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ORIGINAL PAPER

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CYTOTOXICITY OF SAPONIN NANOPARTICLES FROM QUINOA (*CHENOPODIUM QUINOA* WILLD.) IN THE MC-F7 CELL LINE

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ABSTRACT

Background. The effect of saponin nanoparticles from *Chenopodium quinoa* Willd. on MC-F7 (breast cancer) cell lines was evaluated. The ionic gelation method with chitosan and pentasodium tripolyphosphate was used to synthesize nanoparticles. Saponins were extracted from quinoa industry residues using ultrasound.
Materials and methods. The effect of pH (5, 6, 7) and pentasodium tripolyphosphate concentration (1, 2 and 3 mg/mL) was evaluated; the treatment with pH 6 and pentasodium tripolyphosphate concentration of 3 mg/mL obtained the best encapsulation efficiency. The nanoparticles had a Z potential of –32.9 V, a polydispersity of 0.282, and a particle size of 758.75 nm. The cytotoxicity of these nanoparticles formed in MC-F7 cells was tested and an IC_{50} value of 4.5 ug/mL was obtained.
Conclusion. The in vitro cytotoxic activity of *Chenopodium quinoa* Willd. saponin nanoparticles in MCF-7 cells was demonstrated using chitosan and pentasodium tripolyphosphate as a cross-linking material at a concentration of 3 mg/mL and pH 6, with an IC_{50} value of 4.5 mg/mL. These findings suggest its potential high bioactivity, which should be followed by further in vitro studies on cellular and molecular effects on cancer and normal cells.

Keywords: ionic gelation, saponin, nanoparticles, anticancer

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.), cultivated in Peru and various other countries in Latin America, is renowned for its nutritional attributes. Nevertheless, the quinoa seed is equipped with an outer layer that necessitates removal before being made available for consumption, as it is characterized by a bitter flavour (Bonfiglio et al., 2020), and additionally impacts the digestibility of the grain (Jiang et al., 2021). Nevertheless, the significance of this industrial waste lies in its various functional attributes, such as amphiphilic, anticarcinogenic, hepatoprotective, and antioxidant properties, among others (Sharma et al., 2023). Consequently, it offers an alternative approach for the treatment and prevention of cancer, the underlying mechanisms of which are currently being elucidated (Tan et al., 2022; El Hazzam et al., 2020).

Twenty triterpene saponins have been identified in quinoa in different parts of the plant, among which the following are found in the highest concentrations: 3β-[(O-β-d-glucopyranosyl-(1→3)-α-l-

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-arabinopyranosyl)oxy]-23-oxo-olean-12-en-28-oic acid β-d-glucopyranoside, 3β-[(O-β-d-glucopyranosyl-(1→3)-α-l-arabinopyranosyl)oxy]-27-oxo-olean-12-en-28-oic acid β-d-glucopyranoside, 3-O-α-l-arabinopyranosyl serjanic acid 28-O-β-d-glucopyranosyl ester, and 3-O-β-d-glucuronopyranosyl organic acid 28-O-β-d-glucopyranosyl ester (Kuljanabhagavad et al., 2008). These in turn were tested on Caco-2 cancer cells, with good inhibitory characteristics (Taherian et al., 2021). In the present work, it was tested on breast cancer cells, considered one of the main types of cancer that causes death. Treatment often involves a combination of chemotherapy, radiotherapy, and other modalities; nevertheless, challenges arise from issues like non-specific targeting, cytotoxicity, and the development of resistance to multiple drugs, thereby complicating efforts to enhance cancer therapy. Nanoparticles present a promising alternative for treating cancer due to their advantageous characteristics, including biocompatibility, reduced toxicity, stability, enhanced permeability, prolonged retention, and the ability to precisely target specific areas. The application of nanotechnology to optimize drug delivery for both cancer treatment and detection has attracted attention in several preclinical investigations (Taherian et al., 2021). The synthesis of nanoparticles utilizing chitosan is prevalent, given its status as a natural polymer. Chitosan, a linear polysaccharide derived from the deacetylation of chitin, is widely utilized for nanoparticle production due to its abundance, cost-effectiveness, biodegradability, and natural origins (Zaboon et al., 2021). Chitosan and its derivatives show good anticancer activity (Chen and Gong, 2016); the successful application of cancer therapy and the reduction of non-specific harmful effects are attributed to the gradual release of the drug. Nevertheless, a comprehensive investigation into the formation of nanocapsules is imperative due to variations in the chemical compositions of the encapsulated agent, warranting a detailed examination of nanoparticle formation. Chitosan, being a cationic polyelectrolyte, exhibits responsiveness to external stimuli such as pH, temperature, and ionic strength of the medium. This responsiveness allows for enhanced sensitivity of the excipients via ionic gelation, leading to the crosslinking of polymeric chains structured in nanoforms through intermolecular interactions of either covalent or non-covalent nature. The facilitation of such crosslinking is achieved through the utilization of crosslinking agents that enable the formation of this linkage (Mohammed et al., 2013).

