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Anticancer, antioxidant and antibacterial activities of selenium nanoparticles preserved by spray drying

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Abstract. Selenium nanoparticles (SeNPs) have gained considerable attention due to their antioxidant, anticancer, and antibacterial activities. For the development of SeNP-based products, storage conditions play a critical role in preserving their functional properties. Among approaches to maintain SeNPs' particle size, stability, and bioactivity, spray drying (SD) is a rapid, cost-effective, scalable technique. In this study, the SeNPs synthesized via electron beam irradiation and stabilized with gum arabic were preserved by spray drying (SeNP/SD), then evaluated for biological activities by MTT cytotoxicity, DPPH radical-scavenging, and agar well diffusion assays, compared with SeNPs stored at 4 °C. Specifically, the SeNP/SD effectively suppressed the proliferation of HeLa cervical cancer cells (IC_{50} = 2.99 µg/mL) while exerting minimal effects on normal BJ-5ta fibroblasts (selectivity index (SI) = 6.06). In addition, the SeNP/SD demonstrated notable DPPH radical-scavenging activity (IC_{50} = 15.3 µg/mL) and inhibited the growth of *Escherichia coli* and *Listeria monocytogenes*. In contrast, non–spray-dried SeNPs completely lost antibacterial activity and exhibited a 2.9-fold reduction in antioxidant capacity. Overall, these findings highlight spray drying as a promising strategy for preserving SeNP bioactivity.

Keywords: selenium nanoparticles, spray drying, anticancer, antioxidant, antibacterial.

Classification numbers: 2.4.3, 1.2.1.

1. INTRODUCTION

Selenium is an indispensable dietary trace element for humans, owing to its role in enhancing immune function, preventing cancer and cardiovascular diseases, and its remarkable antioxidant characteristics [1–3]. It constitutes selenoproteins and antioxidant selenoenzymes such as glutathione peroxidase (GPX), thioredoxin reductase (TXNRD), selenoprotein P (SELENOP), selenoprotein F (SELENOF) taking part in cellular metabolism processes [4, 5]. The selenium exists in non-organic, organic forms or selenium nanoparticles (SeNPs). Due to the fact that selenium has a narrow margin between the threshold of activity and toxicity, recently, the SeNPs with notable properties including good biocompatibility, bioavailability, and lower toxicity become a focused target in numerous studies [1, 6]. Interestingly, the SeNPs displayed valuable bioactivities consisting of antibacterial [7–10], antiviral [11], antioxidant [12, 13], and anticancer abilities [14]. For example, the SeNPs have been shown to suppress the growth of several pathogenic bacteria, including *Vibrio parahaemolyticus, Staphylococcus aureus, Enterococcus faecalis, and Pseudomonas aeruginosa* [7–10]. The SeNPs have also been reported to exhibit remarkable anticancer activity by effectively killing cancer cells while causing minimal harm to normal cells; this selectivity may be attributed to differences in intracellular redox balance and osmotic pressure between these cell types [15, 16]. Until now, the toxic effects of SeNPs have been reported on prostate, colorectal, breast, lung, cervical cancer cells, etc. [14].

The SeNPs could be produced by chemical, biological, and physical methods. Chemical methods commonly used ascorbic acid, sodium borohydride, hydrazine as the reducing agents [17–19], while biological methods utilized the plant extracts or microorganism biomass [20, 21]. However, only limited studies have reported the preparation of SeNPs using physical methods [22–24], especially by electron beam irradiation [25]. Compared with other methods, irradiation offers several benefits, including the elimination of toxic reducing agents, facile control over particle size, high product purity, and suitability for cost-effective large-scale production [25]. Besides, irrelevant to synthetic methods, bare SeNPs without stabilizers usually aggregate, enlarge and transform into a gray/black analog which is more thermodynamically stable but has lower bioactivities due to the increase in SeNP diameter [26–29]. Thus, coating agents to stabilize the particle diameter are necessary in this case, namely chitosan [30], oligochitosan [31], dextran [32], β-glucan [33], and gum arabic [34]. Gum arabic composed of highly branched polysaccharides is widely used in food and pharmaceutical applications, which makes it potential for utilization as a stabilizing agent for nanoparticles [5]. Therefore, SeNPs synthesized via electron beam irradiation and stabilized with gum arabic represent a promising approach for SeNP production with potential for further applications.

