Anthocyanin enhancement of pitaya-induced callus via green synthesized selenium nanoparticles for lung anticancer activity

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Background
Lung cancer has the highest mortality rates and is a major public health concern. It is the primary cause of cancer-related deaths globally. Patients at various stages of lung cancer urgently need access to natural experimental therapies that do not cause any negative side effects.
Objective
The present study increased the anthocyanin content in the induced callus of Pitaya species *Hylocereus polyrhizus* and *Hylocereus costaricensis*. It positively affected the viability and proliferation/migration of wi38 lung cells, resulting in lung anticancer activity in in vitro bioassay evaluation.
Materials and methods
For induction of callus of pitaya plant *spp.* (*H. polyrhizus,* and *H. costaricensis*), different combinations of growth regulators combined with Selenium nanoparticles were experimented. Anthocyanin accumulation in the selected callus was enhanced through bio-synthesized selenium nanoparticles at different concentrations (10, 20, 30, and 40) ppm.
Results and conclusion
High-performance liquid chromatography analysis revealed the presence of the two active compounds; Delphinidin-3-O-glycosides and Pelargonidin-3-O-glycosides at (3.18, 6.54 μg/gm), respectively from pitaya callus *sp. H. polyrhizus* and three active compounds; Delphinidin-3-O-glycosides, Peonidin-3-O-glycosides, and Cyanidin-3-O-glycosides at (4.66, 10.85 and13.79 μg/gm), respectively from pitaya callus *sp. H. costaricensi.* The lung anticancer activity was observed with sp's highest growth inhibition activity. *H. polyrhizus* extract on lung cancer cells (wi38) with IC50 of 309.54±2.69 μg/ml. and the highest growth inhibition activity of the *sp. H. costaricensis* extract was with IC 50 of 348.2±6.71 μg/ml.

Keywords:
anthocyanins, callus induction, lung cancer, pitaya spp, selenium nanoparticles

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Introduction

In vitro culture methods, including callus induction and micro-propagation, offer promising avenues for the mass production of anthocyanins. These techniques allow for the controlled synthesis of secondary metabolites, such as anthocyanins, without the need to harvest from natural plant sources, thus preserving biodiversity [1] Callus culture, in particular, is effective in producing high yields of anthocyanins. Recent studies have highlighted the importance of optimizing culture conditions to enhance anthocyanin production. Factors such as nutrient medium composition, light quality, temperature, and the use of biotic and abiotic elicitors play crucial roles in influencing anthocyanin synthesis in vitro [2] Moreover, advancements in metabolic engineering and the scale-up process further support the industrial application of these techniques, enabling the large-scale production of anthocyanins [3] Overall, the induction of anthocyanins from callus and micro-propagated plants represents a significant advancement in plant biotechnology, offering sustainable and efficient means of producing these valuable compounds which act as antioxidant [4], anti-inflammatory [5] and antitumor [6]. The ongoing research and development in this field continue to optimize and refine these techniques, paving the way for their broader application in various industries. Selenium nanoparticles (SeNPs) have a special role in the living organisms' growth and development [7].

SeNPs were used for seed germination resulting in a high germination index with the addition of 10 ppm. The addition of SeNPs was found to have higher antioxidant activity. Furthermore, were applied as fertilizers in several studies. The exact mechanisms

by which selenium (Se) influences anthocyanin accumulation remain unclear. Se improved photosynthesis by increased sucrose production and facilitated the transport of sucrose by up-regulation of the expression genes responsible for sucrose transportation, the increasing of sucrose levels subsequently induced the expression of anthocyanin biosynthesis genes, resulting in greater anthocyanin accumulation [8]. Abiotic-SeNPs had better performance than chemical-SeNPs. Various studies recorded the role of SeNPs for developing plant growth and for enhancement of bioactive compounds having significant effects towards anti-inflammation, antimicrobial, and anticancer. Cancer is considered a multifactorial and multistep disease that involves complex cascades of signaling pathways. As is known, cell growth is controlled by dysregulation and disruption of cell division. Both chemotherapy and radiation therapy are two common strategies for treating inoperable cancer. Patients with malignant solid tumors benefit significantly from chemotherapy drugs and/or ionizing radiation administered to the malignant site. Chemotherapy regimens are associated with significant pulmonary toxicities, which include the development of pneumonitis and fibrosis.

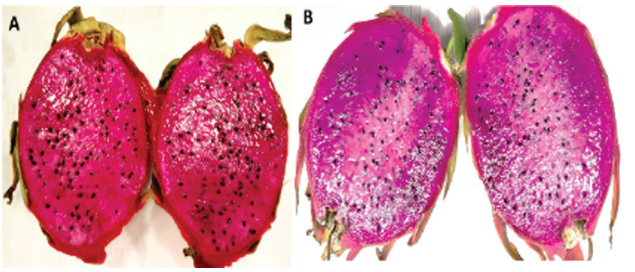
In addition, acute chemo-radiation pneumonitis is characterized by spreading inflammation of the lungs, typically occurring within a few weeks of completion of therapy in up to 15% of all irradiated patients. There is no drug to reverse existing pulmonary fibrosis and the main option in the late stages of the disease is a lung transplant, which is often complicated by immunological problems. Many clinical studies have been conducted on various plants that have anti-cancer agents that induce apoptosis. Cancer cells are eradicated and their growth is prevented by the anti-cancer effect. Cell death can be significantly induced by natural apoptosis, even more so than by natural anticancer agents [9]. The main aim of this study is to develop new lung anti-cancer agents via enhancing bioactive anthocyanins from induced callus of two species of pitaya plants rich in bioactive compounds using a novel protocol combining usual tissue culture techniques with nanotechnology products represented by in vitro biosynthesized SeNPs.