This study aimed to determine the effect of pH and concentration of pentasodium tripolyphosphate on the formation of saponin nanoparticles from *Chenopodium quinoa* Willd. and to test their effect on the viability of MCF-7 (breast cancer) cell lines.

EXPERIMENTAL PROCEDURE

Materials and chemicals

Residues obtained from the dry scarification of quinoa grains of the Hualhuas variety, which were provided by a local company, were used. They were collected in polyethylene bags, sealed and labelled, and taken to the laboratory immediately. The samples were dried at a temperature of 40°C for 12 hours to a humidity of 10%. Then the samples were homogenized to a size of about 0.4 mm.

The reagents and culture media were DMEM (Dulbecco's Modified Eagle's Medium, Cellgro, Herndon Sigma), glutamine (Sigma-Aldrich, St. Louis, USA), fetal bovine serum (FBS, SIGMA), gentamicin (Sigma-Aldrich, St. Louis, USA), trypan blue (Sigma-Aldrich, St. Louis, USA), medium molecular weight chitosan (Sigma-Aldrich), and pentasodium Tripolyphosphate (Sigma-Aldrich).

Extraction and quantification of saponins

Saponin extraction was carried out using a laboratory ultrasonic apparatus UP 100H (Hielscher Ultrasound Technology, Teltow, Germany), with an amplitude of 59% and an ethanol concentration of 70% for 12 minutes. Quantification was carried out by hydrolysis of saponin and quantification of oleanolic acid by the following methods (Espinoza et al., 2021).

Preparation of saponin-loaded nanoparticles

It was prepared by the ionic gelation method using pentasodium tripolyphosphate (Rejinold et al., 2011) with some modifications. 5 mg of dehydrated saponin was suspended in 1 ml of ethanol and chitosan and added to chitosan solution (50 mg in 5 ml of 1% acetic acid) and shaken at 500 rpm for 5 min. The whole system was mixed with pentasodium tripolyphosphate

solution at different concentrations of 1, 2, and 3 mg/mL and pHs of 5, 6 and 7 (adjusted with 0.1 N hydrochloric acid). It was then agitated for 20 min at 500 rpm. The nanoparticle suspension was centrifuged at 12,000 rpm for about 45 min and the residue was resuspended in Milli-Q water and dehydrated by lyophilization.

Encapsulation efficiency (EE)

To determine the encapsulation efficiency, the formed nanoparticles were dissolved in ethanol, centrifuged at 30,000 rpm and the supernatant was separated for the determination of saponins (Rejinold et al., 2011). The following formula was used to calculate the encapsulation efficiency (EE).

$$EE = \frac{\text{Solution total saponins}}{\text{Total supernatant saponins}} \times 100$$

Determination of the z potential, polydispersity, and particle size

This was carried out on a Brookhaven DLS model 90Plus instrument using BIC Zeta Potential Analyser software. A pre-treatment was performed by preparing a dispersion (100 mL) of 0.02% chitosan nanocapsules loaded with quinoa saponin with a 1 mM KCl solution. The dispersion was then prepared by sonication for 30 min at a temperature of 25°C, pH 6. It was analysed with laser light at 659 nm for an analysis time of 1.30 min.

The analysis was carried out with the best encapsulation treatment.

