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Biological and Antioxidant Potential of Alginate-Based Rambutan Ellagitannins Encapsulates: Approach to the Development of Dietary Supplements

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Abstract

Ellagitannins (ETs) derived from rambutan (*Nephelium lappaceum* L.) peel are polyphenols that have gained prominence in the food and nutraceutical industries due to their high bioactivity. The objective of this study was to evaluate the encapsulation efficiency (EE) of ETs obtained from rambutan peel in sodium alginate beads, as well as their effect on cell viability and antioxidant potential. ETs were extracted and semi-purified using Amberlite XAD-16 resin and identified by HPLC/ESI/MS. To determine the encapsulation efficiency, a 3^k Box–Behnken experimental design was employed, evaluating the influence of ET concentration (1000, 2000, and 3000 ppm), sodium alginate (1, 2, and 3%), and $CaCl_2$ (5, 10, and 15 mM). Cell viability was assessed in HEK-293 and 3T3 cell lines, and antioxidant potential was determined through ABTS, DPPH, and FRAP assays. The results showed a maximum EE of 55% under conditions of 3000 ppm ETs, 2% sodium alginate, and 5 mM $CaCl_2$. Cell viability assays confirmed that both the crude extract and the ET-loaded capsules possess excellent biocompatibility, maintaining values above 70%. Furthermore, the capsules demonstrated significant antioxidant potential (ABTS 76%, DPPH 65%, FRAP 1.11 mmol TE/L) compared to the control (sodium alginate). These findings position the ET-loaded rambutan peel capsules as a promising alternative for the development of functional products in the food industry.

Keywords: ellagitannins; encapsulation efficiency; cell viability

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1. Introduction

In recent years, the development of functional foods and dietary supplements enriched with polyphenols from natural sources has gained significant momentum due to their broad biological potential associated with disease prevention and the reduction in physiological disorders [1]. A rich source of polyphenols is the rambutan peel (*Nephelium lappaceum*), an

agro-industrial residue discarded after consumption. Due to its high polyphenol content, particularly ellagitannins (ETs) such as geraniin, corilagin and ellagic acid, it represents a source of great interest for producing functional ingredients [2–4]. In this sense, these compounds have been widely recognized for their antioxidant potential, exhibiting diverse biological activities, including anti-inflammatory, antimicrobial and cardiovascular effects [5].

However, ETs are susceptible to degradation under environmental conditions such as light, temperature, oxygen, and humidity, as well as pH variations in acidic and alkaline media, which can alter their stability and reduce their biological activity [6,7]. An alternative to minimize these effects and preserve their properties is encapsulation. This process consists of incorporating bioactive compounds within a matrix or coating material designed to protect them, maintain their stability, and ensure their preservation [8,9]. A biopolymeric matrix widely used to encapsulate polyphenols is sodium alginate, a natural polysaccharide obtained from certain brown algae and bacteria. It is generally recognized as safe (GRAS), indicating its suitability for food applications; furthermore, it is biodegradable, stable, and widely used in various sectors including the pharmaceutical, cosmetic, dental, and food industries [10–12]. Polyphenol encapsulation with sodium alginate is carried out via ionic gelation, which has demonstrated enhanced functionality and stability of the encapsulated polyphenols, in addition to promoting controlled release during digestion, thereby increasing their potential as a functional ingredient [7].

Therefore, encapsulation efficiency and preservation of biological activity are considered fundamental parameters for determining their viability as dietary supplements. In this sense, the evaluation of antioxidant potential and cell viability is of utmost importance to establish the bioactive potential, as well as the safety of the developed polyphenol-loaded encapsulates [13,14]. Based on the foregoing, the objective of this study was to evaluate the encapsulation efficiency (EE) of ETs obtained from rambutan peel in sodium alginate beads, as well as their effect on cell viability in HEK-293 and 3T3 cell lines and their antioxidant potential.

2. Materials and Methods

2.1. Raw Material Pretreatment

Rambutan (*Nephelium lappaceum* L.) peel was collected from the Soconusco region, Chiapas, Mexico. The plant material was disinfected. Subsequently, it was dried at 50 °C for 48 h. The dried peels were ground to a particle size less than 2 mm. The ground plant material was stored in plastic bags protected from light at room temperature.

