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Antioxidant activity, enzyme inhibition, photoprotection, cytotoxicity, and phytochemical profiling of sea lavender (*Limonium algarvense* Erben) seed extracts for dermo-cosmetic use

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Abstract

Background Despite sea lavender being a medicinal species, research on its seeds' biological properties and chemical composition is unexplored. Thus, this study evaluated the effect of different extraction solvents on the biological activities and chemical profile of greenhouse-cultivated sea lavender seeds, aiming at their potential use as a dermo-cosmetic ingredient. Therefore, ethyl acetate, acetone, ethanol, and water extracts were examined for their antioxidant activity, enzyme inhibition, photoprotection, and cytotoxicity, followed by phytochemical analysis through spectrophotometric methods, further detailed by Ultrahigh-Performance Liquid Chromatography Coupled with Electrospray Ionization Mass/Mass Spectrometry (UHPLC-Esi-MS/MS).

Results The water extract demonstrated significant antioxidant activity, evidenced by low half maximal effective concentration (EC_{50}) values in scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals, reducing iron and chelating copper (296, 478, 230 and 678 µg/mL, respectively). The ethanol extract was more effective in inhibiting cosmetic-related enzymes, particularly elastase and hyaluronidase (2.18 and 3.21 µg/mL, respectively). The water and acetone extracts had the highest sun protection factors (23.2 and 18.9, respectively). All the extracts had nil to weak cytotoxicity (70–120% cell viability) towards mammalian cell lines. The water extract had the highest phenolics and condensed tannins (115 and 78.30 mg/g extract, respectively), while the ethanol contained the most flavonoids (62.73 mg/g extract). UHPLC-ESI–MS/MS analysis identified ethyl gallate, myricetin, rutin, and quercetin as major components of the ethanol extract, whereas myricetin-O-rutinoside isomers are predominant in the water extract.

Conclusions These findings highlight the potential of greenhouse-cultivated sea lavender seeds as potential dermo-cosmetic ingredients, with ethanol and water extracts demonstrating superior biological activities and chemical profiles, significantly contributing to general skin health and protection.

Keywords Halophytes, Salt tolerant plants, Skincare, Photoprotective properties, Anti-hyperpigmentation, Anti-ageing, Flavonoids

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1 Introduction

The global dermo-cosmetic market has been experiencing a remarkable resurgence in recent years, driven by a growing awareness of the importance of skin health and a desire for products that enhance beauty and prioritize overall well-being [1, 2]. This resurgence is emblematic of a broader societal shift towards embracing natural and sustainable solutions, not only for health and wellness but also for personal care and cosmetics [3, 4]. In this context, the demand for innovative dermo-cosmetic products, particularly those derived from natural sources, has gained substantial momentum [4]. Consumers are actively seeking formulations that align with their values, reflecting a deeper understanding of the power of nature in nurturing and preserving skin health. This trend is fuelled by growing concerns about the environmental impact of natural cosmetics, the potential side effects associated with synthetic compounds, and the desire for greater transparency in product formulations. As a result, the beauty and skincare industry is at the forefront of research and development, actively exploring novel natural ingredients to meet the evolving needs of dermo-cosmetics [5].

In the exploration of botanical resources for cosmetic applications, plant seeds have emerged as a rich source of bioactive compounds with considerable potential for dermatological benefits. For example, almonds (*Prunus dulcis*) contain an edible seed encased in a brown skin rich in antioxidants such as phenolics, flavonoids, phytosterols, lipid-soluble vitamins, and fatty acids [6]. The hydroethanolic extract from *Vitis vinifera* seed is rich in polyphenols, namely proanthocyanidins, reported with many cosmetics uses, including properties such as antioxidant, antifungal, antimicrobial, anti-caries, antidandruff, flavouring, light stabilizer, and sunscreen agent. In fact, it has been reported in 495 cosmetic formulations [6]. These examples highlight the potential of plant seeds in addressing dermatological concerns and demonstrate a careful integration of nature into precisely formulated, science-driven cosmetics.

Sea lavender (*Limonium algarvense* Erben) is native to the southwest region of the Iberian Peninsula and thrives in diverse salinity conditions, including coastal dunes and salt marshes [7]. Moreover, this medicinal species endows highly bioactive flavonoids with biological properties relevant to skincare products, namely antioxidant, anti-inflammatory, and anti-melanogenic properties [8–12]. However, its seeds have never been studied for their potential dermatological benefits. Thus, this work investigates the promising attributes of sea lavender seeds and their role in crafting innovative dermo-cosmetic formulations that cater to the modern consumer's desire for natural, sustainable, and efficacious skincare solutions. To achieve this, seeds from greenhouse-grown plants were extracted using cosmetic-grade solvents, including ethyl acetate, acetone, ethanol, and water. These extracts were then subjected to a comprehensive assessment of their antioxidant potential through both radical-based and metal-related assays; their capacity to inhibit enzymes relevant to cosmetic uses, including elastase, tyrosinase, hyaluronidase, and collagenase, and photoprotective properties were also evaluated. Subsequently, the extracts' cytotoxicity was determined towards four mammalian cell lines. The extracts' phytochemical profile was evaluated by spectrophotometric methods for their total contents of phenolics, flavonoids, and condensed tannins, followed by a detailed chemical profiling via Ultrahigh-Performance Liquid Chromatography Coupled with Electrospray Ionization Mass/Mass Spectrometry (UHPLC-ESI–MS/MS) analysis.

2 Methods

2.1 Chemicals

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals, mushroom tyrosinase (EC 1.14.18.1), porcine elastase (EC 3.4.21.36), *Streptomyces hyaluronlyticus* hyaluronidase (EC 4.2.2.1), *Clostridium histolyticum* collagenase (EC 3.4.24.3), L-tyrosine, kojic acid, N-Succinyl-Ala-Ala-Ala-p-nitroanilide (AAAPVN), Epigallocatechin gallate (EGCG), hyaluronic acid, tannic acid, N-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA), 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) were purchased from Merck (Darmstadt, Germany). Biowest (Nuaillé, Belgium) provided Dulbecco's Modified Eagle Medium (DMEM), foetal bovine serum (FBS), trypsin, L-glutamine and penicillin/streptomycin. Additional reagents and solvents were obtained from VWR International (Leuven, Belgium).

2.2 Plant material

The plants were cultivated in a greenhouse, using 1-L pots filled with a peat and perlite mixture (3:1 ratio) as the growing substrate, and were watered with fresh water every other day. Additionally, the irrigation water was supplemented weekly with a liquid fertilizer (NPK 7–5-6). The light cycle ranged from 13 to 14 h of daylight to 11–10 h of night, and the temperatures fluctuated between 10 and 20 °C at the lowest and 40–50 °C at the highest. The relative humidity levels varied from a low of 10–25% to a high of 70–80% [11]. After 14 weeks, dried flowers from *L. algarvense* containing the seeds were collected, and the seeds were separated, dried, powdered, and stored at room temperature.

2.3 Extraction

The seed powder was individually combined with water, ethanol (96%), acetone, and ethyl acetate (1:40, w/v) and subjected to extraction in an ultrasonic bath for 30 min. The resulting extracts were filtered through Whatman No. 4 paper, evaporated in a rotary evaporator, resuspended in dimethyl sulfoxide (DMSO) at 10 mg/mL, and stored at − 20 °C until further use.