Cytotoxicity test

Saponin nanoparticles were evaluated in the MCF-7 cell line (breast cancer) (Chaudhary et al., 2015). Cells were grown in DMEM (Dulbecco's modified Eagle's medium) containing 2 mL glutamine, 10% fetal bovine serum (FBS), and 50 mg/L gentamicin. Cell numbers were quantified by taking 10 uL of cell suspension, diluting them with 90 uL trypan blue (0.4%), counting them in a Neubauer chamber, and diluting them to 5×105 cells/mL. Subsequently, 5,000 cells/well were added to a volume of 100 µL DMEM medium in 96-well microplates (Corning Inc. Costar). These plates were incubated for 24 h at 37°C in a humid atmosphere and 5% CO_2 in a culture incubator. Dilutions of the extracts were then prepared in DMEM to a final concentration of 6, 12, 24, 48, and 96 µg/mL, 50 µL/well of each dilution was added and the assay was performed in quadruplicate. DMSO was used as a negative control and 5-FU (fluorouracil – chemotherapeutic drug) as a positive control. Cytotoxicity was assessed individually in a similar manner to the extracts, and the microplates were incubated for 72 hours in a 5% CO_2 atmosphere at 37°C. After 72 h, 20 µL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium salt was added. These microplates were incubated for 72 h in a 5% CO_2 atmosphere at 37°C. The medium was decanted, 200 µL/well of DMSO was added, and the optical density (OD) was read on an ELISA microplate reader at wavelengths of 560 nm and 630 nm as ref.

Statistical analysis

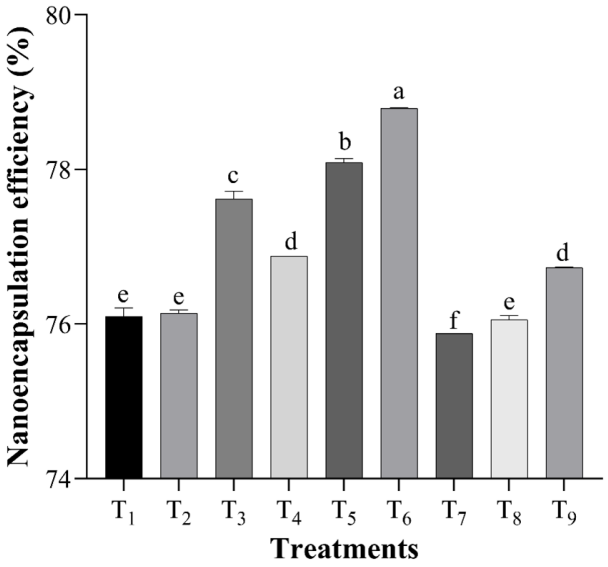
The Completely Randomised Design (CRD) was used with a factorial arrangement, three replicates, 5% significance level ($p < 0.05$), where the independent variables in the case of nanoencapsulation were pH and pentasodium tripolyphosphate concentration, and the dependent variable was encapsulation efficiency. The Minitab 16 program was used for the statistical evaluation of the data obtained and subsequent hypothesis testing.

RESULTS AND DISCUSSION

Encapsulation efficiency

Figure 1 shows the results of saponin encapsulation efficiency at different pH and concentrations of pentasodium tripolyphosphate. It was observed that there is a higher efficiency at pH 6 and 3 mg/mL of pentasodium tripolyphosphate.

The results obtained followed a normal distribution ($p > 0.05$), so the analysis of variance was performed, and the results showed a *p*-value of less than 0.05 for the model, pH, and concentration of pentasodium tripolyphosphate. The Tuckey test with a confidence interval of 95% shows that there is a significant difference between the treatments and that the highest encapsulation efficiency was obtained at a pH of 6 and a pentasodium tripolyphosphate concentration of 3 mg/ml. It is observed that varying the pH and the concentration



T_1 = pH 5, ccTPP 1 mg/mL; T_2 = pH 5, ccTPP 2 mg/mL; T_3 = pH 5, ccTPP 3 mg/mL; T_4 = pH 6, ccTPP 1 mg/mL; T_5 = pH 6, ccTPP 2 mg/mL; T_6 = pH 6, ccTPP 3 mg/mL; T_7 = pH 7, ccTPP 1 mg/mL; T_8 = pH 7, ccTPP 2 mg/mL; T_9 = pH 7, ccTPP 3 mg/mL.