2.2. Extraction and Semipurification of Rambutan Peel Polyphenols

The polyphenolic extract of rambutan peel used in this study was previously obtained and characterized. The quantification of total polyphenol and condensed tannin content was performed using the Folin–Ciocalteu and HCl-butanol methods, respectively. Subsequently, the extract was partially purified by column chromatography with Amber-lite XAD-16 resin, and the phenolic compounds were identified by HPLC-ESI-MS. The detailed methodology and characterization results have been previously described [15].

2.3. Experimental Design for Ellagitannin Encapsulation

To define the optimal conditions for encapsulation efficiency, a Box–Behnken 3^k experimental design was implemented. The independent variables evaluated were the concentration of sodium alginate (1%, 2%, and 3% w/v), $CaCl_2$ (5, 10, and 15 mM), and the concentration of rambutan peel ellagitannins (1000, 2000, and 3000 ppm). Each variable was

evaluated at three levels (low, medium, and high) (Tables 1 and 2). The response variable was encapsulation efficiency (EE%).

Table 1. Factors and levels of the Box–Behnken experimental design.

Independent Variables	Levels		
	Low (−1)	**Medium (0)**	**High (1)**
Sodium alginate (% *m*/*v*)	1	2	3
$CaCl_2$ (mM)	5	10	15
Rambutan peel total polyphenols (ppm)	1000	2000	3000

Table 2. Condensed experimental matrix of the Box–Behnken experimental design.

Treatments	Alginate (% *m*/*v*)	$CaCl_2$ (mM)	RPP (ppm)
1	1	0	1
2	1	0	−1
3	0	1	1
4	0	0	0
5	0	−1	−1
6	0	1	−1
7	0	−1	1
8	−1	0	1
9	−1	1	0
10	0	0	0
11	0	0	0
12	1	1	0
13	−1	−1	0
14	1	−1	0
15	−1	0	−1

All treatments were performed in triplicate.

The selected levels for sodium alginate and $CaCl_2$ were based on previous encapsulation studies of phenolic and bioactive compounds [16–18]. Meanwhile, the rambutan peel ellagitannin concentrations (1000, 2000, and 3000 ppm) were selected based on preliminary solubility assays, which demonstrated optimal compatibility and homogenization with the alginate matrix without inducing saturation or structural collapse of the encapsulates.

2.4. Encapsulation Efficiency Calculation

To calculate the encapsulation efficiency, the concentrations of hydrolyzable and condensed polyphenols present in the crude extract and the rambutan peel ellagitannin encapsulates were first determined. A 3% (*w*/*v*) sodium citrate solution was prepared to dissolve the rambutan peel ellagitannin encapsulates (RPEE). Subsequently, 5 g of RPEE was dissolved in 20 mL of the sodium citrate solution, and the resulting liquid was used to determine the hydrolyzable and condensed tannin content using the Folin–Ciocalteu and HCl-Butanol methodologies. The encapsulation efficiency was calculated according to Equation (1).

$$EE(\%) = \frac{L}{L_0} \times 100 \quad (1)$$

where L represents the amount of rambutan peel polyphenols encapsulated, and L_0 is the total amount of polyphenols contained in the rambutan peel extract [19].

2.5. Cell Viability Assay

The cytotoxicity of the crude rambutan peel extract and the RPEPs was evaluated at concentrations ranging from 125 to 1000 ppm using HEK-293 (human embryonic kidney cells; ATCC: CRL-1573) and NIH/3T3 (mouse embryonic fibroblasts; ATCC: CRL-1658) cell lines. Both cell lines were obtained from the ATCC (Manassas, VA, USA) and were provided by the Faculty of Medicine at the Autonomous University of Nuevo León, Mexico.

Cell viability was determined by the MTT assay following a previously reported method with slight modifications [20]. Prior to the assay, the polyphenolic extract was homogenized in an ultrasonic bath and filtered through a 0.25 µm nylon membrane. Sodium citrate and water-loaded alginate capsules, mixed with DMEM medium at a 1:1 (v/v) ratio, served as controls. Both cell lines were seeded in 96-well plates at a density of 1×10^5 cells/mL in DMEM medium and incubated for 24–48 h in a controlled CO_2 incubator, with daily medium replenishment and microscopic morphology verification. Once cell confluence and adherence were confirmed, the medium was replaced with treatments and controls, followed by a 24 h incubation period. Subsequently, the medium was exchanged for fresh DMEM medium containing the MTT reagent, and the plates were incubated for 4 h. Finally, the medium was removed, and 50 µL of DMSO was added to dissolve the formazan crystals. Absorbance was measured using an ELISA microplate reader at 650 nm under constant agitation.