In addition, preservation of SeNPs in an aqueous solution is not an optimal strategy as reported in our previous studies that the SeNPs stored at room temperature aggregated into bigger particles, though the particles kept at a low temperature (4 °C) could restrain this phenomenon. However, the cold storage is rather costly and inconvenient for transportation and commercial applications [31]. For those reasons, the SeNP solution should be dried into SeNP powder by spray drying, freeze drying, or coagulation to maintain the particle size and their stabilities as well as to ensure bioactivities [31–33]. Spray drying method atomizes/sprays SeNP suspension into droplets which are dried by heated gas in a drying chamber to finally collect solid particles [35]. The spray drying is fast, simple, cost-effective, scalable, and successfully applied in pharmaceutical and food manufacturing for the production of dry powder [36, 37]. The freeze-drying of SeNPs was described in our recent work [25], but its efficiency in preservation of these SeNPs has not been evaluated. Therefore, to broaden the applicability of these SeNPs, our work

was conducted to investigate the effect of spray drying method in maintaining diameter and conserving bioactivities of the particles. In this study, bioactivities of the SeNPs powder generated by electron beam irradiation, coated by gum arabic then preserved by spray drying including anticancer, antioxidant, and antibacterial ability will be investigated and compared with the original SeNP solution stored at 4 °C temperature.

2. MATERIALS AND METHODS

2.1. Selenium nanoparticles

The selenium nanoparticles (SeNPs) used in this study were provided by the Applied Research Institute of Natural Resources, Materials and Environment (Ho Chi Minh City, Viet Nam). They were synthesized via electron beam irradiation and stabilized with a gum arabic coating following previously reported methods [25]. After synthesis, the materials were handled in two different ways: A portion was processed into a dry powder using spray-drying (SD), while the remainder was kept in its original solution form (S) and stored at 4 °C. These two preparations were designated as SeNP/SD and SeNP/S, respectively. Following a storage period of approximately 3–4 months, both sample types were diluted with distilled water to obtain the required concentrations for experimentation.

2.2. Bacteria strains

In the present study, *Listeria monocytogenes* (ATCC 15313) and *Escherichia coli* (ATCC 25922), obtained from the American Type Culture Collection (ATCC), were propagated in Tryptone Soy Broth (TSB) medium (Himedia, India) under standardized growth conditions.

2.3. Human cell lines

The human cancer cell lines MCF-7 (breast adenocarcinoma, ATCC HTB-22) and HeLa (cervical adenocarcinoma, ATCC CRM-CCL-2), together with the normal human fibroblast cell line BJ-5ta (ATCC CRL-4001), were obtained from the American Type Culture Collection and used for cytotoxicity assessment.

The HeLa cells were cultured in Eagle's Minimum Essential Medium (EMEM; Himedia, India), whereas MCF-7 and BJ-5ta cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich, USA), with both media supplemented with 10 % fetal bovine serum (FBS). All cultures were incubated at 37 °C in a humidified atmosphere containing 5 % CO_2, and the culture medium was renewed every 2–3 days.

2.4. MTT cytotoxicity assay

For this assay, adherent cells were detached using 0.25 % trypsin–0.53 mM EDTA, counted with a Neubauer hemocytometer, adjusted to 1×10^5 cells/mL, and seeded into 96-well plates. After overnight incubation for cell attachment, the cells were treated with various concentrations of SeNPs and incubated for 48 h. Subsequently, 5 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 h. The resulting formazan crystals were dissolved with 60 µL of lysis buffer (30 % w/v SDS, 0.03 N HCl) and 90 µL of DMSO (99.9 %, v/v), followed by shaking at 800 rpm for 10 min. Absorbance was then measured at 550 nm using a microplate reader. The

assay was performed according to the method of Nga *et al.* [38], including untreated control and blank groups. The percentage of growth inhibition was calculated using the equation [39]:

$$I\ \% = 100\ \% - (OD_{conc.} - OD_{blank})/(OD_{control} - OD_{blank}) \times 100\ \%$$

All experiments were performed in triplicate. Dose–response relationships were analyzed by non-linear regression using GraphPad Prism software to determine IC_{50} values, defined as the concentration required to inhibit 50 % of cell viability. Additionally, the selectivity index (SI) was calculated as the ratio between the IC_{50} value in normal fibroblast cells and that in cancer cell lines.