Fig. 1. Encapsulation efficiency of saponin at different pH and concentration of pentasodium tripolyphosphate

of pentasodium tripolyphosphate varies the encapsulation efficiency. This is supported by (Mohammed et al., 2013), who discuss the properties of chitosan and note that it is a cationic polyelectrolyte known for its bioactive characteristics and its ability to react to various external stimuli such as pH, temperature, and ionic strength within the surrounding environment. This polymer has been shown to enhance the reactivity of excipients, leading to the creation of nanocapsules through a process known as ionic gelation. This method involves the linking of polymeric chains in an ordered manner to form nanostructures, facilitated by interactions that can either be covalent or non-covalent. The formation of these nanocapsules is achieved through the use of cross-linking agents that aid in the bridging process (Dong et al., 2013). As with pentasodium tripolyphosphate, which is negatively charged, this property explains why it can be used to prepare cross-linked chitosan nanoparticles. This is confirmed by Hashad et al. (2016), who mention that chitosan is a hydrophilic polycationic polymer, which gels spontaneously at pH 7 in the presence of polyanions such as pentasodium tripolyphosphate (TPP) to form submicron particles. In that study, results similar to those found in the present work were reported, in which it was found that increasing the pH of the chitosan and TPP solution increased the encapsulation efficiency, due to the reduction of repulsive electrostatic forces and the strengthening of H-bonds between chains. Also, (Al-nemrawi et al., 2018) and (Grehna et al., 2008) reported similar results concerning the concentration of pentasodium tripolyphosphate, mentioning that as the amount of TPP increased, the nanoparticle suspension became increasingly turbid due to the formation of chitosan nanoparticles. When the amount of TPP was very low, the mixture was transparent and viscous like a pure chitosan solution, possibly because the amount of TPP was not sufficient to form a cross-linked chitosan structure (Al-nemrawi et al., 2018). It was also found that as the amount of pentasodium tripolyphosphate increased above 1.5 mg, the NPs started to form, and as the amount of TPP increased, the particle size decreased. Consequently, we can say that the size is related to the concentration of pentasodium tripolyphosphate. This may be due to the increased inter- and intra-crosslinking between chitosan and TPP. The pH level significantly influences the chemical properties of chitosan and pentasodium tripolyphosphate (TPP).

Chitosan shows the highest radiolabel performance at pH = 9.3–10.4, while water-soluble chitosan shows the highest performance at pH > 5 (Kamali et al., 2022). The formation and protein encapsulation efficiency of chitosan nanoparticles is affected by pH, with smaller particles formed at lower pH and higher encapsulation efficiency observed at higher pH (Nejabat et al., 2022). Pentasodium tripolyphosphate (TPP) also plays a crucial role in the cross-linking of chitosan. The pH of the TPP solution influences the properties of chitosan nanoparticles, such as size, yield, and encapsulation efficiency (Nguyen et al., 2023).

Physical characterisation of nanoparticles loaded with saponins

Table 1 and Figure 2 show the results of the physical characterization of saponin nanoparticles by ionic gelation using chitosan and pentasodium tripolyphosphate.

It was observed that the developed nanoencapsulation method was successfully applied to obtain saponin-loaded nanoparticles with an average diameter of 58.75 nm.

Table 1. Physical properties of saponin nanoparticles obtained by the ionic gelation method

Physical characteristics	Result
Potential Z	–32.9 V
Polydispersity	0.282
Average particle size	58.75 nm