2.4 Phytochemical analysis

2.4.1 Determination of total phenolic content (TPC)

The Folin−Ciocalteu reagent (FC) was used to assess the TPC, as outlined in a previously described method [8]. Extracts (5 µL at 10 mg/mL) were combined with diluted FC, followed by a 10-min incubation at room temperature (RT) in the dark. Sodium carbonate solution (75 g/L in water) was then added, and samples were incubated for 90 min at RT in the dark. The absorbance was then recorded at 650 nm (Biochrom EZ Read 400, Cambridge, UK), and results were presented as milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g, DW), based on a calibration curve with gallic acid (0.003–1 mg/mL; $r^2 = 0.9848$).

2.4.2 Determination of total flavonoid content (TFC)

The aluminium chloride ($AlCl_3$) method was used to estimate the TFC of the extracts [8]. Extracts (30 µL at 10 mg/mL) were combined with 50 µL of 2% $AlCl_3$ (w/v, in 98% ethanol) and incubated for 10 min at RT. The absorbance was recorded at 420 nm (Biochrom EZ Read 400, Cambridge, UK), and the results were presented in milligrams of quercetin equivalents per gram of dry sample (mg QE/g, DW), based on a calibration curve of quercetin (0.003–1 mg/mL; $r^2 = 0.9859$).

2.4.3 Determination of total condensed tannins content (CTC)

The 4-dimethylaminocinnamaldehyde (DMACA)–hydrochloric acid (HCl) method was used to determine the CTC of the samples [8]. Extracts (10 µL at 10 mg/mL) were combined with 200 µL of 1% DMACA (w/v, in methanol) and 100 µL of 37% HCl. After a 15-min incubation, the absorbance was recorded at 640 nm (Biochrom EZ Read 400, Cambridge, UK), and the results were presented as milligrams of catechin equivalents per gram of dried sample (mg CE/g DW), following a catechin calibration curve (0.003–1 mg/mL; $r^2 = 0.9914$).

2.4.4 UHPLC-Esi–MS/MS analyses

The extracts were analysed with a Dionex Ultimate 3000RS UHPLC system paired with a Thermo Q Exactive Orbitrap mass spectrometer (Thermo Scientific, Waltham, USA) [13]. Extracts were filtered through 0.22-µm PTFE syringe filters before injection. Compounds were separated using a Thermo Accucore C18 column (100 mm × 2.1 mm, 2.6 µm) (Thermo Scientific, Waltham, USA) set at 25 °C. The mobile phase consisted of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in methanol (Solvent B). The flow rate was maintained at 200 µL/min with the following gradient program: starting with 95% A for 0–3 min, transitioning to 0% A over 3–43 min, maintaining 0% A from 43 to 61 min, shifting back to 95% A over 61–62 min, and holding at 95% A from 62 to 70 min. The mass spectra were acquired in both positive and negative ion modes, utilizing a resolution of 70,000, collision energy of 30 NCE, and a scan range of 100–1500 m/z. Data analysis was done using Trace Finder 3.1 software, and the metabolites were identified using exact molecular mass, isotopic pattern, fragmentations, and retention time, with a mass accuracy within 5 ppm, referencing previous works and online databases.

2.5 In vitro radical-based antioxidant assays

2.5.1 Radical scavenging activity (RSA) on DPPH radical

The RSA of the extracts on the DPPH radical was assessed as previously described [8]. Samples (22 µL, at concentrations of 80–10000 µg/mL) were combined with 200 µL DPPH solution (120 µM in 96% ethanol) and incubated in the dark at RT for 30 min. Absorbance was recorded at 492 nm (EZ Read 400, Biochrom, Cambridge, UK), and the RSA was calculated as percentage inhibition compared to the negative control (DMSO) and as the half maximal inhibitory concentration (EC_{50}, µg/mL). Gallic acid served as the standard.

2.5.2 RSA on $ABTS^{\cdot+}$ radical

The RSA on the $ABTS^{\cdot+}$ radical was determined using the method detailed by Rodrigues et al. [8]. ABTS (7.4 mM in water) was mixed with potassium persulfate (2.45 mM) to form the $ABTS^{\cdot+}$ radical and incubated overnight in the dark at 4 °C. Then, the solution was diluted with ethanol to an absorbance of ~ 0.7 at 725 nm. Samples (10 µL, 80–10000 µg/mL) were combined with 190 µL $ABTS^{\cdot+}$ solution, followed by a 6-min incubation. The absorbance was recorded at 725 nm (EZ Read 400, Biochrom, Cambridge, UK), and the results were presented as percentage inhibition relative to a DMSO negative control and as EC_{50} values (µg/mL). Gallic acid worked as the positive control.

2.6 In vitro metal-based antioxidant assays

2.6.1 Copper chelating activity (CCA)

The CCA was evaluated using chelate titration with pyrocatechol violet (PV) as an indicator, following the method described previously [8]. Thirty µL of samples (80–10000 µg/mL) was combined with 200 µL sodium acetate buffer (50 mM, pH 6), 6 µL PV (4 mM in the same buffer) and 100 µL copper sulphate ($CuSO_4$, 50 µg/mL in water). The absorbance was recorded at 620 nm (EZ Read 400, Biochrom, Cambridge, UK), and the results were presented as percentage inhibition relative to the control (DMSO) and as EC_{50} values (µg/mL). Ethylenediaminetetraacetic acid (EDTA) served as the standard.

2.6.2 Iron chelating activity (ICA)

The ICA was determined using the ferrous ion (Fe^{2+})-ferrozine complex formation method as described previously [8]. Samples (30 µL, 80–10000 µg/mL) were combined with 200 µL deionized water and 30 µL iron (II) chloride ($FeCl_2$) solution (0.1 mg/mL in water). After a 30-min incubation, 12.5 µL ferrozine (40 mM in water) was added. Following a 10-min incubation in the dark at RT, the absorbance was recorded at 562 (EZ Read 400, Biochrom, Cambridge, UK). Results were presented as percentage inhibition relative to the control (DMSO) and as EC_{50} values (µg/mL). EDTA was used as the standard.

2.6.3 Ferric reducing antioxidant power (FRAP)

The FRAP was measured using the protocol outlined by Rodrigues et al. [8]. Samples (50 µL, 80–10000 µg/mL) were combined with 50 µL distilled water and 50 µL 1% potassium ferricyanide and then incubated at 50 °C for 20 min. Following this, 50 µL 10% trichloroacetic acid (TCA) and 10 µL 0.1% ferric chloride were added and incubated for another 10 min at 50 °C. Absorbance was recorded at 700 nm (EZ Read 400, Biochrom, Cambridge, UK). BHT was used as the standard, and results were presented as percentage inhibition relative to the positive control, and as EC_{50} values (µg/mL).

2.7 Enzyme inhibitory activity

2.7.1 In vitro inhibition of tyrosinase

The anti-tyrosinase activity of the extracts was assessed according to previously outlined methods [12]. Samples (10 µL, 62.5–1000 µg/mL) were combined with enzyme solution (333 U/mL) and then incubated at room temperature for 10 min. Then, 20 µL of 1.5 M L-tyrosine and 110 µL of 0.1 M sodium phosphate buffer were added to the samples, followed by a 20-min incubation at 25 °C. The absorbance was measured at 490 nm (Varioskan LUX, Thermo Scientific, Waltham, USA). Kojic acid was employed as the standard, and the results were calculated as percentage inhibition, relative to the negative control, and as EC_{50} values (µg/mL).

