Research Article

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Drimia maritima flowers as a source of biologically potent components: Optimization of bioactive compound extractions, isolation, UPLC–ESI–MS/MS, and pharmacological properties

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Abstract: An examination and study were carried out in order to investigate the beneficial advantages of *Drimia maritima* aqueous and hydroalcoholic extracts (DmAE/DmHE). The chemical profile was determined using rapid ultrahigh performance liquid chromatography–electrospray ionization tandem mass spectrometry analysis, revealing the presence of 31 bioactive components. Four different methodologies were employed to conduct a series of antioxidant testing. The DmAE and DmHE have exhibited a noteworthy antioxidant capacity, as evidenced by their significant half maximal inhibitory concentration values across several activities, comparable to the performance of DmAE. However, *D. maritimia* does not exhibit any reduction in power activity. Furthermore, a notable suppression of the critical enzyme (urease), which serves a role in various health conditions, including hyperpigmentation and pathological functional abnormalities, was discovered for the first time. A significant inhibition of the urease enzyme was observed for DmAE at a value of 122.04 ± 1.42. *D. maritima* had strong sun protection properties and demonstrated effective anti-inflammatory effects. The pharmacological activities of DmAE and DmHE were also conducted *in vivo* using analgesic and anti-inflammatory tests, and the oral toxicity was determined in accordance with the Organisation for Economic Cooperation and Development 425 Guideline. Intoxication symptoms were not observed in any of the treated animals following oral administration of DmAE and DmHE at 2,000 mg/kg. Conversely, the oral administration of DmAE and DmHE at varying concentrations inhibits the development of paw edema at different concentrations, which results in a substantial reduction in the acetic acid-induced writhing test. Chemical and spectroscopic inves-

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tigation of *D. maritima* flowers resulted in the isolation of sucrose, and it was never isolated in this plant species and Scilliphäosidin-3-*O*-*β*-D-glucoside, using proton nuclear magnetic resonance and carbon 13 nuclear magnetic resonance spectroscopy. As indicated by these findings, *D. maritima* has the potential to be utilized in a wide range of applications, including as a nutritional supplement, as an alternative treatment for a variety of physiological conditions and pathologies, and as a preventive and healing agent.

Keywords: anti-inflammatory, *Drimia maritima*, OECD 425, RP-UPLC–ESI-MS/MS, sun protection factor, NMR

1 Introduction

Medicinal plants have long been regarded as a significant source of compounds with potential therapeutic uses. Therefore, researching herbs and spices that have historically been used as analgesics makes sense as part of an effort to develop novel medications [1,2]. Natural compounds isolated from medicinal plants have long been a significant reservoir of medicinal compounds, with around 50% of medications originating from them [3]. Scientific inquiry has shown significant interest in natural plants in the past few years, and their interest has steadily increased. Therefore, during the past two decades, a substantial increase in the number of various databases and collections has occurred serving as comprehensive or specialized scientific sources on natural products. A considerable number of natural products databases and collections have been released and reused since 2000, with over 120 different ones identified [4].

The genus *Drimia* is known as bulbous plants [5,6]. In more recent taxonomic revisions, *Drimia* and related genera have been placed in the family *Asparagaceae*, subfamily *Scilloideae* [5,7]. *Drimia* has been associated with several synonyms. *Urginea* is indeed one of the primary synonyms of *Drimia* [5]. The name of the genus *Urginea* comes from the Algerian tribe of Ben Urgin [8–10]. These are bulbous perennial plants. After the leaves begin to grow, an upright blooming stem emerges. In dense clusters, the flowers are placed. With smooth edges, the leaves are lanceolate. Some species' pear-shaped bulbs can grow up to 30 cm [5]. The medicinal component of the *Drimia maritima* plant is primarily comprised of either dried or fresh bulbs, and it is widely distributed in the Mediterranean area, Africa, and India [11].

D. maritima bulb has been used in folk medicine to cure a variety of conditions, including jaundice, chronic bronchitis, asthma, pneumonia, wounds, hemorrhoids, and vipers bites [11,12]. Additionally, it is used to treat hepatitis, whooping cough, digestive disorders, ear ache, and whooping cough [1,13]. The *D. maritima* bulb extract also has strong anticancer, antioxidant, head lice, asthma, musculoskeletal pain, antimalarial, anti-insecticidal, and antifungal properties. Until this time, numerous investigations have been implemented to isolate and identify the structure of chemical components of this species' bulbs, such as bufadienolides [12,14,15], monoacylglycerol, fructan sinistrin, and lignan. Although significant examination has been performed on the bulbs, the chemical profile and biological activity of *D. maritima* flower extracts have yet to be well investigated.

The purpose of selecting *D. maritima* harvested from the Setif region-Algeria, considering previous research on other subspecies of *Drimia*, was to validate the consistent biological effects of *D. maritima*, as well as to thoroughly examine and categorize novel qualities and chemicals that specifically describe the plant of interest. Given this context, the aim of our investigation is to analyze the chemical composition and conduct a thorough assessment of the biological properties of extracts derived from the flowers of *D. maritima*. This study aims to fill a substantial gap in knowledge by investigating the antioxidant, anti-enzymatic, anti-inflammatory, and sun protection properties of flower extracts *in vitro*. Furthermore, it assesses these extracts' acute toxicity, analgesic, and anti-inflammatory properties *in vivo*. Our study aims to highlight the significant therapeutic potential of *D. maritima* flowers through a thorough analysis. This will enhance their use in developing new medications and natural health products.

2 Material and methods

2.1 Plant material

Urginea maritima (L.) Baker ssp. Numidica is known as Feraoun in the northeast of Algeria. During the flowering season (October 2020), the flowers of *D. maritima* were harvested in the Bougaa region (Setif 36°19′57″ north, 5°05′19″ east) in the northeast of Algeria. After 15 days of drying at room temperature in the shade, the plant material was shielded from light and moisture. With the accession number 202010CB/DrimMa, the voucher sample has been placed in the herbarium of the laboratory.

2.2 Extraction procedure

2.2.1 Preparation of extracts for isolation

The dried flowers of *D. maritima* (173.47 g) were macerated in hexane (270 mL) for 24 h to remove chlorophylls and

lipids. Subsequently, it was desiccated and filtered. The resulting material was macerated in a hydroalcoholic solution containing MeOH and H_2O (80:20) × 3 at ambient temperature for 24 h. The filtrate was concentrated to dryness following filtration, resulting in a viscous residue. It was suspended in H_2O and subsequently partitioned using $CHCl_3$, EtOAc, and then *n*-butanol. The organic fractions were condensed under reduced pressure.

2.2.2 Preparation of extracts for biological activities

Traditional medicine frequently employs aqueous extraction of *D. maritima*, hot or cold water based). *Drimia*'s aqueous and hydromethanolic extracts are utilized to treat skin maladies, calculous infections, and inflammation caused by injury [16,17]. For this reason, we have selected the aqueous and hydromethanolic extracts to conduct the pharmacological activities to confirm the traditional uses of *D. maritima*. Furthermore, phytochemical profiling is conducted to identify the specific bioactive chemicals that are accountable for the observed biological activities.

An agate mortar and pestle were employed to grind the dried flowers of *D. maritima* to a powdery consistency. By subjecting a solution of methanol and water (80:20, v/v) to continuous agitation while macerating overnight in the dark, the hydroalcoholic extract of *D. maritima* (DmHE) was acquired. Following the filtration of the maceration exudate, the obtained solution was subsequently evaporated at 35°C under vacuum utilizing a rotary evaporator. The procedure was iterated three times every 24 h. The aqueous extract of *D. maritima* (DmAE), which was utilized to assess biological activities, was prepared via the decoction method. To achieve this, 42 g of *D. maritima* flowers was grounded and steeped in 360 mL of H_2O for a duration of 30 min. Then, the resulting solution was dried with a freeze dryer and filtered through vacuum filtration.

2.2.3 Isolation and purification

The n-BuOH extract (7.74 g) was subjected to column chromatography (CC) on silica gel with gradient elution (CH_2Cl_2MeOH, 98:2–0:100); a total of 70 fractions were gathered and tested using a thin layer chromatography (TLC) plate. The plate was visualized using ultraviolet (UV) light (254 and 365 nm), and the results were revealed through the use of an acid mixture and heating.

F19–F20 fractions were combined and separated sequentially using C18 column using $H_2$0: MeOH (100:0–0:100) as eluent. **F′4–F′12** sub-fractions were combined and separated sequentially by using C18 column using $H_2$0: MeOH (40:1–20:1) as eluent. **F″19–F″27** sub-fractions were purified using a TLC plate successively with the system: CH_2Cl_2: MeOH: CH_3COOH – 10:1:0.01 to give pure compound ***C1*** (3 mg).