Materials and methods

Plant materials and source of explants

Seeds of Dragon or pitaya fruit were extracted from fresh ripe fruit. Red (*Hylocereus costaricensis*) and purple (*Hylocereus polyrhizus*) (Fig. 1). The fresh fruits were purchased from a cultivated field in Samarkand Ranch Farm in Egypt, Seeds of two dragon fruit species were used as a source of explants for *in vitro* cultures.

Figure 1



The purchased Dragon or pitaya fruit A. Red Pitaya species (*Hylocereus costaricensis)* and B. Purple Pitaya species (*Hylocereus polyrhizus)*.

Sterilization, germination and culture conditions

After being separately extracted, seeds were surface-sterilized by washing with tap water for 10 min and sterilizing with a solution of 2% sodium hypochlorite for 5 min, and then washing by distilled water three times. The sterilized seeds were grown on a free MS medium containing 3% sucrose and 0.6% agar and incubated at 25–27°C in a growth chamber with a photoperiod of 16 h light and 8 h dark. After 6 weeks of incubation, the sterilized shoots were obtained on MS basal medium without any hormones.

Micropropagation of Pitaya plant species (*H. polyrhizus*, and *H. costaricensis*)

Emerging shoots were separated from seedlings and cultured on MS basal medium containing BA (0.5 mg/l) to obtain the highest average number of shoots per explant as recorded by [10] with little modification by the addition of a low concentration of naphthalene acetic acid (NAA) at 0.1 to promote cell elongation during the micropropagation stage.

Callus induction of pitaya shoots

For mass induction of callus, semi-solid MS media containing 2.5 g/l Agar combined with 1 g/l Phytagel was used to solidify the medium, consisting of full-strength MS with vitamins [11], and 30 g/l sucrose. For callus induction, various treatments were prepared using combinations of Plant growth regulators (GRs); BA (6-benzyl- amino- purine), and NAA with – previously biosynthesized SeNPs, all treatments were shown in Table 1, five jars of each treatment were cultured. The analysis of callus growth was conducted according to [12,13]. The relative fresh weight of the callus was measured after the first month (W1) of culture on the induction treatments and re-measured after the second month (W2). The relative growth weight was calculated as W2-W1/W1.

Table 1 Effect of various treatments on the callus growth of pitaya spp

	Growth Regulators		
Treatments	BA (mg/l)	NAA (mg/l)	SeNPS (mg/l)
S0	0.0	0.0	0.0
S1	1.5	1.0	0.2
S2	2.0	1.0	0.2
S3	1.5	1.0	0.3
S4	2.0	1.0	0.3
S5	1.5	1.0	0.0
S6	2.0	1.0	0.0
S7	0.0	0.0	0.3

Enhancement of anthocyanin accumulation and selenium nanoparticles (SeNPs) treatments

Four concentrations of biosynthesized SeNPs;10, 20, 30, and 40 ppm were added to the best callus induction medium which was determined after callus induction experiments, to enhance the accumulation of Anthocyanin compounds. All media were prepared using the idealistic SeNPs with spherical-like shape and an average diameter of 17.9±1.34 were biosynthesized before in the previous study by using extract of *in vitro*-derived *Plectranthus aboinicus* leaves (data not shown in this study), all cultures were incubated for 21 days in a growth chamber under dark conditions at 25±2°C.

High-performance liquid chromatography (HPLC) analysis

High-performance liquid chromatography (HPLC) analysis for the determination of Anthocyanin compounds was conducted according to the common protocol mentioned by [14]. Two samples of yellow-reddish extracts were used for the determination of the presence of active anthocyanins. The extracts were prepared from the selected callus obtained from the best-enhanced callus that showed an obvious change in color to reddish color pigmentation as a result of anthocyanin accumulation. The purified anthocyanins were used as standards for HPLC analysis.

Anticancer activity analysis

Viability assay

Determination of cytotoxicity on lung cancer cells (wi38) was conducted using MTT protocol, the selected samples of two pitaya callus extracts which revealed the existence of active anthocyanins were used in different concentrations for the Viability assay as described by [15].

Morphological assay

The morphological changes that occur on the surface of the cell could be attributed to cell viability. By large decrease in secondary volume, the damage was identified as losses in protein and intracellular ions as a result of the alteration of the permeability of sodium or potassium.

Necrotic cells: nuclear swelling, chromatin flocculation, loss of nuclear basophile

Apoptotic cells: cell shrinkage, nuclear condensation, nuclear fragmentation.

Statistical analysis

Statistical analyses were performed using IBM SPSS (SPSS Inc; IBM Corporation, New York, USA) Statistics program, version 25 (2017), Windows. Data analysis was carried out using the Shapiro–Wilk test [16,17]. Data were entered into ANOVA with a P value of less than 0.05. The average values of all treatments were compared using the least significant difference as a post hoc test with a P value of less than 0.05 considered statistically significant.