2.5. DPPH radical scavenging assay

The free radical scavenging activity was evaluated using a DPPH assay in 96-well plates, adapted from a previously reported method with minor modifications [40]. Prior to analysis, the two SeNP formulations and L-ascorbic acid (Sigma-Aldrich, USA) were diluted in distilled water to the desired concentrations. A freshly prepared DPPH solution in absolute ethanol (99.9 %, v/v) was added to obtain a final concentration of 350 μM in each well, while a mixture of DPPH solution and distilled water served as the negative control. The reaction mixtures were incubated at 30 °C in the dark with shaking at 800 rpm for 60 min, after which absorbance was measured at 492 nm using a microplate reader. The percentage of inhibition (I %) was calculated using the following equation [41]:

$$I\ (\%) = 100\ \% - \frac{(\text{OD sample} - \text{OD blank}) \times 100\ \%}{\text{OD control} - \text{OD blank}}$$

Each experiment was performed in triplicate. The IC_{50} value, representing the concentration required to neutralize 50 % of DPPH radicals (conversion of DPPH• to DPPH-H), was determined by linear regression analysis of the relationship between inhibition percentage and sample concentration using GraphPad Prism software.

2.6. Agar well diffusion assay

Bacterial strains *Escherichia coli* and *Listeria monocytogenes* were first propagated in Tryptone Soy Broth (TSB) at 37 °C under shaking conditions overnight. The cultures were then refreshed by inoculating into new medium at a 1:20 (v/v) ratio and incubated for 2–3 h to restore active growth. Subsequently, the bacterial suspensions were standardized to an optical density of 0.1 and incorporated into 0.5 % (w/v) agar, which was carefully overlaid onto a pre-solidified 2 % (w/v) agar base in Petri dishes. After solidification, wells with a diameter of 7 mm were aseptically punched into the agar surface, and 50 μL of each test sample was dispensed into the wells. The plates were then incubated at 37 °C for 24 h, followed by measurement of the inhibition zone diameters. Ampicillin (500 μg/mL) served as the positive control, while distilled water was used as the negative control. The assay procedure was adapted from the method reported by Anh Thu *et al.* [42].

2.7. Data analysis

Statistical analyses were conducted using GraphPad Prism software. Experimental results are presented as mean ± standard deviation (SD) from independent replicates. Differences between the two groups were evaluated using an unpaired two-tailed Student's *t*-test, with a *p*-value ≤ 0.05 considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Size of SeNPs preserved by spray drying

Table 1. Size of SeNPs preserved by spray drying.

Sample	Preservative method	Diameter
SeNP/SD	Spray drying	~40 - 50 nm
SeNP/S	Maintained as original solution	~40 - 60 nm

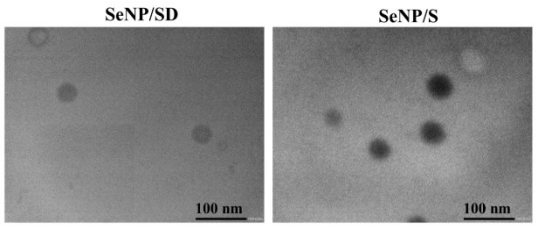

Figure 1. SeNP samples observed under transmission electron microscopy (TEM).

The diameter of SeNPs was observed and recorded by transmission electron microscopy (TEM) (Table 1, Figure 1). The measuring results indicated that the average size of SeNPs preserved by spray drying method was approximately 40 - 50 nm, slightly smaller than the original SeNPs, which were kept in aqueous solution, with the diameter fluctuating in the range from 40 to 60 nm (Table 1). This result implied that the preservative method affected the size of SeNPs.

Recently, electron beam irradiation has been considered as a time-saving and economical technique to produce SeNPs with a small diameter (27.3 ± 4.8 nm), which may expose better bioactivities [25]. Although it is reported that the preservation of SeNP solution at cold temperature (4 – 5 °C) helped to maintain the stability of particle size up to 6 months and prevent the aggregation of SeNPs when compared with being left at ambient temperature, this storage method could be costly and inconvenient for transportation and commercialization [25]. In addition, the SeNP powder could be more easily sterilized by irradiation than SeNPs in aqueous solution [32].