2.7.2 In vitro inhibition of elastase

The inhibition of the samples against porcine elastase was assessed using the method outlined by Barak et al. [14]. Samples (50 µL, 62.5–1000 µg/mL) were mixed with 1 µg/mL elastase enzyme (3.33 mg/mL) and incubated at 37 °C for 15 min. Afterwards, the 1.6 mM AAAPVN (0.2 mM Tris–HCl buffer) substrate was added and incubated for 20 min. The absorbance was then recorded at 410 nm (Varioskan LUX, Thermo Scientific, Waltham, USA). EGCG was used as the standard, and the results were presented as percentage inhibition, relative to the negative control, and as EC_{50} values (µg/mL).

2.7.3 In vitro inhibition of hyaluronidase

The anti-hyaluronidase activity of the samples was evaluated based on the principle of precipitation with setylpyridinium chloride [14]. Extracts (50 µL, 62.5–1000 µg/mL) were combined with hyaluronidase enzyme solution (0.02 M phosphate buffer containing sodium chloride and bovine serum albumin) and 0.1 M acetate buffer (pH 3.5), followed by a 20-min incubation. Then, 10 µL of hyaluronic acid was added and incubated for another 20 min at 37 °C, and absorbances were measured at 600 nm (Varioskan LUX, Thermo Scientific, Waltham, USA). Tannic acid was used as the standard, and the results were calculated as percentage inhibition, relative to the negative control, and as EC_{50} values (µg/mL).

2.7.4 In vitro inhibition of collagenase

The effect of the samples on collagenase inhibition was assessed according to the method described previously [15]. Samples (50 µL, 62.5–1000 µg/mL) were combined with collagenase enzyme from *Clostridium histolyticum* (0.8 unit/mL in 50 mM Tricine buffer) and incubated for 15 min. Following, 150 µL of tricine buffer and 0.8 mM FALGPA were added and incubated for 20 min and analysed at 340 nm (Varioskan LUX, Thermo Scientific, Waltham, USA). EGCG was used as the standard, and the results were presented as percentage inhibition, relative to the negative control, and as EC_{50} values (µg/mL).

2.8 Photoprotective properties

2.8.1 In vitro protection against ultraviolet (UV) radiation

The plant extract's capacity as a natural UV filter was assessed using a UV–visible spectrophotometer. Extracts at 0.250 mg/mL were analysed across the UV spectrum from 250 to 400 nm, with measurements taken at 2 nm intervals (Biotek Synergy 4, Agilent, Winooski, USA). The extraction solvent was used as a blank.

2.8.2 Determination of sun protection factor (SPF)

The samples were diluted in the respective solvent to various concentrations (0.0625–1 mg/mL), with the extraction solvent serving as a blank. Spectrophotometric scanning was conducted using a UV–visible spectrophotometer from 290 to 320 nm, with measurements taken at 5 nm intervals (Biotek Synergy 4, Agilent, Winooski, USA). SPF values were calculated using the equation previously established [16].

$$\mathrm{SPF} = \mathrm{CP} \times \sum_{290}^{320} \mathrm{EE}\,(\lambda) \times I(\lambda) \times \mathrm{Abs}(\lambda)$$

CF: correction factor (=10); EE (λ): erythemal effect spectrum; $I(\lambda)$: solar intensity spectrum; Abs(λ): absorbance.

2.9 Cellular viability

HepG2 (human hepatocellular carcinoma), RAW 264.7 (murine macrophages), and S17 (murine bone marrow stromal) and murine melanoma (B16 4A5) cell lines were grown as previously detailed [17, 18]. Briefly, after seeding and reattachment, the samples were tested at 100 µg/mL for 72 h of incubation. Then, the cell viability was assessed by the MTT colourimetric method [17]. The absorbance was recorded at 595 nm (EZ Read 400, Biochrom, Cambridge, UK), and the results were calculated as the percentage of cell viability relative to a control sample containing 0.5% DMSO.

2.9.1 Statistical analyses

The data were presented as the mean ± standard error of the mean (SEM), with each experiment performed in triplicate. Significant differences were evaluated using an Analysis of Variance (ANOVA) followed by Dunnett's tests, considering P values below 0.05 as significant. All statistical analyses were carried out using the XLSTAT statistical software for Microsoft Excel (version 2013) (Addinsoft, Paris, France).

3 Results

3.1 Phytochemical analysis

The extracts from *L. algarvense* seeds were evaluated for their total contents of phenols, flavonoids, and condensed tannins, and the results are depicted in Table 1.

Results are expressed in mg/g of dried extract. Values represent the mean ($n=6$) ± standard error (SE). A different letter (a–d) denotes significant differences by Tukey's multiple comparisons test ($p<0.05$).

The obtained results showed a tendency between the polarity of the extraction solvent and the concentration of total phenols detected, which decreased as the solvent polarity decreased. For instance, the aqueous extract presented the highest concentration of total phenols (115 mg GAE/g DW), followed by ethanol (95.60 mg GAE/g DW), acetone (45.22 mg GAE/g DW), and ethyl acetate (29.79 mg GAE/g DW). Similarly, the TCT was the higher concentration in the water extract (78.30 mg CTN/g DW), followed by ethanol (66.44 mg CTN/g DW), ethyl acetate (61.37 mg CTN/g DW), and acetone (26.13 mg CTN/g DW). In turn, the ethanol extract showed the highest levels of TFC (62.73 mg QCT/g DW), followed by the ethyl acetate (50.99 mg QCT/g DW), water (36.25 mg QCT/g DW), and acetone (8.72 mg QCT/g DW).

Table 1 Total phenolic (TPC), total flavonoids (TFC), and total condensed tannins (CTC) of extracts from *L. algarvense* seeds

Extract	Phenolics	Flavonoids	Condensed tannins
Water	115 ± 0.5^{a}	36.2 ± 0.2^{c}	78.3 ± 1.4^{a}
Ethanol	95.6 ± 0.3^{b}	62.7 ± 0.5^{a}	66.4 ± 1.2^{b}
Acetone	45.2 ± 0.2^{c}	8.72 ± 0.33^{d}	26.1 ± 1.6^{c}
Ethyl acetate	29.8 ± 0.3^{d}	51.0 ± 0.6^{b}	61.4 ± 2.5^{b}

Table 2 presents the UHPLC-ESI–MS/MS putative identification of metabolites in extracts from *L. algarvense* seeds, analysed using different solvents (water, ethanol, acetone, and ethyl acetate).

A total of 43 compounds were identified, with flavonoids being the most represented group [e.g. ampelopsin (4), taxifolin (9), dihydrokaempferol (16)], and their glycoside derivatives [e.g. myricetin-O-hexoside (11), rutin (20)], followed by some phenolic acids [e.g. gallic (1), gentisic (2), and caffeic acid (3)]. The ethanol extract exhibits the highest abundance and diversity of compounds. Specific compounds such as ethyl gallate (7), myricetin (26), rutin (20), and quercetin (36) were predominantly found in the ethanol extract. In contrast, dihydrokaempferol (16), eriodictyol (29), and chrysoeriol (42) were more abundant in acetone and ethyl acetate extracts. Myricetin-O-rutinoside isomers 1 (12) and 2 (14) were highly abundant in the water extract but absent in acetone and ethyl acetate extracts. This analysis highlights the significant impact of the extraction solvent on the metabolite profile of *L. algarvense* seeds, with ethanol and water, proving to be the most effective solvents for extracting a broader range of phenolic metabolites.