Results and discussions

Germination and shooting

The germinated shoots of the two pitaya species were observed after two weeks (Fig. 2a and c). This study recorded the same germination result as recorded in the previous study by [18] when the viability of pitaya seeds decreased after 3 months of storage at room temperature, it is recommended as well as store seeds under cooling to save their viability without any affecting the germination percentage. Besides, the method of seed extraction from fresh fruits may affect seed germination, which must be considered in in vitro pitaya experiments. In this study, seeds were extracted and cultured immediately without any storage time to avoid the reduction of viability.

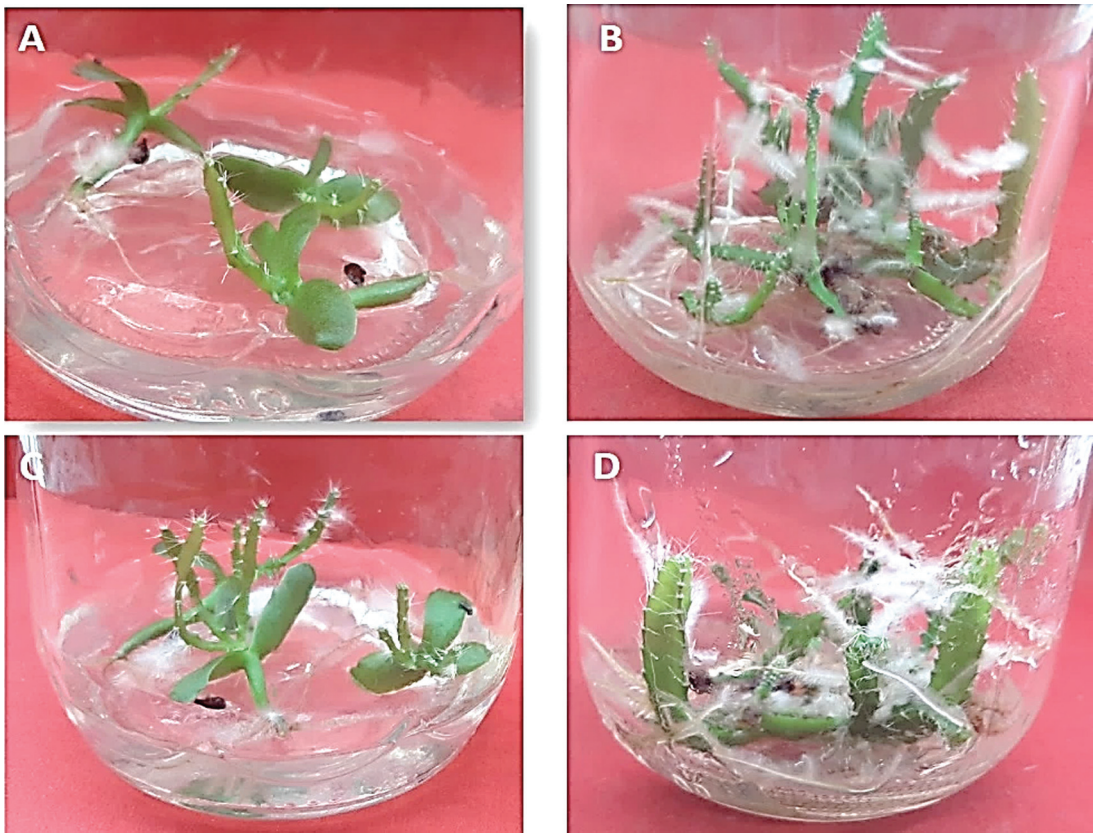
Multiplication of pitaya species

After germination, the emerging shoots were cultured on the modified multiplication medium supplemented with 0.5 g/L BA and 0.1 g/L NAA, resulting in a faster multiplication of shoots than medium containing BA alone (Fig. 2b and d). Besides, observing little difference in shoot length with the addition of NAA, the shoot length was about 3.0–3.5 after the second week of the first culture on the medium whereas the shoot length was about 2.0–2.7 with the supplementation of BA alone without NAA.

Induced callus of pitaya species

These experiments were conducted to verify the possibility of using Nanotechnology techniques to be compatible with plant tissue culture techniques for

Figure 2

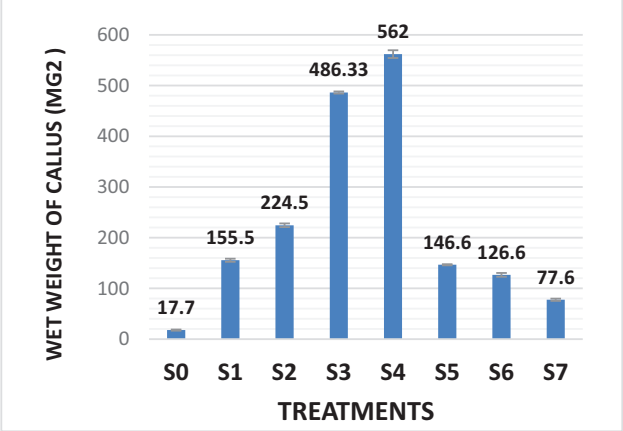


Germination and multiplication stages of Pitaya species. (a): Germinated seeds sp. *Hylocereus polyrhizus*. (b): Micropropagated shoots sp. *Hylocereus polyrhizus*. (c): Germinated Seeds sp. *Hylocereus costaricensis.* (d): Micropropagated shoots sp. *Hylocereus costaricensis*.

