Short Communication

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Phytostimulant properties of highly stable silver nanoparticles obtained with saponin extract from *Chenopodium quinoa*

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Abstract

BACKGROUND: Quinoa (*Chenopodium quinoa* Willd) is an Andean original pseudocereal with high nutritional value. During quinoa processing, large amounts of saponin-rich husks byproducts are obtained. Quinoa saponins, which are biologically active, could be used for various agriculture purposes. Silver nanoparticles have increasingly attracted attention for the management of crop diseases in agriculture. In this work, silver nanoparticles are synthesized by a sustainable and green method, using quinoa husk saponin extract (QE) to evaluate their potential for application in agriculture as biostimulants.

RESULTS: Quinoa extract was obtained and characterized by liquid chromatography with tandem mass spectrometry (LC–MS/MS). Sixteen saponin congeners were successfully identified and quantified. The QE obtained was used as a reducing agent for silver ions to synthesize silver nanoparticles (QEAgNPs) under mild conditions. The morphology, particle size, and stability of Ag nanoparticles were investigated by transmission electron microscopy (TEM), ultraviolet–visible spectroscopy (UV-visible), energy-dispersive X-ray (EDS), zeta potential, and Fourier transform infrared spectroscopy with attenuated total reflection (FTIR-ATR). Ultraviolet–visible spectroscopy measurements confirmed the formation of silver nanoparticles in the presence of QE, with estimated particle sizes in a range between 5 and 50 nm. According to the zeta potential values, highly stable nanoparticles were formed. The QE and QEAgNPs (200–1000 μg/mL) were also tested in radish seed bioassay to evaluate their phytotoxicity. The seed germination assays revealed that QEAgNPs possessed a phytostimulant effect on radish seeds in a dose-dependent manner, and no phytotoxicity was observed for both QE and QEAgNPs.

CONCLUSION: Silver nanoparticles obtained by a so-called 'green' method could be considered as good candidates for application in the agricultural sector for seed treatment, or as foliar sprays and plant-growth-promoters.

Keywords: silver nanoparticles; *Chenopodium quinoa*; saponin extracts; HPLC-MS(MS) analysis; phytostimulant properties

INTRODUCTION

One of the biggest problems in agriculture is the protection of crops from biotic and abiotic stress. Each year, enormous economic losses are reported due to the harmful effects of plant pathogens and issues associated with climate change (such as drought, high ultraviolet radiation, high temperature fluctuations). To minimize the stress effect on crops, a wide range of inorganic fertilizers and synthetic compounds have been tested. Due to the production practices used, residues of toxic synthetic compounds remain in plants, fruits, and seeds. These could be introduced into the human body through the food chain. Recently, the design of new eco-friendly and nontoxic natural compounds has been studied, to increase the quality and yield of the crops. Quinoa (*Chenopodium quinoa* Willd) is a pseudo-cereal that has been cultivated since ancient times in the Andean mountains of South America.[1] Due to its high nutritional value (i.e., its high level of proteins, minerals, and vitamins) it has gained worldwide attention and has become one of the most commonly exported crops

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from South America. However, there is a large accumulation of quinoa husks, which need to be eliminated before the final preparation and transfer of quinoa to the market. At an industrial scale, quinoa husk is removed from quinoa grains by mechanical treatment, becoming residual biomass with no added value. It is known that quinoa husk contains saponins and polyphenolic compounds, which are highly valuable plant secondary metabolites that have shown high potential as fungicides, insecticides, and nematicides.[2,3] So, quinoa husk as agriculture residuals can be valorized by extracting saponins, and they can potentially be exploited in the agriculture sector to protect and increase crop yields. Several works have described saponin structures from different quinoa varieties, and their surfactant properties.[4] Saponins are a family of compounds consisting of a sapogenin core linked to one or more oligosaccharide chain(s).[5] In previous work, saponin and saponin-rich extracts from different plants were used as an environmentally friendly method for silver nanoparticle preparation. Such nanoparticle conjugates display bactericide activity.[6] However, in most cases, data about the saponin chemical structure are not presented. Knowing the saponin composition in extracts would allow the chemical interactions between saponins and silver nanoparticles to be understood during both metallic reduction and nanoparticle stabilization processes. Saponins-containing extract from quinoa husk could therefore be used to reduce silver ions, thus providing a green, nontoxic methodology to obtain a product with synergic properties. Silver nanoparticles are commonly used in the food and agriculture industry at specified concentrations due to their well-known antimicrobial activity and root-regeneration property.[7–9] There has been a substantial amount of research into the use of natural extracts derived from plants to prepare silver nanoparticles by a green method.[10–12] However, to the best of our knowledge, there has been no previous report on the valorization of quinoa husk agricultural waste to give value-added products, or the use of quinoa husk extract to prepare silver nanoparticles using a green method. Hence, the main objective of the present work is to extract saponins from quinoa husk and to use the resulting extract to obtain silver nanoparticles that are characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy with attenuated total reflection (FTIR-ATR). The phytotoxicity of the nanoparticles was also assessed through a bioassay, to evaluate their potential application in the agriculture sector as foliar sprays or plant growth biopromoters.