Cell viability is then calculated using the following formula:

$$Cell\ viability\ (\%) = \frac{Ac - As}{Ac}(100)$$

where Ac represents the absorbance of the untreated cells (positive control) and As represents the absorbance of the sample.

2.6. Antioxidant Assays

The antioxidant potential of the rambutan peel ellagitannin encapsulates (RPEE), control encapsulates (blank alginate), and crude rambutan peel extract was determined using ABTS, DPPH, and FRAP assays. Samples were prepared at concentrations ranging from 7 to 1000 ppm (specifically 7, 15, 31, 62, 125, 250, 500, and 1000 ppm), while the bioactive compounds trapped within the capsules were evaluated at their maximum concentration of 3000 ppm. For the ABTS and DPPH assays, results were expressed as inhibition percentage (%). For the FRAP assay, a commercial Trolox standard curve (0–1000 ppm) was prepared under identical conditions to determine and compare the antioxidant potential of the samples, with results reported as Trolox equivalents (mmol TE/L).

2.6.1. DPPH

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was measured using a previously described methodology [21]. A solution of 60 µM of DPPH was diluted in ethanol in a dark room. Then, 2.9 mL of this DPPH solution was mixed with 100 µL of the sample, and the mixture was kept in the dark for 30 min to be able to properly measure the radical scavenging activity. After that time, absorbance at 517 nm of the samples with DPPH reagent was measured using a spectrophotometer using a 1 cm cell. The results were then measured as inhibition percentage according to the following formula:

$$DPPH\ inhibition\ \% = \frac{Ac - As}{Ac}(100)$$

where Ac represents the control absorbance of DPPH + ethanol and As the sample absorbance.

2.6.2. ABTS

The ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity of the rambutan peel extract was measured using a previously described method [22]. The ABTS radical was generated by oxidation of 7 mM ABTS with 2.45 mM potassium persulfate, then covered adequately in an amber recipient to protect from light and stored in the dark for 16 h. Then, the ABTS reactive was adjusted to have an absorbance of 0.70 ± 0.02 at 734 nm in a spectrophotometer Biomate 3, (Thermo Spectronic, Waltham, MA, USA). To measure the scavenging potential, 1 mL of ABTS was mixed with 10 µL of the sample in a 1 cm quartz cell. The absorbance was then measured at 734 nm, the same as the adjustment of the ABTS radical. The results were then expressed as inhibition percentage with the following formula:

$$ABTS\ inhibition\ \% = \frac{Ac - As}{Ac}(100)$$

where Ac represents the control absorbance of ABTS + ethanol and As the sample absorbance.

2.6.3. FRAP

The ferric reducing antioxidant power (FRAP) assay was performed using a previously described methodology [23]. The FRAP reagent was prepared freshly by mixing 10 mM of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) dissolved in 40 mM HCl, 20 mM $FeCl_3$, and 300 mM acetate buffer (pH 3.6) in a 1:1:10 ($v/v/v$) ratio. For the analysis, 10 µL of the sample was transferred to a 96-well microplate, followed by the addition of 290 µL of the FRAP reagent. The mixture was incubated at 37 °C for 15 min under dark conditions. The absorbance was subsequently measured using an Epoch microplate reader (BioTek Instruments, Winooski, VE, USA). Results were expressed as mmol TE/L.

2.7. Statistical Analysis

Statistical analysis was made from the means of three replicates using one-way ANOVA and Tukey mean comparison test with a significance level of $p < 0.05$ using the software OriginPro 8.5, and experimental design and analysis were made using the Statistica 7.0 software.

3. Results and Discussion

3.1. Tannin Content and Compound Identification

In a study reported in 2025 by Hernández-Hernández et al. [15], a partially purified extract was obtained from rambutan peel. This partial purification was carried out by column liquid chromatography using Amberlite XAD-16; the total polyphenol content was 378.48 mg/g. In addition, Table 3 presents the identification of the phenolic compounds by HPLC-ESI-MS, as previously reported in a study conducted by our research group [15]. It is important to note that this semipurified extract was the material used in the present study.

Table 3. Retention time of the compounds present at the EERP [15].