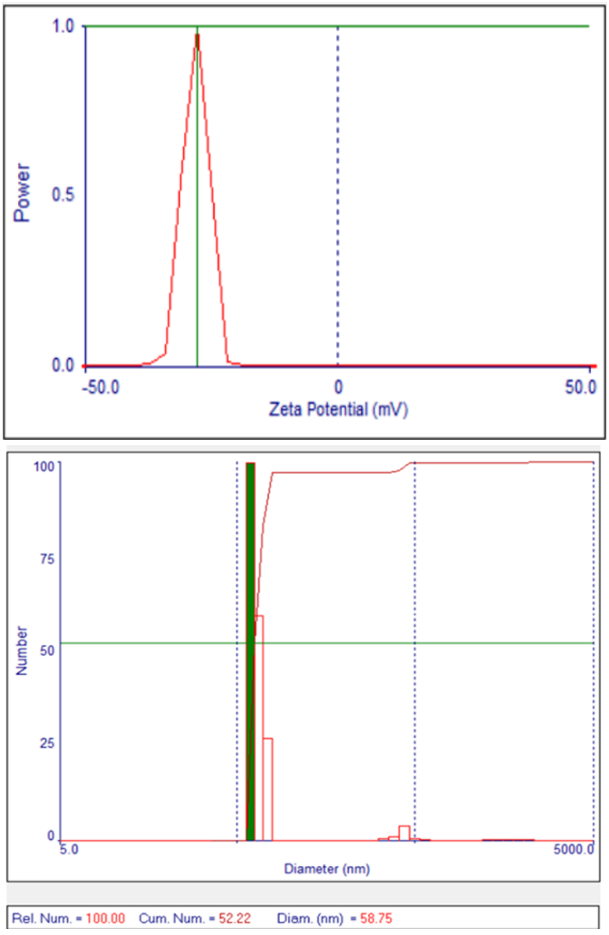


Fig. 2. Dynamic Light Scattering (DLS) z-potential and particle size

The relationship between zeta potential (ζ), particle size, and polydispersibility of nanoparticles significantly influences the stability and performance of nanoparticles. The zeta potential reflects the surface charge and plays a crucial role in colloidal stability (Sengottiyan et al., 2023). Particle size affects viscosity and stability. Smaller nanoparticles improve stability and increase viscosity (Chakraborty and Panigrahi, 2020). The polydispersibility of nanoparticles, which is influenced by factors such as the power of the sonication, affects aggregation and stability, and low-power sonication is recommended to maintain stability (Toropov et al., 2018). Computational models using structural descriptors can predict the potential and stability of Zeta, which helps in the initial assessment of biological results (Payzullaev et al., 2023). These interrelationships are essential for optimizing nanoparticle properties such as aggregation state, composition, shape, and surface chemistry to improve stability and performance in various applications (Phan and Haes, 2019).

The obtained value of polydispersity is a low parameter and an index that gives information about the sample: values close to 0 indicate that the sample is monodisperse, whereas values close to 1 indicate that the sample presents a great variety of sizes; the results shown for the saponin nanoparticles show a low value; therefore, we can say that the uniformity of the particles is good (Rodríguez and Zea, 2014).

Saponin nanoparticle cytotoxicity

After obtaining the saponin-loaded nanoparticles, the cytotoxicity assay was performed on MCF-7 cells using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method, which is based on the ability of mitochondrial enzymes in viable cells to convert MTT into blue crystals known as formazan. Table 2 shows the viability of MC-F-7 cells treated with nanoencapsulated saponins at different concentrations.

Table 2. Viability of the MCF-7 cells after treatment with nanoencapsulated saponins different concentrations

Saponin concentration ug/mL	Viability
6.1	25.23 ±0.12
12.2	15.75 ±0.12
24.4	10.51 ±0.12
48.8	7.29 ±0.06
97.6	5.24 ±0.06
Negative control	100

Viability was measured as a function of the negative control without extract, taken as 100%. The lowest cell viability was observed at a saponin concentration of 97.6 mg/mL and the IC_{50} value was determined.

IC_{50} determination

The percentage cell viability values were plotted, and the doce-response relationship curve was established to determine the half-maximal inhibitory concentration (IC_{50}) value.

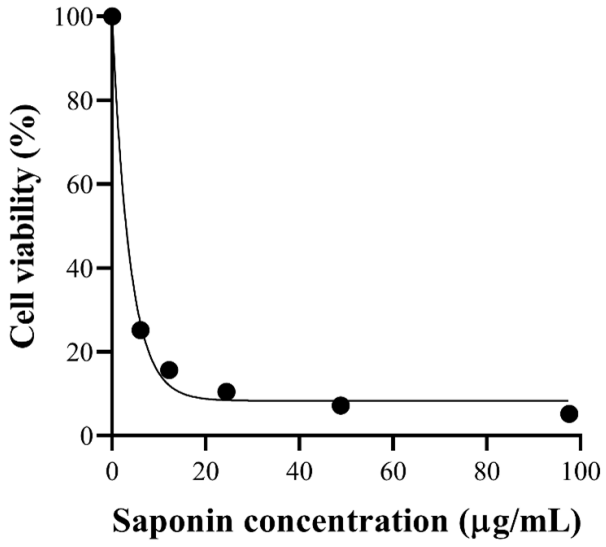


Fig. 3. Dose-response relationship curve plotted for saponins and MCF-7 cells.

The IC_{50} value calculated was 4.5 ug/mL. The value found is low, compared to other bioactive compounds (Grewal et al., 2022). (Podolak et al., 2022) explained the characteristic amphiphilic activity of the triterpene saponins. Espinoza et al. (2021) identified the sapogenins in the applied extract, consisting mainly of oleanolic acid, hedegerin, and sejanic acid. The carbohydrate chain has an effect on membrane permeability (Woldemichael et al., 2001) explained by its amphiphilic structure (Zouaoui et al., 2018). The lipophilic triterpene aglycone allows the saponins to enter membranes, while the carbohydrate chain unit helps the molecule to bind to extracellular glycolipids and glycoproteins. They can interact with membrane lipids and cholesterol as active molecules on the surface of cells, breaking them up and destabilizing the membrane. Similarly, Podolak et al. (2022) mention that the main component present in this type of quinoa saponin, oleanolic acid, affects other cancer cell lines (Podolak et al., 2022).