3.2 In vitro antioxidant activity

In this study, the antioxidant potential of water, ethanol, acetone, and ethyl acetate extracts of *L. algarvense* seeds was evaluated through their RSA on DPPH and ABTS, FRAP, ICA, and CCA. The results are presented in Table 3.

Table 2 Ultrahigh-Performance Liquid Chromatography Coupled with Electrospray Ionization Mass/Mass Spectrometry (UHPLC-ESI–MS/MS) putative identification of metabolites present in the extracts from *L. algarvense* seeds

No	Name	Formula	Rt	$[M+H]^+$	$[M-H]^-$	Water	Ethanol	Acetone	Ethyl acetate
1[1]	Gallic acid (3,4,5-Trihydroxybenzoic acid)	$C_7H_6O_5$	4.65		169.01370	+ + +	+ + +	+	+
2	Gentisic acid (2,5-Dihydroxybenzoic acid)	$C_7H_6O_4$	9.16		153.01879	+ +	+ + +	+	–
3[1]	Caffeic acid	$C_9H_8O_4$	17.50		179.03444	+ +	+ + +	+	+
4	Ampelopsin (Dihydromyricetin)	$C_{15}H_{12}O_8$	17.70		319.04540	+ +	+ + +	+	–
5[1]	Vanillin (4-Hydroxy-3-methoxybenzaldehyde)	C8H8O3	18.28	153.05517		+	+ + +	+	–
6	Unidentified sugar derivative	$C_{17}H_{30}O_{10}$	18.88		393.17607	+ + +	+ + +	+	–
7	Ethyl gallate	$C_9H_{10}O_5$	19.67		197.04500	–	+ + +	-	–
8[1]	*p*-Coumaric acid	$C_9H_8O_3$	20.61		163.03952	+	+ + +	+	+
9[1]	Taxifolin (Dihydroquercetin)	$C_{15}H_{12}O_7$	21.71		303.05048	+	+ + +	+	+
10[1]	Ferulic acid	$C_{10}H_{10}O_4$	21.75		193.05009	+ +	+ + +	+	+
11	Myricetin-O-hexoside	$C_{21}H_{20}O_{13}$	23.19		479.08257	+ + +	+ + +	+	+
12	Myricetin-O-rutinoside isomer 1	$C_{27}H_{30}O_{17}$	23.28		625.14048	+ + +	+	–	–
13	N-trans-Feruloyloctopamine	$C_{18}H_{19}NO_5$	23.36		328.11850	+	+ + +	+ +	+
14	Myricetin-O-rutinoside isomer 2	$C_{27}H_{30}O_{17}$	23.81		625.14048	+ + +	+	–	–
15	Prunin (Naringenin-7-O-glucoside)	$C_{21}H_{22}O_{10}$	24.15		433.11347	+ +	+ + +	+	+
16	Dihydrokaempferol (3,4′,5,7-Tetrahydroxyflavanone)	$C_{15}H_{12}O_6$	24.23		287.05557	+	+ + +	+	+
17[1]	Myricitrin (Myricetin-3-O-rhamnoside)	$C_{21}H_{20}O_{12}$	24.32		463.08765	+ + +	+ + +	+	+
18	N-cis-Feruloyltyramine	$C_{18}H_{19}NO_4$	24.33	314.13924		+	+ + +	+ +	–
19	Myricetin-O-pentoside	$C_{20}H_{18}O_{12}$	24.41		449.07201	+ +	+ + +	+	–
20[1]	Rutin (Quercetin-3-O-rutinoside)	$C_{27}H_{30}O_{16}$	25.22		609.14557	+ + +	+	–	–
21	Eriodictyol-O-hexoside	$C_{21}H_{22}O_{11}$	25.63		449.10839	+ +	+ + +	+	+
22	Lumichrome	$C_{12}H_{10}N_4O_2$	26.02	243.08821		+	+ + +	+ +	+
23[1]	Cosmosiin (Apigenin-7-O-glucoside)	$C_{21}H_{20}O_{10}$	26.10	433.11347		+	+ + +	+ +	+
24	Acetyl-malonylmyricitrin	$C_{24}H_{22}O_{15}$	26.17		549.08805	+ + +	+ +	+	–
25	Acetylmyricitrin	$C_{23}H_{22}O_{13}$	26.47		505.09822	+ +	+ + +	+	+
26[1]	Myricetin (3,3′,4′,5,5′,7-Hexahydroxyflavone)	$C_{15}H_{10}O_8$	26.69		317.02974	–	+ + +	+	–
27[1]	Quercitrin (Quercetin-3-O-rhamnoside)	$C_{21}H_{20}O_{11}$	26.71		447.09274	+ +	+ + +	+	+
28	N-trans-Feruloyltyramine	$C_{18}H_{19}NO_4$	26.73	314.13924		+	+ + +	+ + +	+ +
29[1]	Eriodictyol (3′,4′,5,7-Tetrahydroxyflavanone)	$C_{15}H_{12}O_6$	26.80		287.05557	+	+ + +	+ + +	+
30[1]	Apigenin-7-O-glucuronide	$C_{21}H_{18}O_{11}$	26.90		445.07709	+ + +	+ + +	+ +	+
31	Luteolin-O-hexoside	$C_{21}H_{20}O_{11}$	27.99		447.09274	+	+ + +	+ +	+
32	Diacetylmyricitrin isomer 1	$C_{25}H_{24}O_{14}$	28.63		547.10879	+	+ + +	+	+
33	Acetyl-malonylquercitrin	$C_{24}H_{22}O_{14}$	28.75		533.09314	+ + +	+ +	+	–
34[1]	Naringenin (4′,5,7-Trihydroxyflavanone)	$C_{15}H_{12}O_5$	29.06		271.06065	+	+ + +	+ + +	+ +
35	Acetylquercitrin	$C_{23}H_{22}O_{12}$	29.27		489.10331	+	+ + +	+	+
36[1]	Quercetin (3,3′,4′,5,7-Pentahydroxyflavone)	$C_{15}H_{10}O_7$	29.44		301.03483	–	+ + +	+	–
37	Diacetylmyricitrin isomer 2	$C_{25}H_{24}O_{14}$	29.65		547.10879	+	+ + +	+	+
38[1]	Luteolin (3′,4′,5,7-Tetrahydroxyflavone)	$C_{15}H_{10}O_6$	30.15		285.03991	+	+ + +	+ + +	+
39	Diacetylquercitrin isomer 1	$C_{25}H_{24}O_{13}$	31.28		531.11387	+	+ + +	+	+
40[1]	Apigenin (4′,5,7-Trihydroxyflavone)	$C_{15}H_{10}O_5$	31.87		269.04500	+	+ +	+ + +	+ +
41	Diacetylquercitrin isomer 2	$C_{25}H_{24}O_{13}$	31.96		531.11387	+	+ + +	+	+
42	Chrysoeriol (3′-Methoxy-4′,5,7-trihydroxyflavone)	$C_{16}H_{12}O_6$	32.14		299.05556	–	+	+ + +	+
43[1]	α-Linolenic acid	$C_{18}H_{30}O_2$	45.16		277.21676	–	+	+ + +	+

[1] Confirmed by standard. Compound occurrences were distinguished between –, +, + + or + + + for nil, low, medium, or high abundance, respectively

Results are presented as half maximal effective concentration (EC_{50}, μg/mL). Values indicate the mean ($n=6$) ± standard error of the mean (SEM). Within the same column, different letters (a–d) indicate significant differences according to Tukey's multiple comparisons test ($p<0.05$). –: not tested; Na: non-active (activity lower than 50% up to 10,000 μg/mL).