F29–F31 fractions were combined and then rechromatographed on a column of silica gel using CH_2Cl_2: MeOH as eluent with the proportions: 45:1–0:100. **F′19–F′37** sub-fractions were combined and then rechromatographed on a column of silica gel using CH_2Cl_2: MeOH as eluent with the proportions: 100:10–0:100. **F″5–F″21** sub-fractions were purified by using a TLC plate successively with the system: (CH_2Cl_2: MeOH: CH_3COOH – 4:1:0.01) to give pure compound ***C2*** (4 mg).

2.3 NMR spectroscopy

NMR spectra were recorded with a Bruker AMX-400 spectrometer (1H frequency: 400.13 MHz, 13C frequency: 100.62 MHz). Solvent: MeOD. Temperature: 303.1 K. An Aspect computer employing MestReNova software was used for data processing.

2.4 Qualitative and quantitative analyses

Ultra-performance liquid chromatography–mass spectrometry (UPLC/MS-MS) analysis of *D. maritima* extracts

The UPLC/MS-MS analysis of DmAE and DmHE involved using direct injection of 5 µL without a column (specifically, the Restek Ultra C18 3 µm 150 mm× 4.6 mm) on a SHIMADZU 8040 Ultra-High sensitivity instrument with UFMS technology. The instrument was equipped with a binary bump Nexera XR LC-20AD to optimize polyphenol standards. Gradient elution was used with a mobile phase consisting of solvent A (water), solvent B (methanol), and 0.1% formic acid at a total flow rate of 0.4 mL/min. The electrospray ionization (ESI) parameters were set as follows:

- The DL temperature: 250°C.
- The nebulizing gas flow rate: 3.00 L/min.
- The heat block temperature: 400°C.
- The drying gas flow rate: 15.00 L/min.
- The collision-induced dissociation gas pressure: 230 KPs.
- The conversion dynode voltage: – 6.00 kV °C.

2.4.1 Total bioactive components assessment

2.4.1.1 Total phenolic content

The quantification of total phenolic compounds (TPCs) in the DmHE and DmAE was conducted using spectrophotometric

analysis, precisely employing the Folin–Ciocalteu process with slight adjustments. To succinctly illustrate the experimental protocol, 100 µL of Folin–Ciocalteu reagent (1:10) with 75 µL of Na_2CO_3 (7.5%) was added to 20 µL of DmAE and DmHE, respectively. Following a period of incubation under limited lighting conditions for 2 h at room temperature, the absorbance at a precise wavelength of 765 nm was measured using a Perkin Elmer Enspire microplate reader. The quantity of polyphenol was ascertained by utilizing gallic acid (µg GAE/mg extract) [18].

2.4.1.2 Total flavonoid content (TFC)

The TFC was determined employing a modified aluminum colorimetric procedure. A microplate comprising 96 wells was utilized to meticulously dispense 130 µL of methanol. Then, 50 µL of DmHE and DmAE was introduced into the microplate. Furthermore, a volume of 10 µL each of $C_2H_3KO_2$ (1 M) with $Al(NO_3)_3$ at a 10% was added to the micro-plate. After an incubation period of 40 min at room temperature, the absorbance was measured utilizing a Perkin Elmer Enspire microplate reader with a 415 nm wavelength. In order to quantify your outcomes, the findings were expressed as micrograms of quercetin equivalent per milligram of extract (µg QE/mg) [19].

2.5 *In vitro* biological activities

2.5.1 Antioxidant activity

2.5.1.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical tapping test

The experiment utilized the methodology described by Blois to evaluate the impact of DmHE and DmAE on the DPPH radical [20]. About 160 µL of a pre-prepared DPPH solution was added to a 96-well microplate containing 40 µL of each extract and standard at a fluctuating concentration. The investigation utilized standard antioxidants, specifically α-tocopherol and butylated hydroxyanisole (BHA), and the experimental conditions were identical. The measurement of absorbance was conducted at a precise wavelength of 517 nm using a control solution consisting of a methanolic solution of DPPH. This measurement was obtained following an incubation period of 30 min. The outcomes were subsequently expressed as % of DPPH inhibition, which was measured utilizing the following equation:

$$\% = [(A_{Cnt} - A_{Spl})/A_{Cnt}] \times 100,$$

where A_{Cnt} is the absorbance of control and A_{spl} is the absorbance of the sample.

2.5.1.2 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) scavenging activity

The scavenging activity of $ABTS^{\cdot+}$ was evaluated in accordance with the method described by Re et al. [21]. About 7 mM ABTS and 2.45 mM potassium persulfate were utilized in a chemical reaction to produce the cation $ABTS^{\cdot+}$. After 24 h, the absorbance of the ABTS solution was adjusted to a value of 0.700 ± 0.020 at a wavelength of 734 nm. Then, a solution containing $ABTS^{\cdot+}$ was combined with 40 µL of DmHE, DmAE, and BHA in different concentrations into a mixture of 160 µL. The measurement of absorbance was performed at a precise wavelength of 734 nm, following a duration of 10 min in darkness.

2.5.1.3 Reducing power assay

Oyaizu [22] conducted the reducing power assay using potassium ferricyanide and then measured the absorbance at 700 nm. Subsequently, 1% potassium ferricyanide ($K_3Fe(CN)_6$) was added to a 0.2 M phosphate buffer solution containing standards and samples at varying concentrations. The resulting solution had a pH of 6.6. The obtained mixture was incubated at 50°C for 20 min. Then, a solution was introduced, which comprised 10% trichloroacetic acid and 10 µL of ferric chloride $FeCl_3$ (0.1%). The results were symbolically represented as $A_{0.5}$, where $A_{0.5}$ is the concentration at which an absorbance of 0.5 is obtained.

2.5.1.4 Silver nanoparticle (AgNP)-based assay

The reduction of Ag^+ to spherical AgNPs was developed by Özyürek et al. [23]. Briefly, a solution containing 130 µL of AgNPs (the solution was created by heating 50 µL of 1.0 mM $AgNO_3$ for 10 min. Then, 5 µL of $Na_3C_6H_5O_7$ (1%) was added slowly, drop by drop, until a pale-yellow color appeared) and 20 µL of sample solution was combined. About 50 µL of H_2O was also added to the mixture. The microplate was incubated for 30 min at 25°C, and the absorbance was read at 423 nm. The obtained value was denoted as $A_{0.5}$.

2.5.2 Enzymatic activity

2.5.2.1 Urease inhibition potential

The inhibitory capacity of urease was assessed through the quantification of ammonia production utilizing the indophenol method [24]. In brief, 25 µL of enzyme solution (5 U/mL) (Jack bean urease), 10 µL of DmHE and DmAE solution, and 50 µL of urea substrate solution comprised the reaction mixture, which was then incubated at 30°C for 15 min. Subsequently, 70 µL of primary reagent (0.7125 g of NaOH

in 25 mL H_2O + 1.175 mL of NaOCl in 25 mL H_2O) and 45 µL of phenol reagent (2 g of phenol (C_6H_5OH) in 25 mL H_2O + 25 mg of $Na_2[Fe(CN)_5NO]$, $2H_2O$ in 25 mL H_2O) were added. Upon incubation for 50 min, the absorbance at 630 nm was quantified, and the resulting value was designated as the half maximal inhibitory concentration (IC_{50}).

2.5.3 Anti-inflammatory activity (inhibition of bovine serum albumin [BSA] denaturation)

For the purposes of this study, 100 µL of different concentrations of DmAE and DmHE at 1,500, 3,000, and 6,000 µg/mL was mixed with 100 µL of BSA (0.2% w/v) dissolved in Tris-buffered saline with pH of 6.6. About 100 µL of a 0.2% BSA solution was mixed with 100 µL of water to form the control group. To the contrary, the standard group contained a solution that was a mixture of 500 µL of BSA solution and 100 µg/µL of ibuprofen suspended in water. The test containers were incubated at 37°C for 15 min and kept for 5 min in a 72°C water bath. Solutions' absorbance values were measured at 660 nm using a microplate reader (Perkin Elmer, Enspire).

The quantification of denaturation *I*% of BSA was accomplished utilizing the subsequent equation [25]:

$$\% = [\text{Absorbance}_c - (\text{Absorbance}_s - \text{Absorbance}_w) / \text{Absorbance}_c] \times 100$$

Absorbance_c: Absorbance of control = 100 µL H_2O + 100 µL BSA; Absorbance_s: Absorbance of sample = 100 µL DmHE/DmAE + 100 µL BSA; Absorbance_w: Absorbance of white = 100 µL DmHE/DmAE + 100 µL Tris-phosphate (pH: 6.8).