Work on saponin cytotoxicity in other cell lines has shown that saponin nanoparticles significantly affect several cell lines in different studies. However, the activity of saponins is highly dose-dependent, and higher doses may induce significant toxic effects on normal cells. Saponin-based nanoemulsions of vitamins A and E showed protective effects against oxidative stress-induced cell damage in AML-12 hepatocytes isolated from the normal liver (Salvioni et al., 2022). In addition, green tea saponins induced cytotoxic effects on cancer cells by promoting apoptosis and anti-angiogenesis, while exhibiting antioxidant and anti-inflammatory properties (Elekofehinti et al., 2021). Quillaja saponina saponaria saponin fractions formulated into nanoparticles showed cytotoxic activity against cancer cells, inducing cell cycle arrest, differentiation, and apoptosis, with potential as anticancer agents (Choudhry et al., 2016). Furthermore, saponin-conjugated colloidal nanoparticles, such as those with the saponin, exhibited selective cytotoxicity against cancer cell lines through functional ribosomal inactivation, leading to apoptosis, while sparing healthy fibroblasts (Hu et al., 2010).

CONCLUSIONS

The in vitro cytotoxic activity of *Chenopodium quinoa* Willd. saponin nanoparticles in MCF-7 cells was demonstrated using chitosan and pentasodium tripolyphosphate as cross-linking material at a concentration of 3 mg/mL and pH 6, with an IC_{50} value of 4.5 mg/mL, positioning it as a potential bioactive, thus it is recommended to continue carrying out more in vitro and in vivo studies.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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REFERENCES

Zouaoui, S. A., Megherbi-Benali, A., Toumi B. F., Ouaar, D. (2018). Contribution à l'étude du pouvoir antifongique des graines du *Chénopodium quinoa Wild* vis-à-vis de deux champignons phytopathogène de l'orge: *Pyrenophora tritici-repentis et Rhynchosporium secalis*. Bulletin de la Société Royale des Sciences de Liège [En ligne], 87, 100–111. DOI: 10.25518/0037-9565.8127

Al-nemrawi, N. K., Alsharif, S. S. M., Dave, R. H. (2018). Preparation of Chitosan-Tpp Nanoparticles: The Influence of Chitosan Polymeric Properties and Formulation Variables. Int. J. Appl. Pharmac., 10, 60. https://doi.org/10.22159/ijap.2018v10i5.26375

Bonfiglio, G.V., Wierna, R. V., Bonini, N. A., Armada, M., Goldner, C. (2020). Study of bitterness perception of quinoa (*Chenopodium quinoa* Willd.) saponin extracts. J. Cer. Sci., 95(June), 103032. https://doi.org/10.1016/j.jcs.2020.103032

Grenha A., Remuñán-López, C., Carvalho E. L., Seijo, B. (2008). Microspheres containing lipid/chitosan nanoparticles complexes for pulmonary delivery of therapeutic proteins. Eur. J. Pharm. Biopharm.,69(1), 83–93. https://doi.org/10.1016/j.ejpb.2007.10.017

Chakraborty, S., Panigrahi, P. K. (2020). Stability of Nanofluid: A Review. Appl. Therm. Eng., 174, 115259. https://doi.org/10.1016/j.applthermaleng.2020.115259

Chaudhary, S., Chandrashekar K. S., Karkala Pai, S. R., Manganahalli, Setty, M. M., …, Shoja, M. H. (2015). Evaluation of Antioxidant and Anticancer Activity of Extract and Fractions of Nardostachys Jatamansi DC in Breast Carcinoma. BMC Complement. Altern. Med., 15(1), 1–13. https://doi.org/10.1186/s12906-015-0563-1

Chen, G., Gong, R. (2016). Study on fluorouracil-chitosan nanoparticle preparation and its antitumor effect. Saudi Pharm. J., 24(3), 250–53. https://doi.org/10.1016/j.jsps.2016.04.008

Choudhry, Q. N., Kim, M. J., Kim, T. G., Pan, J. H., Kim, J. H., …, Kim, Y. J. (2016). Saponin-Based Nanoemulsification Improves the Antioxidant Properties of Vitamin A and E in AML-12 Cells. Int. J. Mol. Sci., 17, 1406. https://doi.org/10.3390/ijms17091406