METHODOLOGY

Materials and reagents

Absolute ethanol, Folin–Ciocalteu reagent, $AgNO_3$, and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) were purchased from Sigma-Aldrich (Santiago, Chile). For saponin extractions and mass spectrometry analyses, technical-grade methanol, hexane, dichloromethane, chloroform, and isobutanol, as well as high-performance liquid chromatography (HPLC)-grade water, acetonitrile, and methanol were purchased from CHEM-LAB NV (Somme-Leuze, Belgium). *N,N*-Dimethylaniline (DMA), Hederacoside C and 2,5-dihydroxybenzoic acid (DHB) were provided by Sigma-Aldrich (Diegem, Belgium).

Quinoa husk extract (QE) preparation

Quinoa samples were supplied by the Chilean Quinoa Breeding Program from the National Institute of Agriculture Research (INIA Intihuasi, La Serena, Chile). To separate kernels from the outer husk, the seeds were subjected to physical shearing to obtain quinoa grains for human consumption. The remaining husks powder (particle size <1 mm) was stored in a dry place until the extraction process. Quinoa husk (50 g) was suspended in 130 mL of ethanol solution (50% v/v) under stirring at 20 °C for 24 h. Then the sample was centrifuged at 1000 g, and the solid phase was discarded. The liquid phase was concentrated using a rotary evaporator. Dried quinoa husk extract (QE) was further used to isolate saponins according to a method that has been described previously.[13] Briefly, the weighed QE powder was stirred in methanol for 24 h at room temperature and then filtrated. The extracts were diluted to 70% methanol/Milli-Q water. These methanolic extracts were partitioned (v/v) successively against *n*-hexane, dichloromethane, and chloroform. Finally, the hydromethanolic solution was evaporated using a rotary evaporator. The dry extract was diluted in water to undergo the last partitioning against isobutanol (v/v). The butanolic phase was washed twice with water to remove salts and impurities. The organic solution obtained was lyophilized for further characterization.

Mass spectrometry analysis of quinoa saponin

Matrix-assisted laser desorption / ionization-time of flight (MALDI-ToF) analyses

Quinoa extract was analyzed with a Waters Q-ToF Premier mass spectrometer (Waters Corp., Milford, USA) in the positive ion mode.[14] All the ions that were detected corresponded to Na^+ adducts on saponins and were therefore detected at m/z = M+ 23. The MALDI source consisted of a Nd-YAG laser, operated at 355 nm with a maximum pulse energy of 104.1 μJ delivered to the sample at 200 Hz repeating rate. All samples were prepared using a mixture of 25 mg of 2,5-dihydroxybenzoic acid (DHB) in water / acetonitrile (v/v) with 6 μL of DMA (*N,N*-dimethylaniline) as the matrix. The dry droplet method was selected to prepare the sample / matrix co-crystal on the target plate. A sample droplet (1 μL) was applied on top of a fast-evaporated matrix-only bed. For the recording of the single-stage matrix-assisted laser desorption / ionization – mass spectrometry (MALDI-MS) spectra, the quadrupole (rf-only mode) was set to pass ions between m/z 250 and 1500, and all ions were transmitted into the pusher region of the time-of-flight analyzer where they were mass analyzed with 1 s integration time. Accurate mass measurements were performed at 10 000 FWHM resolution to afford ion compositions.

Liquid chromatography with tandem mass spectrometry (LC–MS/MS) analyses

The second step of saponin quantification was performed with an online LC–MS/MS analysis with a Waters Alliance 2695 liquid chromatography device coupled to a Waters Synapt G2-S*i* mass spectrometer (Waters Corp., Milford, USA).[15] Hederacoside C, a commercially available saponin purified from *Hedera helix*, was used as the internal standard. The integration of the LC–MS signals was used to estimate the concentration of each saponin in the extract. The global mass was further compared to the mass of extract submitted to the extraction procedure (see above) to estimate the saponin contained in the Quinoa husk extract.