In this investigation, the control group comprises denatured proteins, accounting for the entire sample. The acquired outcomes are subsequently contrasted with those of ibuprofen.

2.5.4 Sun protection factor (SPF)

The SPF of *D. maritima* has been evaluated according to the method described by Mansur et al. [23]. A methanol stock solution containing DmHE and DmAE was prepared at a concentration of 2,000 ppm. The absorbance was subsequently calculated at seven wavelengths with a 5 nm separation between each; the range was 290–320 nm. The SPF was computed utilizing the subsequent equation, with all measurements being performed in triplicate:

$$\text{SPF spectrophotometric} = \text{Correction factor} \times \sum_{290}^{320} \text{erythemal effect spectrum } (\lambda) \times \text{solar intensity spectrum } (\lambda) \times \text{absorbance of sunscreen product } (\lambda)$$

Correction factor: (=10); erythemal effect spectrum (λ) × solar intensity spectrum (λ): is a constant determined by Sayre et al. and is displayed in Table 1.

2.6 *In vivo* biological activities

2.6.1 Preparation of animals

The *in vivo* investigation of DmAE and DmHE was conducted using male and female albino mice ((*Mus musculus*) with a weight range of 20–25 g. The mice stemmed from the Pasteur Institute (Elevage Centre, Kouba, Algeria) and were preserved in plastic cages in the animal facility of ASSB faculty (FSB/USTHB). The animals were kept in a controlled setting with a temperature range of 20–24°C, humidity maintained at 50–65%, and a daily lighting schedule of 12 h. Prior to the experiments, they were granted free access to water and regular rodent diet for a duration of 16 h.

The experimental procedures were approved by the Ethical Committee of Animal Experimentation (CEEA) of University of Sciences and Technology Houari Boumediene (USTHB) with approved Ref N°: CEEA-USTHB-08-2023/11118, Algeria.

2.6.2 Acute toxicity

Female albino mice were selected for acute toxicity testing. The experiment was conducted in accordance with the guidelines outlined in International Organisation for Economic Cooperation and Development (OECD) guideline 423 [26]. Prior

Table 1: SPF computation uses normalized product function

Wave length λ (nm)	290	295	300	305	310	315	320	Total
EE (λ) × *I* (λ) (Normes)	0.0150	0.0817	0.2874	0.3278	0.1864	0.0837	0.0180	1

to receiving the extracts, a total of 12 mice were subjected to a fasting period of 16 h. Then, they were divided into two groups, each consisting of six female mice. Every individual mouse was administered a solitary oral dosage of 2,000 mg/kg of each extract. Food was forbidden for an additional 1–2 h following the administration.

2.6.3 Anti-inflammatory test

Implementing the method laid forth by Koster [27], we subjected male albino mice to the Carrageenan-induced paw inflammatory test, to evaluate the *in vivo* anti-inflammatory activity of DmAE and DmHE. Before delivering the drugs, the mice experienced a 16 h period of fasting. Subsequently, the mice were segregated into four distinct groups, with each group including three male specimens. In the experiment, the two groups that were put to the test were given 100 and 500 mg/kg of DmAE and DmHE, separately. In contrast, the two groups that were supplied as a control were given distilled water and ibuprofen (500 mg/kg body weight). 30 min afterward, a sub-plantar tissue injection of 0.05 mL of 1% (w/w) carrageenan solution was given into the right hind paw to cause paw edema. The mice were courteously killed 4 h later. Their legs were next eradicated at the tarsal joint and weighed using an analytical scale. %Edema and %Inhibition, two measures of paw weight growth and its reduction in mice treated with different substances, were calculated using the following formula:

$$\%\text{Edema} = [(WA_L – WA_R/WA_R)] \times 100,$$

where WA_L is the weight average of left paw, and WA_R is the weight average of right paw.

$$\%\text{Inhibition} = [(\%\text{Edema}_C – \%\text{Edema}_T)/\%\text{Edema}_C] \times 100,$$

where $\%\text{Edema}_C$ is the %Edema in the control group, and $\%\text{Edema}_T$ is the %Edema in the tested group.

2.6.4 Analgesic test

The analgesic effectiveness of both DmAE and DmHE was examined using the acetic acid writhing method, featuring supraspinal nociceptors. The test was conducted employing the torsion process, according to Vogel protocol [28]. The method involved inducing stomach cramps. The mice were split into four groups, with three mice in each. They were underwent a 16 h period of fasting prior to the start of the examination. Each extract was orally administered to the two test groups at doses of 100 and 500 mg/kg, while the reference and control groups were given aspirin (500 mg/kg) and physiological water, respectively. In order to generate cramping pain, all mice were injected intraperitoneally with 0.6% (1 mL/kg) acetic acid 30 min after oral administration of the substances. After 5 min, the extent of cramping in each mouse was determined by closely observing the animal for 15 min. For each group, the percentage reduction in cramps (% protection) was calculated using the formula, which allowed us to assess the analgesic impact of DmAE and DmHE.

$$\%\text{Protection} = [(1 – AW_E/AW_C)] \times 100,$$

where AW_T represents the average writhe value in the tested group, while AW_C represents the average writhe value in the control group.

3 Statistical analysis

The average values derived from three independent analyses are presented as the data related to phytochemical studies and biological activity tests. The mean outcomes of the three tests are represented by the values reported with the standard deviation. The mean values related to each treatment were statistically compared using the one-way analysis of variance (ANOVA) test, specifically implemented using the XLSTAT software. Concentrations that exceeded four distinct values were employed in the tests. The IC_{50} and $A_{0.50}$ values were calculated using linear regression analysis, and significant differences were identified using one-way computation of variance (ANOVA) with a value level of $p < 0.05$.

4 Results and discussion

4.1 Isolation and purification

For n-butanol extract from flowers of *D. maritima,* one bufadienolide name scilliphäosidin-3-*O*-*β*-D-glucoside (C1) and sucrose name β-D-fructofuranosyl-(2 → 1)-D-fructofuranose (C2) were isolated by repeated CC (Figure 1). The C1 and C2 compounds were obtained as colorless. Their structures were characterized using NMR spectroscopic analysis methods, which are 1D: Proton nuclear magnetic resonance and carbon 13 nuclear magnetic resonance and 2D: heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), and COSY. Its NMR spectra are recorded in CD3OD. The C1 compound has been identified in several plants, but for the first time in *D.*

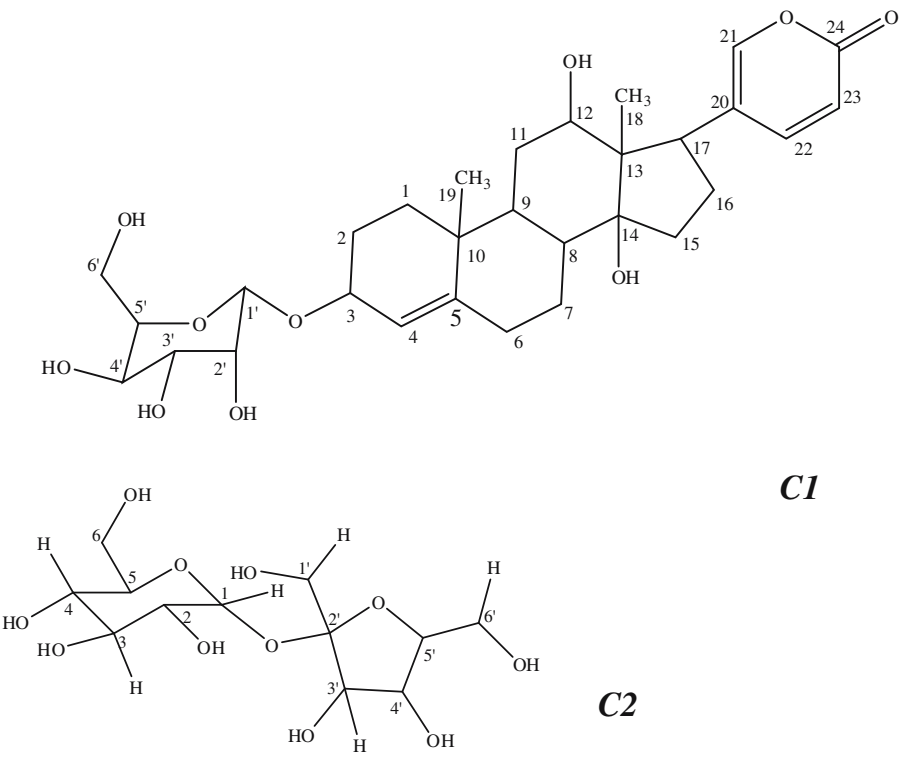

Figure 1: Chemical structures of ***C1*** and ***C2*** compounds.

maritima, while the C2 compound was identified from the bulb of this species [29].