Table 3 Antioxidant properties of *L. algarvense* extracts, including activity against DPPH and ABTS radicals, ferric reducing power (FRAP), and metal chelating effects on copper (CCA) and iron (ICA)

Extract	DPPH	ABTS	FRAP	CCA	ICA
Water	296 ± 12^{a}	478 ± 53^{a}	230 ± 4^{a}	678 ± 28^{b}	Na
Ethanol	352 ± 16^{a}	508 ± 60^{a}	337 ± 6^{a}	865 ± 29^{b}	Na
Acetone	1238 ± 29^{b}	7714 ± 342^{b}	635 ± 16^{b}	4408 ± 44^{c}	Na
Ethyl acetate	2971 ± 70^{c}	Na	2790 ± 71^{c}	6783 ± 277^{d}	Na
Positive control					
Gallic Acid	485 ± 4^{a}	723 ± 23^{a}	–	–	–
BHT	–	–	1267 ± 26^{d}	–	–
EDTA	–	–		212 ± 30^{a}	637 ± 57

Notably, the aqueous extract demonstrated the highest antioxidant activity in all assays, followed by ethanol, acetone, and ethyl acetate extracts. The aqueous sample presented the lowest EC_{50} values against the DPPH (296 µg/mL) and ABTS (478 µg/mL) radicals, but also in the metal-based assays FRAP and CCA with EC_{50} values of 230 and 677 µg/mL, respectively. The second most active sample was the ethanol extract with EC_{50} values of 352, 508, 337, and 865 µg/mL for RSA of DPPH and ABTS, FRAP, and CCA, respectively. In turn, the acetone and ethyl acetate extracts were generally the least active, exhibiting EC_{50} values above 1000 µg/mL on DPPH RSA (1238–2971 µg/mL) and 7000 µg/mL towards the ABTS radical. The same tendency was found for the FRAP and CCA, where ethyl acetate extract presented an EC_{50} value of 2790 µg/mL on FRAP, whereas the EC_{50} values on CCA were found to be over 4000 µg/mL. Regarding the ICA, none of the extracts at the maximum concentration tested (10,000 µg/mL) were able to achieve 50% chelating activity.

3.3 In vitro enzyme inhibitory activity

Table 4 summarizes the enzyme inhibitory activities of different extracts from *L. algarvense* seeds, showing varying EC_{50} values (µg/mL) across collagenase, elastase, hyaluronidase, and tyrosinase.

Results are presented as half maximal effective concentration (EC_{50}, µg/mL). Values indicate the mean ($n = 6$) ± SEM. Within the same column, different letters (a-d) indicate significant differences according to Tukey's multiple comparisons test ($p < 0.05$). –: not tested.

The ethanol extract showed the most potent inhibitory activity across all enzymes, with particularly low EC_{50} values for elastase (2.18 µg/mL) and hyaluronidase (3.21 µg/mL). Acetone and ethyl acetate extracts showed intermediate activity, where the best results for the acetone extract were observed in its hyaluronidase inhibitory activity, with an EC_{50} value of 4.82 ± 0.42 µg/mL, while for the ethyl acetate extract, the most notable inhibitory activity was seen against elastase, presenting an EC_{50} value of 5.29 µg/mL. In contrast, the water extract generally exhibited higher EC_{50} values, indicating minor inhibitory activity, especially for tyrosinase (17.72 µg/mL).

3.4 Photoprotective properties

To evaluate the potential of *L. algarvense* as a UV-protecting agent, the extracts were assessed for their ability to absorb UV-A (320–400 nm) and UV-B (290–320 nm) radiation using spectrophotometric methods. The SPF was calculated for concentrations varying from 0.06 to 1 mg/mL, as illustrated in Fig. 1.

All extracts demonstrated the ability to absorb UV radiation, with the water and acetone extracts exhibiting the highest capacity. While the water extract presented the highest UV-B (290–320 nm) absorption capacity (Abs = 1.800–2.500), the acetone one exhibited a slightly higher UV-A (320–400 nm) absorption (Fig. 1). In addition, the SPF values of all extracts of *L. algarvense* seeds increased proportionally with concentration (Fig. 2),

Table 4 Enzyme inhibitory activity of *L. algarvense* extracts towards collagenase, elastase, hyaluronidase, and tyrosinase

Extract	Collagenase	Elastase	Hyaluronidase	Tyrosinase
Water	14.0 ± 1.1^{c}	7.11 ± 0.63^{c}	9.05 ± 1.07^{d}	17.7 ± 2.1^{d}
Ethanol	5.95 ± 0.83^{a}	2.18 ± 0.11^{a}	3.21 ± 0.61^{a}	8.6 ± 0.09^{a}
Acetone	10.9 ± 1.0^{d}	5.07 ± 0.72^{b}	5.01 ± 0.85^{b}	11.2 ± 1.0^{b}
Ethyl acetate	11.6 ± 1.2^{d}	5.37 ± 0.06^{b}	5.63 ± 0.01^{b}	12.1 ± 1.3^{b}
Positive control				
Epigallocatechin gallate	8.71 ± 2.95^{b}	7.04 ± 1.05^{c}	–	–
Tannic acid	–	–	7.73 ± 1.06^{c}	–
Kojic acid	–	–	–	15.3 ± 1.3^{c}

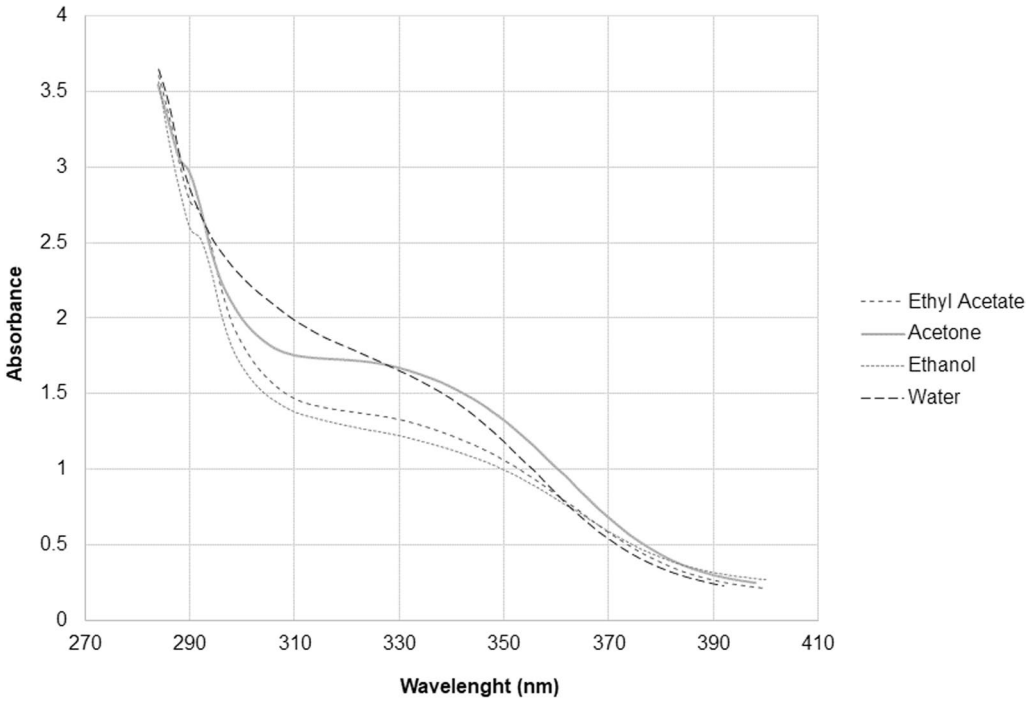

Fig. 1 Ultraviolet–visible absorption spectra of the extracts at 1 mg/mL from *L. algarvense* seeds