Quinoa extract silver nanoparticle (QEAgNPs) preparation

Silver nanoparticles were synthesized by aqueous phase reduction of $AgNO_3$ with QE. The silver solution was prepared by mixing 0.017 mg of silver nitrate with 50 mL of deionized water; 0.5 g of the quinoa extract was dissolved in 50 mL of deionized water.

Once both compounds were well dissolved, they were mixed and kept under magnetic stirring. The final concentration of silver nitrate was 10^{-3} mol L^{-1}. The resulting mixture was then kept under agitation at room temperature and in dark conditions for 24 h to 1 week. The suspension was separated by centrifugation at 5000 g × 20 min, and the solid was dried by lyophilization for further characterization. The supernatant was collected, and the silver excess was determined by titration using a $1{,}2 \times 10^{-4}$ mol L^{-1} KSCN solution and $Fe(NO_3)_3$ as indicator.

Nanoparticle characterization

Ultraviolet-visible (UV-visible) spectrometry

The UV-visible spectra of QEAgNPs samples (1 mL of mixture taken after 1 h, 1 day, 3 days, 5 days, and 7 days) were recorded with a Shimadzu UV2600UV-visible spectrophotometer (Shimadzu Latinamerica S.A., Santiago, Chile).

Fourier transform infrared spectroscopy with attenuated total reflection (FTIR-ATR) analysis

Fourier transform infrared spectroscopy with attenuated total reflection spectra was recorded using a Thermo–Nicolet IS10 FTIR spectrophotometer connected to a PC with Omnic software (Thermo Electron Corp., Woburn, MA, USA) for data processing. The lyophilized samples of QE and QEAgNPs were analyzed after 1 week of mixing. The ATR spectra were recorded at 4 cm^{-1} resolution and 64 scans.

Transmission electron microscopy (TEM)

After 1 week of mixing, the QEAgNPs solution was analyzed on JEOL JEM 1200 EX II TEM equipment (JEOL, Tokyo, Japan). Prior to analysis, one drop of the sample was placed in a Cu grid of 100 mesh and left to dry at room temperature.

Scanning electron microscopy (SEM)

The samples were fixed in a sample holder and covered with a gold layer for 3 min using an Edwards S150 sputter coater (BOC Edwards, São Paulo, Brazil) before analysis. The spectroscopic studies of energy dispersion were carried out using an ETEC auto-scan Model U-1 scanning electron microscope (University of Massachusetts, Worcester, MA, USA) coupled to an energy-dispersive X-ray (EDS) device with an X-MAX silicon drift detector (Oxford Instruments, Oxford, UK). The spectra with characteristic signals for each element using the Kα energy level were obtained.

Particle size distribution and Z potential

The zeta potential of QEAgNPs was determined using a dynamic light-scattering (DLS) analysis using a Zetasizer Nano Series analyzer (Malvern Instruments Ltd, Malvern, UK).

Phytotoxicity assay

A radish seed germination assay[15] was conducted using the QE and QEAgNPs. For this purpose, radish seeds obtained from Anasac Garden S.A. (Santiago, Chile) were sterilized for 5 min in 70% ethanol solution. The seeds were washed three times with distilled water and dried at room temperature for 1 h. Then 5 ml solutions with a concentration of 2 mg/mL of QE extract and an equivalent concentration of QEAgNPs were prepared. The seeds (30 per treatment) were soaked for 20 min in each of the solutions and dried at room temperature for 1 h. Then the seeds were placed on damp filter paper inside Petri dishes: ten seeds in each petri dish for three trials of each extract. Trials were also performed using sample concentrations from 200–1000 µg/mL and with pure water as a control. Petri dishes were kept at 25 °C in darkness and checked daily for seed germination count. After 5 days, the root length was also recorded. The germination index (GI) was calculated from SG (seed germination) and RG (root growth) parameters, according to the following formulas:

SG = N° germinated seed (sample)/N° germinated seed (control)*100%; RG = root length (sample)/root length (control) *100% and GI = SG*RG/100.

Statistical analysis

Data collected were analyzed using ANOVA and the SPSS 11.0 statistical package. Duncan's multiple range test with a 0.05 significance level was used to compare treatment means for each parameter.