The ^{1}H NMR spectrum of the ***C1*** showed: 0.57 (s, 3H, C-18); 0.97 (s, 3H, C-19); 4.16 (t, 11-I, *J* = 7.73 Hz, C-3); 5.38 (d, 1H, *J* = 1.74.74 Hz, C-4); 6.18 (dd, 1H, *J* = 9.66–1.06 Hz, C-23); 7.35 (dd, 1H, *J* = 2.65–1.22 Hz, C-21); 7.84 (dd, IH, *J* = 9.74–2.56 Hz, C-22); 4.32 (d, 1H, *J* = 7.69 Hz, C-1′); 3.05 (m, 1H, C-2′); 3.28 (1, 1H, *J* = 7.69 Hz, C-3′); 3.19 (m, 1H, C-4′); 3, 18(m, 1H, C-5′); 3.54–3.74 (d, 1H, *J* = 7.69 Hz, C-6′). The ^{1}H NMR and ^{13}C-NMR chemical shifts of the compound *C1* are summarized in Table 2.

The HMBC experiment (Figure 2) established the connectivity between methyl group H_3-18 at δH 0.57ppm and C-12, C-13, C-17, and C-14. It also showed the connectivity between methyl group H_3-19 at δH 0.97 ppm and C-1, C-5, C-9, and C-10.

Table 2: ^{1}H NMR and ^{13}C NMR data for *C1*

Position	δ^{13}C	δ^{1}H	Position	δ^{13}C	δ^{1}H
1	35.34	1.68–1.26	16	28.7	2.02–1.56
2	26.89	1.94–1.52	17	46.01	3.05
3	75.47	4.16 (t, 11-I, *J* = 7.73)	18	9.23	0.57 (s, 3H)
4	121.02	5.38 (d, 1H, *J* = 1.74.74)	19	17.95	0.97 (s, 3H)
5	146.57	—	20	128.97	—
6	32.02	2.02–1.56	21	149.66	7.35 (dd, 1H, *J* = 2.65–1.22)
7	28.30	2.02–1.56	22	148.09	7.84 (dd, IH, *J* = 9.74–2.56)
8	41.47	1.57	23	113.98	6.18 (dd, 1H, *J* = 9.66–1.06)
9	46.90	0.99	24	163.42	—
10	37.07	—	1′	102.06	4.32 (d, 1H, *J* = 7.6)
11	29.42	1.95–1.27	2′	73.75	3.05 (m, 1H)
12	75.09	3.25	3′	76.55	3.28 (1, 1H, *J* = 7.69)
13	54.37	—	4′	70.62	3.19 (m, 1H)
14	84.65	—	5′	76.75	3.18 (m, 1H)
15	29.42	2.02–1.56	6′	61.42	3.54 - 3.74 (d, 1H, *J* = 7.69)

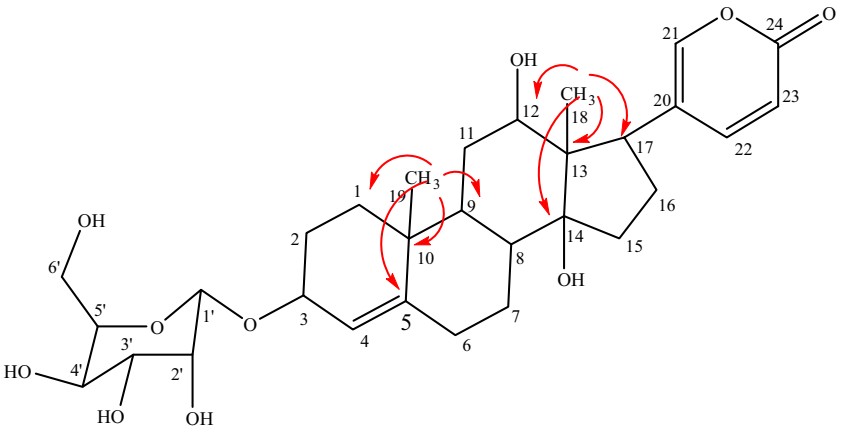

Figure 2: Correlation of H-18, H-19, and H-3′ according to HMBC spectrum of compound C1.

The ^{13}C NMR spectrum of ***C2*** showed 12 signals (Table 3), and the appearance of these peaks in the field from 60.85 to 103.96 ppm may confirm that the compound ***C2*** was saccharide compound as we mentioned previously. The spectrum also shows two signals (δC 92.30 and 103.96) in anomeric spectral region [30].

Depending on the HSQC experiment, each proton is assigned to its corresponding carbon as shown in Table 2. The furanose type could be confirmed by the chemical shift value of C-1″ 62.68 ppm a free CH_2OH [30].

The HMBC experiment (Figure 3) of ***C2*** showed cross-peaks between H-1 of glucose unit at δH 5.40 ppm and C-2 at δC 71.82 ppm and also between H-3 at δH 3.72ppm and C-2 at δC 71.82 ppm, as well as the presence of correlation spot between H-4 at δH 3.35 ppm and C-5 at δC 73.02 ppm; a correlation spot between H-1′ δH 3.65 ppm and C-2′ at δC 103.96 ppm; also, between H-3′ at δH 4.09 ppm and C-4′ at δC 74.36 ppm; as well as the presence of correlation spot between H-6′ at δH 3.76 ppm and C-5′ at δC 82.39 ppm of fructose; and a correlation spot between H-1 of glucose unit at δH 5.40 ppm and C-2′ of fructose unit at δC 103.96 ppm.

Table 3: 1H NMR and ^{13}C NMR data for ***C2***

Position	δ^1H	δ^{13}C
1	5.40 (d, 1H, *J* = 3.76)	92.30
2	3.43 (dd, 1H, *J* = 9.78–3.79)	71.82
3	3.72 (dd, IH, *J* = 7.34–2.45)	73.22
4	3.35 (dd, 1H, *J* = 10.03–8.98)	70.01
5	3.84 (m, 1H)	73.02
6	3.80 (m, 2H)	60.85
1′	3.65 (m, 2H)	62.68
2′	—	103.96
3′	4.09 (d, 1H, *J* = 4.0)	78.09
4′	4.03 (d, IH)	74.36
5′	3.76 (m, 1H,)	82.39
6′	3.76 (d, 2H, *J* = 4.15)	62.01

4.2 Qualitative and quantitative analysis

4.2.1 Ultra-performance liquid chromatography–mass spectrometry (UPLC–ESI–MS/MS) analysis of *D. maritima* extracts

DmAE and DmHE were analyzed using UPLC–ESI–MS/MS analysis, resulting in the tentative detection and characterization of 23 phytochemical compounds on the DmAE and 26 phytochemical compounds on the DmHE. This was achieved by comparing their retention durations with those of standards. The results of the molecules detected in DmAE and DmHE by UPLC/MS-MS are shown in Table 4 and Figure 4. The UPLC/MS-MS analysis of *D. maritima* extracts confirms the detection of various phytochemical compounds in both extracts. These include phenolic acids such as coumaric acid, ferulic acid, cinnamic acid, gallic

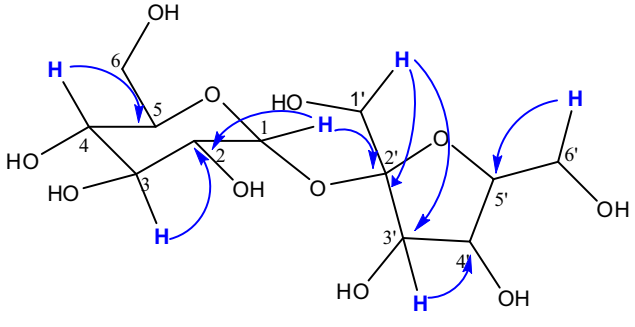

Figure 3: Correlation of H-1, H-3, H-4, H-1′, H-4,′ and H-6′ according to the HMBC spectrum of compound ***C2***.