improving and developing eco-friendly Nano Growth Regulators instead of the harmful synthetic herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) known as the most dangerous herbicides still used (The exposure of 2,4-D causes non-Hodgkin's lymphoma (a blood cancer) and sarcoma (soft-tissue cancer), Even though, it's usually used as a plant growth regulator in most research experiments for various tissue culture purposes as recorded before in multiplication protocols [19], callus induction methods [12,20], Elicitation of different secondary metabolites from each part of plants [19]. Instead of using 2, 4- D for callus induction of pitaya plant as recorded in the several previous studies, new biosynthesized SeNPs from *in vitro*-derived *Plectranthus amboinicus* leaves were used in combination with NAA and BA for induction of callus Table 1. The biosynthesized reduction methods produced SeNPs have more advantages than SeNPs produced by chemical reduction methods. Besides, its reduction is economical, eco-friendly, and safe. The chemical reduction methods require downstream processing of the produced hazardous [21]. The comparative treatments were conducted between the combination of GRs treatment (S5 and S6) and the same treatment with the addition of two concentrations of SeNPs (0.2 and 0.3) mg/l, with S7 the addition of SeNPs at 0.3 without growth regulators and S0 MS free without any GRs or SeNPs. The growing callus was separated and weighed for measuring fresh weight. Three jars containing calluses were selected for each treatment (Table 1).

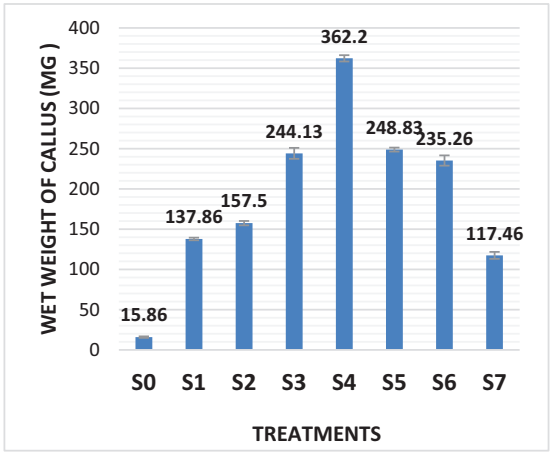
The calluses were separated and sub-cultured on the same treatment after 21 days without delay as a reason for the prevention of coloring changes occurring (browning). After the second subculture, callus was obtained. The fresh weight of the callus species *H. costaricensis* increased about 10 times after 30 days, the second subculture on treatments S3 and S4 when GRs were supplemented combined with 0.3 mg/L SeNPs obtained 486.3 and 562 mg callus, respectively (Fig. 3), compared with treatments containing lower concentrations of SeNPs at 0.2 mg/l (S1 and S2) which obtained 155.5 mg and 224.5 mg callus, respectively. The supplementation of SeNPs at 0.3 mg/l without the main factor which responsible for callus proliferation (S7) obtained 77.66 mg callus wet weight. Both treatments supplemented with GRs without SeNPs S5 and S6 obtained 146.66 and 126.66 mg callus, respectively.

Figure 3



Effect of GRs (BA and NAA) alone and combined with two different concentrations of SeNPs (0.2 and 0.3) mg/l. on wet weight of the proliferated callus of pitaya species (*Hylocereus costaricensis*). Presenting the maximum wet weight on treatment S4 (2.0 mg/l BA, 1.0 mg/l NAA and 0.3 mg/l SeNPs) followed with treatment S3 (1.5 mg/l BA, 1.0 mg/l NAA and 0.3 mg/l SeNPs) compared with treatments with the addition of GRs combined with 0.2 mg/L SeNPs (S1 and S2), and treatments S5, S6 (GRs without SeNPs), and S7 (SeNPs at 0.3 mg/L without GRs), S0 control on MS (without GRs or SeNPs).

Figure 4



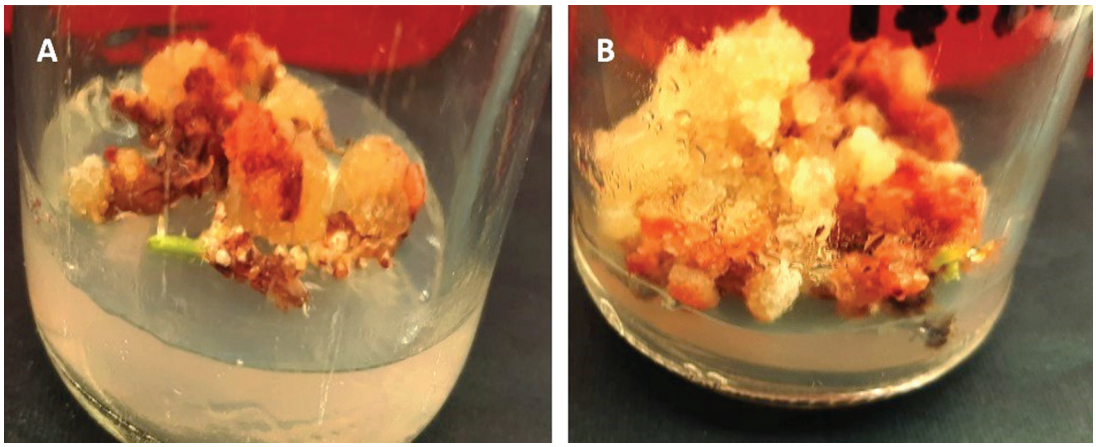
Effect of GRs (BA and NAA) alone and combined with two different concentrations of SeNPs (0.2) and (0.3) mg/l. on wet weight of the proliferated callus of pitaya species (*Hylocereus polyrhizus*). Presenting the maximum wet weight on treatment S4 (2.0 mg/l BA, 1.0 mg/l NAA and 0.3 mg/l SeNPs) followed with treatment S3 (1.5 mg/l BA, 1.0 mg/l NAA and 0.3 mg/l SeNPs) compared with treatments with addition of GRs combined with 0.2 mg/L SeNPs (S1 and S2), and treatments S5, S6 (GRs without SeNPs), and S7 (SeNPs at 0.3 mg/L without GRs), S0 control on MS (without GRs or SeNPs).