No	Retention Time	Mass (*m*/*z*)	Compound	Family
1	20.148	630.7	Dimmers of tergallagic-0-hexoside	Ellagitannins
2	22.334	950.5	Geraniin	Ellagitannins
3	26.589	475.8	Acetyl-arabinoside ellagic acid	Dimmers of hydroxybenzoic acid
4	27.518	432.8	Arabinoside ellagic acid	Dimmers of hydroxybenzoic acid
5	29.51	300.8	Ellagic acid	Dimmers of hydroxybenzoic acid

3.2. Polyphenolic Encapsulation (Box–Behnken Design)

Table 4 presents the encapsulation efficiency results along with the coded values of the variables evaluated using the Box–Behnken 3^k experimental design. Treatment 7 exhibited the highest encapsulation efficiency (55%) using a 2% alginate concentration, 5 mM of $CaCl_2$, and 3000 ppm of RTP. However, since treatments 3 and 8 reached values similar to treatment 7, the mean comparison analysis confirmed that there were no statistically significant differences among them; therefore, the conditions of treatment 7 were selected for subsequent experiments.

Table 4. Results from each combination of independent variables, EE estimate stands for the encapsulation efficiency obtained by the experimental design estimation in Statistica 7.0, EE stands for the results obtained in each run and the mean of the triplicate. Different letters mean significant differences among treatments (Tukey $p < 0.95$).

Treatments	Alginate (% *m*/*V*)	$CaCl_2$ (mM)	RPP (ppm)	EE (%)
1	3	10	3000	$42 \pm 4_{\ b}$
2	3	10	1000	$27 \pm 4_{\ de}$
3	2	15	3000	$55 \pm 8_{\ a}$
4	2	10	2000	$32 \pm 3_{\ cde}$
5	2	5	1000	$23 \pm 3_{\ e}$
6	2	15	1000	$21 \pm 0_{\ e}$
7	2	5	3000	$55 \pm 4_{\ a}$
8	1	10	3000	$54 \pm 2_{\ a}$
9	1	15	2000	$38 \pm 3_{\ bc}$
10	2	10	2000	$38 \pm 3_{\ bc}$
11	2	10	2000	$34 \pm 2_{\ bcd}$
12	3	15	2000	$30 \pm 2_{\ cde}$
13	1	5	2000	$34 \pm 3_{\ bcd}$
14	3	5	2000	$24 \pm 1_{\ de}$
15	1	10	1000	$23 \pm 3_{\ e}$

Figure 1 shows the Pareto chart from the Box–Behnken 3^k experimental design, identifying the factors that significantly influenced the encapsulation efficiency. The RTP (ppm) factor was found to be significant in both its linear (L) and quadratic (Q) forms, while alginate was significant in its linear (L) form, with all of them crossing the dashed line ($\alpha = 0.05$). Conversely, the quadratic form of alginate and both forms of calcium chloride were non-significant.

The alginate concentration exhibited a pronounced negative effect on the EE. As shown in Figure 1, increasing the biopolymer concentration from 1% to 3% resulted in a significant reduction in the retention of the phytochemicals. This adverse behavior at higher polymer percentages can be attributed to the concomitant increase in the viscosity of the formulation. Highly viscous solutions restrict mass transfer and hinder the homogeneous dispersion of rambutan peel polyphenols within the polymeric matrix prior to cross-linking. From a macromolecular perspective, elevated alginate concentrations increase the droplet size during the extrusion process, yielding larger beads that paradoxically reduce core retention.

This phenomenon is closely linked to the rheological limitations of sodium alginate solutions outlined in a previous study [24]. While concentrations between 1% and 3% are generally preferred to avoid incomplete cross-linking (which typically occurs below 1%), concentrations reaching or exceeding 3% significantly increase viscosity, which prevents proper droplet formation during the dripping process. Under these highly viscous conditions, the extrusion mechanism fails to produce spherical matrices, leading instead to the

formation of long, irregular, and continuous structures that compromise the entrapment and uniform retention of the core material.