Table 4: Results of the molecules detected in DmAE and DmHE by UPLC/MS-MS analysis

Compound	Molecular formula	Molecular weight	ESI charge (+/−)	*m/z*	RT	
					DmAE	DmHE
Thymol	$C_{10}H_{14}O$	150.22	(+)	151.7500 > 88.1000	11.382	20.985
Coumaric acid	$C_9H_8O_3$	164.16	(+)	165.1000 > 69.1500	21.080	13.745
4-Mythoxybenzoic acid	$C_8H_8O_3$	152.15	(+)	153.0500 > 70.7500	13.789	13.041
Naringenin	$C_{15}H_{12}O_5$	272.25	(+)	272.9500 > 209.1500	13.038	15.451
Kojic acid	$C_6H_6O_4$	142.11	(+)	143.0000 > 38.9500	2.139	19.151
Myricetin	$C_{15}H_{10}O_8$	318.23	$(+NH^{4+})$	336.2500 > 46.1500	19.155	18.244
Beta-carotene	$C_{40}H_{56}$	536.87	(+)	537.2000 > 23.1000	17.489	11.624
Ferulic acid	$C_{10}H_{10}O_4$	194.18	(+)	194.9000 > 177.1500	11.648	19.864
Folic acid	$C_{19}H_{19}N_7O_6$	441.14	(+)	442.9000 > 323.4500	19.666	12.184
Vitexin	$C_{21}H_{20}O_{10}$	432.4	(+)	433.0000 > 312.9500	12.196	19.420
Esculin hydrate	$C_{15}H_{18}O_{10}$	358.3	(+)	359.1000 > 295.1500	19.271	ND
Quercetin	$C_{15}H_{10}O_7$	302.23	(+)	302.9000 > 270.9000	16.326	18.534
Rutin	$C_{27}H_{30}O_{16}$	610.5	(+)	611.2000 > 73.2000	18.470	1.764
Catechin	$C_{15}H_{14}O_6$	290.27	(+)	291.1000 > 123.0500	1.763	9.521
Curcumin	$C_{21}H_{20}O_6$	368.4	(+)	369.0000 > 177.0000	11.035	10.692
Vanillin	$C_8H_8O_3$	152.15	(+)	153.1000 > 65.1500	10.710	8.383
Chrysin	$C_{15}H_{10}O_4$	254.24	(+)	195.1000 > 137.9000	ND	11.401
Caffeine	$C_8H_{10}N_4O_2$	194.19	(+)	195.1000 > 137.9000	11.449	10.170
8-Hydroxyquinoline	C_9H_7NO	145.16	(+)	149.0500 > 84.7500	ND	21.217
Cinnamic acid	$C_9H_8O_2$	148.16	(+)	149.0500 > 84.7500	21.228	11.312
Luteolin	$C_{15}H_{10}O_6$	286.24	(−)	153.1000 > 109.1000	ND	7.052
3,5-Dihydroxybenzoic acid	$C_7H_6O_4$	154.12	(−)	153.1000 > 109.1000	7.705	ND
Syringic acid	$C_9H_{10}O_5$	198.17	(−)	300.9000 > 255.2500	ND	19.408
Hespertin	$C_{16}H_{14}O_6$	302.28	(−)	160.8000 > 117.1000	ND	0.506
4-Hydroxy coumarin	$C_{27}H_{30}O_{16}$	162.14	(−)	160.8000 > 117.1000	1.532	11.355
Cis-p-coumaric acid	$C_9H_8O_3$	164.16	(−)	163.1500 > 119.1500	11.394	ND
Gallic acid	$C_4H_4O_4$	170.12	(−)	353.0500 > 191.1000	ND	9.914
Chlorogenic acid	$C_{16}H_{18}O_9$	354.31	(−)	353.0500 > 191.1000	9.904	ND
Salicylic acid	$C_7H_6O_3$	138.12	(−)	179.1000 > 135.1500	ND	10.186
Caffeic acid	$C_9H_8O_4$	180.16	(−)	179.1000 > 135.1500	10.258	ND
Vanillic acid	$C_8H_8O_4$	168.15	(−)	151.7500 > 88.1000	ND	11.271

ND: Not detected. Rt: Retention time.

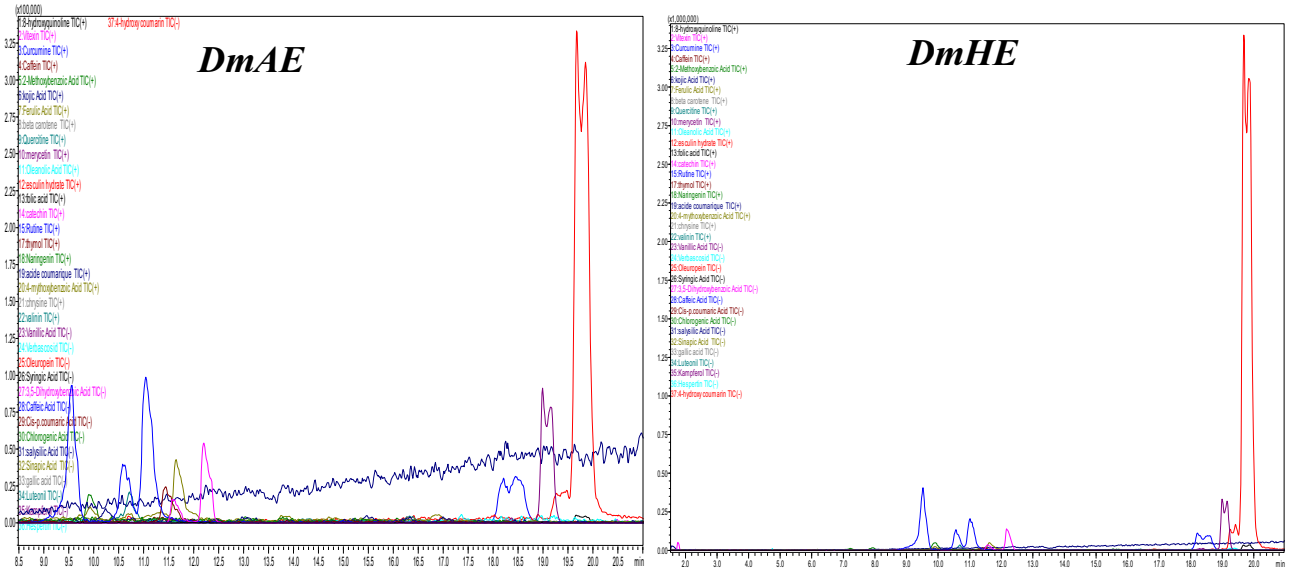

Figure 4: UPLC–ESI–MS/MS chromatograms of DmAE and DmHE.

acid, cis-p-coumaric acid, chlorogenic acid, salicylic acid, and caffeic acid, along with flavonoids such as naringenin, rutin, catechin, quercetin, myricetin, chrysene, luteolin, and hespertin. Coumarins such as 4-hydroxy coumarin were also present. Polyphenols such as thymol, kojic acid, and curcumin were also identified. Other compounds detected include esculin hydrate, 8-hydroxyquinoline, vanillin, folic acid, vitexin, 4-mythoxybenzoic acid, caffeine, and 3,5-dihydroxybenzoic acid. In a study conducted by Zhang et al. in 2022, it was demonstrated that distinctive polyphenols were annotated for each section of *D. maritima*. Significantly, the extracts obtained from the aerial section were primarily concentrated with anthocyanins. The bulb extracts contained a high concentration of lignans, mainly composed of matairesinol derivatives. Sesamolinol, pinoresinol, and conidendrin were also present, albeit in smaller amounts. Furthermore, flavones are present in *D. maritima* bulb extracts alongside lignans [31]. In a work carried out by Yadav et al. in 2021, they used reverse-phase HPLC to estimate the levels of cardiac glycoside (Scillaren A) in the bulbs of eight different genotypes of *Drimia*. The presence of a bufadienolide compound known as Scillaren A was verified using Fourier transform infrared spectroscopy (FTIR) and high-resolution liquid chromatography–mass spectrometry (HR-LCMS). In addition, the HR-LCMS analysis detected three cardenolides (convallatoxin, digoxigenin monodigitoxoside, and peruvoside) as well as 160 significant metabolites in the bulbs of *Drimia* species [32]. In addition to our UPLC/MS-MS results and the results achieved by Yadav et al. in 2021 and Zhang et al. in 2022, these findings suggest a rich phytochemical profile in the plants belonging to the *Drimia* genus, which may contribute to its pharmacological activities and potential therapeutic uses.

4.2.2 Total bioactive component assessment

The measurement of bioactive constituents found in extracts of *D. maritima* is presented in Table 5. By employing a calibration curve, the concentrations of total phenol and flavonoids in the extract were determined to be (µg GAE/mg and µg QE/mg, respectively): $y = 0.0035x$ for phenol and $0.0048x$ for flavonoids (R^2 = 0.9972 and 0.997, respectively). The highest concentrations of phenolic and flavonoid compounds were found in DmHE (175.14 ± 0.85 µg GAE/mg; 29.30 ± 1.06 µg QE/mg, respectively). In contrast, the analysis of the DmAE fraction revealed a significantly higher concentration of phenolic compounds in comparison with the analysis of the DmHE fraction, which exhibited a flavonoid content of 28.12 ± 1.45 µg QE/mg.