Concerning species *H. polyrhizus,* wet weight was recorded as well after the second subculture for each treatment (Fig. 4), S3 and S4 when GRs were provided combined with 0.3 mg/L SeNPs obtained 244.13 and 362.2 mg callus, respectively, compared with treatments S1 and S2 which obtained 137.86 and 157.5 mg callus, respectively. The addition of SeNPs at 0.3 mg/L without GRs (S7) obtained 117.46 mg callus wet weight. Both treatments with GRs and without SeNPs in S5 and S6 obtained 248.83 and 235.26 mg callus, respectively, and S0 (control) obtained 15.86 mg callus. The fresh weights of all replicates were recorded in different treatments for statistical analysis of the data.

Effects of *in-vitro* bosynthesized selenium nanoparticles (SeNPs) on the enhancement of anthocyanins

SeNPs act as elicitors for the stimulation of the secondary metabolites' synthesis, involving antioxidants and various phytochemicals in plants. The enhanced production of using SeNPS is a result of the stress caused by the nanoparticles in plant tissues, increasing bioactive compound levels. Besides, the selenium element is an essential micronutrient for all plants [22], working as a dual-purpose elicitor by the enhancement of nutrient uptake and biosynthesis of secondary metabolite. The certain mechanism by which selenium (Se) affects anthocyanin accumulation remains unknown. Via improvement of photosynthesis, Se up-regulates the expression genes responsible for sucrose transportation which leads to increases in sucrose production and facilitates the transportation of sucrose, and increasing of sucrose levels consequence induces the expression of anthocyanin biosynthesis genes, resulting in the accumulation of anthocyanin [8].The maximum weight of the induced callus from the two species (*H. polyrhizus*, and *H. costaricensis*) was revealed in treatment S4 on (2.0 mg/l BA, 1.0 mg/l NAA and 0.3 mg/l SeNPs). The induced callus on S4 was separated to be enhanced for Anthocyanins accumulation by exposure to various concentrations of the biosynthesized SeNPs at (10, 20, 30, and 40 ppm) under dark conditions. SeNPs could enhance anthocyanins under certain light regimes [23]. After 7 days reddish regions were observed in the differentiated callus, after 21 days of culture the calluses were collected and extracted for HPLC analysis. The highest reddish pigmentation on the different regions was observed with the exposure to SeNPs at 30 ppm (Fig. 5). The result indicate that SeNPs at certain concentrations and under an appropriate culture condition are considered a suitable elicitor to be applied for tissue culture protocols for enhancement of various anthocyanin compounds having significant medical effects as recorded by several studies.

Figure 5



The elicitation of anthocyanins on the Induced callus of pitaya plant species treated with SeNPs at 30 ppm A. The selected elicited callus of (*Hylocereus polyrhizus).* B. The selected elicited callus of (*Hylocereus costaricensis*).

