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Enhancing bioactivity and bioavailability of *Limonium bellidifolium* via cyclodextrin-based inclusion complexes: a new strategy in drug discovery from halophyte source

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In the present study we investigated the enhancement of solubility, stability, and bioavailability of phytochemicals from *Limonium bellidifolium* using α- and β-cyclodextrin-based encapsulation. Ethanol/water extracts were encapsulated at 25 °C, 40 °C, and 60 °C, and their performance was evaluated through simulated in vitro gastrointestinal digestion. Optimal results were observed at 25 °C for β-cyclodextrin and at 40 °C for α-cyclodextrin. Structural analysis with FTIR and SEM confirmed minimal chemical changes and well-formed particles. Bioavailability tests revealed significantly higher recovery of major compounds, such as quercetin, catechin, and ferulic acid, in encapsulated samples compared to pure extracts. Both α- and β-cyclodextrin systems provided strong protection during digestion and retained a higher proportion of bioactives. Antioxidant assays showed increased activity after digestion in encapsulated forms, especially in aerial and root extracts. Enzyme inhibition tests demonstrated notable tyrosinase and acetylcholinesterase activity, particularly in β-cyclodextrin-encapsulated root extracts. These results confirm the effectiveness of cyclodextrin encapsulation in improving compound retention and biological activity. To our knowledge this is the first detailed report on *L. bellidifolium* encapsulation using cyclodextrins and offers a promising strategy for developing phytochemical delivery systems for therapeutic and nutraceutical applications.

Keywords Limonium bellidifolium, Halophyte plants, Cyclodextrin inclusions, Bioavailability

Abbreviations

CD	Cyclodextrin
α-CD	Alpha-cyclodextrin
β-CD	Beta-cyclodextrin
γ-CD	Gamma-cyclodextrin
SGF	Simulated gastric fluid
IN	Intestinal (bioavailable) fraction
PG	Post-gastric fraction
PI	Post-intestinal fraction
TPC	Total phenolic content
TFC	Total flavonoid content
DPPH	1,1-diphenyl-2-picrylhydrazyl
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

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FRAP	Ferric reducing antioxidant power
CUPRAC	Cupric ion reducing antioxidant capacity
MCA	Metal chelating activity
PBD	Phosphomolybdenum assay (total antioxidant capacity)
AChE	Acetylcholinesterase
BChE	Butyrylcholinesterase
EGCG	Epigallocatechin gallate
HIA	Human intestinal absorption

Halophyte plants, which are famous for their robustness in salty and coastal conditions, have drawn great scientific attention from researchers because of their unusual phytochemical composition and potential health-promoting qualities[1]. The *Limonium* genus, part of the Plumbaginaceae family, commonly known as sea lavender (in Turkey known as "deniz lavantası") and the genus encompasses approximately 24 genera and approximately 800 species globally[2,3]. Historically, *Limonium* species have been used in traditional medicine, to treat respiratory (bronchitis), gastrointestinal problems, menstrual disturbances, fever and skin disorders[4–6]. The genus is rich in secondary metabolites like flavonoids, tannins and phenolic acids, which exhibit notable antioxidant, antimicrobial and anti-inflammatory properties[7]. In addition to their medicinal uses, the members of the *Limonium* genus are valued ornamental appeal due to their long-lasting flowers[8]. In Turkey, the familyis represented by six genera and 68 species [3].

Phytotherapy or plant-based treatments are essential in healthcare because of their high efficiency, low cost, greater accessibility, and minimal adverse effects[9]. However, industrial application of bioactive compounds in plant extracts is associated with challenges such as poor water solubility and instability under various conditions. To overcome these challenges, encapsulation techniques (particularly cyclodextrin complexes) have been developed to enhance stability, controlled release and health benefits[10–12]. Among the various encapsulation techniques, the most widely used method for enhancing solubility is cyclodextrin encapsulation, which utilizes the distinctive properties of these cyclic oligosaccharides. Cyclodextrins (CDs) and their derivatives have become valuable pharmaceutical excipients for improving the therapeutic effectiveness of various drugs[13]. CDs are cyclic lipopolysaccharides that include hydrophobic inner cavities and hydrophilic surfaces and their unique structure, makes them exceptional encapsulation agents[13–15]. CDs are biocompatible and biodegradable, produced through the enzymatic breakdown of starch, and are widely used in industries such as medicine, food, and cosmetics due to their ability to enhance compound stability, bioavailability, solubility, and distribution. CDs, unlike polysaccharides, do not create viscous hydrogels and can penetrate mucus layers, hence improving drug absorption. Natural CDs are three primary types of α-CD, β-CD, and γ-CD and among them, β-cyclodextrin is the most commonly used due to its ease of production and low cost[16]. All CDs are considered safe and meet the GRAS (Generally Recognized as Safe) standards[17].

The bioavailability, bioaccessibility and bioactivity of phytochemicals are one of the most remarkable topics of scientific research. These terms generally include the parameters such as digestion, absorption, metabolism, tissue distribution and the body's physiological responses[18]. To maximize the health benefits of bioactive compounds, nutraceuticals or drugs, these substances must be efficiently absorbed in the gastrointestinal tract, with prolonged residence time in the body[9,19]. Bioavailability analyses play a critical role in assessing how effectively active compounds are absorbed and utilized. While in vitro test systems for bioactive compounds often yield positive results, these outcomes may vary under in vivo conditions[20]. In vitro digestion models are widely employed to investigate structural changes and the release of phytochemicals under simulated gastrointestinal conditions[20].

However, animal models or clinical studies offer the most precise insights into bioavailability, although they are time-consuming, expensive and can raise ethical concerns. Thus, in vitro models are frequently used to approximate oral bioavailability by examining the impact of diverse phytochemical factors[20]. Bioavailability can be enhanced through advanced delivery systems, including liposomes, glycerosomes, niosomes, β cyclodextrins, nanoparticles, and lipid-based systems that improve release rates and membrane penetration capabilities[19].

L. bellidifolium has long been recognized in traditional medicine; however, its pharmacological potential-particularly in terms of drug delivery and bioavailability-remains insufficiently studied in scientific literature. In this study, ethanol/water extracts from both the aerial and root parts of the plant were encapsulated using α- and β-cyclodextrins to develop optimized delivery systems for potential therapeutic applications. A series of temperature-based encapsulation experiments were conducted to investigate the effects of thermal conditions on particle morphology, compound stability, and host–guest interactions. Bioavailability was assessed using an in vitro simulated gastrointestinal digestion model, and the recovery of key bioactive compounds in the post-gastric and post-intestinal phases was quantified through on-line HPLC analysis. Thanks to its remarkable resilience in extreme environmental conditions and its potential for sustainable cultivation, *L. bellidifolium* emerges as a promising candidate for future research in plant-derived drug development. This study not only enhances the current scientific understanding of the *Limonium* genus but also highlights the broader pharmaceutical relevance of halophytic medicinal plants in the development of innovative formulation strategies.

Materials and methods

Preparation of cyclodextrin complexes with limonium extracts

Limonium bellidifolium (Gouan) Dumort samples were collected during field studies conducted in Balıkesir in 2021 (B1 Balıkesir: Ayvalık, Sarımsaklı, Badavut, 39°16'26.97"N, 26°37'36.37"E, 1 m, 15.08.2021. The taxonomic identification of the plant samples was carried out by Prof. Dr. Selami Selvi, a faculty member of the Medicinal and Aromatic Plants Program at Balıkesir University, Altınoluk Vocational School. The voucher specimen number is SV 3455, and it has been deposited in the Balıkesir University Herbarium.

Five grams of plant material were weighed and added to 100 mL of an ethanol/water solution (70:30 v/v). The mixture was homogenized at 5000 rpm x g for 5 min. Subsequently, the cyclodextrin complex was prepared in the ratio 1:1. And 25 mg of cyclodextrin and 25 mg of plant extract were accurately weighed to achieve a final concentration of 1 mg/mL. These were mixed in 50 mL of water and stirred for 24 h in the dark to protect from light-induced degradation. The resulting samples remained in liquid form and were used within three days of preparation, without undergoing any freezing process.

Chemicals

The chemicals were purchased from Sigma-Aldrich (Darmstadt, Germany) and included: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, electric eel acetylcholinesterase (AChE) (type-VI-S, EC 3.1.1.7), horse serum butyrylcholinesterase (BChE) (EC 3.1.1.8), galantamine, acetylthiocholine iodide (ATChI), butyrylthiocholine chloride (BTChI), 5,5-dithio-bis(2-nitrobenzoic) acid (DTNB), tyrosinase (EC 1.14.18.1, from mushroom), α-glucosidase (EC 3.2.1.20, from *Saccharomyces cerevisiae*), α-amylase (EC 3.2.1.1, from porcine pancreas), α-cyclodextrin (CAS:10016-20-3), β-cyclodextrin (CAS:7585-39-9, sodium carbonate, Folin-Ciocalteu reagent, hydrochloric acid, sodium hydroxide, trolox, ethylenediaminetetraacetate (EDTA), neocuproine, cupric chloride, ammonium acetate, ferric chloride, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), ammonium molybdate, ferrozine, ferrous sulphate hexahydrate, kojic acid, and acarbose. All chemicals were of analytical grade.

HPLC analyses

We conducted detailed chemical analyses to characterize the composition of the pure extract. The measurements were performed with an Agilent 1260 chromatograph equipped with 1260 diode array (DAD)[21].

The experimental procedure has been described in our previous study[22]. Antioxidant activity was evaluated using ABTS, DPPH, FRAP, and CUPRAC[22–25] assays, providing complementary insights into radical scavenging and reducing power. Detailed procedure and instrumental specifications, including the thermostable column cabinet, autosampler, secondary syringe pump, and UV-DAD detector configuration, are provided in the supplementary material.

Simulated digestion model for human bioavailability testing

The gastrointestinal digestion simulation was carried out following a modified protocol described previously[20,26]. To mimic the gastric environment, 2.5 mL of the sample solution was mixed with 17.5 mL of simulated gastric fluid (SGF), containing pepsin enzyme and appropriate electrolytes dissolved in water at pH 2. The mixture was then placed in a shaking water bath at 37 °C for 2 h to mimic peristaltic movement. After this stage, samples were immediately cooled in an ice bath to halt enzyme activity, and a 2 mL aliquot was taken as the "post-gastric" (PG) sample. The simulated digestion model is shown Fig. 1.

For the next step, designed to mimic the intestinal absorption process, a dialysis membrane containing enough $NaHCO_3$ was added to the remaining solution to neutralize acidity. Additionally, bile salts and pancreatin were prepared by dissolving 250 mg of bile and 40 mg of pancreatin in 0.1 M $NaHCO_3$. These solutions were mixed to a final volume of 10 mL, then added to the digestion mixture. This mixture was incubated during an additional 2 h at 37 °C. After the incubation, a portion of the solution diffused into the dialysis membrane was considered the "serum-available" or "bioavailable" content (IN).

To complete the setup, a cellulose tube approximately 15 cm in length was filled with the prepared solution, and sufficient $NaHCO_3$ was added to maintain pH stability. The ends of the tube were tightly secured, and all samples were stored at − 20 °C until further analysis. This detailed procedure allowed for the in vitro assessment of bioavailability, effectively simulating human digestive conditions.

Biological activity assessments of all extracts

In our study, we tested the total phenolic and total flavonoid contents[27] and the biological activity of samples using different antioxidant assays. Specifically, radical scavenging activity was tested using four different methods: ABTS[28], DPPH[29], FRAP, and CUPRAC[30], with each allowing us to capture different aspects of antioxidant potential. The ABTS and DPPH assays measured the ability of samples to neutralize radicals, with absorbance recorded at respective wavelengths. The FRAP method provided an indication of the ferric reducing antioxidant power, while CUPRAC evaluated the sample's capacity to reduce copper ions, reflecting broader antioxidant capacity. In addition to radical scavenging activity, we measured metal chelating activity to examine the sample's ability to bind metal ions, further contributing to its antioxidant profile[31]. Total antioxidant capacity was also evaluated as an overarching measure of the combined activities[32]. These methods were well-established and validated before our study, with detailed procedures provided in previously published methods[27,33].

Enzyme Inhibition profiles of all extracts

The AChE and BChE inhibition assays[34], the α-amylase[35], α-glucosidase[36], and tyrosinase inhibition[34] assays, were conducted following previously published protocols.

On-line HPLC analysis for post- bioavailability assay

For HPLC analysis, an Agilent-1100 system with autosampler, UV-DAD, and thermostable column cabinet was used for gradient analysis with a Purospher Star C18 column (5 µm, 4.6 × 250 mm), an injection volume of 20 µL, and a 30-minute run time[22]. Mobile phase composition and further setup details can be found in the supplementary materials.

Fig. 1. Schematic representation of the in vitro gastrointestinal digestion procedure. Stage 1: Preparation of the simulated gastric fluid (SGF) by dissolving 1.6 g pepsin and 1 g NaCl in 500 mL distilled water. Stage 2: Gastric digestion of plant extracts (2.5 mL extract with cyclodextrins + 17.5 mL SGF) at 37 °C under continuous shaking (100 rpm) for 2 h. Stage 3: Cooling of gastric digestion samples in an ice water bath (10–15 min) to stop enzymatic reactions. Stage 4: Adjustment of pH for the intestinal phase by dissolving 250 mg bile and 40 mg pancreatin in 5 mL of 0.1 M $NaHCO_3$ and combining them in a round-bottom flask. Stage 5: Intestinal digestion with the addition of 4.5 mL pancreatin solution and $NaHCO_3$; dialysis membrane (15 cm length) was immersed and incubated for 2 h at 37 °C with stirring. Stage 6: Cooling of intestinal digestion samples in an ice bath to stop enzymatic activity; samples were collected for antioxidant assays and stored at − 20 °C.