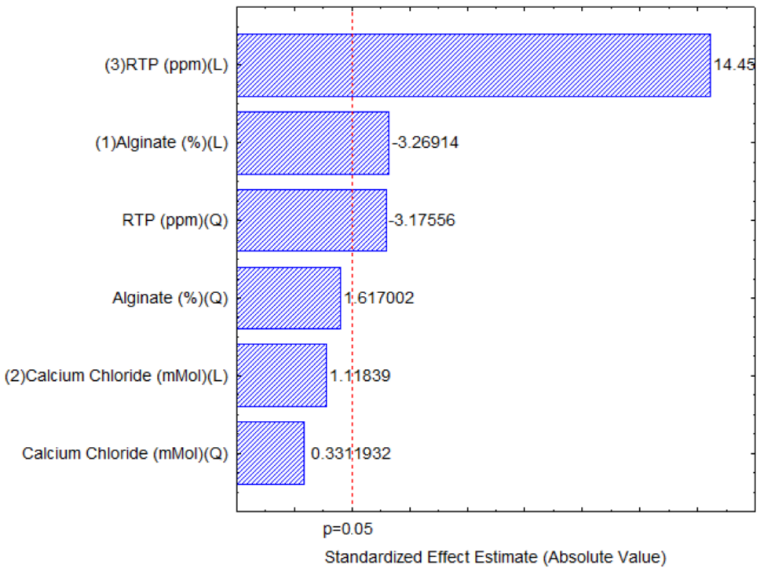

Figure 1. Pareto chart of Box–Behnken experimental design.

Another variable that was evaluated was the concentration of calcium chloride ($CaCl_2$), which was found to have no significant effect on the encapsulation process, as none of the concentrations tested affected it; therefore, any concentration of $CaCl_2$ can be used to achieve satisfactory encapsulation results. In theory, higher concentrations of calcium chloride should allow for better encapsulation, since there are more calcium ions that can interact with sodium alginate. However, in a previous study [25], they noted that an excess of $CaCl_2$ induces severe compaction of the polymeric matrix of the encapsulate, which reduces the size of the encapsulate, potentially leading to the expulsion of the encapsulated material due to osmotic or contraction forces. However, in this study, it is also possible that when dealing with rambutan polyphenols, these interact molecularly with the encapsulation matrix. According to previous research [26], when evaluating alginate capsules loaded with polyphenol-rich extracts of *Mesona chinensis*, FTIR analysis of the alginate capsules showed that there were no harmful chemical interactions between the extract and the polymer, but rather, there was the formation of intermolecular hydrogen bonds via their hydroxyl (OH) groups. These hydrogen-bond interactions are capable of stabilizing the spherical structure of the bead and effectively retaining the active compounds within it. This helps explain why variations in $CaCl_2$ concentration did not significantly affect encapsulation efficiency, since retention depends not only on ionic cross-linking with calcium but also on physical affinity and hydrogen bonding between the polyphenols and the alginate chains.

Conversely, the initial concentration of RTP exerted a strong, positive, and highly significant effect on the EE, representing the most influential variable in the experimental design. As the RTP concentration increased from its lowest to its highest level, a sharp enhancement in the entrapment of bioactive compounds was observed (Figure 1). This behavior is governed by the high water-solubility of the rambutan peel extract, which ensures an excellent initial homogenization with the sodium alginate mixture before the ionic gelation takes place. Mechanistically, a higher concentration of the core material (RTP) increases the concentration gradient between the internal and external phases, acting as a powerful driving force that enhances the loading yield within the biopolymer network. Nevertheless, it is critical to note that an indefinite increase in the core-to-wall ratio could

potentially exceed the spatial loading capacity and binding sites of the alginate network, eventually leading to a decline in the EE% due to matrix overload [27].

3.3. Cell Viability Assay

Evaluating cell viability, defined as the proportion of healthy, metabolically active cells within a population relative to an untreated control, is a fundamental parameter for assessing the biological safety and proliferation-modulating capacity of bioactive compounds intended for food or nutraceutical applications [28]. In encapsulated delivery systems, this assessment is paramount to guarantee the non-toxicity and biocompatibility of both the core active ingredients (such as rambutan peel ellagitannins) and the structural wall matrix (sodium alginate), ensuring that the final encapsulates do not induce cytotoxic damage upon administration. In the present study, cell viability was determined via the colorimetric MTT assay, which is based on the capability of active cells to reduce the yellow tetrazolium salt (MTT) into insoluble purple formazan crystals. Cytotoxicity was evaluated using two cell lines: HEK-293 (human embryonic kidney cells) and 3T3 (mouse embryonic fibroblasts), selected due to their high proliferation rates and widespread use as in vitro models in cytotoxicity studies [29,30].

Although the highest encapsulation efficiency was achieved at an RTP concentration of 3000 ppm, preliminary tolerance and biological response assays indicated that concentrations above 1000 ppm compromised the stability of the evaluated cellular system. To ensure biocompatibility and prevent the cytotoxic effects associated with excessive exposure to the compound, 1000 ppm was selected as the working concentration. Consequently, the 3000 ppm capsules were subjected to a serial dilution protocol using sodium citrate to adjust the final concentration to 1000 ppm, thereby preserving the integrity of the experimental model.

