ($IC_{50} = 4.25 \pm 0.17$ mg/mL) (Table 6).

Since the extract with the lowest IC50 value exhibited the highest AIA, it is evident that the leaves and flowers have the most potent effects. The *Globularia alypum* L. leaf extracts demonstrated the greatest activity among the four plant parts, with IC50 values of 0.15 ± 0.05 mg/mL for Neber, followed by Seres and Kalaat Snan. Additionally, flower extracts showed IC50 values of 0.38 ± 0.04 mg/mL for Neber, then Kalaat Snan and Seres. In Neber, the roots had an IC50 value of 0.95 ± 0.16 mg/mL, while the values were higher in the other provenances. The IC50 of AIA in the stem extracts was 1.36 ± 0.15 mg/ml in Seres, followed by Neber and Kalaat Snan.

Table 6 IC_{50} (mg/mL) of the AIA of the four organs of *Globularia alypum* L. (leaves, flowers, stems and roots) from the studied regions (Neber, Seres and Kalaat Snan) on the enzymatic activity of LOX-5

Provenances/ Part of plant	Flower	Leaves	Stem	Root
Neber	$0.38^{a^*} \pm 0.04$	$0.15^{a^*} \pm 0.05$	$2.47^{a} \pm 0.18$	$0.95^{a} \pm 0.16$
Seres	$0.96^{b} \pm 0.17$	$0.46^{a^*} \pm 0.023$	$01.36^{b} \pm 0.15$	$2.87^{b} \pm 0.12$
Kalaat Snan	$0.54^{a} \pm 0.17$	$0.22^{a^*} \pm 0.05$	$4.25^{c} \pm 0.17$	$1.16^{a} \pm 0.07$

n = 3, different letters within the same column indicate significant differences (one-way ANOVA and Tukey test), with $p < 0.01$, except for the, *, where $p < 0.05$

3.4.3 Effect of leaves aqueous extracts and gels on Collagenase activity

The AIA of the aqueous extracts of the leaves of *Globularia alypum* L. and their corresponding hydrogels was assessed based on their ability to inhibit collagenase activity (Fig. 5). The aqueous extracts demonstrated significant inhibitory activity, with values of 41.57 ± 1.38% for Neber, 44.14 ± 1.14% for Seres, and 39.38 ± 2.22% for Kalaat Snan. These results suggest a promising potential for utilising these extracts in the treatment of skin disorders.

The hydrogels, comprising only 5% of the aqueous leaf extract, exhibited a notable reduction in inhibitory activity. The recorded values were 10.02 ± 2.72% for Neber, 18.95 ± 5.19% for Seres, and 12.64 ± 3.59% for Kalaat Snan. Although these values are lower than those of the aqueous extracts, they remain significant, suggesting that the hydrogels preserve AIA, even in a diluted form.

It is noteworthy that the extracts and gel with the highest phenolic content and most effective biological activity were obtained from Globularia plants collected in Kalaat Snan. These findings can be attributed to the site's highest altitude (1048 m) and its location in the southern part of the governorate.

3.5 Rheological test

Regarding the Viscosity as a Function of Shear Rate (Fig. 6), the control gel (GT) exhibits an initial viscosity of 550 mPa s at a low shear rate (1 s^{-1}), which gradually diminishes to 400 mPa s at a shear rate of 100 s^{-1}, indicating non-Newtonian behaviour. Meanwhile,

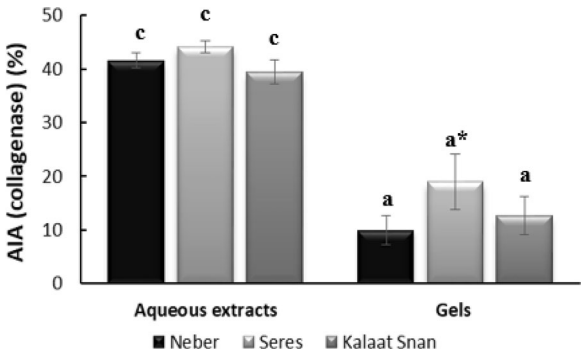

Fig. 5 The AIA of aqueous leaf extracts and the corresponding hydrogels *Globularia alypum* L. in the studied regions (Neber, Seres and Kalaat Snan)