FTIR analyses for material characterization

The Fourier transform infrared (FT-IR) spectra of all samples were recorded using a Bruker Vertex 70 spectrophotometer (Rheinstetten, Germany) with a resolution of 4 cm^{-1}. The spectra were measured over a wavenumber range of 400–4000 cm^{-1}[37].

SEM (scanning electron microscope)

The surface morphology of the extract was examined using a Zeiss EVO LS10 scanning electron microscope (SEM) (Oberkochen; Germany) The extracts were placed on an aluminum stub and images were obtained using the sputtering technique at a magnification of 20.000 x and an accelerating voltage of 1 and 15 kV[37].

Bioinformatic analysis

The graphs were created using GraphPad Prism 9.0.0, and bioinformatic analyses were performed using the Swiss ADME program. (http://www.swisstargetprediction.ch/result.php? job=764501993&organism=Homo_sapiens)

Encapsulation efficiency calculation

To evaluate the encapsulation efficiency (EE%) of *Limonium bellidifolium* extracts, the amount of non-encapsulated compound was determined after complexation with β-cyclodextrin at 25 °C and α-cyclodextrin at 40 °C. EE% was calculated using the following formula:

EE%=(Total compound used – Free compound/Total compound used)×100.

Assessment of the potential bioavailability index of major phenolic compounds

The bioavailability index (BAvI) were evaluated using the theoretical formula provided below:

% BAvI = (amount in bioaccessible fraction/amount in undigested extract) × 100.

Result and discussion

To understand the process in this study, it is essential to have a thorough understanding of the structure of CDs. To give a brief overview of the materials, CDs are surrounded by primary and secondary OH^- groups that enable H bonding with water and other polar molecules[38]. Each type of CDs has great number of H bond donors and acceptors. But some studies reported that CDs have varying solubility rates at the same degree of heat[14,38,39]. For example, it was reported that, at 25 °C in 100 ml CD had complete dissolving rate; α-CDs:14.5 g, β-CDs: 1.85 g and γ-CDs: 23.2 g[39]. The rates were so different, however when it comes to the delivery of the molecules, β-CD has been reported as the most suitable structure. β-CD is primarily for the delivery of molecules, because it has an ideal cavity size that makes it highly effective for forming stable inclusion complexes with a wide range of molecules. The cavity size allows for the encapsulation and protection of various molecules, enhancing their stability and bioavailability[14,38,39].

The effectiveness of cyclodextrin-based encapsulation depends on the compatibility between cavity size and molecular structure. This study aimed to determine the most suitable cyclodextrin type and preparation conditions for encapsulating the diverse bioactive compounds in *L. bellidifolium*. Aerial and root extracts were prepared using an ethanol/water solvent and encapsulated with α- and β-cyclodextrins. Temperature was selected as at 25 °C, 40 °C, and 60 °C to identify the most effective conditions for each formulation.

Evaluation of Temperature Change Effects on Total Phenolic, Flavonoid, and Antioxidant Activity

Total phenolic content (TPC) and total flavonoid content (TFC) assays provide a general measure of the abundance of bioactive compounds with strong antioxidant potential, since phenolics and flavonoids act primarily as electron or hydrogen donors and contribute to radical scavenging, redox balance, and enzyme modulation[40,41]. For this purpose we conducted TPC and TFC assays to evaluate the bioactive compound profiles of *L. bellidifolium*, providing baseline information for further testing. Among α-CD complexes, the highest activity was observed in the aerial part at 25 °C and 40 °C, while the root complex formed at 25 °C exhibited the highest activity.

Similarly, for β-CD complexes, the aerial part at 40 °C showed the greatest activity, whereas the root complex at 25 °C demonstrated superior results. The values are represented Table 1. These findings are consistent with previous studies on the members of the *Limonium* genus, which were reported high phenolic and flavonoid contents, particularly in root extracts, contributing significantly to their antioxidant properties. Zengin and colleagues[42] found that six *Limonium* species had phenolic levels ranging from 83.62 to 135.74 mg GAE/g and flavonoid levels between 6.90 and 35.38 mg/g. The distribution of these compounds in *L. bellidifolium* extracts

Samples	TPC (mg GAE/g)	TFC (mg RE/g)	ABTS (mg TE/g)	DPPH (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mg TE/g)	MCA (mg EDTAE/g)	PBD (mmol TE/g
1-*L.bellidifolium AP* α-CD 25	42.38 ± 0.54	15.01 ± 1.90	159.46 ± 0.24	215.29 2.08	193.44 ± 5.06	179.03 ± 4.53	6.50 ± 0.52	0.99 ± 0.09
2-*L.bellidifolium AP* α-CD 40	42.25 ± 0.22	15.58 ± 0.83	162.92 ± 1.95	266.30 ± 2.10	199.34 ± 6.85	177.85 ± 2.09	7.55 ± 0.45	0.99 ± 0.05
3-*L.bellidifolium AP* α-CD 60	42.00 ± 0.42	14.83 ± 1.10	161.67 ± 0.94	253.34 ± 0.52	196.48 ± 1.12	168.59 ± 4.36	7.66 ± 0.12	1.03 ± 0.04
4-*L.bellidifolium R* α-CD 25	52.56 ± 1.11	3.56 ± 0.22	174.95 ± 6.35	314.10 ± 2.14	257.85 ± 1.73	226.49 ± 5.11	4.81 ± 0.94	1.58 ± 0.06
5-*L.bellidifolium R* α-CD 40	51.51 ± 0.32	3.44 ± 0.41	162.00 ± 7.26	311.82 ± 0.90	257.04 ± 2.29	226.95 ± 2.95	4.30 ± 0.79	1.64 0.01
6-*L.bellidifolium R* α-CD 60	50.27 ± 0.29	2.42 ± 0.40	185.96 ± 4.77	296.46 ± 5.74	223.71 ± 1.21	194.27 ± 2.68	1.51 ± 0.75	1.59 ± 0.10
7-*L.bellidifolium AP*β-CD 25	40.90 ± 0.59	13.52 ± 1.80	167.26 ± 2.00	280.69 ± 1.71	198.86 ± 1.10	178.02 ± 0.51	7.60 ± 0.67	0.81 ± 0.06
8-*L.bellidifolium AP*β-CD 40	41.81 ± 0.58	13.77 ± 3.95	166.72 ± 1.89	270.56 ± 1.72	198.39 ± 1.28	180.84 ± 0.37	8.53 ± 1.28	0.84 ± 0.05
9-*L.bellidifolium AP*β-CD 60	41.02 ± 0.46	12.59 ± 1.26	163.26 ± 0.84	255.56 ± 2.05	199.28 ± 0.56	171.78 ± 1.22	11.87 ± 0.96	0.77 ± 0.02
10-*L.bellidifolium R*β-CD 25	51.94 0.88	3.77 ± 0.10	207.37 ± 0.71	282.58 ± 0.14	259.26 ± 1.11	241.70 ± 3.39	3.42 ± 0.76	1.44 ± 0.02
11-*L.bellidifolium R*β-CD 40	49.12 ± 1.22	2.80 ± 0.22	191.51 ± 1.79	313.01 ± 1.09	247.43 ± 1.78	218.63 ± 3.46	7.62 ± 0.77	1.29 ± 0.01
12-*L.bellidifolium R*β-CD 60	48.93 ± 1.26	3.29 ± 0.62	191.26 ± 1.76	303.58 ± 1.63	230.95 ± 1.47	200.07 ± 2.12	1.51± 2.12	1.43 ± 0.02
13-*L.bellidifolium free AP*	72.65 ± 0.92	18.84 ± 3.37	226.29 ± 0.13	322.43 ± 0.13	396.34 ± 1.89	330.53 ± 7.27	19.51 ± 1.00	2.62 ± 0.17
14-*L.bellidifolium free R*	110.26 ± 1.63	6.91 ± 1.66	226.25 ± 0.09	323.18 ± 0.02	605.66 ± 8.69	525.96 ± 8.84	11.45 ± 0.22	4.04 ± 0.18

Table 1. Biological activities of all heat-optimized extracts. **GAE* Gallic acid equivalent, *RE* Rutin equivalent, *TE* Trolox equivalent, *EDTAE* EDTA equivalent, *AP* Aerial part, *R* Root part, *α -CD* α cyclodextrin, *β-CD*β cyclodextrin.

reflects both plant tissue differences and the impact of encapsulation conditions. Cyclodextrin complexes can influence the measured values by improving the solubility and stability of phenolics, thereby enhancing their extractability, although in some cases partial masking of hydroxyl groups within the cavity may result in lower apparent values. These findings suggest that the rich phenolic and flavonoid composition of roots underlies their stronger biological activity and that cyclodextrin encapsulation can serve as an effective tool to stabilize these compounds without major loss of bioactive potential.

Investigation on other species, such as *L. delicatum* and *L. quesadence*, further highlights the strong bioactivity of methanolic extracts compared to aqueous ones, emphasizing the potential of these species as valuable natural antioxidant sources[43]. Additionally, in the literature, TPC values in the roots of *L. bellidifolium*, *L. globuliferum*, *L. gmelinii*, *L. lilacinum*, *L. sinuatum*, and *L. iconicum* have been reported to range between 43.64 and 238.18 mg GAE/g, highlighting the phenolic richness of root tissues[42]. Among the Turkish species, *L. gmelinii* roots showed the highest flavonoid content with 129.69 mg RE/g, while other species displayed TFC values ranging from 1.61 to 129.69 mg RE/g (Senizza et al., 2021). Various studies have confirmed significant phenolic and flavonoid content in *Limonium* extracts obtained using different solvents[44–46]. However, comprehensive studies specifically on *L. bellidifolium* remain limited, underscoring the need for further research to fully explore its phytochemical and antioxidant potential.

Following the determination of phenolic and flavonoid contents, radical scavenging assays such as DPPH and ABTS were performed. These assays were employed with the aim of assessing the modulatory effect of cyclodextrin encapsulation on the antioxidant activity of *Limonium* extracts. Since the DPPH radical is soluble in organic solvents, it mainly interacts with lipophilic or moderately polar antioxidants, while the ABTS radical is soluble in both hydrophilic and lipophilic environments, enabling a broader evaluation of antioxidant potential[47,48]. When the results are examined, in ABTS assays, the highest value was observed in the non-encapsulated aerial part extract, followed by the β-CD-encapsulated root extract at 25 °C. Among α-CD applications, the root extract encapsulated at 60 °C exhibited the greatest activity, surpassing the aerial part extract encapsulated at 40 °C. These results suggest that both plant parts origin and encapsulation temperature contribute to the preservation or enhancement of antioxidant potential, with root extracts consistently showing higher activity. Similarly, DPPH assay results supported these observations. The non-encapsulated root extract recorded the highest activity overall. Among α-CD forms, the root extract at 25 °C displayed the highest DPPH value, while β-CD encapsulation of the root at 40 °C yielded a comparably high result. The aerial part extracts showed lower DPPH values in both α-CD and β-CD formulations. As a result , cyclodextrin inclusion complexes, especially those formed with β-cyclodextrin, improved the solubility and stability of phenolic compounds, which in turn made these molecules more available to interact with free radicals. This effect was particularly evident in the DPPH assay, where encapsulation enhanced the ability of moderately polar phenolics to react with the radical. In contrast, the ABTS assay (because it can detect both hydrophilic and lipophilic antioxidants) showed that the broad antioxidant activity of the extracts was not only preserved but in some cases even increased after encapsulation. Taken together, these findings indicate that cyclodextrin structures help maintain and strengthen the radical scavenging potential of *Limonium* extracts, demonstrating that encapsulation is an effective strategy for modulating antioxidant mechanisms.

To evaluate the antioxidant capacity of the samples, we performed the FRAP and CUPRAC assays. FRAP and CUPRAC assays are electron transfer–based methods that assess the ability of antioxidants to reduce metal ions ($Fe^{3+} \rightarrow Fe^{2+}$ in FRAP and $Cu^{2+} \rightarrow Cu^{+}$ in CUPRAC). These assays provide insight into the redox potential of phenolic compounds, which depends largely on the number and position of hydroxyl groups[49–51]. In both assays, the non-encapsulated root extract showed the highest antioxidant activity, indicating strong electron donation ability. Among the encapsulated samples, the β-CD-encapsulated root extract at 25 °C exhibited the highest activity, followed closely by the α-CD -encapsulated root extract. These results highlighted the superior antioxidant potential of root-derived extracts and suggest that β-CD may offer slightly improved preservation of redox-active compounds under optimal conditions. In contrast, aerial part extracts displayed lower antioxidant values, with comparable results in both α-CD and β-CD treatments.