In fact, spray drying has been utilized in many previous reports to preserve SeNPs which still well-performed in antioxidant assays and on irradiated mouse models after spray drying process [31, 32]. However, the effect of spray drying on SeNPs generated by electron beam irradiation has not been investigated yet. In this study, the TEM images revealed that when compared with SeNP samples preserved by this process (diameter of 40 - 50 nm), the SeNPs in the original solution exhibit fluctuations with a wider diameter range (40 - 60 nm) which might be due to the aggregation of particles during the storage time which was 7 - 10 days prior to the TEM analysis. In relation to a previous work, the SeNPs created by electron beam accelerator then preserved by freeze drying exhibited the size of approximately 25 - 30 nm which is a bit smaller than those preserved by spray drying method in this study [25]. However, the SeNP size of 40 - 50 nm in this study still belongs to the usual diameter range (10 - 300 nm) of SeNPs produced by different methods such as chemical, biological, and physical ones [43].

3.2. Anticancer activity of SeNPs preserved by spray drying

The growth-inhibition capacity of SeNPs on the cancer cells was tested by MTT cytotoxicity assay. The results showed that the toxicity of SeNP samples towards HeLa cells was strong due to their low IC_{50} values, and especially they were accompanied by good selectivity with SI values greater than 6 (Table 2). However, there was no difference in killing effect between the SeNP/SD and SeNP/S samples (2.99 vs. 1.06 μg/mL) (Figure 2). On the other hand, regarding MCF7 cells, the inhibition of SeNP/SD (IC_{50} 29.37 μg/mL) was 1.8 times higher than SeNP/S samples (52.55 μg/mL), however the selectivity was not exhibited in this cancer cell line when compared with normal fibroblasts (SI < 1).

Table 2. IC_{50} values and selectivity index (SI) of SeNP samples.

		SeNP/SD	SeNP/S
IC_{50} (μg/mL)	HeLa	2.99 ± 1.86	1.06 ± 0.21
	MCF-7	29.37 ± 10.76	52.55 ± 19.13
	BJ-5ta	18.13 ± 7.33	50.05 ± 5.41
Selectivity index (SI)	HeLa	6.06	42.22
	MCF-7	0.62	0.95

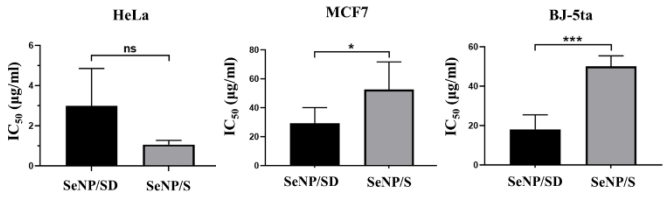

Figure 2. Comparative analysis of IC_{50} values of SeNP samples in HeLa and MCF-7 cells. Data are expressed as mean ± SD from at least three independent experiments. Statistical significance was assessed using a two-tailed Student's t-test ($p \leq 0.05$, $^{**}p \leq 0.001$; ns, not significant).

The results demonstrated that HeLa cells exhibited greater sensitivity to SeNPs than MCF-7 cells, irrespective of the differences in particle size between the two samples. Interestingly, the SeNPs preserved by spray drying not only facilitated the maintenance of inhibition activity against cancer cell growth in vitro, but also exhibited higher activity, comparable to other reports in which the IC_{50} values of SeNPs on HeLa cells ranged from 4 to 30 μg/mL [44–46] or on MCF7 cells ranged from 2.5 to 160 μg/mL [20, 47–50]. And importantly, the SeNP/SD sample also showed high selectivity (SI value of 6.06) towards cancer cells when compared with normal fibroblasts. In *in-vitro* cytotoxicity assays, SI values greater than 2 indicate specific inhibitory capacity against cancer cells [51], upon which SeNPs show potential effects in cancer treatment to restrain unexpected harmful side effects on normal tissues and organs of the human body.

The cell morphology observations were consistent with the MTT results, as HeLa cells treated with SeNPs at concentrations above 1.25 μg/mL exhibited abnormal features, including the accumulation of intracellular vesicles (Figure 3A). In agreement with previous studies, vacuolization was also observed in SeNP-treated HeLa cells and has been attributed to the cellular endocytosis of SeNPs [52]. Meanwhile, MCF-7 cells maintained a morphology comparable to the negative control at concentrations below 20 μg/mL. However, at higher concentrations (20 μg/mL for SeNP/SD and 40 μg/mL for SeNP/S), a reduction in cell density and disruption of membrane

integrity were evident (Figure 3B). The observation of cell morphology suggested that SeNPs elicited distinct responses across different cancer cell lines, indicating variations in their underlying cytotoxic mechanisms.