RESULTS AND DISCUSSION

Saponin extract preparation and characterization

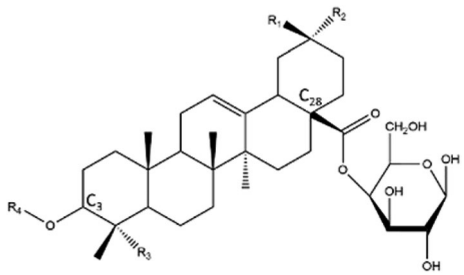
This work is focused on Chilean quinoa saponins that were identified by Madl *et al.*[1] These saponins are triterpene based, and the aglycon moiety is a β-amyrin covalently linked to two oligosaccharide chains at the C_3 and the C_{28} positions, allowing the saponins to be classified in the so-called bidesmosidic family. The reported monosaccharides consist of sugar chains, glucose (Glc), galactose (Gal), arabinose (Ara), xylose (Xyl), and glucuronic acid (GlcA).[1] The determination of the elemental compositions, accurate molecular weight, separation, and relative quantification of isomeric saponins was achieved by the combination of different analytical techniques (MALDI-MS, LC–MS, and LC–MS/MS), as previously reported.[14] The detailed characterization of QE is presented in Table 1.

Mass spectrometry data confirm the presence of 14 elemental compositions of saponins in the Chilean quinoa extract. Based on the side chain differences in R_1, R_2 and R_3, aglycon structures have been detected: oleanic acid (**M**, **R**, **S**, **70**, $\mathbf{M_{ac}}$), hederagin (**I**, **F**, **61**), AG489 (**19,19a**), AG487 (**37**), serjanic acid (**H**, **G**), and phytolaccagenic acid (**B**, **O**).[1] Regarding oligosaccharide chain analysis, the sugar attached at C_{28} is always glucose, whereas the linear chain of two or three sugars is present at C_3. Based on semi-quantitative LC–MS experiments with hederacoside C as the internal standard, it was estimated that the quinoa extract contained around 60% weight of saponins. The relative content of all the detected saponins are presented in Table 1. As can be seen from Table 1, Saponin **B** (51% weight) is by far the dominant congener within the extract.

Silver nanoparticles (AgNPs) preparation and characterization

Production of silver nanoparticles in the presence of quinoa saponin-rich extract is confirmed by different methods. Figure 1(a)-(c) shows TEM images of silver nanoparticles (QEAgNPs) obtained after a 1 week reaction of quinoa extract (QE) at room temperature. In general, the particles obtained exhibit irregular shapes, but some of them showed faceted profiles (truncated triangles and hexagons), as can be observed in Figs. 1(b) and (c). The statistical analysis of the particle size is presented in the frequency histogram of Fig. 1(d). The mean diameter of the measured sample is around 19 nm, and the particles are distributed mainly in sizes between 5–50 nm with a maximum between 10–15 nm. Muniyan *et al.*[6] obtained the same range of silver nanoparticles, which was synthesized with the use of fenugreek saponin. Images of the main solution after 1 h and 1 week are presented in Fig. 1. After

Table 1 Compilation of MALDI-MS and LC–MS/MS analysis of the quinoa saponin extract and schematic structure of quinoa saponin (I suggest to remove or put the line (-) symbol before all saponin groups R1, R2 and R3 column for homogenity)



Code	Composition	m/z (M + Na)	ppm	R_1	R_2	R_3	R_4	Tr	Proportion (wt%)
M	$C_{47}H_{76}O_{17}$	935.4980	2.2	- CH_3	- CH_3	- CH_3	Glc – Ara -	8.3	1.2
R	$C_{47}H_{74}O_{18}$	949.4773	4.4	- CH_3	- CH_3	- CH_3	Xyl – GlcA -	5.6	0.7
S				- CH_3	- CH_3	- CH_3	Ara – GlcA -	8	0.6
I	$C_{47}H_{76}O_{18}$	951.4929	2.1	- CH_3	- CH_3	CH_2OH	Glc – Ara -	7.2	18.4
X	$C_{47}H_{74}O_{19}$	965.4722	0.2	- CH_3	- CH_2OH	CH_2OH	Glc – Ara -	5.6	3,5
F				- CH_3	- CH_3	CH_2OH	Xyl – GlcA -	7.7	0.5
19 [a]	$C_{47}H_{76}O_{19}$	967.4878	4.9	- CH_2OH	- CH_3	CH_2OH	Xyl - Glc -	4.7	2.1
19a [a]				- CH_2OH	- CH_3	CH_2OH	Xyl – Glc -	5.6	0.4
M_a	$C_{49}H_{78}O_{18}$	977.5	NA	- CH_3	- CH_3	- CH_3	Glc – GlcAcetyl -	5.9	1.1
H	$C_{48}H_{76}O_{19}$	979.4878	1.5	- CH_3	$COOCH_3$	- CH_3	Glc – Ara -	7.1	2.5
70				- CH_3	- CH_3	- CH_3	Glc – GlcA -	7.5	1.4
Q	$C_{48}H_{78}O_{19}$	981.5035	4.3	- CH_3	- CH_3	CH_2OH	Glc – Gal -	5.5	1.2
B	$C_{48}H_{76}O_{20}$	995.4828	3.2	- CH_3	$COOCH_3$	CH_2OH	Glc – Ara -	6.1	51.1
61	$C_{53}H_{86}O_{23}$	1113.5458	1.6	- CH_3	- CH_3	CH_2OH	Glc – Glc – Ara -	7.1	1.9
37	$C_{53}H_{86}O_{24}$	1127.525	2.1	- CH_3	- CH_2OH	CH_2OH	Glc – Glc – Ara -	5.6	1.8
G	$C_{54}H_{86}O_{24}$	1141.5407	3.6	- CH_3	$COOCH_3$	- CH_3	Glc – Glc – Ara -	7	4.1
O	$C_{54}H_{86}O_{25}$	1157.5356	0.6	- CH_3	$COOCH_3$	CH_2OH	Glc – Glc – Ara -	6.1	8.5