So, the results showed cyclodextrin encapsulation can influence these outcomes by stabilizing redox-active molecules and modulating the accessibility of their functional groups. While β-CD generally favors the preservation of electron-donating capacity by improving solubility and preventing degradation, certain inclusion orientations may also mask reactive hydroxyls, slightly reducing activity under specific conditions. In our study, this was reflected by the higher reducing capacity of root extracts, which are naturally richer in phenolic compounds, while β-CD encapsulation under favorable conditions was able to preserve much of this activity compared to the non-encapsulated forms.

The metal chelating test may give different results from other antioxidant tests because this test specifically aims to prevent the binding of metal ions and their participation in oxidative processes. Other antioxidant tests (DPPH, ABTS, FRAP, CUPRAC) usually focus on free radical neutralization or electron/hydrogen donating capacity. Thus, while some compounds may be free radical scavengers, they may form a weak bond with metal ions or vice versa. Due to these different mechanisms, the results of the metal chelation test may differ from other antioxidant tests.

The metal chelating activity (MCA) and total antioxidant capacity (PBD) of *L. bellidifolium* extracts exhibited distinct performance depending on the plant part, cyclodextrin type, and encapsulation conditions. Among all samples, the β-CD-encapsulated aerial part at 60 °C showed the highest MCA value. The α-CD encapsulated aerial part at 60 °C and β-CD root at 40 °C displayed moderate chelating capacities, while the α-CD and β-CD root extract at 60 °C showed the lowest activity. These results suggest that aerial parts, especially when encapsulated with β-CD at higher temperatures, exhibit enhanced metal-chelating performance.

Similarly, in the PBD assay, the non-encapsulated root extract demonstrated the highest total antioxidant capacity, significantly outperforming all encapsulated formulations. Among the encapsulated samples, the

α-CD-encapsulated root extract at 40 °C and β-CD root at 25 °C showed the highest values. In contrast, aerial part samples exhibited lower PBD values, with α-CD and β-CD treatments at 60 °C and 40 °C, respectively. These findings were confirmed that root extracts showed higher antioxidant potential, while encapsulation especially with β-CD can partially preserve or enhance the activity, depending on temperature and plant tissue. So, the differences observed in MCA and PBD assays can be attributed to their distinct mechanisms of action and the modulatory role of cyclodextrin encapsulation. While MCA reflects the ability of compounds to chelate transition metals and thus inhibit metal-catalyzed radical formation, PBD measures the overall reducing capacity of all redox-active constituents[52–54]. Cyclodextrin inclusion can influence these outcomes by altering the accessibility of functional groups: in MCA, encapsulation (particularly with β-CD at elevated temperatures)may orient hydroxyl groups outward, enhancing their interaction with metal ions, whereas in PBD, some electron-donating sites may become partially masked within the cyclodextrin cavity, leading to lower total reducing signals compared to non-encapsulated extracts. These findings suggest that cyclodextrins do not uniformly increase antioxidant responses but rather modulate specific mechanisms depending on plant tissue, functional group orientation, and encapsulation conditions.

In summary, the results of the antioxidant assays show that different encapsulation conditions and the type of cyclodextrin (α-CD or β-CD) influence the antioxidant activity of *L. bellidifolium* extracts. In particular β-CD complexes formed with the root extract at 25 °C and α-CD complexes with the aerial part at 40 °C showed high antioxidant capacities in several assays, including ABTS, DPPH, FRAP, CUPRAC, and phosphomolybdenum (PBD). Therefore, using multiple assays is essential to comprehensively evaluate the antioxidant potential of the samples. Overall, root extracts demonstrated stronger antioxidant activity, especially when encapsulated under optimal conditions, while cyclodextrin encapsulation helps preserve antioxidant capacity, β-cyclodextrin showed better interaction with phenolic compounds, particularly in root samples. Although β-cyclodextrin appeared slightly more effective in some cases, the differences between β-CD and α-CD treatments were not substantial. This suggests that both types of cyclodextrins can be effectively used for encapsulation. The absence of large variations in activity indicates that the phytochemicals in *L. bellidifolium* are compatible with both α- and β-cyclodextrin structures. Nevertheless, the slightly higher activity observed in non-encapsulated extracts suggests that some loss of antioxidant compounds may occur during encapsulation, possibly due to partial entrapment or limited release of active compounds.

Furthermore, encapsulation with β-CD significantly enhanced the antioxidant activity of plant extracts. This finding is consistent with previous reports. For example, β-CD complexes with tea catechins (EGCG and GCG) doubled the antioxidant activity compared to free catechins, as measured by the DPPH assay. This result showed the ability of β-CD to improve the bioactivity of plant polyphenols[55]. Similarly, a study on the rutin-β-CD complex showed increased antioxidant activity depending on the solvent diversity[56]. Moreover, encapsulation of *Origanum compactum* essential oil in β-CD metal-organic frameworks resulted in enhanced DPPH scavenging activity and improved thermal stability[57]. In our findings, antioxidant activity was higher with β-CD encapsulation, while lower values were observed with α-CD. The results are supported by previous studies. For instance, in red clover and *Actinidia* leaf extracts, α-CD showed weaker antioxidant capacity than β- or γ-CD in ABTS, DPPH, and FRAP assays[58]. On the other hand, many studies also support finding, reporting that β-CD enhances solubility, stability, and antioxidant function. For example, encapsulated garlic oil, turmeric extract, and other essential oils showed increased DPPH activity and longer-lasting antioxidant effects after complexation with β-CD[59–61]. Overall, our results confirmed by previous evidence that β-CD is an effective encapsulation agent for enhancing the antioxidant potential of plant-derived compounds. In contrast, α-CD may contribute to molecular protection but can reduce antioxidant activity under certain conditions. This difference highlights the influence of cyclodextrin cavity size and binding interactions on the antioxidant performance of inclusion complexes.

Phytochemical content analysis

Detailed phytochemical analyses were carried out to identify the bioactive compounds in the pure plant extracts. A total of 32 compounds were found in the aerial and root parts of the plant. These analyses were done only on pure extracts to understand the natural potential of the plant's bioactive molecules. In the next step, to better understand how the compounds behave after bioavailability, we selected 11 major compounds for further analysis. These included gallic acid, rutin, apigenin, quercetin, kaempferol, epigallocatechin gallate (EGCG), ferulic acid, caffeic acid, myricetin, and vanillic acid. The selection was based on the results of our study, previous literature, and data from other *Limonium* species[2,62–67]. A literature review was also conducted to support and validate the selection of these compounds[2,42,64,65]. In particular, the aerial parts were rich in epigallocatechin gallate (EGCG) and myricetin derivatives.

As shown in Table 4, *L. bellidifolium* primarily contained myricetin and catechin derivatives as the main compound groups. As a result, different compounds were detected in the aerial and root parts. Our findings especially, in the aerial parts, epigallocatechin gallate (EGCG), myricetin derivatives were identified as major compounds. The results, presented in Table 4.

Further analyses were conducted after encapsulation to better understand the recovery of the molecules. After the encapsulation process, bioavailability studies were carried out, and post-gastric and post-intestinal samples were obtained. These samples were analyzed using the 11 identified standard compounds to measure the recovery rates of the molecules.

An on-line HPLC method was applied for more detailed evaluation. This method aimed to provide clearer results regarding the protection and release of the compounds after encapsulation. The on-line HPLC antioxidant tests showed that quercetin, ferulic acid, catechin, and caffeic acid had the highest antioxidant activities among the tested phenolic compounds. All of this results were given in supplementary materials. (Table S1–S18). The *L. bellidifolium* root and aerial part samples, encapsulated with β-cyclodextrin at 25 °C and collected after

the post-intestinal phase, showed the strongest antioxidant responses across all test methods. These samples outperformed all others in terms of antioxidant activity (Table S1–S18).

A general decrease in antioxidant activity was observed across the datasets, with the highest values presented supplementary material Table S18 and the lowest in Table S1, corresponding to the pure extract. Figures 7 and 8 summarizes the differences in molecule recovery rates. Among the analytical techniques, HPLC-CUPRAC yielded the highest antioxidant values, likely due to its sensitivity to compounds with strong reducing ability (Tables S1–S18). Interestingly, although vanillic acid was present in all samples, it showed no activity in the on-line HPLC-FRAP test, possibly due to the short reaction time within the capillary system, which may have limited its ability to reduce iron. These findings support the improved antioxidant performance of β-cyclodextrin-encapsulated *L. bellidifolium* extracts, particularly after the intestinal phase, suggesting enhanced compound stability and bioavailability. Furthermore, analysis of the on-line HPLC-based FRAP, CUPRAC, ABTS, and DPPH results confirmed which molecules were more active in specific antioxidant systems and under which encapsulation conditions. Although β-cyclodextrin generally provided higher antioxidant activity, both α- and β-cyclodextrin showed comparable effectiveness in preserving the compounds, with β-CD offering slightly better protection.

This indicates that both types of cyclodextrins may be useful for compound delivery, depending on the structure of the target molecule, and supports literature findings suggesting that β-CD offers higher encapsulation efficiency[68]. Therefore, both α- and β-cyclodextrins can be considered suitable carriers for future applications, with selection tailored to the specific phytochemical composition of the plant extracts.

Evaluation of the enzyme inhibition effects of the complexes

The cholinesterase inhibitory effects of *L. bellidifolium* were evaluated through both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition assays. The highest AChE inhibition was observed in the root extract, followed by the aerial part extract. Encapsulation with β-CD at 25 °C resulted in moderate activity in the root, while α-CD encapsulation at 25 °C with 2.25 ± 0.22 mg GALAE/g. In the aerial parts, β-CD (40 °C) and α-CD (40 °C) encapsulated samples showed lower activities respectively. All of the values presented Table 2. For BChE inhibition, the root extract exhibited the strongest activity with 4.26 ± 0.79 mg GALAE/g, while the aerial part extract showed a lower value of 1.86 ± 0.20 mg GALAE/g. However, no detectable BChE inhibition was observed in any of the cyclodextrin-encapsulated samples, regardless of plant part or encapsulation condition.

These results indicate that the pure extracts of *L. bellidifolium*, particularly the root, possess strong cholinesterase inhibitory potential, while cyclodextrin encapsulation tends to reduce or eliminate this activity, especially for BChE. As a result, AChE and BChE enzymes catalyze the hydrolysis of acetylcholine and butyrylcholine, and their inhibition increases synaptic acetylcholine levels, providing therapeutic effects in neurodegenerative diseases[69,70]. The root part of *L. bellidifolium* exhibited strong AChE and BChE inhibitory activity, whereas cyclodextrin encapsulation, particularly for BChE, markedly reduced or even eliminated this effect. This outcome can be explained by the entrapment of phenolic or flavonoid compounds with inhibitory potential inside the cyclodextrin cavity or by the masking of their accessible functional groups. In the case of AChE inhibition, the β-CD (25 °C) encapsulated root sample retained moderate activity, indicating that cyclodextrins may partially preserve certain enzyme–substrate interactions. Moreover, the reduced inhibition observed after encapsulation might not necessarily reflect a loss of bioactivity but could instead suggest that the active compounds were more effectively protected within the cyclodextrin structure, limiting their immediate availability for enzyme binding while enhancing their stability for potential long-term effects.

Samples	AChE inhibition (mg GALAE/g)	BChE inhibition (mg GALAE/g)	Amylase inhibition (mmol ACAE/g)	Glucosidase inhibition (mmol ACAE/g)	Tyrosinase inhibition (mg KAE/g)
1-*L.bellidifolium* AP α-CD 25	0.88 ± 0.10	na	0.10 ± 0.01	5.34 ± 0.00	48.89 ± 4.01
2-*L.bellidifolium* AP α-CD 40	1.22 ± 0.18	na	0.10 ± 0.01	5.19 ± 0.17	50.92 ± 0.04
3-*L.bellidifolium* AP α-CD 60	0.95 ± 0.02	na	0.11 ± 0.01	5.10 ± 0.46	52.10 ± 2.21
4-*L.bellidifolium* R α-CD 25	2.25 ± 0.22	na	0.16 ± 0.04	5.35 ± 0.06	74.11 ± 1.21
5-*L.bellidifolium* R α-CD 40	2.05 ± 0.19	na	0.13 ± 0.02	5.23 ± 0.28	75.57 ± 3.04
6-*L.bellidifolium* R α-CD 60	1.62 ± 0.19	na	0.11 ± 0.02	5.13 ± 0.46	71.30 ± 2.48
7-*L.bellidifolium* AP β-CD 25	1.32 ± 0.19	na	0.10 ± 0.02	5.34 ± 0.00	65.07 ± 1.09
8-*L.bellidifolium* AP β-CD 40	1.60 ± 0.04	na	0.10 ± 0.01	4.44 ± 0.20	66.07 ± 0.62
9-*L.bellidifolium* AP β-CD 60	1.24 ± 0.37	na	0.10 ± 0.06	5.35 ± 0.01	48.26 ± 1.40
11-*L.bellidifolium* R β-CD 25	2.44 ± 0.32	na	0.19 ± 0.01	5.40 ± 0.01	85.33 ± 0.54
11-*L.bellidifolium* R β-CD 40	2.03 ± 0.19	na	0.12 ± 0.02	5.29 ± 0.11	67.21 ± 1.44
12-*L.bellidifolium* R β-CD 60	1.86 ± 0.20	0.12 ± 0.93	0.12 ± 0	4.95 ± 0.39	67.09 ± 0.88
13-*L.bellidifolium* AP	4.07 ± 0.10	1.86 ± 1.40	0.96 ± 0.04	5.35 ± 0.01	129.56 ± 1.31
14-*L.bellidifolium* R	4.85 ± 0.07	4.26 ± 0.79	0.89 ± 0.05	5.37 ± 0.17	135.83 ± 0.33

Table 2. Enzyme inhibitory effects of the tested extracts. *Values are reported as mean ± SD of three parallel measurements. *GALAE* Galantamine equivalent, *KAE* Kojic acid equivalent, *ACAE* Acarbose equivalent, *AP* aerial part, *R* root part, *α -CD* α cyclodextrin, *β-CD*β cyclodextrin, *na* not active.