Dong, Y., Ng, W. K., Shen, S., Kim, S., Tan, R. B. H. (2013). Scalable Ionic Gelation Synthesis of Chitosan Nanoparticles for Drug Delivery in Static Mixers. Carbohydr. Polym., 94(2), 940–45. https://doi.org/10.1016/j.carbpol.2013.02.013

Elekofehinti, O. O., Iwaloye, O., Olawale, F., Ariyo, E. O. (2021). Saponins in Cancer Treatment: Current Progress and Future Prospects. Pathophysiol., 28(2), 250–72. https://doi.org/10.3390/pathophysiology28020017

Espinoza, C. R., Jaime Ruiz, C. A., Flores Ramos, O. P., Quispe Solano, M. A., Hinostroza Quiñonez, G., Saavedra Mallma, N. E. (2021). Optimization of the ultrasoud-assisted extraction of saponins from quinoa (*Chenopodium quinoa* Willd.) using response surface methodology. Acta Sci. Pol. Technol. Aliment., 20(1), 17–23. http://dx.doi.org/10.17306/J.AFS.2021.0859

Grewal, J., Kumar V., Rawat, H., Gandhi, Y., Singh, R., …, Mishra, S. K. (2022). Cytotoxic Effect of Plant Extract-Based Nanoparticles on Cancerous Cells: A Review. Environ. Chem. Lett., 20(4), 2487–2507. https://doi.org/10.1007/s10311-022-01422-z

El Hazzam, K., Hafsa, J., Sobeh, M., Mhada, M., Taourirte, M., Kacimi, K. E. L., Yasri, A. (2020). An Insight into Saponins from Quinoa (*Chenopodium Quinoa* Willd.): A Review. Molecul., 25(5), 1–22. https://doi.org/10.3390/molecules25051059

Hu, K., Berenjian, S., Larsson, R., Gullbo, J., Nygren, P., Lövgren, T., Morein, B. (2010). Nanoparticulate Quillaja Saponin Induces Apoptosis in Human Leukemia Cell Lines with a High Therapeutic Index. Int. J. Nanomed., 5(1), 51–62. https://doi.org/10.2147/IJN.S7879

Jiang, F., Ren, Y., Du, C., Nie, G., Liang, J., Yu, X., Du, S.-k. (2021). Effect of pearling on the physicochemical properties and antioxidant capacity of quinoa (*Chenopodium quinoa* Willd.) Flour. J. Cereal Sci., 102(April). https://doi.org/10.1016/j.jcs.2021.103330

Kamali, N. D., Alishahi, A., Heidarieh, M., Rajabifar, S., Mirsadeghi, H., Kordjazi, M. (2022). Effect of PH Variation on Cross-Linking of Water-Soluble and Acid-Soluble Chitosan with Sodium Tripolyphosphate and Gallium-67. Curr. Radiopharmac.,15(3), 249–255. DOI: 10.2174/1874471015666211220094433

Kuljanabhagavad, T., Thongphasuk, P., Chamulitrat, W., Wink, M. (2008). Triterpene saponins from *Chenopodium quinoa* Willd. Phytochem., 69(9), 1919–26. https://doi.org/10.1016/j.phytochem.2008.03.001

Hashad, R. A., Ishak, R. A., Fahmy, S., Mansour, S., Geneidi, A. S. (2016). Chitosan-tripolyphosphate nanoparticles: Optimization of formulation parameters for improving process yield at a novel pH using artificial neural networks. Int. J. Biol. Macromol., 86, 50–58. https://doi.org/10.1016/j.ijbiomac.2016.01.042

Mohammed, M. H., Williams, P. A., Tverezovskaya, O. (2013). Food Hydrocolloids Extraction of Chitin from Prawn Shells and Conversion to Low Molecular Mass Chitosan. Food Hydrocoll., 31(2), 166–171. https://doi.org/10.1016/j.foodhyd.2012.10.021

Nejabat, M., Kalani, M. R., Nejabat, M., Hadizadeh, F. (2022). Molecular dynamic and in vitro evaluation of chitosan/tripolyphosphate nanoparticles as an insulin delivery system at two different pH values. J. Biomolec. Struct. Dynam., 40(20), 10153–10161. https://doi.org/10.1080/07391102.2021.1940280