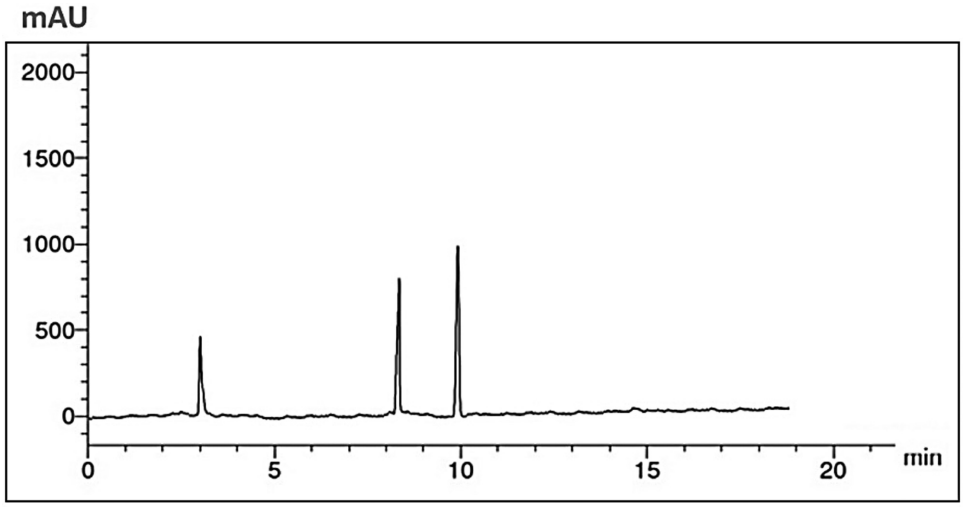
Determination of anthocyanins

HPLC data (Figs. 6 and 7) indicate the enhancement of the active anthocyanins; Delphinidin-3-O-glycosides, peonidin-3-O-glycosides, and cyanidin-3-O-glycosides at (4.66, 10.85 and13.79 µg/gm), respectively in the extract of pitaya callus species *H. costaricensi*, and Delphinidin-3-O-glycosides and pelargonidin-3-O-glycosides at (3.18, 6.54 µg/gm), respectively in callus pitaya species *H. polyrhizus*. Several factors could affect anthocyanins accumulation during culturing on media containing SeNPs such as the type of plant tissue, culture conditions, age, temperature, and exposure to salinity.

Evaluation of lung anticancer activity

The anticancer activities promoted by plant extracts of various species belonging to the *cactaceae* family were previously demonstrated against PC3 prostate and breast Mcf7-7 cancer cells [24], and treatment with SeNPs enhanced the active compounds in the *Opuntia*

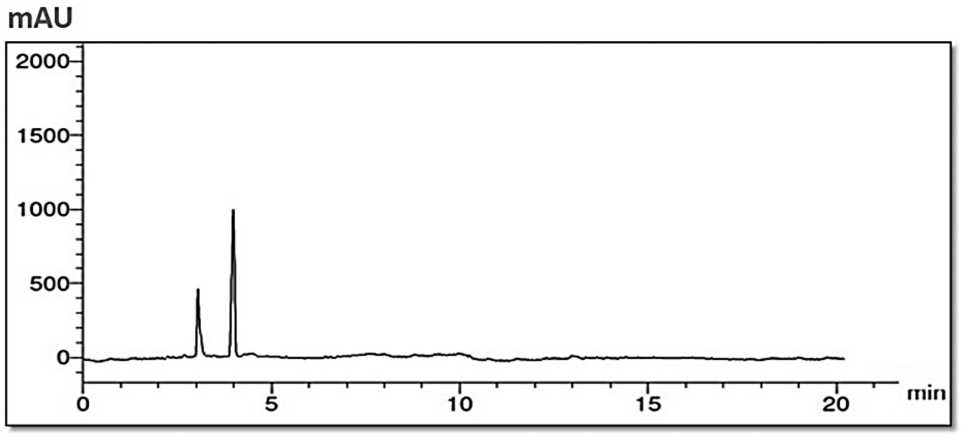
Figure 6



RT	Compound	Concentration (µg/gm)
3.0	Delphinidin-3-O-glycosides	4.66
8.0	peonidin-3-O-glycosides	10.85
10.0	cyanidin-3-O-glycosides	13.79

HPLC analysis of Anthocyanins on the extract of the. Induced callus of pitaya species *Hylocereus costaricensis*.

Figure 7



RT	Compound	Concentration (µg/gm)
3.0	Delphinidin-3-O-glycosides	3.18
4.0	pelargonidin-3-O-glycosides	6.54

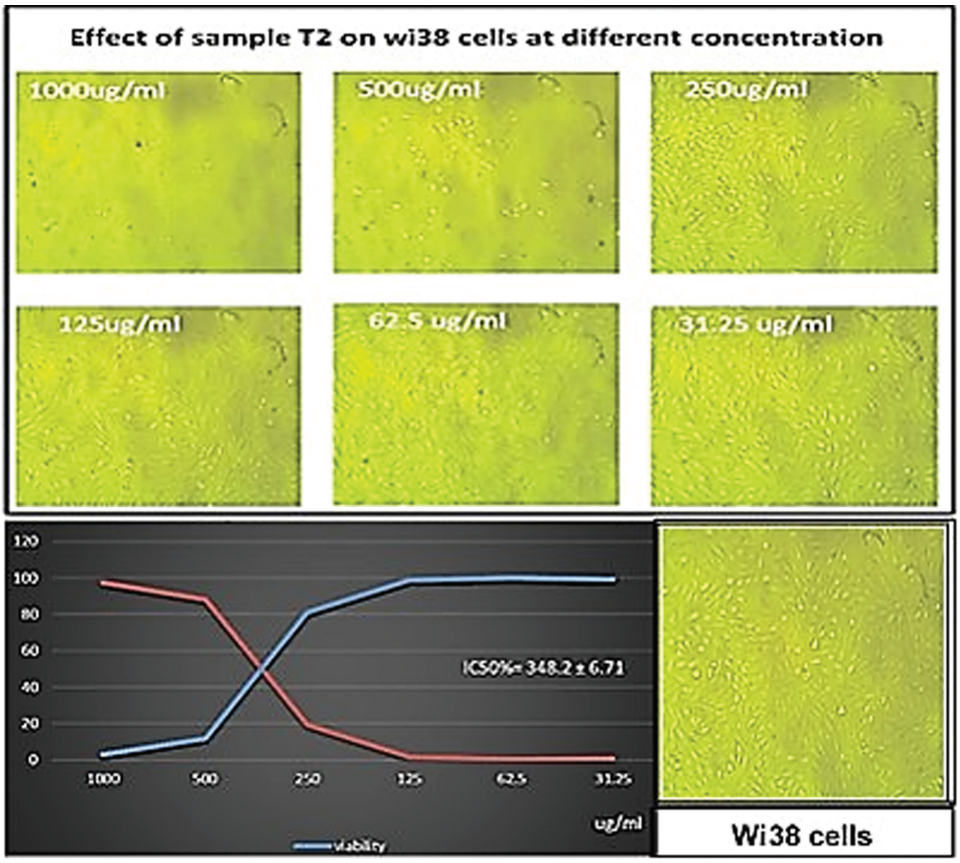
HPLC analysis of Anthocyanins on the extract of the. Induced callus of pitaya species. *Hylocereus polyrhizus*.