In a previous study, AChE inhibitory activity was observed in *L. effusum* and *L. sinuatum*, with the aqueous fraction of *L. sinuatum* showing strong inhibition (IC_{50} = 0.199 ± 0.009 µg/ml)[62]. In our study, ethanol/water extracts were used, and the observed AChE and BChE inhibition activities support the findings reported in the literature. Previous study, cholinesterase inhibition activities of six different *Limonium* species were investigated, and strong inhibition was found in almost all extracts[42]. These findings confirm that cholinesterase inhibitory activities are commonly observed in *Limonium* species, which is consistent with the results of our study.

The α-amylase inhibitory activity of *L. bellidifolium* extracts was generally low across all samples. The highest inhibition was recorded in the pure aerial part extract, followed by the root extract. Encapsulated samples showed considerably lower activity. Among these, the β-cyclodextrin encapsulated root extract at 25 °C exhibited the highest inhibition value, while the lowest activity was observed in the β-CD encapsulated aerial part at 60 °C (0.10 ± 0.06 mmol ACAE/g). These results suggest that encapsulation reduced the amylase inhibitory effect of the extracts.

In contrast, α-glucosidase inhibition was strong and consistent across all samples. The highest activity was observed in the β-CD encapsulated root extract at 25 °C, followed closely by the pure root extract and β-CD encapsulated aerial part at 60 °C. Both α-CD encapsulated aerial and root samples at 25 °C also demonstrated high inhibition. These findings indicated that *L. bellidifolium* extracts, particularly those encapsulated with β-cyclodextrin, are potent α-glucosidase inhibitors and may have potential in managing postprandial hyperglycemia.

When the results are examined together, the inhibitory effects of *L. bellidifolium* extracts on carbohydrate-hydrolyzing enzymes can be explained by the different mechanisms of α-amylase and α-glucosidase[71]. While α-amylase catalyzes the breakdown of starch into oligosaccharides, its inhibition generally requires a direct and accessible interaction of active compounds with the enzyme's catalytic site[71,72]. Encapsulation with cyclodextrins likely masked some of these functional groups, reducing the inhibitory effect compared to the free extracts. In contrast, α-glucosidase, which catalyzes the hydrolysis of disaccharides into glucose, was strongly inhibited across all samples, and encapsulation—particularly with β-CD—appeared to enhance this activity by improving the solubility and stability of phenolic compounds, thereby increasing their availability to the enzyme[71,72]. These findings indicate that cyclodextrins can modulate enzyme inhibition in a mechanism-dependent manner, reducing activity when access to catalytic sites is hindered (α-amylase) but enhancing it when solubility and stability favor enzyme binding (α-glucosidase).

The enzyme inhibition results obtained in this study are in line with previous reports on various *Limonium* species. In particular, the relatively low α-amylase and moderate α-glucosidase inhibitory activities observed in *L. bellidifolium* agree with studies on *L. contortirameum*, *L. virgatum*, and *L. axillare*, which also demonstrated dual enzyme inhibition but with moderate potency (IC_{50} values ranging from 0.6 to 1.2 mg/mL for α-amylase and lower values for α-glucosidase)[73,74].

Moreover, the stronger α-glucosidase inhibition compared to α-amylase observed in our samples, especially in β-CD encapsulated aerial parts, supports the selective inhibitory behavior reported for *L. spathulatum*, whose hydroethanolic extract showed potent α-glucosidase inhibition (IC_{50} = 0.04 mg/mL), even stronger than that of the standard drug acarbose[67]. This suggests that *Limonium* species may selectively inhibit glucosidase, which is particularly valuable in managing postprandial hyperglycemia[67].

The tyrosinase inhibitory activities of *L. bellidifolium* extracts were notably high in both pure and encapsulated forms. The pure extracts exhibited the strongest activities, with the root extract showing the highest inhibition, followed closely by the aerial part extract. Among the encapsulated samples, the root extract encapsulated with β-cyclodextrin at 25 °C showed the highest activity, followed by the β-CD encapsulated aerial part at 40 °C. The α-CD encapsulated root and aerial part extracts displayed lower inhibition respectively. These findings demonstrate that *L. bellidifolium* possesses strong tyrosinase inhibitory potential, particularly in its pure form. However, encapsulation with cyclodextrins, especially α-CD, appears to reduce this activity. The root extracts consistently exhibited higher inhibition than the aerial parts, suggesting that root-derived compounds may play a more prominent role in tyrosinase inhibition.

The tyrosinase inhibition findings in our study are also supported by existing literature. Several *Limonium* species, including *L. tetragonum*, *L. boitardii*, and *L. cercinense*, have been reported to exhibit strong tyrosinase inhibitory activity due to their high phenolic and flavonoid content[75,76]. Notably, isolated flavonoid glycosides such as myricetin 3-galactoside and quercetin 3-O-β-galactopyranoside from *L. tetragonum* have shown effective inhibition of tyrosinase and melanogenesis[75,76]. Similarly, the high inhibitory activity of *L. bellidifolium* observed in this study, particularly in the pure root extract, is likely linked to the presence of structurally related phenolic compounds. These findings further support the potential of *Limonium* species as natural sources of bioactive agents with antidiabetic and skin-protective properties.

Tyrosinase is a copper-containing enzyme that catalyzes key steps in melanin biosynthesis, and its inhibition can occur through competitive binding at the active site, chelation of the catalytic copper ions, or by phenolic compounds acting as antioxidants that intercept quinones and prevent melanin polymerization[77–79]. The strong inhibitory effects of *L. bellidifolium*, particularly in the root extracts, are likely related to their high content of flavonoids and phenolic acids bearing catechol or hydroxyl groups capable of interacting with the enzyme. Cyclodextrin encapsulation modulated these effects, with β-CD maintaining relatively high activity under favorable conditions, probably by stabilizing phenolics and preserving their functional integrity, whereas α-CD encapsulation appeared to reduce activity by partially masking reactive groups within its cavity. These findings suggest that the tyrosinase inhibition observed is closely linked to both the structural features of phenolic compounds and the microenvironment provided by cyclodextrins.

All of these results showed a moderate decrease in enzyme inhibition activity was observed in the α-cyclodextrin (α-CD) encapsulated samples. This reduction was not significant in most cases. One possible explanation is that α-CD has a smaller cavity compared to other cyclodextrins, which may limit the full

inclusion of larger bioactive compounds. However, partial inclusion or surface interactions may still allow these compounds to retain a considerable portion of their biological activity. Moreover, the encapsulation can help protect sensitive compounds from degradation or oxidation, which may support the preservation of their inhibitory potential. Therefore, although α-CD encapsulation may slightly limit the interaction between active compounds and enzymes, it does not completely impair their function, resulting in only a moderate loss of activity. This interpretation is further supported by the bioavailability results presented in the following section, which indicate that the active compounds remain relatively accessible even after encapsulation with α-CD.

In vitro bioavailability assays for encapsulated *L. bellidifolium* extracts

In vitro bioavailability assays are essential for developing effective plant-based therapies, as they predict how bioactive compounds behave during digestion, absorption, and metabolism. These assays help improve formulations to overcome the common challenge of low bioavailability in plant-derived compounds. The primary purpose of performing this test within the scope of the given acknowledgment of the project is to generate foundational data required for subsequent advanced anticancer studies. Specifically, this stage aims to evaluate how encapsulated compounds behave in different simulated digestive environments and to determine the efficiency of molecular recovery. Understanding these parameters is critical for optimizing the formulation process and ensuring that the active molecules retain their stability and therapeutic potential through the gastrointestinal tract. The data obtained from this step will guide the selection of the most promising formulations for future in vitro and in vivo anticancer efficacy assessments. Based on this knowledge, we conducted in vitro bioavailability tests on all encapsulated extracts, with results presented in Table 3.

The tests revealed that antioxidant activities generally decreased after gastric and intestinal digestion (PG and PI phases), although certain encapsulated samples exhibited increased activity due to the protective effects of α-cyclodextrin and β-cyclodextrin. Compound levels following digestion were quantified using an on-line HPLC system, with Figs. 7 and 8 illustrating the detected amounts in both aerial and root parts. The analysis demonstrated that encapsulation was effectively preserved, and in some cases enhanced, the levels of major bioactive compounds such as epigallocatechin gallate, ferulic acid, catechin, and caffeic acid. The highest compound retention was observed in root extracts encapsulated with β-cyclodextrin at 25 °C, whereas the lowest levels were found in unencapsulated samples.

Following these results, it was also evident that samples encapsulated with α-cyclodextrin retained significantly higher amounts of compounds compared to the pure forms. This finding supports the earlier statements outlined in previous sections, confirming that despite relatively lower antioxidant activities, α-cyclodextrin provided a level of compound protection comparable to β-cyclodextrin. Therefore, if the main goal is just to protect sensitive bioactive compounds during digestion, α-cyclodextrin can be a useful option. However, if the aim is both protection and good delivery, β-cyclodextrin seems to be a better choice. These results also show that future

Samples	ABTS (mg TE/g)	DPPH (mg TE/g)	CUPRAC (mg TE/g)
1-*L.bellidifolium* AP	472.7 ± 0.22	118.49 ± 0.26	310.0 ± 6.8
2-*L.bellidifolium* R	472.3 ± 0.36	119.00 ± 0.27	384.1 ± 4.3
3-*L.bellidifolium* AP/PG	97.2 ± 0.15	30.99 ± 2.77	52.9 ± 1.7
4-*L.bellidifolium* R/PG	120.6 ± 8.08	39.06 ± 1.38	58.5 ± 2.5
5-*L.bellidifolium* AP/PI/IN	198.5 ± 3.60	3.32 ± 1.32	26.1 ± 0.7
6-*L.bellidifolium* AP/PI/OUT	124.9 ± 4.16	2.01 ± 1.88	28.0 ± 1.6
7-*L.bellidifolium* AP/α-CD 40	404.7 ± 1.33	118.12 ± 0.21	188.6 ± 2.0
8-*L.bellidifolium* R/α-CD 40	341.2 ± 2.89	116.22 ± 0.95	154.5 ± 11.0
9-*L.bellidifolium* AP/α-CD 40/PG	87.7 ± 0.74	20.63 ± 0.76	42.6 ± 0.9
10-*L.bellidifolium* R/α-CD 40/PG	86.4 ± 1.18	14.32 ± 2.79	40.0 ± 1.3
11-*L.bellidifolium* AP/α-CD 40/PI/In	472.5 ± 0.11	10.95 ± 1.78	41.9 ± 0.6
12-*L.bellidifolium* AP/α-CD 40/PI/OUT	472.2 ± 0.45	7.40 ± 0.84	107.9 ± 2.6
13-*L.bellidifolium* R/α-CD 40/PI/In	472.1 ± 0.23	10.67 ± 1.40	39.5 ± 0.8
14-*L.bellidifolium* R/α-CD 40/PI/OUT	472.3 ± 0.46	8.25 ± 1.65	96.6 ± 5.1
15-*L.bellidifolium AP*β-CD 25	406.3 ± 3.00	117.89 ± 0.13	168.0 ± 6.4
16-*L.bellidifolium* R/β-CD 25	449.6 ± 0.34	118.93 ± 0.30	220.3 ± 4.4
17-*L.bellidifolium* AP/β -CD 25 PG	86.4 ± 2.40	23.85 ± 0.49	38.0 ± 0.7
18-*L.bellidifolium* R/β-CD 25 PG	100.6 ± 0.13	25.63 ± 0.88	44.2 ± 0.5
19-*L.bellidifolium* AP/β-CD 25/PI/IN	472.6 ± 0.57	10.28 ± 1.05	42.2 ± 1.1
20-*L.bellidifolium* AP/β-CD 25/PI/OUT	473.2 ± 0.72	8.71 ± 1.34	102.6 ± 1.7
21-*L.bellidifolium* AP/β-CD 25/PI/IN	473.2 ± 0.23	12.27 ± 1.67	41.4 ± 0.6
22-*L.bellidifolium* R/β -CD 25/PI/OUT	472.6 ± 0.26	7.89 ± 1.96	108.1 ± 2.3

Table 3. Results of the radical scavenging activity of samples after the bioavailability assay. **AP* Aerial part, *R* Root part, *PG* Post gastric, *PI* Post ıntestınal, *α -CD* α cyclodextrin, *β-CD*β cyclodextrin.