[a]Double bond between C_{21} and C_{22}.

1 hour, the solution remained transparent. After 1 week, the solution became pale yellow, indicated that silver nanoparticles had been formed.

The spectral characterization of QEAgNPs formed by the reduction of silver ions with quinoa extract is shown in Fig. 2. Ultraviolet-visible spectroscopy is a technique that is widely used to measure the optical properties of nanoparticles. Figure 2(a) shows the UV-visible spectra of samples taken from the reaction vessel after 1 h, 1 day, 3 days, 5 days and 7 days from the initial mixture. From 1 h to 3 days, no significant changes were detected in the recorded spectra. After 5 days, an incipient maximum appeared at 430 nm in the visible region, which is more pronounced on the 7th day, indicating the formation of silver nanoparticle colloidal suspension.[16] This absorption corresponds to the well known surface plasmon resonance (SPR). The presence of a wide strip of the surface plasmon implies a wide distribution of particles size. In the first 3 days no changes in the coloration were observed and the SPR effect at UV-visible diagram was not manifested, TEM images were taken only for samples under 1 week reaction, and it was decided to stop the reaction process at that time. In the present work, the formation of silver nanoparticles using quinoa saponin-rich extract took a longtime regarding previous results from literature.[6] It could be due to the experimental conditions employed, in which the initial Ag+ solution was ten times more concentrated than reported by these authors, and the initial quinoa extract was more diluted. After one week, titration determines the excess of Ag+ in the supernatant and allows it to obtain the QEAgNPs yield. The results showed that the excess of silver ions was close to 6% of initial concentration, confirming that a large part of the silver ions (94%) was converted into QEAgNPs.

The chemical composition of quinoa extract and silver nanoparticles was determined by EDS (Fig. 2). The EDS diagram of quinoa extract (Fig. 2(b)) displays strong signals around 0.25 keV and 0.55 keV, related to C and O elements, respectively. On the other hand, the EDS diagram of QEAgNPs (Fig. 2(c)) reveals two signals at around 2.6 and 3.4 keV, confirming the presence of the crystalline silver nanoparticles.[12] Moreover, the spectral signals of carbon and oxygen have also been detected, thus confirming that QE components are adsorbed on the surface of the silver nanoparticles. The remaining signals at 1.8 and 2.2 keV, respectively, belong to the Au coating layer.[12]

In an attempt to identify the groups from the quinoa extract that could be responsible for the reduction of Ag^+ ions and the possible influence of quinoa extract on stabilization of synthesized silver nanoparticles, FTIR-ATR measurements were performed. The ATR spectra of the QE and the QEAgNPs are shown in Fig. 2(d).