cladodes against lung cancer A549 cells [25]. Anthocyanins have shown various anti-toxic and anti-carcinogenic properties, including: directly neutralizing reactive oxygen species (ROS), enhancing the oxygen-radical absorption capability of cells, promoting the expression of Phase II detoxification enzymes, diminishing the formation of oxidative adducts in DNA, lowering lipid peroxidation, hampering mutagenesis caused by environmental toxins and carcinogens, and curtailing cellular growth by influencing signal transduction pathways. The majority of the protective effects of Anthocyanins are conjugated to the capacity to scavenge ROS and also act through chelating metals and binding to proteins directly [26,27]. Pitaya is associated with several bioactive compounds such as phenolic acids, flavonoids, and betacyanin, especially betacyanin [28]. These compounds have antioxidant and anti-inflammatory properties that may help prevent some oxidative stress-related diseases associated with malignancies [29]. The effects of callus extracts on the viability and proliferation/migration of wi38 lung cancer cells were used to evaluate the enhanced active anthocyanin compounds as potential lung cancer agents. The effects of sample T2 (pitaya callus extract species *H. costaricensis* containing the bioactive anthocyanins (delphinidin-3-O-glycosides, peonidin-3-O-glycosides and cyanidin-3-O-glycosides) (Fig. 6) on wi38 cells, the wi38 cells were evaluated using MTT assay (Table 2) treated with different concentrations; wi38 with an IC50 value of 348.2±6.71 µg/ml (Fig. 8) from *Oryza sativa L. indica* were investigated as treatment options for various cancer cells, among which the breast cancer cell

Table 2 Effect of various concentrations of Pitaya callus extracts on viability and proliferation/migration of wi38 cells

ID	ug/ml		O.D		Mean O.D	±SE	Viability %	Toxicity %	IC50±SD
Wi38	–	0.712	0.705	0.719	0.712	0.004041	100	0	ug
T2	1000	0.018	0.02	0.019	0.019	0.000577	2.668539326	97.33146067	
	500	0.075	0.094	0.082	0.083667	0.005548	11.75093633	88.24906367	
	250	0.548	0.597	0.584	0.576333	0.014655	80.94569288	19.05430712	348.2±6.71
	125	0.709	0.711	0.685	0.701667	0.008353	98.54868914	1.451310861	
	62.5	0.72	0.709	0.705	0.711333	0.004485	99.90636704	0.093632959	
	31.25	0.717	0.701	0.703	0.707	0.005033	99.29775281	0.702247191	
T3	1000	0.019	0.018	0.018	0.018333	0.000333	2.574906367	97.42509363	309.54±2.69
	500	0.063	0.066	0.08	0.069667	0.005239	9.784644195	90.21535581	
	250	0.376	0.396	0.388	0.386667	0.005812	54.3071161	45.6928839	
	125	0.701	0.694	0.711	0.702	0.004933	98.59550562	1.404494382	
	62.5	0.716	0.71	0.709	0.711667	0.002186	99.95318352	0.046816479	
	31.25	0.711	0.702	0.715	0.709333	0.003844	99.62546816	0.374531835	