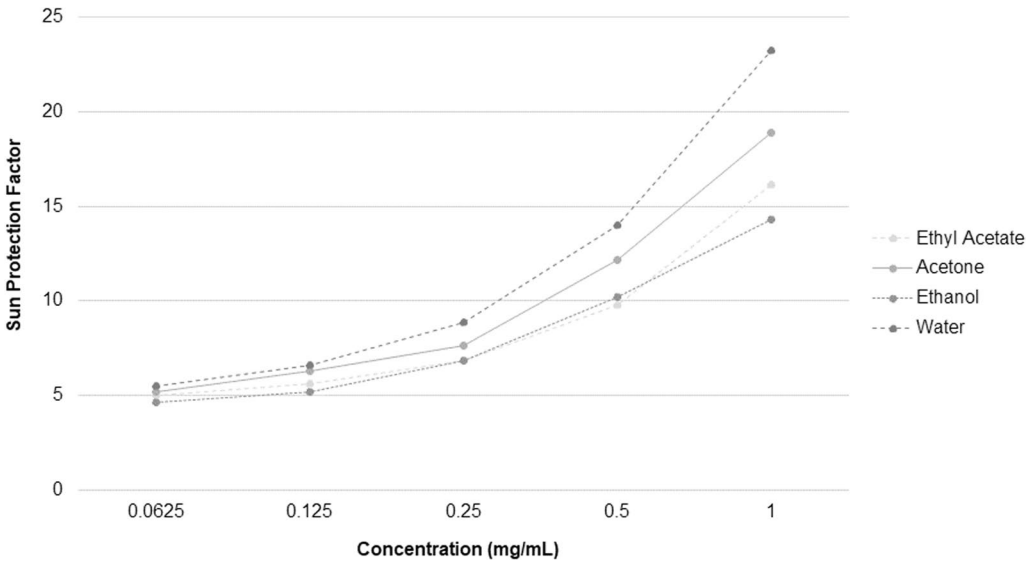

Fig. 2 Sun protection factor (SPF) of the extracts of *L. algarvense* seeds at different concentrations (0.0625–1 mg/mL)

where the water extract showed the highest SPF at 1 mg/mL (23.2), followed by the acetone (18.9), ethyl acetate (16.1), and ethanol (14.3).

3.5 Cellular cytotoxicity

To assess the cytotoxic potential of extracts from *L. algarvense* seeds, a systematic cytotoxicity assay was conducted across four mammalian cell lines, including HepG2 (human liver cancer), S17 (bone marrow stromal), RAW 264.7 (murine macrophage), and B16 4A5 (murine melanoma). Results are shown in Fig. 3.

For the HepG2 cell line, the observed cell viability percentages indicate a gradient of low cytotoxicity across the extracts (69.0–92.6%). Regarding the S17 cells, the

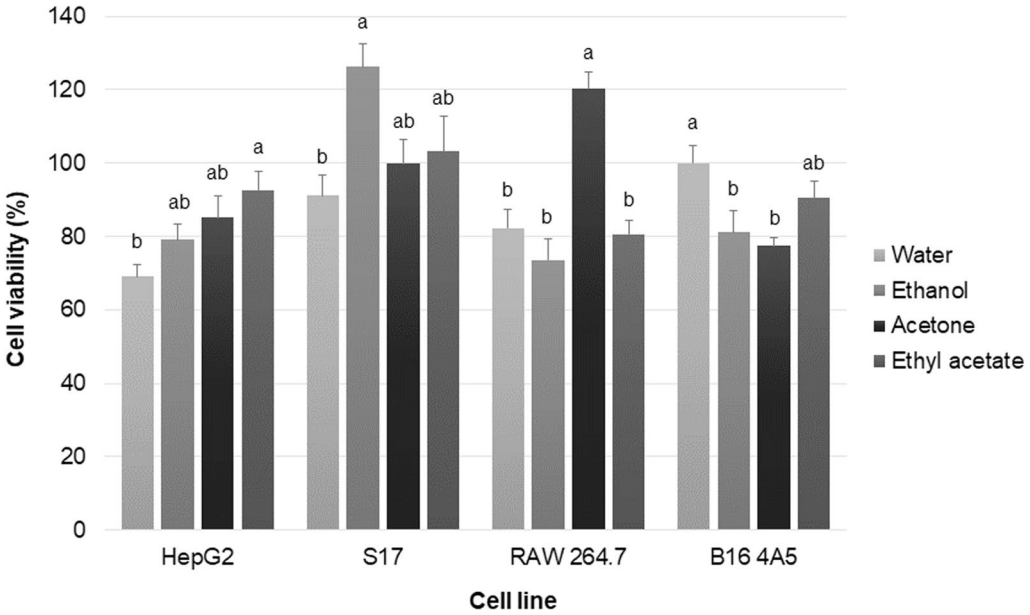

Fig. 3 Cytotoxicity of the extracts of *L. algarvense* seeds against human hepatocellular (HepG2), murine bone marrow stromal (S17), murine macrophage (RAW 264.7) and murine melanoma (B16 4A5) cell lines. Results are expressed as cellular viability (%) at 100 µg/mL after 72 h of incubation. Values represent the mean ± standard error of the mean (SEM) of six independent experiments ($n = 6$). For each cell line, columns marked with different letters (a–b) denote significant differences by Tukey's multiple comparisons tests ($p < 0.05$)

cell viability was also high for both water and acetone extracts (> 90%), with the ethanol extract enhancing cell viability to more than 120%. The analysis of the RAW 264.7 macrophages also revealed low cytotoxicity for all the extracts (> 73% cell viability), with the acetone extract demonstrating a notable proliferative effect of 120%. Following the same trend, all the extracts showed low toxicity towards human skin melanoma B16 cells, with cell viability values ranging from 77 to 100%.

4 Discussion

4.1 Phytochemical analysis

Rodrigues et al. [8] and subsequent studies demonstrate the TPC, TFC, and CTC across various extracts of *L. algarvense*, highlighting significant variability depending on the plant organ, extraction solvent, and species. Our findings indicate that ethanol seed extract of *L. algarvense* seeds possesses notably high TPC and TFC, surpassing methanolic extracts of peduncles and leaves (83 and 54 mg/g extract, respectively) and approaching the TPC of roots (118 mg/g extract) but remain lower than flowers (228 mg/g extract) [8]. The seed's aqueous extract showed superior TCT, outperforming other organ extracts (19–43 mg/g extract) except flowers (145 mg/g extract) [8]. Moreover, comparative studies within the *Limonium* genus reveal a broad range of TPC (43.6–522 mg/g extract) and TFC (1.61–129 mg/g extract) across species, with the highest concentrations typically found in ethyl acetate and acetone extracts, particularly during the flowering stage [19, 20]. This indicates the influence of species, developmental stage, plant organ, extraction method, and solvent on phytochemical content.