Table 5: TPC and TFC of *D. maritima* extracts

Extracts	TPC (µg GAE/mg)	TFC (µg QE/mg)
DmAE	129.23 ± 1.00	28.12 ± 1.45
DmHE	175.14 ± 0.85	29.30 ± 1.06

4.3 *In vitro* biological activities

4.3.1 Antioxidant activities

Due to the complexity of phytochemicals, plant extract antioxidant properties cannot be measured by a single approach. The chemical composition of plant tissues affects antioxidant activity, making it difficult to determine each antioxidant component. *D. maritima's* DPPH, ABTS, reducing power, and AgNPs antioxidant activity are shown in Table 6. The metrics $A_{0.5}$ and IC_{50} are used to measure the antioxidant activity of the studied fractions.

4.3.1.1 DPPH scavenging ability

The standards and plant extracts exhibited a decreasing DPPH scavenging activity in the following order: BHA (IC_{50}: 6.14 ± 0.41 µg/mL) > α-tocopherol (IC_{50}: 13.02 ± 5,17 µg/mL) > DmHE (IC_{50}: 96.71 ± 1.78 µg/mL) > DmAE (IC_{50}: 319.97 ± 0.73 µg/mL). A lower IC_{50} value indicates an improved ability to scavenge DPPH radicals.

4.3.1.2 ABTS scavenging ability

The ABTS scavenging activity was utilized to determine the antioxidant activity of *D. maritima* extracts, as shown in Table 6. The inhibition concentration of ABTS increases as the IC50 value decreases: DmHE (IC50 = 236.88 ± 1.47 µg/mL), followed by DmAE (IC50 = 178.83 ± 2.78 µg/mL) and finally BHA (IC50 = 1.91 ± 0.09 µg/mL). Notably, the findings indicated that the DmAE extract exhibited the most robust antioxidant activity.

4.3.1.3 Reducing power assay

Based on the parameter $A_{0.5}$ (µg/mL), it can be concluded that *D. maritima* exhibited the least potent capacity to reduce antioxidants (IC_{50} > 200 µg/mL) in comparison with standards.

4.3.1.4 AgNP-based assay

DmAE exhibited a moderate inhibitory effect on AgNPs ($A_{0.5}$ = 364.15 ± 0.84 µg/mL) in comparison with ascorbic

Table 6: Antioxidant capacity with IC_{50} and $A_{0.5}$ values

Extracts	IC_{50} (µg/mL)		$A_{0.5}$ (µg/mL)	
	DPPH	ABTS	RP	AgNPs
DmAE	96.71 ± 1.78[b]	178.83 ± 2.78[b]	>200	364.15 ± 0.84[a]
DmHE	319.97 ± 0.73[a]	236.88 ± 1.47[a]	>200	>400
BHA*	6.89 ± 0.12[d]	1.91 ± 0.09[d]	NT	NT
α-Tocopherol*	13.02 ± 5.17[c]	NT	34.93 ± 2.38[a]	NT
Acid ascorbic*	NT	NT	6.77 ± 1.15[b]	7.14 ± 0.05[b]

*Standard compounds. NT: not tested. IC_{50} and $A_{0.50}$ are the 50% inhibition percentage concentration and 0.50 absorbance, respectively. IC_{50} and $A_{0.50}$ were determined via linear regression analysis and presented as the mean ± standard deviation (n = 3). Data with different superscripts ([a, b, c, d]) in the same columns differ significantly (p < 0.05).

acid ($A_{0.5}$ = 7.14 ± 0.05 µg/mL), whereas DmHE demonstrated the least potent inhibitory effect ($A_{0.5}$ > 400 µg/mL).

The interaction between antioxidants and free radicals occurs through various mechanisms, including single electron transfer (SET) and hydrogen atom transfer (HAT). In the HAT approach, the free radical takes away an atom of hydrogen from the antioxidant. In the SET method, the antioxidant donates an electron to decrease substances such as radicals, metals, and carbonyls. An antioxidant reaction can also encompass both the HAT method and the SET approach [33]. Due to the involvement of various pathways in neutralizing free radicals, it is impossible to accurately measure the overall antioxidant capacity using only one assay [34]. Therefore, to more accurately evaluate the overall antioxidant impact, the extract of *D. maritima* was examined in four separate trials (Table 6). In general, the data at hand suggest that *D. maritima* extract exhibited a higher ability to capture radicals. The $DPPH^{\cdot}$, $ABTS^{\cdot+}$, and FRAP activity results are lower than those determined by previous research on the aqueous extract of the Algerian *D. maritima* (IC_{50} = 36.99; 85.96; 55.43 µg/mL, respectively) [35]. Nevertheless, the values obtained in this investigation are significantly higher than the results of a previous study carried out with the same species (IC_{50} = 94.66 ± 1.75; 25.77 ± 0.69; and 53.12 ± 0.017 mg/mL) for $DPPH^{\cdot}$, $ABTS^{\cdot+}$, and RP, respectively. The variation in antioxidant activity results among the cited research can be attributed to differences in the solvent used for extraction, cultivation area, and collecting period. No prior research has been undertaken on the antioxidant activity of *D. maritima* using the AgNPs' antioxidant abilities. Table 6 shows that when compared to standards, DmAE had a higher IC_{50} value for DPPH radical scavenging than DmHE. The $DPPH^{\cdot}$, $ABTS^{\cdot+}$, and AgNPs data indicate that the constituents found in DmAE effectively eliminate free radicals by either donating electrons or hydrogen to stabilize them. Consequently, these components can potentially safeguard biological matrices against oxidative degradation caused by free radicals [36]. Based on the RP data, *D. maritima* exhibited a relatively low level of effectiveness (>200) in reducing Fe^{3+} to Fe^{2+} compared to the positive standards.

The reducing properties of DmAE indicate that its components, primarily chlorogenic acids, flavonoids, and primary metabolites, have the ability to halt a chain reaction through removing free radical intermediates, making them classified as antioxidants [37]. Other species exhibited a strong positive association between their TPC and reducing sugar levels and their powerful antioxidant ability [35]. Moreover, the antioxidant capabilities of flavonoids and phenolic acids are intricately linked to their chemical composition [38], which contain both dual bonds and groups of hydroxyl [39,40]; these compounds are well suited for removing free radicals and binding metal ions. Furthermore, these qualities also provide significant antioxidant capacity in living organisms [41]. The current study conducted an examination of the chemical constituents of DmAE and DmHE, which revealed that phenolic acids are the predominant group detected in this species. Furthermore, the DmAE contains numerous chemicals identified for their exceptional antioxidant properties. For instance, it has been documented that basic hydroxycinnamic acids have strong antioxidant properties against $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ [42]. DmAE and DmHE contains myricetin, which is the primary component responsible for its potent antioxidant activity. A prior investigation documented that myricetin effectively scavenges $HO^{\cdot}$, $DPPH^{\cdot}$, and $ABTS^{\cdot+}$ in antioxidant tests, while also augmenting the relative amounts of Cu^{2+} and Fe^{3+} reduction [43]. Cikman et al. found in their *in vitro* investigation that syringic acid exhibits antioxidative properties and effectively decreases markers of oxidative stress. Additionally, it enhances the antioxidant capacity in rats with L-arginine-induced acute damage of the pancreas [44]. Furthermore, luteolin, which is one the flavonoid detected in liquid chromatography-mass spectrometry/mass spectrometry, has gained significant interest due to its diverse pharmaceutical properties, such as its

ability to antioxidants [45]. It has been extensively utilized in pharmaceuticals and food applications [46,47]. The substantial antioxidant capability reported in the present investigation may be attributed to the presence of these substances, which could function individually or in combination to confer DmAE with its potent antioxidant properties.

4.3.2 Urease inhibition potential

Table 7 provides additional information on *D. maritima's* capacity to inhibit urease. The inhibitory activity decreases in the following order: thiourea (IC_{50} = 11.57 ± 0.68 µg/mL), DmAE (IC_{50} = 122.04 ± 1.42 µg/mL), and DmHE (IC_{50} = 122.04 ± 1.42 µg/mL). Compared to thiourea (reference), DmAE has strong anti-urease action when measuring ammonia generation.