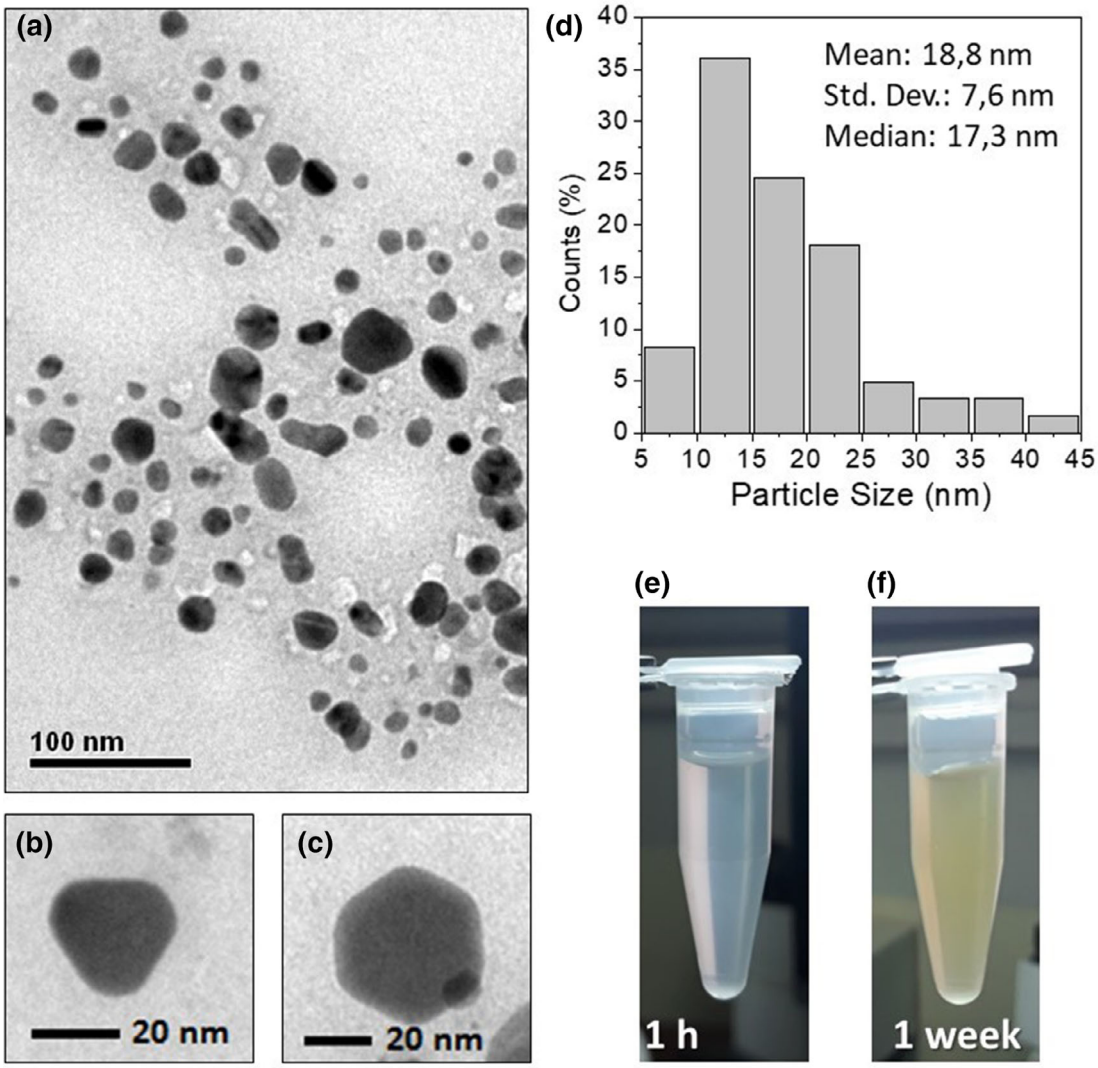


Figure 1 (a) A TEM image of QEAgNPs prepared by reduction of $AgNO_3$ with quinoa extract. (b, c) Zoom images of silver nanoparticles with faceted triangular and hexagonal shapes. (d) Frequency histogram of AgNPs measured from TEM images. (e, f) Optical images of the sample after 1 h and 1 week after mixing silver nitrate with the quinoa saponin extract.

Characteristic bands that correspond to oleanane triterpenoid saponin were detected in the spectrum of quinoa husk extract at 3331 cm^{-1} (stretching vibrations of –OH groups), 2935 and 2873 cm^{-1} (stretching vibration of CH_2/CH_3 groups), 1726 cm^{-1} (stretching vibrations of C═O groups from oleanolic acid/ester), 1611 cm^{-1} (stretching vibrations of C═C group from olefinic group), 1069, and 1024 cm^{-1} (stretching vibrations of C-O-C groups) associated with oligosaccharide linkage to sapogenins.

Considering the spectrum of QEAgNPs, all characteristic bands from quinoa extract were also detected. A dominant shift of –OH bands to higher frequencies was observed, suggesting that these functional groups were mainly involved in the reduction of silver ions. Moreover, a band located around 1383 cm^{-1} related to stretching vibrations of COO^- group shifts to 1387 cm^{-1} and becomes more intense and narrower in comparison with the ATR spectrum of quinoa extract, which indicates the formation of strong interactions with silver ions, making silver nanoparticles more stable. Absorption bands associated with oligosaccharide linkage to sapogenins (stretching vibrations of C—O—C groups) are also shifted to higher frequencies, and a change in their relative intensity and shape is observed. This result indicates that the saponin structure could have some role in silver nanoparticle stabilization, acting as a capping agent. These results agree with results previously achieved using a natural saponin for obtaining silver nanoparticles.[6]