Figure 8



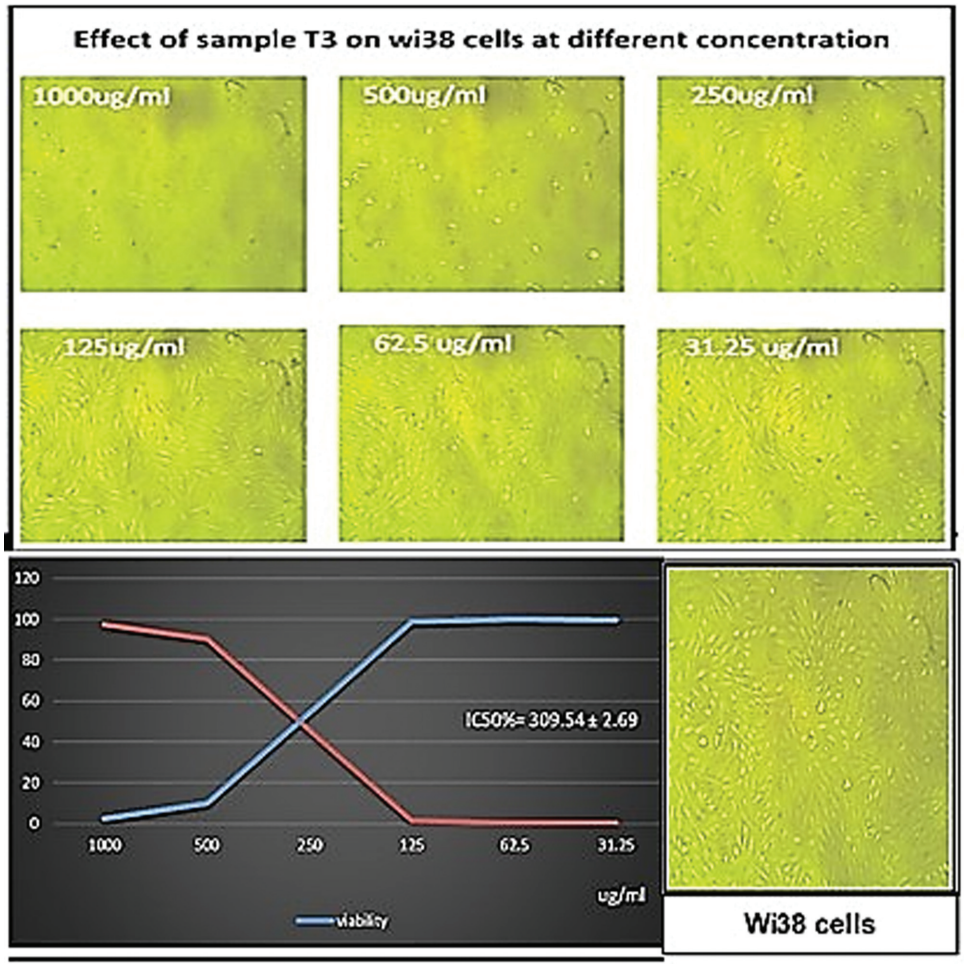
Effects of Different Concentrations of T2 sample (induced pitaya callus extract sp. *Hylocereus costaricensis* on the viability, proliferation/ migration of lung cancer cells wi38.

line HS578T was analyzed with the greatest sensitivity to Peonidin-3-glucoside and cyanidin-3-glucoside. Notably, a strong inhibitory effect was observed when treated with the bioactive anthocyanins peonidin-3-glucoside or cyanidin-3-glucoside drew attention to cell growth through G2/M arrest [30].

Treatment with peonidin-3-glucoside resulted in downregulation of the levels of cyclin-dependent kinase (CDK)-1, CDK-2, cyclin B1, and cyclin E, meaning that cyanidin-3-glucoside decreased the protein levels of CDK. 1, CDK-2, cyclin B1, and cyclin D1, moreover, both bioactive anthocyanins induced the activation of caspase-3 activation, condensation of chromatin leading to cell death [31].

It is noteworthy that these bioactive anthocyanins have an inhibitory effect on the growth of Lewis lung carcinoma cells in the in vivo test. Sample T3 (pitaya callus extract species *H. polyrhizus*) was found to contain anthocyanins (delphinidin-3-O-glycosides and pelargonidin-3-O-glycosides) (Fig. 7), whose proliferative effect on wi38 cells was also estimated using same MTT assay at different concentrations (Table 2). The results showed that the growth inhibition of wi38 IC50 was 309.54±2.69 µg/ml (Fig. 9). Delphinidin acts as a VEGF inhibitor in vascular smooth muscle cells stimulated by PDGF by inhibiting the p38 MAPK and JNK signaling pathways blocked. Delphinidin has antiangiogenic properties in lung cancer cells and reduces the depression of mRNA of $CoCL_2$ and epidermal growth factor, which cause the induction of hypoxia-inducible factor 1 (HIF). The hypoxia response element promoter was blocked by H1F-1, which inhibited VEGF expression [30].A possible method could be to attempt to inhibit signaling via ERK and PI3K/Akt/mTOR/p70S6K signaling pathways, which lead to angiogenesis and inhibition of tumor growth. These new promising results highlight the importance of using SeNPs at specific concentrations as triggers of bioactive compounds that target specific cancer cells and are natural extracts without side effects for the treatment of lung cancer diseases

Figure 9



Effects of Different Concentrations of T3 sample (induced pitaya callus extract sp. *Hylocereus polyrhizus*. on the viability, proliferation/ migration of lung cancer cells wi38.

Conclusion

This study focused on the establishment of new protocols for callus induction and enhancement of bioactive anthocyanins via beneficial properties of SeNPs to develop lung anticancer agents. HPLC analysis of the induced callus extracts of pitaya species *H. polyrhizus*, and *H. costaricensis* determined four various bioactive anthocyanins are known for their lung anticancer activity (Delphinidin-3-O-glycosides, peonidin-3-O-glycosides, pelargonidin-3-O-glycosides and cyanidin-3-O-glycosides). Their intervention positively affects the evaluation assay of lung cancer cell wi38, resulting in growth inhibition of wi38 cells with IC50 of 348.2±6.71 µg/ml in sample T2 (Induced callus extract species *H. costaricensis*) and growth inhibition of wi38 cells was IC50 of 309.54 ±2.69 µg/ml in sample T3 (Induced callus extract species *H. polyrhizus*).

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Author contribution statement: All the authors conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools, or data; wrote the paper.

Conflicts of interest

There are no conflicts of interest.

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