Few reports currently exist regarding the enzymatic inhibition capacities of *D. maritima*. Our objective in this study was to demonstrate the plant's capacity to obstruct the catalytic site of specific enzymes. Consequently, it would be pertinent and useful for the implementation in the backdrop of the enzymes' pathologic dysfunction. The metalloenzyme urease's inhibition has significant pharmacological applications in the development of anti-gastric cancer medications and antiulcer. Urease is implicated in numerous severe infections that are caused by *Helicobacter pylori* in the gastrointestinal system and by Proteus and associated species in the urinary system [48]. DmAE has exhibited a blocking feature of the urease catalytic site, analogous to other enzymatic inhibitory activities.

4.3.3 Anti-inflammatory activity (inhibition of BSA denaturation)

The results of the *in vitro* anti-inflammatory test, inhibition of BSA denaturation, are presented in Table 8. Specifically, DmAE exhibited a favorable activity (IC_{50} = 75.13 ± 0.53 µg/mL), followed by DmHE (IC_{50} = 22.72 ± 1.53 µg/mL). These data points were deemed highly significant ($p < 0.05$) compared to the control group, which consisted of ibuprofen (Table 9).

The conventional utilization of this species in traditional medicine is substantiated by these results. Traditionally, *D. maritima* has been used for the management of heart conditions and infections caused by fungi, as well as for its diuretic properties [49]. Based on our current knowledge, no empirical evidence supports the claim that *D. maritima* extract possesses the inhibition of BSA denaturation properties. This research is the first to outline the potential anti-inflammatory capabilities of this particular species. The anti-inflammatory effect observed in this study is likely attributed to the abundance of phenolic compounds present in DmAE and DmHE. Flavonoids can also be emphasized [50,51]. Previously, myricetin, the primary flavonoid constituent in *D. maritima* extract, exhibited anti-inflammatory properties by suppressing the synthesis of prostaglandins (PGs) generated by lipopolysaccharides [52]. Another flavonoid was identified during the analysis of *D. maritima's* phytochemical profile. This flavonoid is known for its anti-inflammatory properties, as it effectively reduced the chemotaxis of polymorphonuclear neutrophils to fMet-Leu-Phe in a dose-dependent manner, with a significant decrease ($p < 0.05$). In addition, the release of elastase, triggered by both

Table 7: Anti-urease effects of *D. maritima* extracts

Extract	Anti-lipase IC_{50} (µg/mL)
DmAE	122.04 ± 1.42[b]
DmHE	357.42 ± 3.19[a]
Thiourea*	11.57 ± 0.68[c]

*Standard compounds. NT: not tested. IC_{50} is the 50% inhibition percentage concentration. IC_{50} was determined via linear regression analysis and presented as the mean ± standard deviation (n = 3). Data with different superscripts ([a, b, c]) in the same columns differ significantly ($p < 0.05$).

Table 8: Inhibition of BSA denaturation of *D. maritima* extracts

Extracts	Anti-inflammatory activity		
	1,500 µg/mL	3,000 µg/mL	6,000 µg/mL
DmAE*	NA	14.37 ± 1.14[a]	75.13 ± 0.53[a]
DmHE	1.80 ± 0.16[b]	10.50 ± 0.2[b]	22.72 ± 1.53[b]

*Reference compound. NA: Not active. The inhibition % of BSA is expressed as mean ± SD (n = 3). The values with different superscripts ([a, b]) in the same line are significantly different ($p < 0.05$), and the values with the same superscripts ([a, a]) are not significantly different ($p > 0.5$).

Table 9: *In vitro* anti-inflammatory effect of ibuprofen standard

Anti-inflammatory activity	
µg/mL	*I*%
7,500	94.18 ± 1.93
3,750	87.83 ± 1.65
1,875	79.53 ± 1.98
937.5	77.48 ± 0.76
58.59	67.64 ± 0.79
29.29	35.22 ± 1.85
14.64	26.22 ± 1.07

The inhibition % of BSA is expressed as mean ± SD (n = 3).

stimuli, was partially suppressed by rutin at concentrations of up to 25 μM [53]. According to the data presented in Table 9, we assert that this significant impact is attributed to the presence of the confirmed anti-inflammatory substances mentioned in the previous research studies.

4.3.4 SPF

The SPF calculation was utilized to assess the photoprotective activity of *D. maritima*. The sun protection activity is considered to be minimal, moderate, or high, respectively, for SPF values ranging from 2 → 12, 12 → 30, 30 → 50, and greater than 50. Table 10 shows the SPF values of the extracts used in the investigations of *D. maritima*. The values for DmAE and DmHE range from 37.80 ± 0.34 to 38.10 ± 0.82, respectively. According to these findings, both extracts exhibited a significant photoprotective effect.

Prolonged exposure to UV radiation increases the likelihood of developing skin illnesses, such as melanoma and photoallergic responses. UV-B radiation, emitted at a wavelength of 280–320 nm, is the main factor responsible for skin disorders. Recent research has focused on exploring the possibility of natural compounds with antioxidant capabilities to be used as sunscreen resources by studying their absorption in the UV area. A clear and robust association has been demonstrated between the phenolic contents of plant extracts and SPF [54,55], due to that fact the DmAE and DmHE have given an important index of photoscreening estimated by an SPF of 37.80 ± 0.34 and 38.10 ± 0.82, respectively, which is considered high, comparatively with commercial and cosmetic sunscreen SPF values (Table 10).

Table 10: Photoprotective potential of the *D. maritima* extracts

Extracts	DmAE	DmHE	Nivea*	Vichy*
SPF	37.80 ± 0.34	38.10 ± 0.82	50.11 ± 0.53	44.22 ± 0.35
Protection	High	High	High	High

*Reference compound.

4.4 *In vivo* biological activities

4.4.1 Acute toxicity

Acute toxicity occurs when the undesirable consequences of a chemical are felt either instantly or within a short period of time following one or more administrations of the substance throughout a 24 h period [56]. After a single dose of 2,000 mg/kg of DmAE and DmHE, no toxicity or mortality was observed in the mice monitored over a 14 day period. All of the mice showed no abnormalities in their skin, salivation, eyes, diarrhea, or weight loss. Therefore, DmAE and DmHE are deemed relatively safe according to the Globally Harmonised System of Classification and Labelling of Chemicals [57], as their lethal dose 50 transcends 2,000 mg/kg. These outcomes align with studies that were undertaken to evaluate the acute toxicity of the bulbs of several species in the *Drimia* genus. These investigations revealed that rats who ingested methanol bulb extracts of *D. maritima* at doses ranging from 1,000 to 5,000 mg/kg did not have any deaths or observable effects [58]. Furthermore, the results of B. Nighantu et al. study corroborated our own. It turned out that rats given oral doses of 750 and 1.5 g/kg of an ethanol extract of *D. indica* bulbs did not have any toxic reactions [59].

4.4.2 Anti-inflammatory activity

Inflammation, which can be either chronic or acute, is the major immune system's principal defensive reaction against harm. Acute inflammation results in the accumulation of fluid in tissues (edema), the migration of white blood cells into the affected area (leukocyte infiltration), and the infiltration of specialized immune cells called macrophages into the injured muscle [60]. Edema is a prominent sign of inflammation and an important feature to consider when evaluating the ability of a product to reduce inflammation [61]. The *in vivo* anti-inflammatory efficacy of *D. maritima* has been conclusively demonstrated for the first time through our investigation. This research report suggests that DmAE and DmHE at the doses of 100 and 500 mg/kg have the ability to decrease carrageenan-induced paw edema in treated mice. The paw edema test, notably the carrageenan-induced model, is used to assess the effectiveness of possible non-steroidal anti-inflammatory drugs [62]. Table 11 displays the percentage of edema (%Edema) and its degree of decrease (%Inhibition) achieved with DmAE, DmHE, and a reference Diclofenac®. All

Table 11: Anti-inflammatory activity of DmAE, DmHE, and aspirin

	Dose	% edema	% inhibition
Control	—	57	—
Diclofenac®	500	20.52	73.61
DmHE	100	22.10	55.50
	500	17.32	70.40
DmAE	100	36.20	34.27
	500	35.03	39.09

groups that underwent sub-plantar injections of carrageenan solution exhibited a substantial augmentation in paw volume compared to the control group. Mice that were administered Diclofenac, DmAE, and DmHE at both doses (100 and 500 mg/kg) exhibited a reduced increase in paw volume compared to the untreated positive control. This points out that the oral administration of the extracts and Diclofenac effectively suppresses inflammation. The DmHE displayed a 70.40% inhibition rate, which is comparable to Diclofenac's 73.61% at a dosage of 500 mg/kg. On the other hand, the DmAE exhibited a lower inhibition percentage of 34.27% in comparison to the reference. Previous research on the genus *Drimia* (*Urginea*) using the carrageenan-induced inflammatory method is congruent with our results. To assess *Urginea indica*'s anti-inflammatory properties, Akhtar and Shabbir used acute inflammatory models such as carrageenan-, histamine-, and serotonin-induced paw edema models. *U. indica* considerably decreased paw edema through many pathways, pursuant to outcomes of this investigation. One of these essential mechanisms is the suppression of autacoids, as demonstrated by a decrease of histamine- and serotonin-induced paw edema in the animals treated with aqueous and ethanolic extracts of *U. indica* [62].