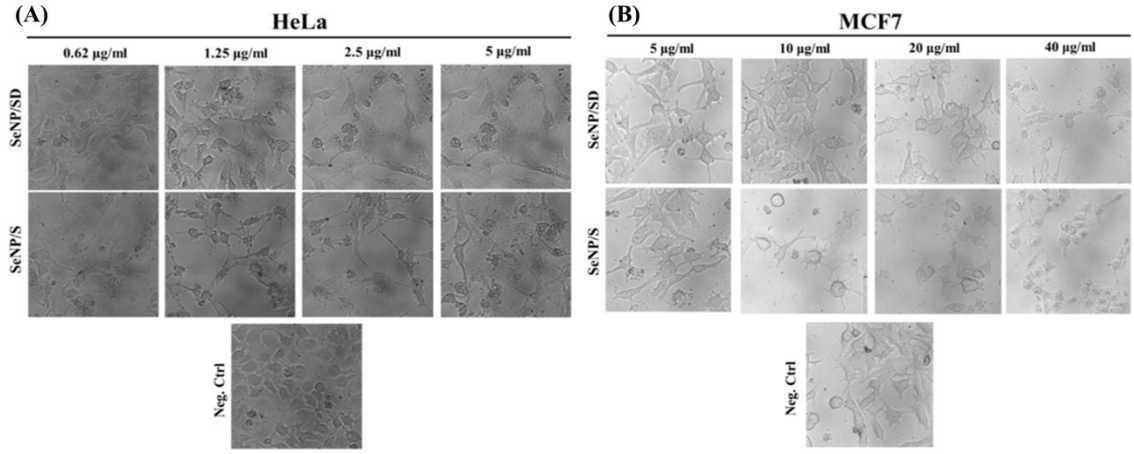

Figure 3. Effects of SeNP samples on the morphology of HeLa (A) and MCF7 (B) cells after 48 h of incubation at various concentrations. The negative control (Neg. Ctrl) was treated with distilled water in the absence of SeNPs. Images were acquired at 400× magnification.

To sum up, the above results point out that the preservation may help maintain the cytotoxic ability of SeNPs against MCF7 breast cancer cells but does not show an obvious contribution towards HeLa cervical cells.

3.3. Antioxidant activity of SeNPs preserved by spray drying

The antioxidant activity of SeNPs was assessed using the DPPH assay (Figure 4). DPPH radical-scavenging activity increased with concentration (1.25–25 µg/mL). Notably, SeNP/SD exhibited an IC_{50} of 15.3 µg/mL, representing a 2.9-fold improvement over SeNPs stored in the original solution (Table 3), highlighting the importance of storage conditions in preserving antioxidant activity. Moreover, SeNP/SD showed stronger antioxidant capacity than previously reported SeNPs (IC_{50} = 22.5–600 µg/mL), which may be attributed to the synthesis method and coating agent used [45, 46, 53–55].

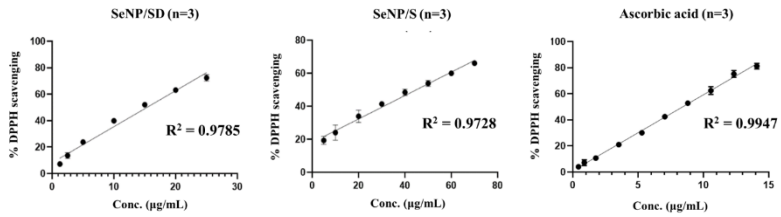

Figure 4. Antioxidant activity of spray-dried SeNPs evaluated by DPPH radical-scavenging assay.

Table 3. IC_{50} values of SeNP samples in DPPH radical-scavenging assay.

Sample	SeNP/SD	SeNP/S	Ascorbic acid
IC_{50} (µg/mL)	15.3 ± 0.2	44.9 ± 1.5	8.4 ± 0.3

3.4. Antibacterial capacity of SeNPs preserved by spray drying

The antibacterial capacity was examined by agar well diffusion assay with SeNPs samples of 50 to 800 µg/mL. Regarding SeNP/SD sample, the inhibition zone appeared at experimental concentrations greater than 200 µg/mL with diameter of 9.7 – 13.0 mm and 8.7 – 11.3 mm towards *E.coli* and *L.monocytogenes,* respectively (Figure 5, Table 4). Interestingly, the SeNP/S sample did not cause inhibition on both bacteria, which underscores the importance of preservation methods for their antibacterial activity besides the antioxidant and anticancer abilities mentioned above.