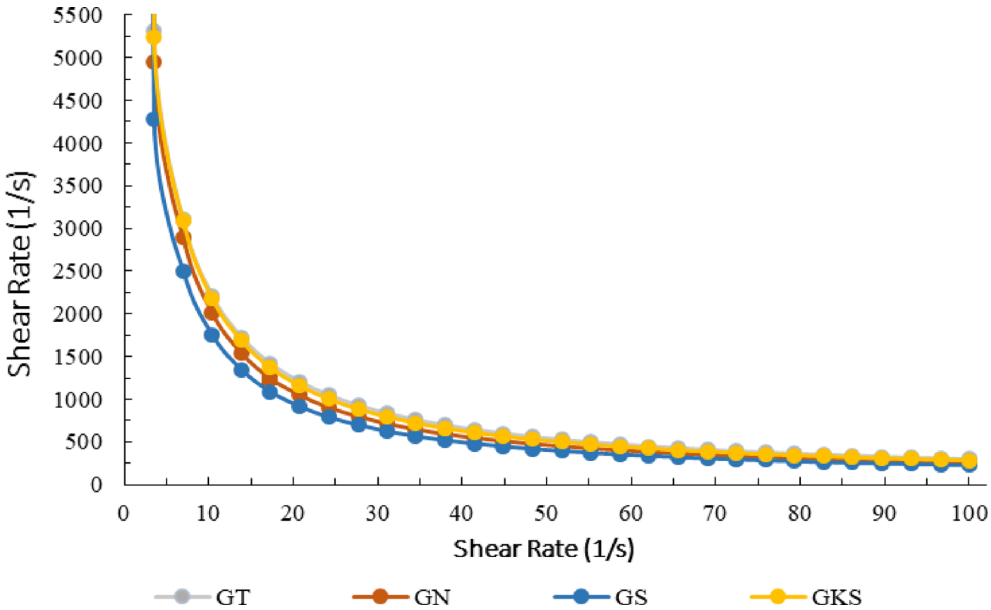

Fig. 6 Viscosity as a Function of Shear Rate for Four gels (Control gel (GT); gel made with leaf extract of Neber (GN); gel made with leaf extract of Seres (GS), and gel made with leaf extract of Kalaat Snan (GKS)

the GN, GS, and GKS gels display similar viscosity profiles with some variations. GN (Neber gel) starts with an initial viscosity of 525 mPa s, decreasing to 375 mPa s at 100 s^{-1}. GS (Seres gel) shows an initial viscosity of 500 mPa s, reducing to 350 mPa·s at 100 s^{-1}. Conversely, GKS (Kalaat Snan gel) has a higher initial viscosity of 575 mPa s, which declines to 425 mPa s at 100 s^{-1}. These findings suggest that GS (Kalaat Snan) possesses a more resilient rheological structure than GN and GC, possibly due to a higher content of bioactive compounds in the Kalaat Snan extract.

Upon analyzing the viscosity as a function of temperature (Fig. 7), we observed that the GT exhibits an initial viscosity of 550 mPa·s at 19 °C, which gradually declines to 400 mPa·s at 37 °C, indicating a slight loss of thermal stability. In comparison, the GN, GS, and GKS