Both the crude polyphenol extract and the RPEE maintained cell viability percentages above 75% in both cell lines, indicating the absence of cytotoxic effects under the evaluated conditions.

In particular, the extract (Figure 2) exhibited a dose-dependent effect on the HEK-293 cell line, reducing cell viability from approximately 100% to 77% as the concentration increased from 125 to 1000 ppm. Conversely, the 3T3 cell line maintained viability percentages above 90% across all treatments, demonstrating lower susceptibility to the extract and suggesting an adequate in vitro safety profile. This behavior may be attributed to the selective cytotoxicity exhibited by rambutan polyphenol extracts; while they display antiproliferative properties against tumor cell lines, they manifest low or negligible toxicity in normal or fibroblastic cell models (such as 3T3 cells), which reinforces the safe potential of the crude extract. Such biocompatibility is associated with the high concentration of ellagitannins characteristic of rambutan extracts, with geraniin being the major compound. These compounds possess a remarkable antioxidant and free-radical scavenging capacity that, at the cellular level, can exert cytoprotective effects against oxidative stress, thereby promoting the maintenance of metabolic integrity in non-tumor cell lines [31].

Cell viability of the HEK-293 and 3T3 cell lines upon treatment with RPEE (Figure 3) decreased gradually with increasing extract concentration, demonstrating a dose-dependent effect [32]. In the HEK-293 line, viability was reduced from approximately 100% to 75% as the concentration increased from 125 to 1000 ppm, while in 3T3 cells, it decreased from 100% to 78%. On the other hand, treatments with alginate and citrate exhibited viability percentages close to 80% in both cell lines, indicating that the materials used in the encapsulation process do not exert significant adverse effects on cell proliferation.

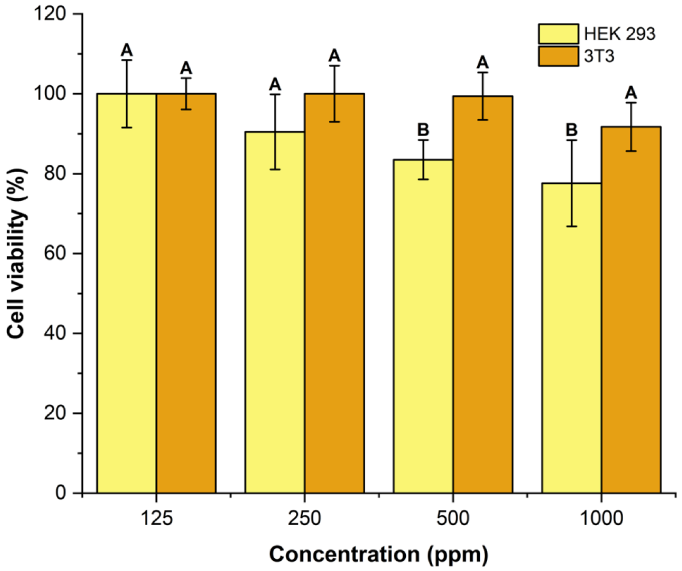

Figure 2. Cell viability assay performance in HEK-293 and 3T3 cell lines with rambutan peel polyphenols (letters A and B indicate significant differences between the treatments).

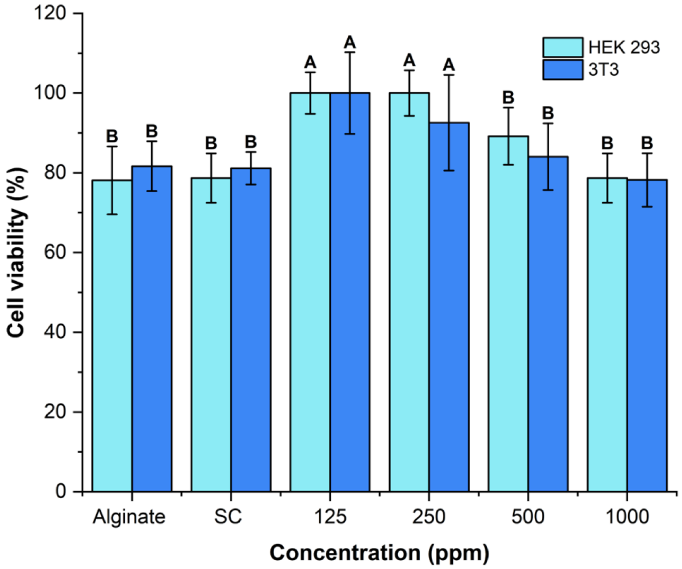

Figure 3. Cell viability assay performance in HEK-293 and 3T3 cell lines EERP and controls (alginate and sodium citrate (SC)) (letters A and B indicate significant differences between the treatments).