To evaluate the surface charge of silver nanoparticles and their stability, the zeta-potential of colloidal nanosilver solution was measured. The magnitude of zeta potential gives a clear image of the stability of silver nanoparticles in colloids. In fact, nanoparticles with zeta potential values more positive than +30 mV or more negative than −30 mV are considered to be highly cationic or anionic, respectively, being highly stable.[17] Nanoparticles with zeta potential in the range of −30 mV to +30 mV are considered to be slightly charged or neutral (+10 to −10 mV). Silver nanoparticles obtained in this work displayed a negative surface charge (−36.8 mV), which is considered as sufficient mutual repulsion to

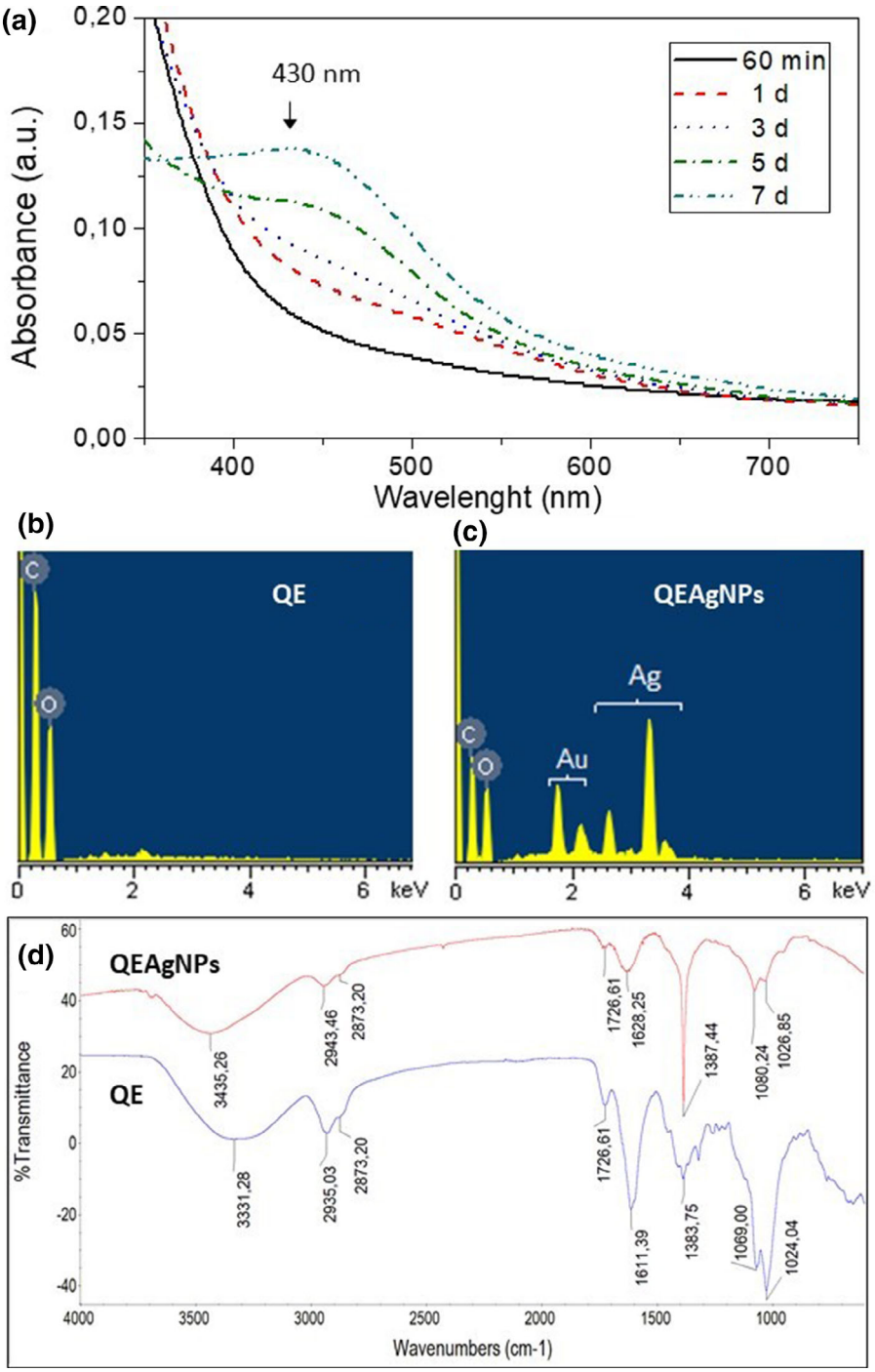


Figure 2 (a) Ultraviolet-visible spectra sample 1 h and 1, 3, 5, and 7 days after mixing $AgNO_3$ with quinoa extract (QE); (b) EDS analysis of QE and (c) EDS analysis of QEAgNPs; (d) FTIR spectra of QE and QEAgNPs. Both, EDS and FTIR of QEAgNPs are for samples lyophilized after 7 days of initial mixing.