Limonium bellidifolium aerial part EtOH/water	mg/g	*L.bellidifolium* root part EtOH/water	mg/g
1. Caffeoyl hexose	-	1. Caffeoyl hexose	3.47
2. (epi)-Gallocatechin-(epi)-gallocatechin	0.34	2. Monogalloyl hexoside	2.96
3. Epigallocatechin	0.95	3. Gallic acid	1.37
4. Hydroxyferulic acid sulfate	0.80	4. Monogalloyl hexoside sulphate	2.41
5. (epi)-catechin-(epi)-gallocatechin-O-methoxy-(epi)-gallocatechin-gallate	2.87	5. (epi)-Gallocatechin-(epi)-gallocatechin	**3.09**
6. Sulfate ferulic acid	0.80	6. Galloyl-shikimic acid	-
7. Catechin gallate polymer	10.55	7. Galloyl pentoside	4.15
8. Gallocatechin gallate	0.40	8. (epi)-Gallocatechin-(epi)-gallocatechin gallate	**10.04**
9. Myricetin hexoside derivative	2.41	9. (epi)-gallocatechin-(epi)-gallocatechin-(epi)-gallocatechingallate	1.92
10. Myricetin-3 O-(galloyl) hexoside	1.71	10. (epi)-gallocatechin-(epi)-gallocatechin-(epi)-gallocatechingallate	2.80
11. Myricetin-7-O-glucuronide	12.13	11. (epi)-catechin-O-methoxy-(epi)-gallocatechin-gallate	**9.41**
12. Myricetin-3-O-hexoside	3.66	12. Vanillic acid hexoside	**3.57**
13. Myricetin C pentoside	3.33	13. Procyanidin tetramer	-
14. Gallic acid derivative	3.99	14. (epi)-catechin-(epi)-gallocatechin-O-methoxy-(epi)-gallocatechin-gallate	2.11
15. Quercetin O (galloyl) hexoside	1.90	15. 4'- methyl-epigallocatechin-3'-sulfate	1.58
16. Myricetin O pentoside	2.86	16. Gallocatechin-3-Ogallate	5.82
17. Quercetin O rutinoside	0.68	17. 3-ogalloyl(epi)catechin- (epi)catechin derivative	0.47
18. Phloretin 3.5 di C hexoside	0.98	18. Myricetin hexoside-gallate	**14.38**
19. Myricetin derivative	1.85	19. Glucosinolate derivative	2.11
20. Syringaresinol sulphate	0.37	20. Saccharide	1.37
21. Myricetin hexoside derivative	17.91	21. Myricetin rhamnoside	**19.31**
22. Pinoresinol sulphate	1.66	22. Phloretin-di-C-hexoside	0.60
23. Myricetin O galloylrhamnoside	13.58	23. Myricetin	**7.66**
24. Myricetin O galloyl rhamnoside	2.41	24. Pinoresinol sulphate	0.07
25. Myricetin O feruloylhexoside	2.00	25. N-trans-caffeoyltyramine	1.63
26. Quercetin O galloyl rhamnoside	0.44	26. Galloyl diglucose	0.38
27. Myricetin derivative	1.35	27. Cannabisin B	0.52
28. Quercetin	0.73	28. Myricetin glucoside	2.97
29. Quercetin derivative	2.43	29. Cannabisin B	0.19
30. Gallic acid derivative	0.18	30. Demethylgrossamide	-
31. Myricerin O (pentoside hexoside)	1.89	31. Cannabisin D	0.09
32. Apigenin	0.22	32. Cannabisin F	0.13

Table 4. Detailed chemical composition analyses for pure extracts.

studies with γ-cyclodextrin could be helpful to better understand how different types of cyclodextrins affect protection and stability.

To evaluate the changes in both the prepared formulations and pure extracts following the in vitro digestion system, three different antioxidant assays—ABTS, DPPH, and CUPRAC—were conducted. Antioxidant capacities assessed using these methods showed that encapsulated samples retained significant activity after intestinal digestion, sometimes surpassing the activity of pure extracts. In the ABTS assay, the highest activities were observed for the *L. bellidifolium* root extract/β-CD 25/PI/IN (473.2 ± 0.23 mg TE/g), followed by the aerial part and other encapsulated forms. Although DPPH and CUPRAC assays indicated a reduction in antioxidant activity after digestion, the HPLC results highlighted improved molecular preservation in encapsulated extracts, supporting the protective role of cyclodextrin-based systems during simulated gastrointestinal conditions.

The encapsulation process, particularly with β-CD at 25 °C, enhanced the bioavailability and stability of the compounds by minimizing their degradation during digestion. Table 6 shows that the recovery rates of major compounds after digestion ranged from 18% to 66%, with kaempferol showing the highest recovery (66.53%). Compounds like epigallocatechin gallate and ferulic acid had lower recovery rates (21–22%), while moderate recovery was observed for caffeic acid (45.78%) and vanillic acid (32.43%).

These findings align with other studies where encapsulation with cyclodextrins improved bioavailability. For example, Radic et al. (2020)[80] demonstrated that encapsulating olive pomace polyphenols enhanced antioxidant activity and gastrointestinal stability during digestion. Similarly[81], reported improved drug bioavailability through cyclodextrin inclusion complexes. Encapsulation of ellagic acid with β-CD also increased its water solubility and controlled release due to the stabilization of its highly lipophilic structure[82]. These studies support the efficacy of our encapsulation method in enhancing the stability and bioavailability of plant compounds.

Results of encapsulation efficiency

The encapsulation efficiency results demonstrated a distinct variation depending on both the type of cyclodextrin used and the plant part from which the extract was obtained. Specifically, β-cyclodextrin (β-CD) at 25 °C resulted

Compounds	*EE AP (%)*	*EE R(%)*
Free	-	-
β-CD 25	45.81	42.65
α-CD 40	39.16	59.78

Table 5. Encapsulation efficiency of *L. bellidifolium* complexes.

Compounds	AP/BAvI(%)	*R*/BAvI(%)
Kaempferol	**66.53**	**28.79**
Epigallocathechin gallate	**27.19**	**21.37**
Ferulic acid	**22.52**	**18.87**
Cathechin	**25.53**	**20.13**
Caffeic acid	**45.78**	**24.15**
Vanilic acid	**32.43**	**25.21**

Table 6. Quantification of major bioactive phenolics by on-line HPLC and their recovery rates. **AP* Aerial part, *R* Root part, *BavI* Bioavailibility index.

in a higher encapsulation efficiency for the aerial part of *L. bellidifolium* (45.81%) compared to α-cyclodextrin (α-CD) at 40 °C (39.16%). Conversely, α-CD was significantly more effective for the root extract, achieving an encapsulation efficiency of 59.78% compared to 42.65% for β-CD. This differential behavior likely arises from the structural compatibility between the cyclodextrin cavity size and the molecular profile of the phytochemicals in different plant parts. The results showed Table 5.

These results align with prior literature indicating that encapsulation efficiency typically ranges between 21% and 83%, depending on the cyclodextrin type, guest molecule size and polarity, and preparation conditions[83–85]. In general, larger guest molecules such as polyphenols and flavonoids are more effectively accommodated by β-CD due to its larger hydrophobic cavity (7 glucopyranose units), whereas smaller or more polar compounds—commonly found in root extracts—demonstrate stronger affinity for α-CD, which contains only 6 glucopyranose units.

Furthermore, temperature plays a key role in cyclodextrin complexation. Moderate heating at around 40 °C has been shown to enhance molecular interaction without compromising the stability of thermolabile compounds. Our selection of this temperature range is consistent with prior research, including global thermodynamic analyses of phenolic acid inclusion and molecular dynamics simulations. These studies have demonstrated that temperatures between 27 °C and 48 °C favor optimal complexation efficiency and help preserve the stability of the formed complexes. This situation is likely due to increased molecular interactions and a decrease in kinetic energy that could disrupt the host-guest system. Conversely, at temperatures exceeding 50 °C, a significant decrease in stability has been observed due to the weakening of non-covalent interactions necessary to maintain complex integrity.

Therefore, maintaining the temperature within this optimal range is critical to ensuring both the efficiency and stability of inclusion complexes. Furthermore, studies confirm that encapsulation conditions, particularly temperature, must be optimised to preserve the structural integrity and bioactivity of natural compounds. For example, the encapsulation of linalyl acetate and certain volatile organic compounds has highlighted that the proper selection of cyclodextrin type and process parameters significantly improves thermal stability, solubility, and controlled release behaviour[86,87].

In summary, the encapsulation efficiency obtained in this study (ranging from 39.16% to 59.78%) fall within the commonly reported literature range and highlight the importance of tailoring the host system and processing conditions to the phytochemical profile of the plant material. Such optimization ensures more effective protection, stabilization, and delivery of bioactive compounds in functional food, cosmetics, and pharmaceutical formulations.

Bioinformatical analyses for the major compounds

Understanding the pharmacokinetic properties of plant-derived compounds, such as absorption, permeability, protein binding, distribution, clearance, and half-life, is essential for evaluating their therapeutic potential and bioavailability. These parameters offer insight into a compounds behavior in the body and guide the development of more effective formulations[88–90]. To reduce animal testing and optimize formulation strategies before in vivo studies, in silico predictions were conducted using the SwissADME tool, and the results are presented in Table 7. And the molecular structures of major compounds (kaempferol, epigallocatechin gallate, ferulic acid, catechin, caffeic acid, and vanillic acid) are given in Fig. 2.

Human intestinal absorption (HIA) values are critical indicators used to estimate how efficiently each compound can be absorbed through the gastrointestinal tract following oral administration. In general, phenolic compounds are known to exhibit variable and often limited intestinal absorption, which directly affects their bioavailability and therapeutic efficacy in vivo. Our study, especially major phenolic compounds demonstrated low predicted HIA values (HIA < 30%), which suggests limited intestinal absorption in their non-encapsulated

Compound	Human intestinal absorption (HIA)	Caco-2 permeability (log unit)	MDCK permeability (log unit)	Plasma protein binding (PPB)	Volume of distribution (Vdss)	Clearance (CL)	Half-life (T1/2)
1. Kaempferol (C5)	0.015	-5.969	-4.909	97.881%	-0.812 L/kg	5.694 mL/min/kg	1.388 h
2. Epigallocatechin gallate (C6)	0.015	-6.894	0	87.3%	0.509 L/kg	8.232 mL/min/kg	2.325 h
3. Ferulic acid (C7)	<30%	-4.989	0	68.6%	0.188 L/kg	8.359 mL/min/kg	1.698 h
4. Catechin (C8)	<30%	-6.346	0	92.9%	1.197 L/kg	14.347 mL/min/kg	2.218 h
5. Caffeic acid (C9)	>=30%	-4.94	0	64.7%	0.248 L/kg	14.358 mL/min/kg	2.07 h
6. Vanillic acid (C11)	<30%	-5.096	0	67.7%	0.213 L/kg	3.04 mL/min/kg	1.978 h

Table 7. This table summarizes the *in silico* pharmacokinetic parameters of kaempferol, epigallocatechin gallate, ferulic acid, catechin, caffeic acid, and vanillic acid.

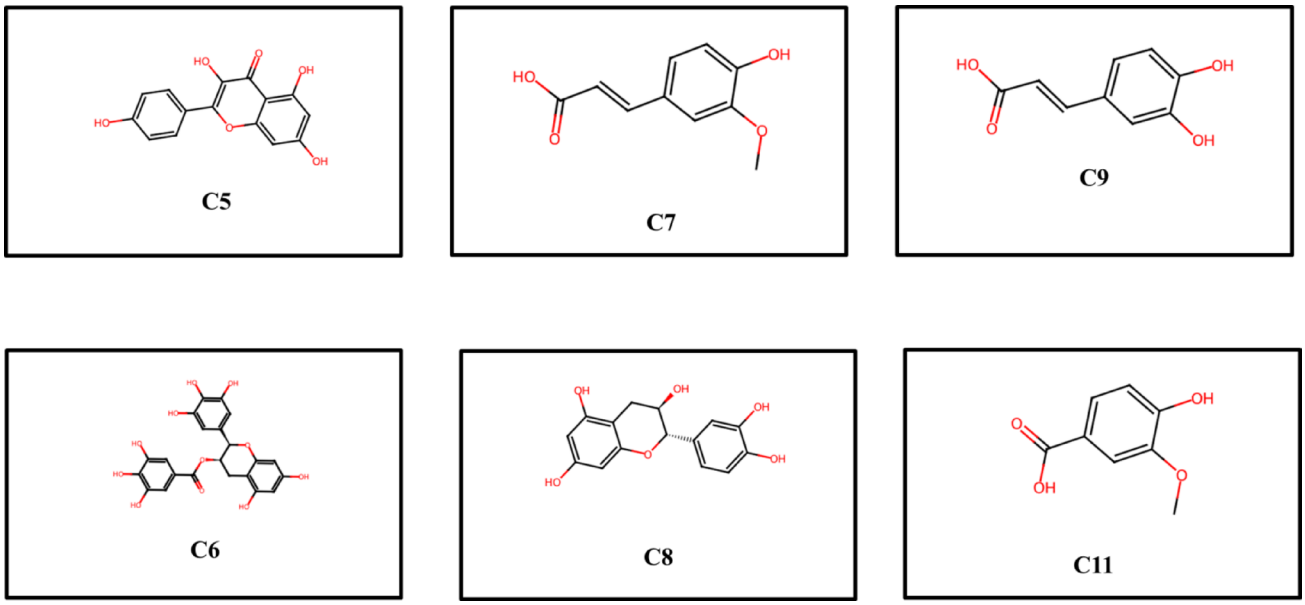

Fig. 2. The structures of major compounds. C5: Kaempferol, C6: Epigallocathechin gallate, C7: Ferulic acid, C8: Cathechin, C9: Caffeic acid, C11: Vanilic acid.

forms. These observations emphasize the necessity of employing innovative delivery strategies such as encapsulation to enhance the stability, permeability, and overall bioavailability of these bioactive molecules in the human body. Accordingly, in the scope of our study, cyclodextrin-based carrier systems were utilized with the aim of enhancing intestinal absorption. Our results clearly demonstrated that the molecules were significantly recovered following in vitro digestion, indicating improved protection and retention of bioactive compounds through encapsulation.