Nguyen, T. A., Bui, H. T., Dang, V. B. H., Trinh, T. B. H., Le, T. H. T., Nguyen, V. T. (2023). Sequent Adsorption of Phosphate Ions by Copper Ions Adsorbed on Tripolyphosphate Chitosan. Orient. J. Chem., 39(2), 295–302. http://dx.doi.org/10.13005/ojc/390209

Payzullaev, A. N., Allaev, B. A., Mirzaev, S. Z., Abdiev, J. M., Urinov, J., Parkash, A. (2023). The Impact of Silicon Dioxide Nanoparticle Size on the Viscosity and Stability of Nanofluids: A Comprehensive Study. ECS Adv., 2(3), 031001. DOI 10.1149/2754-2734/ace121

Phan, H. T., Haes, A. J. (2019). What Does Nanoparticle Stability Mean? J. Phys. Chem. C, 123(27), 16495–16507. DOI: 10.1021/acs.jpcc.9b00913

Podolak, I., Grabowska K., Sobolewska D., Wróbel-Biedrawa D., Makowska-Wąs, J., Galanty, A. (2022). Saponins as Cytotoxic Agents: An Update (2010–2021). Part II – Triterpene Saponins. Phytochem. Rev., 22, 113–167. https://doi.org/10.1007/s11101-022-09830-3

Rejinold, N. S., Muthunarayanan, M., Muthuchelian, K., Chennazhi, K. P., Nair, S. V., Jayakumar, R. (2011). Saponin-Loaded Chitosan Nanoparticles and Their Cytotoxicity to Cancer Cell Lines in Vitro. Carbohydr. Polym., 84(1), 407–416. https://doi.org/10.1016/j.carbpol.2010.11.056

Rodríguez, A. L., Zea, H. R. (2014). Modificación Del Proceso de Reducción Expansiva Para La Síntesis de Nanopartículas de Hierro. Univ. Sci.,19(2), 407–416. https://doi.org/10.1016/j.carbpol.2010.11.056

Salvioni, L., Testa, F., Barbieri, L., Giustra, M., Bertolini, J. A., …, Colombo, M. (2022). Saporin Toxin Delivered by Engineered Colloidal Nanoparticles Is Strongly Effective against Cancer Cells. Pharmac.,14(7), 1517. https://doi.org/10.3390/pharmaceutics14071517

Sengottiyan, S., Mikolajczyk, A., Jagiełło, K., Swirog, M., Puzyn, T. (2023). Core, Coating, or Corona? The Importance of Considering Protein Coronas in Nano-QSPR Modeling of Zeta Potential. ACS Nano, 17(3), 1989–1997. https://doi.org/10.1021/acsnano.2c06977

Sharma, K., Kaur, R., Kumar, S., Saini, R. K., Sharma, S., Pawde, S. V., Kumar, V. (2023). Saponins: A Concise Review on Food Related Aspects, Applications and Health Implications. Food Chem. Adv., 2 (January), 100191. https://doi.org/10.1016/j.focha.2023.100191

Taherian, A., Esfandiari, N., Rouhani, S. (2021). Breast Cancer Drug Delivery by Novel Drug-Loaded Chitosan-Coated Magnetic Nanoparticles. Cancer Nanotechnol., 12(1), 1–20. https://doi.org/10.1186/s12645-021-00086-8

Tan, M., Zhao, Q., Wang, X., Zhao, B. (2022). Study on extraction, isolation, and biological activity of saponins from quinoa bran. J. Food Proc. Preserv. (September), 1–13. https://doi.org/10.1111/jfpp.17155

Toropov, A. A., Sizochenko, N., Toropova, A. P., Leszczynski, J. (2018). Towards the development of global nano-quantitative structure-property relationship models: zeta potentials of metal oxide nanoparticles. Nanomaterials, 8(4), 243. https://doi.org/10.3390/nano8040243

Woldemichael, G. M., Wink, M. (2001). Identification and biological activities of triterpenoid saponins from *Chenopodium quinoa*. J. Agric. Food Chem., 49(5), 2327–2332. https://doi.org/10.1021/jf0013499

Zaboon, M. H., Saleh, A. A., Al-Lami, H. (2021). Synthesis, Characterization and Cytotoxicity Investigation of Chitosan-Amino Acid Derivatives Nanoparticles in Human Breast Cancer Cell Lines. J. Mex. Chem. Soc., 65(2), 178–88. https://doi.org/10.29356/jmcs.v65i2.1265