Nevertheless, according to the ISO 10993-5:2009 standard, none of the evaluated concentrations can be considered cytotoxic, as cell viability remained above the 70% threshold established for cytotoxicity classification [33,34]. This absence of cytotoxic effects is in full agreement with previous findings [35], which evaluated the biological activity and compatibility of a rambutan peel extract rich in ellagitannins (mainly geraniin). In that study, by applying the MTT assay under the international guidelines of the ISO 10993-5 standard, it was demonstrated that the extract was non-toxic, maintaining cell viability above the critical limit of 70%. Additionally, the high tolerance observed in the present study for the 3T3 fibroblast line aligns with the results reported for L929 mouse fibroblasts. Taken together, the high viability recorded across multiple fibroblastic (3T3 and L929) and epithelial (HEK-293) cell lineages solidly validates the cellular safety of these active compounds and confirms their potential biocompatibility for biological and industrial applications without risks of inducing tissue damage. Additionally, a recent study reported via in silico predictions performed with the ProTox-II platform that geraniin is classified

within toxicity class 3, with an estimated LD_{50} of approximately 300 mg/kg. Likewise, it was reported that this ellagitannin does not inhibit the major cytochrome P450 isoenzymes, including CYP1A2 and CYP2C19, suggesting a low potential for metabolic interactions with other compounds [36].

Collectively, these findings demonstrate that both the polyphenolic extract and the RPEE exhibit excellent biocompatibility and negligible cytotoxic potential, thereby supporting their suitability for the development of functional ingredients and targeted delivery systems within the food and nutraceutical sectors.

3.4. Antioxidant Power

The antioxidant activity of the polyphenol-loaded alginate beads, control (blank) beads, and the raw rambutan peel extract was evaluated using DPPH, ABTS, and FRAP assays. These complementary mechanisms were employed to comprehensively determine the radical scavenging potential and reducing power of the formulations.

As illustrated in Table 5, the raw rambutan extract exhibited prominent antioxidant potential, achieving radical inhibition percentages of 99% and 93% for the ABTS and DPPH assays, respectively. These high scavenging efficiencies align with the findings reported by [11], which demonstrated that rambutan peel polyphenols possess substantial antioxidant potential, particularly at elevated concentrations ranging from 500 to 1000 ppm.

Table 5. Antioxidant power for the rambutan peel polyphenols and Trolox as a control.

Concentration [ppm]	ABTS (% Inhibition)	DPPH (% Inhibition)	FRAP (mmol TE/L)
7	0.5 ± 0.02	0 ± 0.00	0.04 ± 0.01
15	6 ± 0.02	7 ± 0.01	0.06 ± 0.01
31	12 ± 0.02	22 ± 0.02	0.18 ± 0.01
62	23 ± 0.01	47 ± 0.01	0.37 ± 0.02
125	46 ± 0.02	78 ± 0.02	0.74 ± 0.02
250	83 ± 0.00	94 ± 0.00	1.47 ± 0.01
500	99 ± 0.00	95 ± 0.00	2.66 ± 0.05
1000	99 ± 0.00	93 ± 0.00	4.04 ± 0.01

This high radical inhibition (exceeding 90%) is primarily attributed to the structural composition of the rambutan peel polyphenols, which are highly abundant in hydroxyl (-OH) groups. Specifically, this antioxidant action is driven by the transfer of a hydrogen atom from the -OH groups of the polyphenols to the nitrogen atom of the radical. Although the reaction mechanism against the ABTS radical is inherently similar to that of the DPPH radical, the ABTS method offers the distinct advantage of concurrently determining the antioxidant potential of both hydrophilic and lipophilic compounds present within the extract [37,38].

In the FRAP assay, the powder obtained from the rambutan peel extract reached a maximum value of 4.04 mmol TE/L. The other concentrations evaluated also exhibited reducing capacity, albeit with values lower than those of the highest concentration. This behavior suggests a concentration-dependent relationship, wherein a higher content of phenolic compounds increases the capacity to donate electrons to reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+})—the mechanism upon which the FRAP assay is based.