For instance, a study on phenolic metabolites derived from cocoa shell reported relatively high HIA values for certain compounds, including phloretic acid, p-coumaric acid, protocatechuic acid, gallic acid, epigallocatechin, and benzoic acid. These compounds showed a wide range of recovery rates following intestinal absorption, with reported values varying between 15% and 95%[91]. In contrast, research on tea-derived phenolics demonstrated that many of these compounds exhibit poor absorption characteristics, with HIA values often below 20%, indicating significant limitations in their native bioavailability[92].

Similarly, a pharmacokinetic evaluation of antidiabetic flavonoids such as chrysin, wogonin, genistein, baicalein, and apigenin showed moderate to good intestinal absorption, with HIA values equal to or exceeding 30%[93]. Additionally, studies on phenolics released during the gastrointestinal digestion of coffee pulp suggested that certain compounds, including protocatechuic acid, are efficiently absorbed in the small intestine following digestion[94].

Like HIA, another important value is permeability. Permeability values as in silico, were assessed using both Caco-2 and MDCK models, which simulate the passage of compounds across the intestinal epithelium[95,96]. MDCK cells (Madin-Darby canine kidney) are also used as a complementary model to assess permeability and the influence of transporters[96,97]. Numerous studies have emphasized the generally low membrane permeability and limited bioavailability of phenolic compounds, which significantly constrain their therapeutic potential[96,97].

Experimental evaluations, such as those conducted using Caco-2 cell models, revealed that only a subset of polyphenols, particularly aglycones like quercetin and N-feruloyl tyramine, exhibit appreciable intestinal

absorption, whereas glycosylated derivatives tend to show much lower permeability[96,97]. This reduced absorption is often attributed not only to molecular size and polarity but also to the involvement of active efflux mechanisms that limit intracellular accumulation. Permeability values also, were also expressed in log units, with values below 5.0 indicating poor membrane permeability and, consequently, reduced potential for oral absorption[96,97].

In current study, the permeability values of the major compounds ranged from −6.894 to −4.94 log units, indicating generally low membrane permeability. However, these values were based on the unencapsulated forms of the compounds. The HPLC results obtained after in vitro digestion simulation demonstrated that permeability can be enhanced through appropriate formulation strategies. Nevertheless, these findings should be further supported by comprehensive in vitro and in vivo studies to confirm the observed improvements in bioavailability and functional activity.

Plasma protein binding (PPB) percentages reflect the extent to which compounds bind to circulating proteins such as albumin[98]. High PPB (> 90%) can prolong the drug's systemic presence by acting as a reservoir but it reduces the free pharmacologically active fraction available immediately[99]. The compounds evaluated in this study displayed variable PPB values, with some exceeding 90%, suggesting limited immediate bioactivity despite longer systemic presence. The PPB values of the major compounds analyzed in this study varied considerably, indicating differences in their potential bioavailability and systemic distribution. Epigallocatechin gallate (EGCG) showed the highest binding affinity with a PPB of 97.88%, suggesting that a large portion of the compound remains bound in plasma, potentially limiting its immediate biological activity. Quercetin and ferulic acid also exhibited high binding levels of 92.9% and 87.3%, respectively. In contrast, gallic acid (68.6%), vanillic acid (67.7%), and caffeic acid (64.7%) displayed moderate protein binding capacities, indicating a relatively higher proportion of free, active compound in circulation. These results highlight the need to consider protein binding behavior in the design of delivery systems, as high PPB may reduce pharmacological effectiveness unless compensated by enhanced release and absorption mechanisms. However, these limitations were largely overcome following the encapsulation process. The HPLC results confirmed that the major compounds were substantially preserved after in vitro digestion, indicating that the applied formulation strategy effectively protected the bioactive molecules and enhanced their stability under gastrointestinal conditions.

The volume of distribution (V < sub > Dss) helps to understand how widely a compound spreads in the body after it enters the bloodstream. When the V < sub > Dss</sub > value is lower than about 0.3 L/kg, it means that the compound mostly stays in the blood or in the fluid around cells[100,101]. This can happen if the compound strongly binds to blood proteins or does not pass easily into tissues. Such a distribution can be useful if the compound is meant to work in the blood or in areas outside the cells[100,101].

On the other hand, V < sub > Dss</sub > values higher than 0.7 L/kg suggest that the compound spreads more into tissues, including inside cells. This is often the case for fat-soluble compounds or those that attach to tissue components. Very high values, such as over 1 L/kg, mean the compound is building up in tissues, which may increase how long it stays in the body but also raise the risk of side effects[100,102,103].

These differences are important when planning how much of a compound to give and how often. A low rate might mean the compound works only for a short time, while a very high volume of distribution might cause it to stay too long in tissues, which could be harmful[102–104]. For plant-based compounds with low bioavailability, controlling the volume of distribution through encapsulation or other formulation strategies could help improve both safety and effectiveness. In our study, the volume of distribution values of the major phenolic compounds varied, indicating differences in how these compounds distribute in the body. EGCG showed a very low rate of −0.812 L/kg, suggesting that it remains mostly in the bloodstream and does not easily distribute into tissues, likely due to its high plasma protein binding and low permeability. Similarly, gallic acid and vanillic acid had shown low values of 0.188 L/kg and 0.213 L/kg, respectively, indicating limited distribution beyond the plasma and extracellular fluid compartments. Caffeic acid (0.248 L/kg) and ferulic acid (0.509 L/kg) showed slightly higher distribution volumes, suggesting moderate tissue penetration. Quercetin, with a rate of 1.197 L/kg, displayed the highest distribution, indicating significant tissue accumulation and a potential for prolonged retention. These findings reveal that while most of the studied compounds tend to stay within the plasma or extracellular space, quercetin behaves differently, distributing more extensively into tissues. Such variation in distribution should be considered in formulation development strategies to optimize therapeutic outcomes and should be resolved with in vivo strategies.

Clearance (CL) rates, are key parameter in understanding how a drug is removed from the body[105]. Previous studies have consistently demonstrated that phenolic compounds generally exhibit low oral bioavailability due to rapid metabolism, fast clearance, and short half-lives. For instance, Fan et al[106]. investigated the pharmacokinetics of phenolic metabolites following mango pulp intake and reported that these compounds undergo extensive phase II metabolism, primarily through sulfation and glucuronidation. Peak plasma concentrations were reached at approximately 2, 4, and 6 h post-consumption, with elimination half-lives typically within a few hours.

Notably, significant inter-individual variability was observed, largely attributed to differences in gut microbiota composition, highlighting the complex interaction between host metabolism and phenolic bioactivity[106]. Similarly, Chang et al[107]. demonstrated that the pharmacokinetics of hawthorn phenolics differed between pure compounds and whole extracts in rats, with the extract form showing reduced clearance and prolonged half-life. This suggests that co-occurring components in plant matrices can modulate the systemic behavior of phenolics, potentially improving their retention and therapeutic effects[107].

In our study, the CL values of the major phenolic compounds were determined using *silico* prediction tools to evaluate their elimination rates. Caffeic acid and quercetin exhibited the highest clearance values, measured at 14.358 mL/min/kg and 14.347 mL/min/kg, respectively, indicating rapid removal from the body and potentially reduced systemic exposure. Gallic acid and ferulic acid also showed relatively high clearance rates of 8.359 mL/min/kg and 8.232 mL/min/kg, suggesting moderate elimination speed. In contrast, epigallocatechin gallate (EGCG) demonstrated a notably lower clearance value of 5.694 mL/min/kg, while vanillic acid had the lowest

clearance among all compounds at 3.040 mL/min/kg. These results suggest that, without formulation support, most of these compounds may be rapidly cleared from the body, thereby limiting their therapeutic duration. However, our HPLC analyses post-digestion indicate that encapsulation may help reduce degradation and slow down clearance, potentially improving overall bioavailability and retention.

Altogether, the encapsulation can reduce the degradation of these compounds during digestion and thereby delay their elimination from the body. This suggests that the bioavailability and therapeutic impact of the encapsulated compounds may be improved. These promising results will be further validated through future studies using in vitro cancer cell lines and in vivo models to confirm the pharmacokinetic advantages and biological efficacy of the developed formulations. These pharmacokinetic characteristics reveal the limitations associated with the oral use of unmodified plant-derived compounds. The findings strongly support the use of advanced delivery systems, such as cyclodextrin-based encapsulation, to improve the absorption, stability, and overall bioavailability of these bioactive molecules. Furthermore, our results indicate that significant progress was achieved in preserving the integrity of key compounds following in vitro digestion simulation. This finding suggests that, although bioinformatic data point to low inherent bioavailability of these molecules within the human body, appropriate encapsulation strategies can markedly enhance their stability and facilitate effective recovery. Thus, under optimized conditions, even compounds predicted to have limited bioavailability can be preserved and delivered more efficiently.

Fourier-transform infrared (FTIR) spectroscopy

FTIR analysis plays a vital role in characterizing cyclodextrin (CD) complexes with plant extracts. Its primary aims include identifying functional groups, characterizing phytochemicals, ensuring quality control, and assessing encapsulation efficiency. These analyses are crucial for optimizing the utilization of plant materials in medicinal and industrial applications[56,108–110].

In this study, the interactions between natural compounds from *L. bellidifolium* (aerial parts and root) and cyclodextrins (α-CD and β-CD) were investigated to confirm the formation of inclusion complexes. FTIR spectra of free extracts and their complexes were recorded within the 4000–500 cm^{-1} range. The solvent choice significantly influenced FTIR signal intensity, impacting the efficiency of β-CD inclusion complex formation. To explore this further, both liquid and dry β-CD-extract formulations were prepared and analyzed for structural differences. The results showed Fig. 3 and the peak areas given Table 8.

At the first stage, FTIR analyses were performed to compare the structural characteristics of β-cyclodextrin inclusion complexes prepared in both liquid and dry forms, aiming to identify the spectral differences between the two formulations and this results are given Fig. 4. These findings demonstrate that the liquid β-CD form undergoes dynamic structural changes, enhancing its functional properties for encapsulation, solubilization, and stabilization of active compounds. Its sharper peaks provide better spectral resolution and clearer identification of functional group interactions. This results shows the liquid form more suitable for applications requiring molecular adaptability, such as delivering bioactive compounds in pharmaceutical and cosmeceutical formulations. In contrast, the dry form, with its stable and rigid structure, is better suited for long-term storage and applications requiring sample stability. In the 500–1500 cm^{-1} region, the liquid sample showed a peak at 1046 cm^{-1}, likely reflecting changes in β-CD conformation due to solubilization. On the other hand, the dry sample exhibited peaks at 1156, 1020, and 574 cm^{-1}, indicating a more rigid and structured conformation[56,108–110]..

According to the after encapsulating results, a broad band between 3304 and 3323 cm^{-1} was related to O–H stretching vibrations from hydroxyl groups in polyphenols. After forming inclusion complexes with cyclodextrins, this band shifted slightly to a lower value (for example, from 3323 to 3312 cm^{-1}). This small shift suggests that hydrogen bonding or interactions happened between the polyphenols and the cyclodextrin. These interactions can weaken the O–H bonds, which causes the band to move and become broader. Similar peak ranges have also been reported in previous studies, showing consistent values and supporting the results observed in our work[111–113].

A weak C–H stretching band was observed around 2700–2900 cm^{-1} in the spectra of the free extracts, which is typical for alkyl and aliphatic groups in plant-based compounds. However, this band disappeared after complexation with cyclodextrins. The absence of this signal suggests that hydrophobic functional groups were encapsulated inside the cyclodextrin cavity. As a result, their vibrational peaks became restricted and less exposed to the IR beam, leading to reduced or undetectable absorption bands. Similar findings have also been reported in previous studies, where this peak disappeared after encapsulation, supporting our results[114–116].

The bands observed in the region of 1632–1642 cm^{-1} corresponds to C = O stretching or aromatic C = C vibrations, commonly found in phenolic structures and flavonoids. This band remained at a similar position after encapsulation, indicating that the aromatic structure of the extract was largely preserved. However, slight shifts in position or changes in intensity suggest some degree of molecular interaction between the phenolic compounds and the cyclodextrin cavity.

Similar results has also been reported in other studies involving the encapsulation of phenolic compounds, showing similar spectral patterns and supporting the consistency of our results[108,110,115].

The bands observed at 1085 and 1042 cm^{-1} correspond to C–O–C and C–O stretching vibrations, which are characteristic of the glucopyranose units in the cyclodextrin structure. In our study, changes in the intensity and slight shifts in these bands were detected after complexation, indicating the successful inclusion of the extract into the cyclodextrin cavity. These spectral changes reflect host–guest interactions and confirm the formation of inclusion complexes. Similar findings have been reported in other studies involving the encapsulation of phenolic and flavonoid compounds, where modifications in the C–O–C region were considered reliable evidence for complex formation[108,110,114,115].