In relation to previous works, SeNP/SD sample displayed the antibacterial capacity although this activity was not really impressive as described in former data [56–59]. More particularly, SeNPs in those works could inhibit *L. monocytogenes* with zone diameter of 14.7 mm at 700 µg/mL and *E. coli* with inhibition zone of 19 mm at 950 µg/mL [57–59], while in this study SeNP/SD restrained the growth of *L.monocytogenes* and *E.coli* with inhibition zone of 11.3 and 13.0 mm, respectively, at the highest experimental concentration of 800 µg/mL. To sum up, the bioactivities of SeNPs preserved by spray drying were kept higher than those of the particles stored in the solution at cold temperature (4 °C).

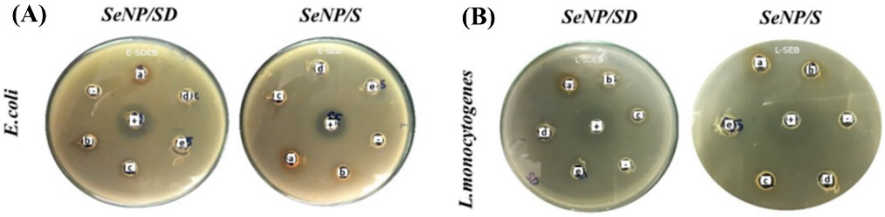

Figure 5. Antibacterial effects of SeNPs assessed using agar well diffusion method against *E. coli* (A) and *L. monocytogenes* (B). (a) 800 µg/mL, (b) 400 µg/mL, (c) 200 µg/mL, (d) 100 µg/mL, (e) 50 µg/mL; (+) ampicillin at 500 µg/mL, (−) distilled water.

Table 4. Inhibition zone diameters (mm) of SeNP samples against *E. coli* and *L. monocytogenes* determined by agar well diffusion assay.

Species	Concentration (µg/mL)	Diameter (mm)			
		SeNP/SD	SeNP/S	Ampicillin (500 µg/mL)	Negative control
E.coli	800	13.0 ± 1.0	-	19.8 ± 1.2	-
	400	11.3 ± 0.6	-		
	200	9.7 ± 0.6	-		
	100	-	-		
	50	-	-		
L.monocytogenes	800	11.3 ± 1.5	-	14.2 ± 1.9	-
	400	10.3 ± 1.5	-		
	200	8.7 ± 2.1	-		
	100	-	-		
	50	-	-		

(-) No inhibition zone.

4. CONCLUSIONS

In this study, SeNPs were synthesized by electron beam irradiation, stabilized with gum arabic, and converted into powder by spray drying. The bioactivities of the original SeNP suspension and spray-dried SeNPs (SeNP/SD) were evaluated under different storage conditions. SeNP/SD exhibited enhanced anticancer, antioxidant, and antibacterial activities compared with SeNPs stored at 4 °C. Although its antibacterial activity was moderate, with inhibition zones of 9.7–13.0 mm against E. coli and 8.7–11.3 mm against L. monocytogenes, SeNP/SD showed promising anticancer activity against HeLa cells (IC50 = 2.99 μg/mL, SI = 6.06) and strong antioxidant activity (DPPH IC50 = 15.3 μg/mL). Overall, these findings demonstrate the importance of appropriate preservation methods in maintaining SeNP bioactivity and support the potential application of spray-dried SeNPs in future healthcare products.

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CRediT authorship contribution statement. Vo Anh Kiet: Conceptualization, Methodology, Investigation, Data curation, Formal analysis. Truong Thi Bich Ngoc, Tran Thi Thanh Ngoc, Nguyen Ngoc Duy: Formal analysis, Writing - original draft. Dang Thi Phuong Thao, Tran Linh Thuoc, Phan Dinh Tuan, Vu Le Van Khanh: Conceptualization, Methodology, Supervision, Writing - review & editing. All authors: Writing - review & editing, Approval of the final manuscript.

Data Availability. All data generated or analysed are included in this published article.

Declaration of competing interest. The authors have no competing interests to declare that are relevant to the content of this article.

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