The values obtained were lower than those reported in a previous study [39]. In this regard, studies conducted with *Myrtus communis* L. reported values of 13.04 and 24.85 mmol TE/L for extracts obtained with 40 and 60% ethanol, respectively, with a higher antioxidant response observed in the extract obtained with 80% ethanol ($p < 0.05$). The differences compared with the present study may be attributed to several factors, including

the nature of the plant matrix, the phytochemical profile of the extracts, and the extraction conditions employed.

In the case of rambutan peel, the reducing activity may be mainly associated with the presence of phenolic compounds such as hydrolyzable tannins, flavonoids, and ellagic acid derivatives, whose concentration and chemical structure determine their ability to reduce the ferric complex. On the other hand, *M. communis* L. contains a wide variety of phenolic compounds and flavonoids, whose extraction may be enhanced by the use of different ethanol concentrations.

Furthermore, the observed differences may be related to the extraction method employed. While the study on *M. communis* L. evaluated hydroalcoholic extracts with different ethanol proportions, the present study used an ultrasound- and microwave-assisted extraction strategy, aimed at promoting the recovery of bioactive compounds from rambutan peel. Therefore, although the values obtained were lower than those reported for other plant extracts, the results confirm the presence of compounds with antioxidant potential in this agro-industrial by-product.

Figure 4 exhibits the maximum antioxidant power obtained by the alginate beads with polyphenols and the control beads; for ABTS and DPPH, the maximum inhibition percentages obtained were 76 and 65%, respectively. Both results were considerably lower than those obtained by its powder counterpart; this is due to the dilution of the alginate solution with sodium citrate and the alcoholic solvents used in both methodologies. For the FRAP assay, the alginate beads with polyphenols obtained a maximum of 1.11 ± 0.04 mmol TE/L; once again, the reaction of the alginate with Fe^{+2} and the alcoholic solvents could be lowering the actual potential of the alginate bead.

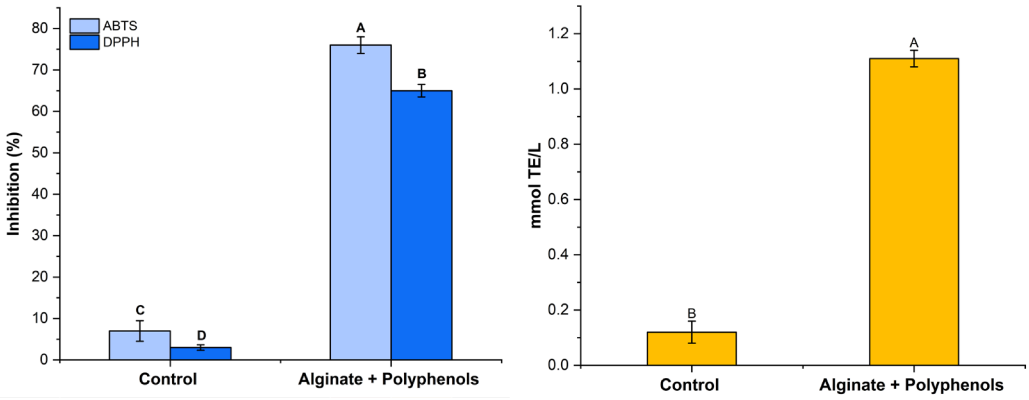

Figure 4. Total antioxidant power of the EERP and the control of only water for comparison (letters A–D indicate significant differences between the treatments).

4. Conclusions

The utilization of rambutan peel for the recovery of polyphenols, particularly ellagitannins, has proven to be a viable strategy for their application as ingredients in dietary supplements, including encapsulated forms. A suitable encapsulation efficiency of 55% for rambutan peel ellagitannins was achieved using sodium alginate. Furthermore, these ellagitannin encapsulates from rambutan peel exhibit a favorable safety profile in HEK-293 and 3T3 cell lines, demonstrating biocompatibility superior to that described in the ISO 10993 standard, which allows for their use at functional concentrations of 1000 ppm. On the other hand, antioxidant potential assays using ABTS, DPPH, and FRAP revealed significant antioxidant power. Therefore, rambutan peel ellagitannin encapsulates in sodium alginate matrices represent a technological alternative for the development of functional foods and dietary supplements, with high potential for the prevention of diseases and physiological disorders.

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Abbreviations

The following abbreviations are used in this manuscript:

EE	Encapsulation efficiency
EERP	Ellagitannin encapsulates from rambutan peel
RTP	Rambutan Total Polyphenols

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