The band observed at 878 cm^{-1} corresponds to C–H bending vibrations, commonly associated with cyclic molecular structures, such as those present in cyclodextrins. The persistence of this band after complexation

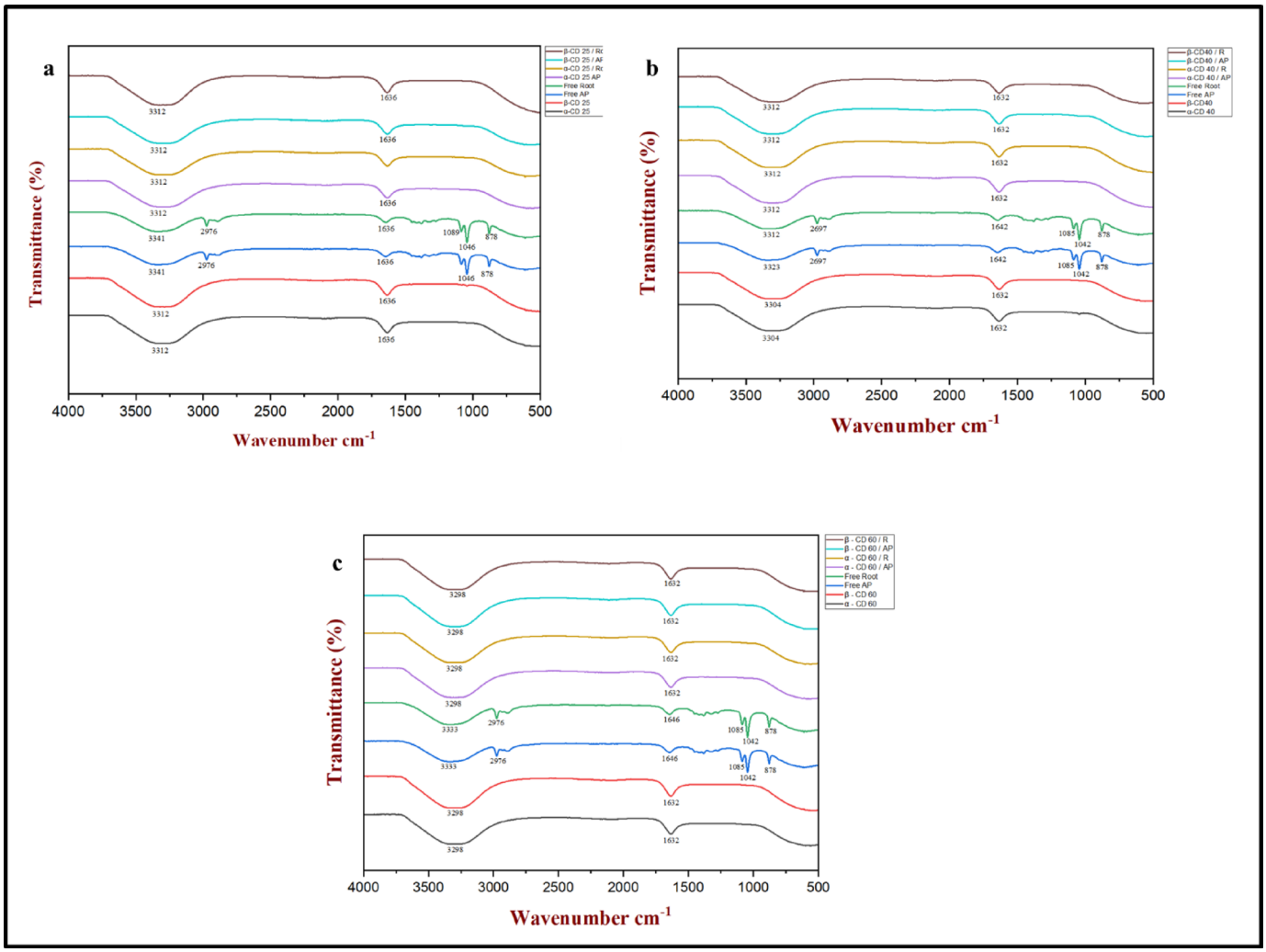

Fig. 3. FTIR analysis results of extracts optimized at different temperatures. (**a**) Aerial (AP)/root (R) parts encapsulated forms optimized at 25 °C, (**b**) Aerial (AP)/root (R) parts encapsulated forms optimized at 40 °C, (**c**) Aerial (AP)/root (R) parts encapsulated forms optimized at 60 °C.

Peak (cm^{-1})	Vibration	Group
3298-3333	O-H	Alcohols, phenols
2976	C-H	Alkanes
1632-1646	O-H-O/ C=O/ N-H bending	Carbonyl/amide groups
1042-1085	C-N	Aliphatic amines of phenolic compounds

Table 8. The peak area for FTIR analysis.

confirms that the cyclic integrity of the cyclodextrin was maintained throughout the encapsulation process. This is important, as the structural stability of cyclodextrin is essential for the formation of effective host–guest complexes. Similar findings have been reported in previous studies, where the presence of the 878 cm^{-1} band was used as a marker for intact cyclodextrin rings in inclusion complexes with various phenolic compounds. These results support our findings and confirm that the complexation did not alter the core structure of the cyclodextrin[108,115,117,118].

As a result, when comparing the spectra of encapsulated samples with pure plant extracts, consistent peaks at wavelengths like 3312 cm^{-1}, 1652 cm^{-1}, and 1046 cm^{-1} were observed across most samples, indicating that the encapsulation process preserved molecular structures without significant degradation. However, some spectral differences, including shifts and intensity changes, suggest that the encapsulation induced partial chemical modifications or interactions within the extract. These results confirm that while encapsulation with α- and β-

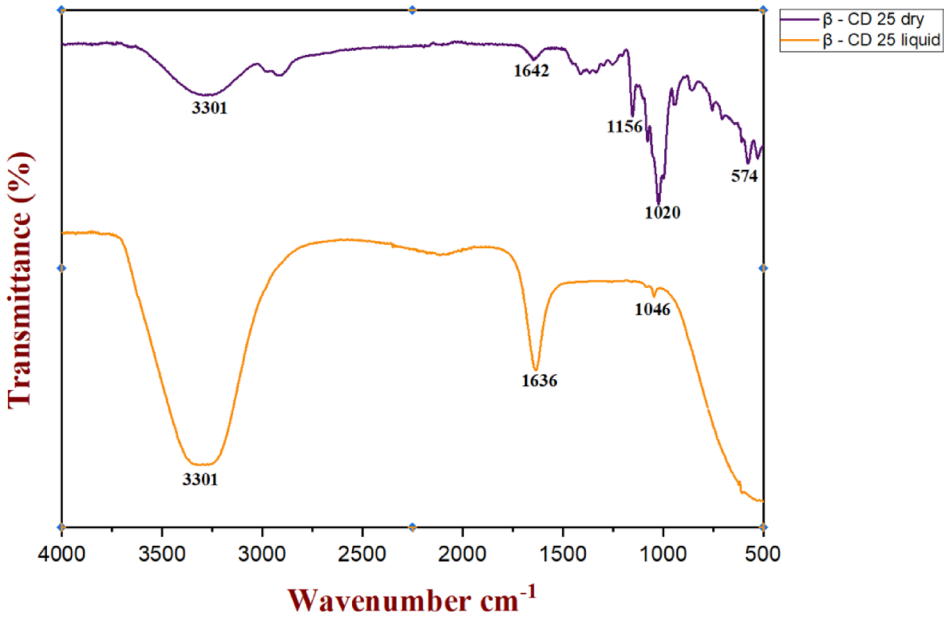

Fig. 4. FTIR analysis results of dry and liquid β -cyclodextrine (β-CD).

CDs stabilized and maintained the structural integrity of bioactive compounds, it also induced minor structural changes, enhancing the functional properties of the complexes.

SEM image results

The SEM images in Figs. 5 and 6 compare the effects of β-CD encapsulation at 25 °C and α-CD encapsulation at 40 °C on the aerial parts and roots of *L. bellidifolium*. The temperatures for encapsulation have been selected based on bioactivity and optimization results to enhance the effectiveness of the encapsulation process. Encapsulation at 40 °C for α-CD yield, more homogenous, smooth, and layered structures, particularly in the aerial parts, suggesting that higher temperatures contribute to better encapsulation efficiency and stability. The particles encapsulated with α-CD at 40 °C have been showing greater uniformity, fewer cracks and a refined surface, indicating that this process has been optimized for protecting and stabilizing bioactive compounds. In contrast encapsulation at 25 °C with β-CD results in rough and more fragmented particles. While β-CD has been improving the stability of the aerial parts and roots compared to their free forms, the particles exhibited more irregularities and fractures than those encapsulated with α-CD.

The lower temperature might have limited the cyclodextrin's ability to fully encapsulate and stabilize the plant material, leading to a more heterogeneous structure. Overall, the optimization results showed that encapsulation at a higher temperature (40 °C with α-CD) is more effective for achieving smoother, more stable particles, while encapsulation at a lower temperature (25 °C with β-CD) results in less uniform structures. This suggests that α-CD at 40 °C is better suited for applications requiring enhanced bioavailability and protection of bioactive compounds for *L. bellidifolium*.

Conclusions

This study clearly showed that using α- and β-cyclodextrins to encapsulate *Limonium bellidifolium* extracts improved the stability, solubility, and bioavailability of important bioactive compounds. FTIR analysis confirmed that the structure of the compounds was protected, and SEM images showed that the shape of the particles changed depending on the temperature. The smoothest particles were formed with α-CD at 40 °C. Among all the conditions, β-CD at 25 °C gave the best results, with the highest levels of quercetin and catechin and the strongest antioxidant and enzyme inhibition effects. α-CD also showed good results, making it a promising alternative. In addition, both types of cyclodextrin coatings protected the molecules much better than the non-encapsulated forms. HPLC analysis confirmed this, showing that about 66% of kaempferol was preserved after encapsulation. These findings clearly prove that cyclodextrin encapsulation plays a key role in protecting sensitive compounds.

As a result, this is the first study to apply this type of encapsulation to a halophytic plant for therapeutic purposes. The results provide valuable data that can help future cancer-related studies, especially those focused on improving solubility and protecting active compounds. This work can guide the development of new pharmaceutical and nutraceutical products using *Limonium*-based extracts.

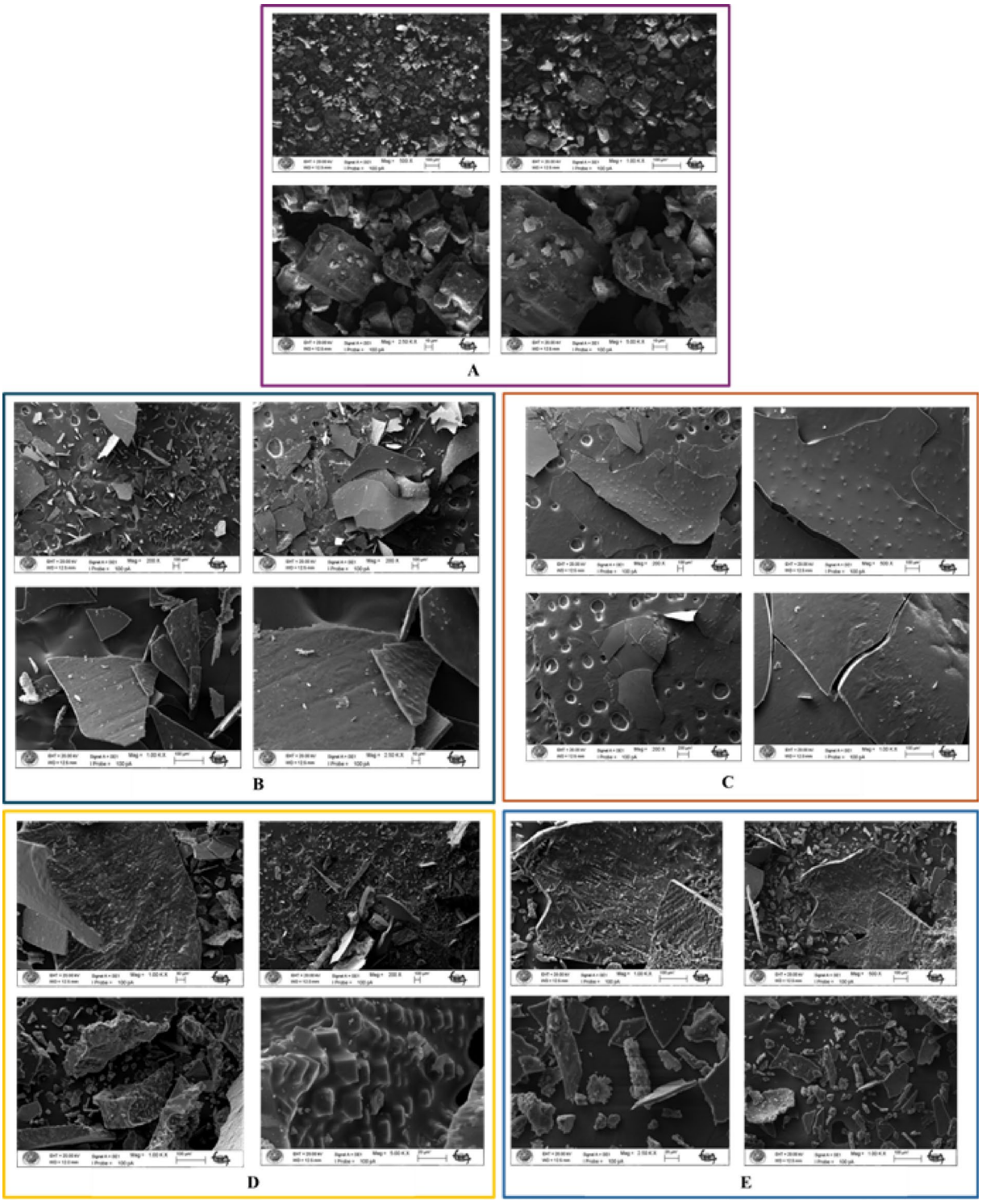

Fig. 5. SEM (Scavenging electron microscope) images results for free α-CD 40 °C/encapsulated form with *L. bellidifoilum* extracts. (**A**) Free α-CD 40 °C, (**B**) *L. bellidifolium* AP α-CD 40 °C, (**C**) *L. bellidifolium* R α-CD 40 °C, (**D**) *L. bellidifolium* AP free, (**E**) *L. bellidifolium* R free.