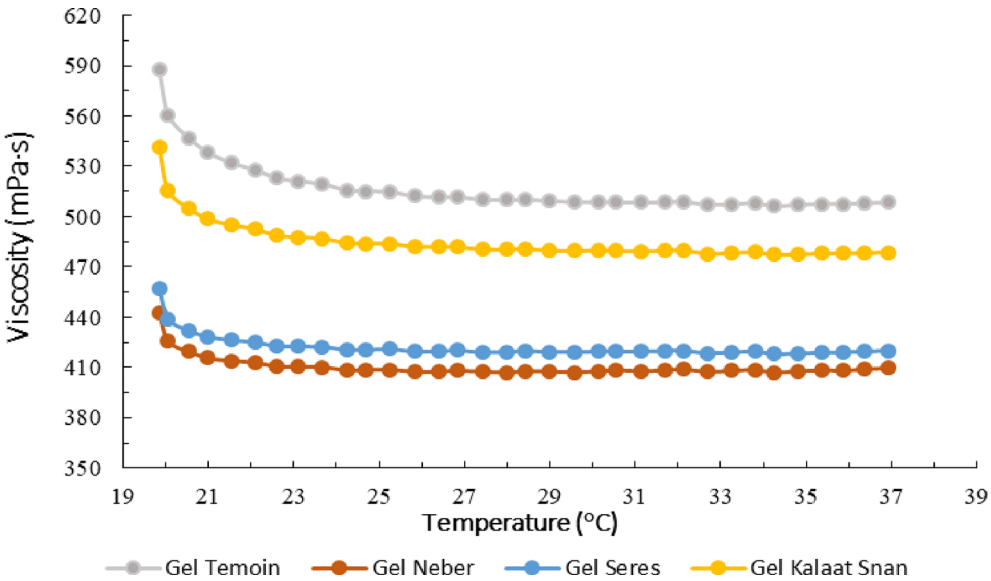

Fig. 7 Viscosity as a Function of temperature of four gels (Control gel (GT); gel made with leaf extract of Neber (GN); gel made with leaf extract of Seres (GS), and gel made with leaf extract of Kalaat Snan (GKS))

gels demonstrate similar behaviors, albeit with some differences. Specifically, GN has an initial viscosity of 525 mPa s at 19 °C, decreasing to 375 mPa s at 37 °C. GS shows an initial viscosity of 500 mPa s at 19 °C and decreases to 350 mPa·s by 37 °C. In contrast, GKS has a higher initial viscosity of 575 mPa s at 19 °C and remains relatively stable at 425 mPa s at 37 °C. These findings suggest that GKS exhibits better thermal stability compared to GN and GS, which may be attributed to a richer composition of polyphenols and flavonoids.

4 Discussion

This study highlights clear differences in the phytochemical richness and bioactivity of *Globularia alypum L.* across plant organs and biogeographical origins. Leaves consistently exhibited the highest extraction yields, TPC, TFC, AOA, and LOX-5 inhibition. These findings are consistent with the known accumulation of secondary metabolites in photosynthetically active tissues.

Among the four studied parts of *Globularia alypum* L., the leaves exhibited the highest yields (EY), with aqueous and hydroethanolic extracts ranging between 37 and 45%. However, these values remain lower than those reported by Sanchez-Moreno [27], who achieved 30.40% EY using the straightforward ethanol maceration. Similarly, Khlifi et al. [25] reported a lower yield (35.52%) for leaf extracts, which contrasts with our findings.

Hydro-ethanolic extracts outperformed aqueous extracts in terms of TPC, likely due to the intermediate polarity of the ethanol–water mixture, which enhances solubilisation of a broader spectrum of phenolic compounds. Similar trends were reported by Khlifi et al. [28] and Moroccan studies on related species.

The TPC of the hydroethanolic extracts was significantly higher than that reported in other studies conducted in Tunisia. For instance, Taghzouti et al. [29] reported a TPC of 157 mg GAE/g DM, while Asraoui et al. found a value of 180.50 ± 2.10 mg GAE/g DM. Both results are notably lower than ours. This trend is also evident in Moroccan research, where Khlifi et al. [25] reported that Soxhlet ethanol extraction yielded a higher TPC of 198 mg GAE/g DM than simple maceration, which achieved only 139 mg GAE/g DM.

Our aqueous leaf extracts and primary hydrogels showed values that surpassed those reported by Khantouche and Abderrabba [30] for ethanolic extracts, which were 25.33 mg GAE/g DM. Additionally, our results exceeded the ethyl acetate extract findings of 56.50 mg GAE/g DM from studies conducted in Algeria [28].

By comparing our results for TFC with those from other studies in Morocco, we found that Khlifi et al. [31] reported a TFC of 30.20 mg QE/g dry matter (DM) in ethyl acetate extracts, which is lower than our findings for hydro-ethanolic extracts. Additionally, various studies from Tunisia, including Asraoui et al. [32], reported a TFC of 10.50 mg QE/g DM, which is also lower than the values we observed for aqueous leaf extracts and gels.

As supported by several scientific studies, including that of Kawee-ai and bourassen et al. [12], 33, herbal hydrogels are recognised for their ability to effectively incorporate and deliver bioactive compounds. In our formulation, the hydrogels retained 50–60% of the phenolic and flavonoid content of the aqueous extracts despite containing only 5% extract, demonstrating good incorporation efficiency. This highlights their potential as promising candidates for topical applications.