ensure the high stability of the colloidal suspension.[18] The negative charge of the QEAgNPs indicates that natural anionic compounds and their counterions are coordinated on the surface of the nanoparticles, acting as capping agents and stabilizers. The zeta potential value that was obtained was higher than previously reported (−18 mV) for QEAgNPs prepared using commercial and naturally obtained saponins from different plant sources, respectively.[6, 19] Hence, the zeta potential value of QEAgNPs from this work could be related to the chemical nature of the reducing source.

Phytotoxicity assays

The radish seed germination assay is of great importance for evaluating the phytotoxic activity of plant extracts. The method consists of the measurement of the average root length and percentage of seeds germinating after seed treatment with water (control sample), and different concentrations of QE and QEAgNPs, as tested samples. The results of tested samples in the radish seeds phytotoxicity are presented in Table 2. The extract is considered as non-phytotoxic if the germination index

Table 2 Phytotoxicity results obtained from radish seed treated with quinoa extract (QE) and QEAgNPs

	Germination (%)		Root length (mm)		GI (%)	
Bioproduct concentration (μg/mL)	QE	QE AgNPs	QE	QE AgNPs	QE	QE AgNPs
Control	87 ± 4 a	87 ± 4 ab	47 ± 5 b	47 ± 5 a	-	-
200	93 ± 4 a	83 ± 5 a	36 ± 5 a	50 ± 6 a	83.2	101.6
500	93 ± 6 a	97 ± 4 c	49 ± 6 b	53 ± 5 a	112.1	126.0
800	89 ± 5 a	93 ± 4 bc	49 ± 5 b	45 ± 5 a	103.7	102.9
1000	90 ± 4 a	93 ± 4 bc	48 ± 6 b	47 ± 4 a	106.1	107.2

Numbers followed by different letters are statistically different ($p \leq 0.05$), according to the Duncan's test.

(GI) is in the range of 66–100%, whereas a GI index above 100% indicates phytonutrient and phytostimulation behavior of extracts.

In this study, the GI for all tested concentrations of QE and QEAgNPs colloidal suspensions was above 66%, confirming that these compounds do not have a phytotoxic effect on radish-seed germination. However, it is interesting to note that the QE treatment of seeds with QE at 200 μg/mL concentration induces a GI value lower than 100%. This may indicate that a lower concentration of QE can have an inhibitory effect on radish root growth. Furthermore, the silver nanoparticle colloid shows the lowest percentage of germination among all treatments, being significantly lower than those obtained at higher concentrations. It seems that 500 μg/mL is the optimal dose to stimulate seed germination and root growth, for both tested products. At this concentration, all the measured parameters, including the GI, are consistently higher than those observed for the other concentrations. The GI value for QEAgNPs is the highest found among the treatments, suggesting a synergistic effect between the saponin compounds and the stabilized silver nanoparticles. According to GI values, QE and QEAgNPs obtained at concentrations from 500 to 800 μg/mL have a phytostimulant effect, which means that, at these concentrations, they could be used as a plant-growth promoter in agriculture. These results confirm that the nature of the green extract use for QEAgNPs preparation / stabilization would have an essential role in determining and fine tuning the biological activity of the silver nanoparticles that are obtained.[20] The phytotoxic analysis of quinoa husk extract and silver nanoparticles prove that these components have a strong potential to be used in the agricultural sector at specified concentrations as foliar sprays or as plant growth bio-promoters.

CONCLUSIONS

A simple and green route was proposed for the synthesis of silver nanoparticles by the use of quinoa husk extract containing well-characterized saponins. The phytotoxicity assay of the synthesized nanoparticles and quinoa husk extract was evaluated using reddish seeds as a model. It was shown that neither of the tested components inhibited the germination of reddish seeds. In fact, the synthesized Ag nanoparticles promoted seed germination at concentrations from 500-1000 μg/mL. The germination index (GI) indicates those seed treatments showed phytostimulant properties. The best dose obtained from silver nanoparticles was 500 μg/mL. These results suggest that silver nanoparticles obtained using quinoa extract could have a synergic effect on plant growth and may find potential applications in agriculture.

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