Recent studies highlight the importance of phenolic compounds, tannins, and flavonoids in cosmetics due to their antioxidant, anti-inflammatory, skin-whitening, and antimicrobial properties. These compounds provide health-promoting benefits for skin preservation and overall skin health, making them valuable in cosmetic formulations [21, 22]. Many of the main compounds found in the water and ethanol extracts have notable cosmetic-related applications. Gallic acid, present in both extracts, is recognized for its antioxidant, anti-inflammatory, and antimicrobial properties, making it valuable in skincare formulations for its anti-ageing and skin-soothing benefits [23]. Gentisic acid, also found in water and ethanol extracts, is known for its antioxidant and anti-inflammatory properties, which are beneficial in cosmetic products aimed at reducing skin irritation and preventing oxidative stress-induced skin damage [24]. Caffeic acid, identified in both extracts, is a potent antioxidant with anti-inflammatory properties, commonly used in cosmetic products for its ability to protect the skin from UV radiation and improve overall skin texture and tone [25]. Ampelopsin (dihydromyricetin), found in ethanol extracts, has antioxidant and anti-inflammatory

properties, making it useful in formulations designed to shield the skin from environmental stressors and reduce signs of ageing [26]. Taxifolin (dihydroquercetin), present in both extracts, is known for its strong antioxidant activity and is used in cosmetics to enhance skin glow, shield it from environmental harm, and diminish the visibility of fine lines and wrinkles [27]. Myricetin, identified in ethanol extracts, possesses anti-inflammatory and antioxidant properties, contributing to improved skin elasticity and hydration in skincare products [28]. Lastly, rutin, found in ethanol extracts, is recognized for its anti-inflammatory and antioxidant effects and is used to strengthen capillaries, reduce dark circles under the eyes, and improve skin texture [29]. The beneficial properties of these compounds make them valuable additions to various cosmetic and skincare products, enhancing skin health, reducing signs of ageing, and protecting the skin from environmental damage.

4.2 In vitro antioxidant activity

Although these are the first specific investigations into the antioxidant activities of *L. algarvense* seeds, existing research already demonstrated the potent antioxidant capabilities of *L. algarvense* [8, 9, 11, 12]. Methanol extracts from various parts of the sea lavender plant exhibit substantial in vitro antioxidant potential, notably, the flowers showed remarkable RSA against DPPH (EC_{50} = 0.09 mg/mL) and ABTS (EC_{50} = 0.27 mg/mL), alongside significant CCA (EC_{50} = 0.29 mg/mL) and FRAP (EC_{50} = 0.01 mg/mL) [9]. This suggests that the *L. algarvense* seeds possess antioxidant properties nearly equivalent to those of the flowers. Besides, investigations into the antioxidant properties of seeds from other halophyte species, such as *C. mariscus*, showed similar antioxidant potency to that of *L. algarvense* seeds. The water acetone and ethanol extracts of *C. mariscus* seeds exhibited high RSA towards DPPH (EC_{50} = 0.21 and 0.38 mg/mL) and ABTS (EC_{50} = 0.13 and 0.22 mg/mL), effective FRAP (EC_{50} = 0.13 and 0.36 mg/mL), and CCA (EC_{50} = 0.83 and 1.14 mg/mL) [30]. Moreover, the observed antioxidant activity correlates with the metabolite profile of the extracts. The aqueous extract, which exhibited the highest antioxidant activity, contains a high abundance of flavonoids (e.g. ampelopsin, taxifolin) and flavonoid glycosides (e.g. myricetin-O-hexoside), both of which are known for their potent antioxidant properties [31–33]. The ethanol extract, which showed the second highest antioxidant activity, also has a high concentration of these compounds, including ethyl gallate, myricetin, and rutin. In contrast, the acetone and ethyl acetate extracts, which were the least active in antioxidant assays, contain fewer flavonoids and phenolic acids. This suggests that the high antioxidant activity of the aqueous and ethanol extracts is primarily due to the presence of these abundant and potent antioxidant metabolites.

Oxidative stress is a major cause of premature ageing and is associated with external factors, such as atmospheric pollution, UV radiation, pathogenic microorganisms, nutrition, smoking, and climate changes, among others [21]. In addition, antioxidants and chelating agents are often used as stabilizers, preventing, or reducing oxidative deterioration of the active components in formulations [21]. As a result, the incorporation of plant extracts in cosmetic formulations as active antioxidant ingredients and/or preservatives is currently a market trend, offering an ecologically sustainable option [34]. For instance, polyphenolic extracts are highlighted for their demonstrated antioxidant, antimicrobial, anti-inflammatory, and anti-ageing properties, as well as their role in enhancing solar photoprotection [21, 35]. Overall, the compounds with RSA and metal-reducing/chelating properties found in sea lavender seeds suggest their potential application in cosmetics, particularly for skin protection. These extracts can function as primary antioxidants, neutralizing free radicals and preventing oxidative stress, thereby reducing skin ageing and damage. Additionally, secondary antioxidants can inhibit the formation of new radicals, providing further protection against oxidative damage. This dual functionality not only promotes skin health but also preserves product integrity and extends shelf life by preventing oxidation.

4.3 In vitro enzyme inhibitory activity

Although these are the first specific investigations into the enzyme inhibitory activities of *L. algarvense* seeds, existing research already demonstrated the bioactive potential of this species. Water extracts from flowers of sea lavender plants cultivated under saline irrigation have previously exhibited substantial in vitro tyrosinase inhibition (EC_{50} = 873 µg/mL) [12]. Additionally, *L. delicatulum* ethanol extracts from the leaves also exhibited considerable tyrosinase inhibitory (EC_{50} = 0.34 mg/mL), while water and hydroethanolic extracts showed lower inhibitory activity (> 1 mg/mL) [36]. Further, 85% aqueous methanol fraction of the whole *L. tetragonum* inhibited cellular melanin production and decreased cellular tyrosinase activity [37]. These studies suggest that the enzyme inhibitory properties observed in *L. algarvense* seeds are consistent with the bioactive potential demonstrated by other members of the *Limonium* genus. Moreover, other studies on the tyrosinase inhibitory activity of halophyte seeds have been described. For example, a study on *C. mariscus* seeds found that medium to high polarity extracts (acetone, ethanol, aqueous acetone, aqueous ethanol, and water) exhibited effective tyrosinase inhibition (55–71 mg KAE/g DW) [30].

Moreover, the presence of bioactive compounds such as gallic acid, gentisic acid, caffeic acid, ampelopsin, taxifolin, myricetin, and rutin in *L. algarvense* seeds' extracts likely contributes to their enzyme inhibitory activities. These compounds are essential for inhibiting enzymes such as tyrosinase, hyaluronidase, elastase, and collagenase, which are responsible for breaking down skin components like melanin, hyaluronic acid, elastin, and collagen. This inhibition may help to maintain skin firmness, elasticity, hydration, and even pigmentation, making these extracts valuable for skin health and anti-ageing formulations [23–29].