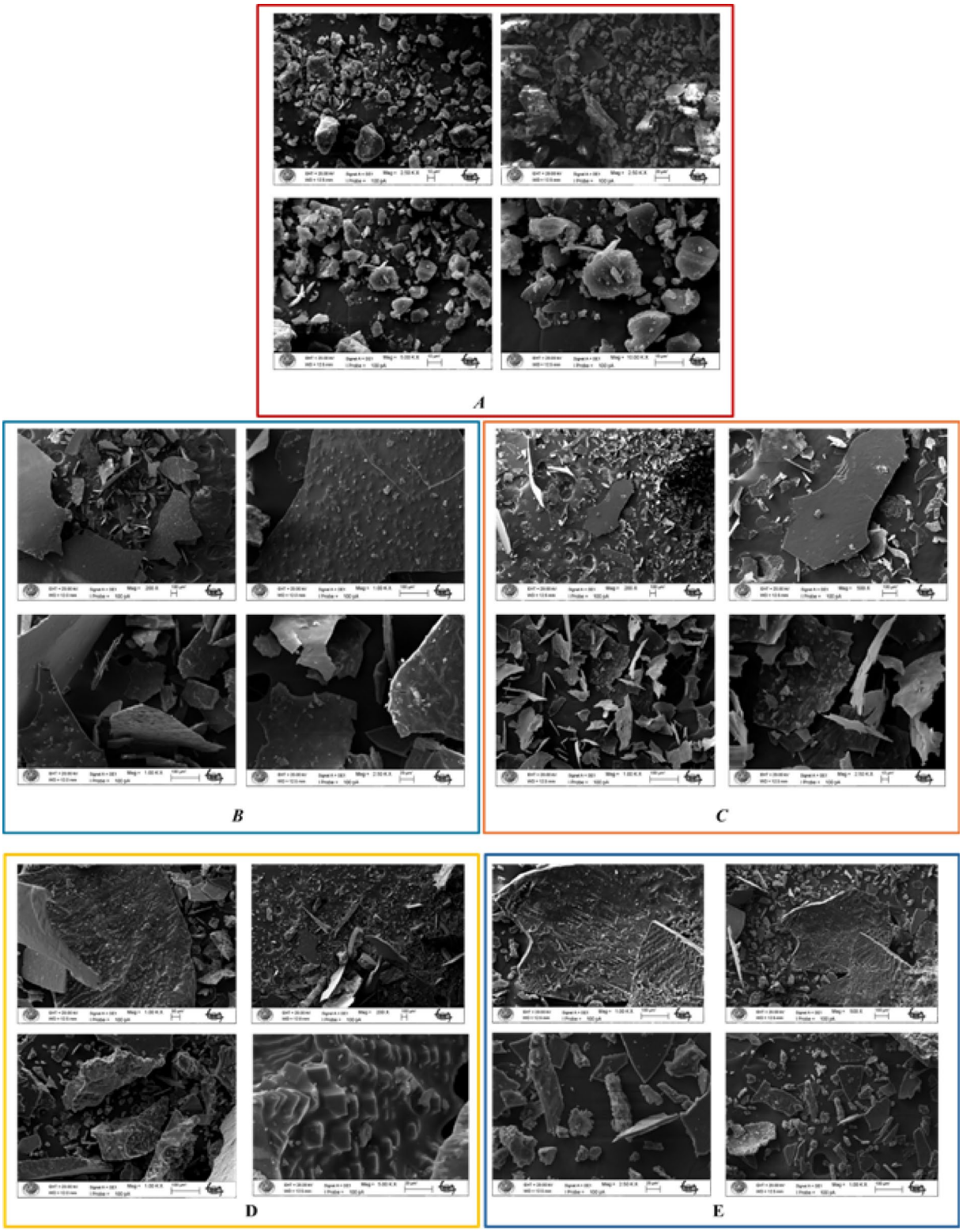

Fig. 6. SEM (scavenging electron microscope) images results for β-CD/encapsulated form with *L. bellidifoilum* extracts. (**A**) Free β-CD 25 °C, (**B**) *L. bellidifolium* AP β-CD 25 °C, (**C**) *L. bellidifolium* R β-CD 25 °C, (**D**) *L. bellidifolium* AP free, (**E**) *L. bellidifolium* R free.

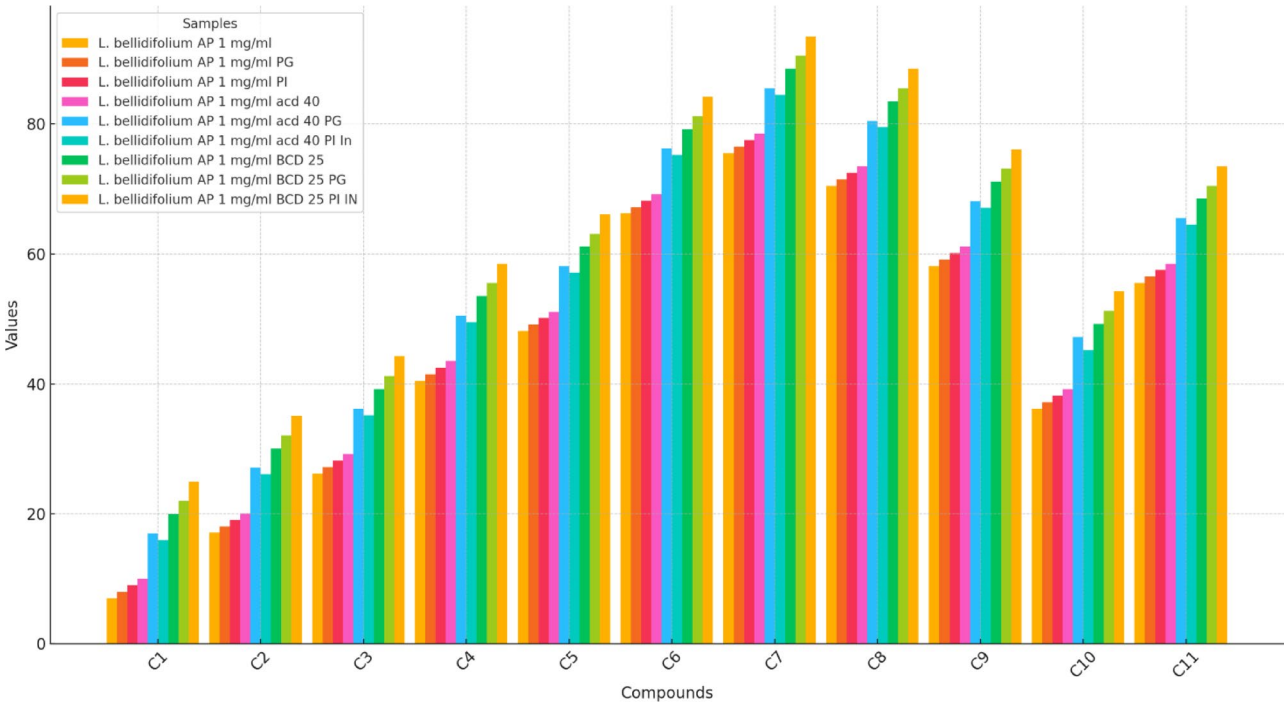

Fig. 7. Recovery rates of bioactive compounds in *L. bellidifolium* aerial part extracts under different conditions, including post-gastric (PG) and post-intestinal (PI) phases, as well as after encapsulation with α and β cyclodextrins, as well as after encapsulation with α and β cyclodextrins. PG: Post gastric, PI: Post intestinal. C1: Gallic acid, C2: Rutin, C3: Apigenin, C4: Quercetin, C5: Kaempferol, C6: Epigallocatechin gallate, C7: Ferulic acid, C8: Cathechin, C9: Caffeic acid, C10: Myricetin, C11: Vanilic acid.

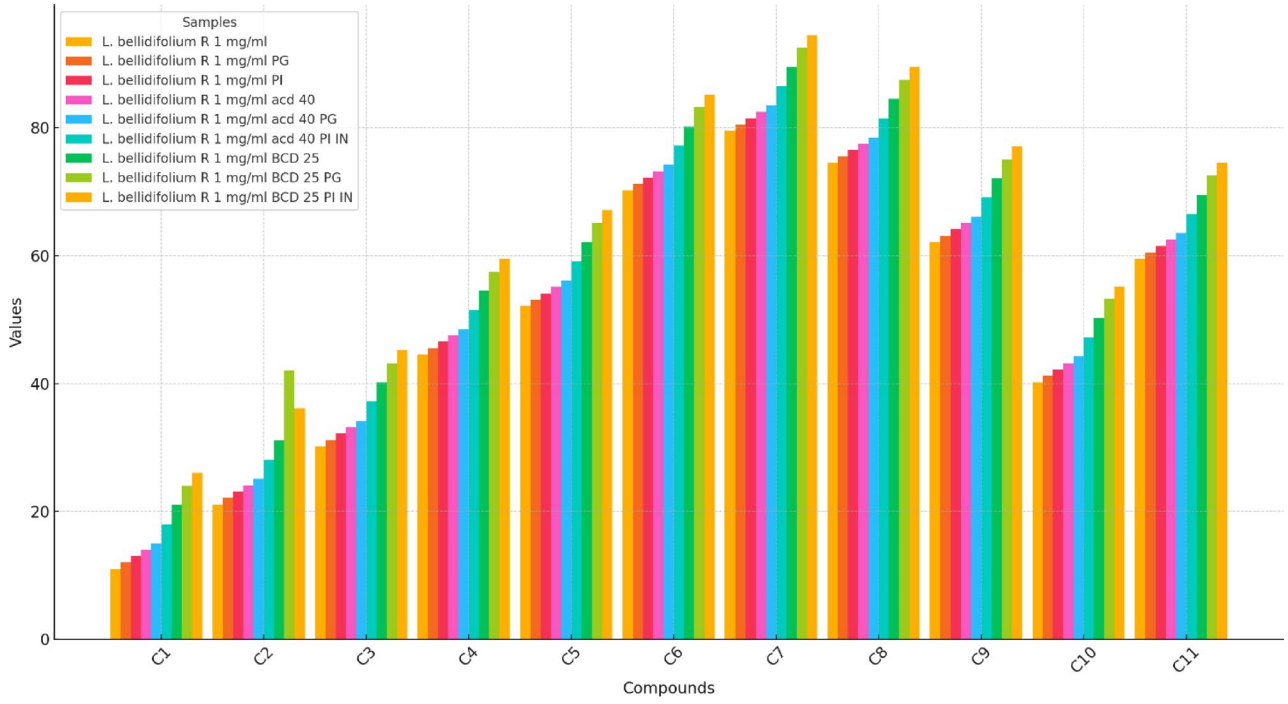

Fig. 8. Recovery rates of bioactive compounds in *L. bellidifolium* root extracts under different conditions, including post-gastric (PG) and post-intestinal (PI) phases, as well as after encapsulation with α and β cyclodextrins. PG: Post gastric, PI: Post intestinal, C1: Gallic acid, C2: Rutin, C3: Apigenin, C4: Quercetin, C5: Kaempferol, C6: Epigallocatechin gallate, C7: Ferulic acid, C8: Cathechin, C9: Caffeic acid, C10: Myricetin, C11: Vanilic acid.

Data availability

Data is provided within the manuscript and supplementary information files.

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Author contributions

Author Contributions: Conceptualization, G.A, G.Z, S.D, S.F; methodology, G.A, S.D, S.F, S.S, O.E.E. software, G.A, S.D; validation, GA, SD, GZ; formal analysis, GZ; investigation, GA, GZ, SD, SF, OEE resources, SS, GZ; data curation, GA, SD, SF.; writing—original draft preparation, GA, SD, SF, GZ; writing—review and editing, SS, OEE.; visualization, GZ, SD, SF; supervision, GZ, SD; project administration, GZ.; funding acquisition, GZ. All authors have read and agreed to the published version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

All experimental procedures involving wild plant materials were carried out in accordance with institutional, national, and international guidelines and regulations. The plant species used in this study are not endangered or protected, and their collection did not require special permission.

Additional information

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