The biological activity, specifically AOA, was compared to other findings in the literature. Loncaric et al. [34] reported that the IC50 value for the aerial parts of *Eryngium planum* L., when tested for LOX-5 activity using a 50% (v/v) ethanol solvent, was 0.0313 mg/mL. This value is lower than the AIA of the hydro-ethanolic extracts from the leaves and flowers of *Globularia alypum* L.. Additionally, Azad et al. [35] found that the IC50 value for the AIA of *Premna integrifolia* was 0.0165 mg/mL, which is lower than that of our hydro-ethanolic extracts from the aerial parts (flowers and leaves).

The significance of addressing skin disorders can be partly achieved by inhibiting a specific enzyme. The phytocompounds identified in our leaf extracts and their gels may be associated with the observed inhibition in these extracts. The inhibition values we measured were comparable to those reported in previous studies. For instance, Zofia et al. [24] tested various extracts for their ability to inhibit collagenase, finding inhibition rates ranging from 10 to 40% for *Meum athamanticum* L., with *Centella asiatica* L. showing an inhibition rate of 30%.

The rheological behaviour of all formulations demonstrated a clear shear-thinning (pseudoplastic) profile, a characteristic commonly reported for plant-based hydrogels intended for dermal application [36]. This behaviour facilitates spreading under mechanical stress while maintaining adequate consistency at rest, which is essential for topical stability and user acceptance, as the hydrogel has good fluid absorption capacity and can provide a moist environment for wound closure and re-epithelialization [37].

5 Conclusion

This study demonstrates that hydroethanolic extracts from *Globularia alypum* L., sourced from northwestern Tunisia, exhibit organ-specific bioactivity. Notably, the leaves and flowers show higher levels of polyphenols and flavonoids, along with improved AOA (DPPH, IC_{50}) and anti-inflammatory effects (LOX-5, IC_{50}), which meet or exceed values reported in previous

studies. Rheological characterisation further confirms the suitability of gel formulations for skincare, indicating potential applications in dermatological and anti-inflammatory treatments.

While the present findings highlight the strong antioxidant and anti-inflammatory potential of *Globularia alypum* hydrogels, future work should investigate their cutaneous bioavailability and in vivo efficacy to better assess their performance under real skin conditions. Evaluating parameters such as skin penetration, stability on the epidermis, and biological response in suitable in vivo models would further validate the relevance of these formulations for dermatological and cosmetic applications.

Abbreviations

A_{blank}	Absorbance values of the blank solution
Al	Aluminum chloride
ANOVA	Analysis of variance
AIA	Anti-inflammatory activity
AOA	Antioxidant activity
A_{sample}	Absorbance values of sample extract
CP50-1	Cone-plate 50 mm diameter, 1° angle (rheometry geometry)
DM	Dry matter
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DPPH (%)	DPPH radical percentage inhibition
EDTA	Ethylenediaminetetraacetic acid
EY	Extract yield
FALGPA	N-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala (collagenase-specific substrate)
GAE	Gallic Acid Equivalent
GKS	Gel from Kalaat Snan
GN	Gel from Neber
GS	Gel from Seres
GT	Control gel
HSD	Honestly Significant Difference
I (%)	Inhibition percentage of the lipoxygenase
IC_{50}	Half maximal inhibitory concentration
LOX-5	Lipoxygenase-5
MCR	Modular compact rheometer
q.s.	*Quantum satis* (As much as needed)
QE	Quercetin equivalent
SAS	Statistical analysis system
SD	Standard deviation
TE	Trolox equivalent
TFC	Total flavonoid content
TPC	Total polyphenol content
V	Reaction velocity
V	Reaction velocity
Va	Reaction velocity in presence of extract
Vc	Negative control's reaction velocity
$\dot{\gamma}$	Shear rate
η	Viscosity
τ	Shear stress

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Author contributions
SN did the experimental tests, wrote and authored almost of the manuscript; KM did authored part of manuscript and reviewed drafts of the paper; JC did the statistical analyses, wrote and reviewed drafts and final paper, MLF and YA reviewed drafts of the paper. All authors read and approved of the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing Interests
The authors declare no competing interests.

Ethics approval
There are no ethics statements.

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