The phytochemicals of DmAE and DmHE contributed for their *in vivo* and anti-inflammatory activity. The presence of rutin and vitexin has been identified in both DmAE and DmHE through the execution of UPLC/MS-MS analysis of these extracts. The anti-inflammatory properties of rutin were examined in the study of Selloum et al. employing a rat paw paradigm caused by carrageenan. The outcomes displayed that the oral administration of 100 mg/kg of rutin had a significant ($p < 0.05$) impact on rat paw edema. This investigation elucidated the anti-inflammatory mechanism of rutin, which involves the suppression of the synthesis of inflammatory mediators. These mediators are crucial in the attraction and activation of neutrophils. Rutin effectively suppressed the activity of phospholipase A2 (PLA2), which is the primary enzyme involved in the arachidonic acid cascade, in human synovial fluid [53]. Applying a carrageenan-induced rat paw approach, Raghu and Agrawal assessed vitexin's anti-inflammatory ability. These findings suggest that this flavonoid has potential as an effective anti-inflammatory drug, since it reduces inflammation when administered orally at a dose of 10 mg/kg [63]. Flavonoids are compounds, which are the main secondary metabolite of *D. maritime,* have several applications, and they have gained significant interest because of their ability to reduce inflammation. They inhibit the production of inflammatory substances such cyclooxygenase-2, interleukin-1beta, NO, and tumor necrosis factor alpha. They also reduce the production of vascular endothelial growth factor and intercellular adhesion molecule, as well as the stimulation of the nuclear factor kappa-light-chain-enhancer of activated B cells, nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3, signal transducer and activator of transcription 3 inflammasome, and MAP kinases processes. Reducing the function of many pathways results in less adverse consequences compared to completely eliminating the action of a single target, as the targets typically have natural physiological functions as well [64].

4.4.3 Analgesic activity

As a sensory modality, pain frequently serves as the sole indicator of a number of diseases [65]. Currently, there is a significant focus on researching the identification of natural pain medications. This is because existing opiate treatments have severe side effects such as gastrointestinal, renal, and respiratory issues [16]. The acetic acid-induced writhing test was implemented to evaluate the analgesic efficacy of *D. maritima* extracts, which has been documented for the first time. The results suggest that giving different amounts of both DmAE and DmHE had a considerable pain-relieving effect in the animals. The observed impact was demonstrated by the dosage-dependent reduction of acetic acid-induced writhing. At a dose of 500 mg/kg of DmHE, the inhibition was 71.28%, which is more significant than the inhibition of aspirin at the same dose (69.44%), as shown in Table 12. Previous study on *D. indica* has used a hot plate assay to evaluate its analgesic effectiveness in rats; our *in vivo* investigation on the analgesic efficacy of *D. maritima* extracts is in agreement with these findings. The study reported that all rats administered with an oral dose of 1.5 g/kg of an ethanol extract of *D. indica* bulbs exhibited analgesic effectiveness. The extract induced a higher level of discomfort in rats exposed to hot plates for a duration of up to three seconds, compared to rats who did not receive the treatment [66].

The use of acetic acid to induce abdominal constriction is a highly sensitive method for evaluating the possible analgesic effects of the examined substances. The pain

Table 12: Analgesic effect of DmAE, DmHE, and aspirin

Treatment	Dose (mg/kg)	Cramp averages	% protection
Control (physiological water)	—	81	—
Aspirin	500	29	69.44
DmHE	100	23	59.31
	500	29	71.28
DmAE	100	43	40.12
	500	40	48.01

experienced in this model was a result of the activation and increased sensitivity of sensory neurons, both in the superficial and central nervous system, caused by inflammatory pain cytokines [67]. Aspirin demonstrates efficacy in experimental approaches where it is used to establish a prior inflammatory state and inhibit the prolonged extending response caused by an intraperitoneal injection of diluted acetic acid in mice [68]. The anti-inflammatory and analgesic effects of aspirin and similar non-steroidal anti-inflammatory medicines have led to their widespread use in clinical practice [69]. These medicines impede the function of the enzyme known as cyclooxygenase, resulting in the production of PGs that induce inflammation, swelling, discomfort, and fever. Nevertheless, the drugs hindered the generation of physiologically significant PGs that safeguard the stomach lining from hydrochloric acid injury, sustain renal function, and promote platelet aggregation when necessary. This inhibition was achieved by targeting a crucial enzyme involved in PG synthesis [68]. Consequently, natural products have the potential to serve as an alternate cure for analgesic effects, offering an alternative to these medications.

Flavonoids are extensively utilized for their pain-relieving action, as well as their proven safety in both preclinical and clinical settings [64]. Naringenin and quercetin were among the many flavonoids detected in DmAE and DmHE when examined through UPLC/MS-MS. Chung et al conducted experiments to evaluate the analgesic properties of naringenin in mice and rats. The antinociceptive effects of naringenin were assessed using hot-plate, acetic acid-induced writhing, and tail-flick methods. The study witnessed that administering naringenin orally at doses of 100 and 200 mg/kg significantly prolonged the time it took for mice to respond to heat stimulation from a hot plate and a tail-flick unit. Additionally, it inhibited the writhing response elicited by acetic acid in mice [70]. Another study executed by Filho et al. scrutinized the pain-relieving effects of quercetin in mice using several models of chemical and thermal pain. According to this study, quercetin, when administered at a dosage range of 10–60 and 100–500 mg/kg, effectively suppressed nociceptive response in the acetic acid-induced pain test. It induces dose-dependent pain relief in various chemical pain models by interacting with the L-arginine-nitric oxide, serotonin, and GABAergic systems [71]. Hence, the present research indicates that the pain-relieving effect of *D. maritima* extracts is caused by their chemical composition, specifically flavonoids, which have the ability to inhibit the production and stimulation of various cellular regulatory proteins such as cytokines and transcription elements. As a result, the sensation of pain is reduced [64].

5 Conclusion

This research has uncovered the vast medicinal potential of *D. maritima* flower extracts, particularly in their antioxidant, anti-inflammatory, anti-urease, and sun protection characteristics. Our comprehensive chemical analysis and biological assessments reveal that these flower extracts contain diverse bioactive compounds, such as phenolic acids, flavonoids, and coumarins, contributing to their pharmacological effects. DmAE demonstrated remarkable antioxidant capabilities: DPPH (IC_{50} = 96.71 ± 1.78), ABTS (1 IC_{50} = 78.83 ± 2.78), and AgNPs (IC_{50} = 364.15 ± 0.84), indicating its potential in alleviating diseases linked to oxidative stress. Furthermore, the *in vitro* (75,13%) and *in vivo* (70.40%) experiments confirmed the DmAE and DmHE's, respectively, potent anti-inflammatory; on the other hand, DmAE (IC_{50} = 122.04 ± 1.42) proved its substantial urease inhibitory actions. Moreover, their high SPF values indicate promising potential for use in natural sun protection for both extracts (37.80 ± 0.34; 38.10 ± 0.82) for DmAE and DmHE, respectively. The acute toxicity experiments provide evidence of the extracts' safety even when administered in high dosages, confirming their traditional use and potential for therapeutic applications. In addition, the isolation and purification methods led to the isolation of two essential compounds: scilliphäosidin-3-*O*-β-D-glucoside (***C1***) and β-D-fructofuranosyl-(2 → 1)-D-fructofuranose (***C2***), which were identified by NMR spectroscopy. These compounds show a significant association with the prominent pharmacological effects of this plant. ***C1***, a chemical in the bufadienolide class, is renowned for its exceptional anti-inflammatory properties. On the other hand, ***C2***, which is a compound derived from sugar, is associated with its capacity to function as an antioxidant. The presence of these compounds in the floral extracts is expected to enhance their pharmacological effectiveness, which aligns with the reported biological activities. To conduct a more thorough examination, it is recommended to extract and purify additional phytochemical compounds from *D. maritima* in order to assess their pharmacological properties, as observed in this study. This could aid in identifying the specific compounds accountable for the therapeutic advantages and potentially reveal new molecules with significant pharmacological effects. In addition, doing a comprehensive investigation of the phytochemicals, carrying out clinical trials, and developing new formulations will be essential in entirely using the therapeutic capabilities of *D. maritima*.

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Data availability statement: The corresponding author can provide the data required to support the findings of this study upon request.

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