The overexpression of elastase, collagenase, and hyaluronidase enzymes leads to the degradation of the extracellular matrix (ECM), contributing to skin ageing and compromising the skin barrier, rendering it susceptible to infections and microbial invasion [38, 39]. This enzymatic imbalance not only results in pathological damage to the skin but also manifests as visible signs of ageing, such as diminished elasticity, and aesthetic concerns, particularly prominent among women [40, 41]. To mitigate ECM degradation and uphold its dynamic structure, it is imperative to regulate the activity of these enzymes by inhibiting them or maintaining them at basal levels [38–40]. Chronic sunlight or UV exposure upregulates the activity of the tyrosinase enzyme, a pivotal player in melanogenesis, exacerbating skin hyperpigmentation [42]. Considering this, inhibiting tyrosinase activity emerges as a strategic approach to mitigate hyperpigmentation problems such as melasma. Overall, our findings underscore the versatile potential of *L. algarvense* seed extracts, not only in delaying skin ageing but also in formulating skin-lightening ingredients and addressing various dermo-cosmetic needs due to their rich content of bioactive phenolic compounds and flavonoids.

4.4 Photoprotective properties

While the research did not yield specific studies on the direct application of *Limonium* species for skin protection in cosmetics, other species within the Plumbaginaceae family have demonstrated related properties. For example, plumbagin, extracted from the roots of *Plumbago zeylanica*, plays a significant role in shielding the skin from ultraviolet radiation damage by reducing the incidence of squamous cell carcinoma and modulating cellular responses to UV radiation. These responses include preventing cell growth, triggering cell death, and reducing the activity of transcription factors like activating protein-1, Stat3, and nuclear factor-kappa B, which are usually stimulated by UV radiation [43]. Additionally, similar to their role in plants—protecting against UV radiation, pathogens, and oxidative stress [44, 45]—the flavonoids found in *L. algarvense* seeds, such as rutin, myricetin, and taxifolin, may significantly enhance the photoprotective effects of the extracts. These molecules have been described as capable of absorbing UV radiation, thereby reducing skin UV-induced damage [46, 47]. Additionally, their antioxidant properties neutralize free radicals generated by UV exposure, preventing oxidative stress, skin ageing, and hyperpigmentation, making these extracts promising for skincare products aimed at UV protection and overall skin health.

Several studies have emphasized the importance and advantages of in vitro models, particularly in the context of evaluating the SPF of cosmetic products. These models offer a practical and ethical alternative to in vivo testing, providing fast, reproducible results without the need for UV exposure to humans or animals. Specifically, in vitro methods have been highlighted for their ability to accurately assess the SPF of products, focusing on UV radiation wavelengths (290–320 nm) that cause the most significant skin damage, including burning, photoaging, and cancer [48, 49]. The SPF rating system is a widely accepted guideline in dermatology and skincare that categorizes the level of protection sunscreen products offer against the sun's UV-B radiation. It ranges from mild (< 15), to moderate (15–30), and high (> 30) protection. Within this classification, water, acetone, and ethyl acetate extracts of sea lavender seeds have shown moderate protective capabilities, with SPF values ranging from 16 to 23. According to The Skin Cancer Foundation, such SPF levels are generally recommended for daily application, especially suitable for individuals with fair to medium skin pigmentation who expect limited to moderate outdoor exposure. This moderate protection category provides significant defence against erythema (sunburn), thereby helping to mitigate acute and cumulative photodamage to the skin [50]. These findings highlight the potential of incorporating sea lavender seed extracts into skincare formulations dedicated to minimizing daily sun-induced skin damage.

4.5 Cellular cytotoxicity

Preliminary toxicity tests on infusions and decoctions of wild sea lavender flowers also have shown no toxicity at 100 µg/mL against HepG2, S17, and N9 (murine microglia) mammalian cell lines (80–100%). Additionally, these extracts were non-toxic to brine shrimp *Artemia salina* [9]. The ethanol extracts from *L. algarvense* flowers, peduncles, and leaves also demonstrated no toxicity against human embryonic (HEK 293) and HepG2 cells, although some extracts had weak cytotoxicity towards RAW 264.7 macrophages (67.4–78.2%) [11].

Medicinal plants are a significant source of novel compounds with potential therapeutic efficacy; however, their utilization is tempered by the possibility of

toxicological effects, posing health hazards. For the development of dermo-cosmetics, ensuring minimal or non-existent toxicity is paramount, rendering cytotoxicity evaluations a critical component in assessing the safety of natural extracts for cosmetic applications [51, 52]. Although dermo-cosmetic products primarily target the epidermis—the skin's outermost layer—substances with high permeability may penetrate the dermis. Given its vascular nature, this layer can facilitate systemic distribution, potentially affecting organs such as the liver [53]. Additionally, stromal cells play a pivotal role in tissue morphogenesis, homeostasis, and responses to injury and immune challenges [54]. Similarly, skin macrophages are essential for both adaptive and innate immunity, as they add in pathogen clearance, infection prevention, and inflammation mitigation, and regulate tissue repair and homeostasis [55]. Therefore, comprehensive toxicity assessments must encompass various cell types to fully reflect the complexity of biological interactions.

Utilizing the ISO 10993–5 criteria for cytotoxicity—where cell viability > 80% indicates non-cytotoxicity, 60–80% suggests weak cytotoxicity, 40–60% moderate cytotoxicity, and < 40% strong cytotoxicity [56]—the cytotoxicity profiles of *L. algarvense* seed extracts across the several cell lines revealed predominantly non-cytotoxic effects. An exception was noted with the water extract, which demonstrated weak cytotoxicity towards hepatic cells. These findings evidence not only emphasize the critical role of solvent selection in the preparation of extracts for dermo-cosmetic applications but also support the integration of *L. algarvense* seeds extracts in the development of dermo-cosmetic products, with nil or minimal cytotoxic impact across various tissues.

5 Conclusions

The comprehensive evaluation of sea lavender (*Limonium algarvense*) seed extracts in this study highlights their promising potential as bioactive ingredients for dermo-cosmetic formulations, showcasing significant antioxidant activity, effective inhibition of skin-relevant enzymes, superior photoprotection capabilities, and nil or minimal cytotoxicity. The differential bioactivity profiles, attributed to the solvent-specific extraction efficiency, underscore the extracts' multifunctionality in promoting skin health and protection. Notably, the high levels of total phenolics, flavonoids, and condensed tannins in specific extracts correlate with their bioactivities, suggesting their substantial contribution to the observed effects. This research paves the way for the innovative use of sea lavender seed extracts in developing natural, effective dermo-cosmetic products, aligning with the growing consumer preference for sustainable and biologically active skincare solutions.

Abbreviations

ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
CTC	Condensed tannins content
DMSO	Dimethyl sulfoxide
DMEM	Dulbecco's Modified Eagle Medium
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EC_{50}	Half maximal effective concentration
EDTA	Ethylenediaminetetraacetic acid
EGCG	Epigallocatechin gallate
FBS	Foetal bovine serum
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalents
ICA	Iron chelating activity
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NPK	Nitrogen, phosphorus, potassium
PV	Pyrocatechol violet
QE	Quercetin equivalents
RSA	Radical scavenging activity
RT	Room temperature
SEM	Standard error of the mean
SPF	Sun protection factor
TCA	Trichloroacetic acid
TFC	Total flavonoid content
TPC	Total phenolic content
UHPLC-ESI-MS/MS	Ultrahigh-Performance Liquid Chromatography Coupled with Electrospray Ionization Mass/Mass Spectrometry
UV	Ultraviolet
UV-Vis	Ultraviolet–visible

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Author contributions
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Availability of data and materials
Data will be made available on request.

Declarations

Ethics approval and consent to participate
Not applicable.

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Not applicable.

Competing interests
The authors declare no competing interests.

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