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

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+Dedicated to the memory of Dr. Danni E. Garcia

Conflict of interest

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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, having biological activity, could be used for different agriculture purposes. Silver nanoparticles have gained increased attention for managing crop diseases in agriculture. In this work, silver nanoparticles are synthesized by a sustainable and green method, using quinoa husk saponin extract (QE), in order to evaluate their applicative potentials in agriculture as biostimulants.

Results. Quinoa extract was obtained and further characterized by LC-MS/MS. Sixteen saponin congeners were successfully identified and quantified. The obtained QE 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 TEM, UV/VIS, EDS, zeta potential, and FTIR-ATR. UV/VIS measurements confirmed the formation of silver nanoparticles in the presence of QE, with estimated particle sizes in the range between 5 and 50 nm. According to zeta potential values, highly stable nanoparticles were formed. Moreover, the QE and QEAgNPs (200-1000 µg/ml) were 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 a good candidate to be applied in the agricultural sector for seed treatment, or as a foliar spray and plant-growth-promoters.

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

1. Introduction

One of the biggest problems in agriculture is the protection of crops from both biotic and abiotic stress. Each year, enormous economic losses are reported due to the harmful effects of plagues and climate change associated issues (drought, high UV radiation, high-temperature fluctuation). In order to minimize the stress effect on crops, a wide range of inorganic fertilizers and synthetic compounds have been tested. Due to the production practices, residues of toxic synthetic compounds remain in plants, fruits, and seeds that could be further introduced into the human body through food chains. Recently, researches have been investigated the design of new eco-friendly and nontoxic natural compounds to increase the quality and yield of the crops. Quinoa (*Chenopodium quinoa Willd*) is a pseudo-cereal cultivated since ancient times in the Andean mountains from South America[1]. Due to its high nutritional value (i.e., high level of proteins, minerals, and vitamins), it has gained worldwide attention and become one of the most exported crops from South America to overseas countries. However, there is a large accumulation of quinoa husks because it needs to be eliminated before the final preparation and transfer of quinoa from agricultural fields to the food chain market. At the 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]. Hence, quinoa husk as agriculture residual can be valorized by extracting saponins, and potentially exploiting in the agriculture sector to protect, and increase the crop yields. There are several works describing quinoa saponin structures from different quinoa varieties and their surfactant properties [4]. Saponins are a family of compounds composed 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 in an environmentally friendly method for silver nanoparticle preparation. Such nanoparticle conjugates present bactericide activity [6]. However, in most cases, data about the saponin chemical structure is not presented. Knowing the saponin composition in extracts would allow understanding the chemical interactions between saponins and silver nanoparticles during both metallic reduction and nanoparticle stabilization processes. Therefore, saponins-containing extract from quinoa husk could be used to reduce silver ions, thus providing a green nontoxic methodology to obtain a product with synergic properties. Namely, silver nanoparticles are commonly used in the food and agriculture application at specified concentrations due to their well-known

antimicrobial activity and root regeneration property [7,8,9]. Up to date, there is numerous research of natural extracts derived from plants to obtain silver nanoparticles by a green method [10, 11, 12]. However, to the best of our knowledge, there is no previous report on the valorization of the quinoa husk agricultural waste to value-added products and usage of quinoa husk extract to form silver nanoparticles with a green method. Hence, the main objective of the present work is to extract saponins from quinoa husk and to further use the obtained extract to obtain silver nanoparticles that are characterized by SEM, TEM, and FTIR-ATR. Moreover, in order to evaluate their potential application in the agriculture sector as foliar sprays or plant growth biopromoters, their phytotoxicity was assessed through a bioassay.

2. Methodology

2.1 Materials and reagents

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

2.2 Quinoa husk extract (QE) preparation

Quinoa samples were supplied by the Chilean Quinoa Breeding Program from the National Institute of Agriculture Research (INIA). In order 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. Afterward, the sample was centrifuged at 4000 rpm, 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 previous method [13]. Briefly, the weighed QE powder was stirred in methanol during 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 in order to undergo the last partitioning against isobutanol (v/v). The butanolic phase was washed twice with water to remove salts and impurities. The obtained organic solution was lyophilized for further characterization.

2.3 Mass spectrometry analysis of quinoa saponin

2.3.1 MALDI-ToF analyses

Quinoa extract was analyzed with a Waters Q-ToF Premier mass spectrometer in the positive ion mode [14]. All the detected ions correspond to Na^+ adducts on saponins and were therefore detected at m/z =M+ 23. The MALDI source was constituted 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 DHB (2,5-dihydroxybenzoic acid) 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 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.

2.3.2 LC-MS(MS) analyses

The second step of saponin quantification was performed by an on-line LC -MS(MS) analysis with a Waters Alliance 2695 liquid chromatography device coupled to a Waters Synapt G2-S *i* mass spectrometer [15]. As the internal standard, Hederacoside C (Sigma-Aldrich), a commercially available saponin purified from *Hedera helix*, was used. 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 2.2) to estimate the saponin contained in the Quinoa husk extract.

2.4 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. On the other hand, 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} M. Afterward, the resulting mixture was kept under agitation at room temperature for 24h to 1 week, at room temperature, and in dark conditions. The suspension was separated by centrifugation at 10.000 rpm x 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 $1{,}2x10^{-4}$ M KSCN solution and $Fe(NO_3)_3$ as indicator.

2.5 Nanoparticle characterization

2.5.1 UV-VIS spectrometry

The UV–Visible spectra of QEAgNPs samples (1 mL of mixture took after 1 h, 1. Day, 3. Day, 5. Day and 7. day) were recorded by Shimadzu UV2600UV–Visible spectrophotometer.

2.5.2 FTIR-ATR analysis

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

2.5.3 Transmission electron microscopy (TEM)

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

2.5.4 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) coupled to an energy-dispersive X-ray (EDS) device with an X-MAX silicon drift detector (Oxford Instrument, UK). The spectra with characteristic signals for each element using the Kα energy level were obtained.

2.5.5. Particle size distribution and Z potential

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

2.6. 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. (Chile) were sterilized for 5 minutes in 70% ethanol solution. The seeds were washed three times with distilled water and dried at room temperature for 1 hour. Five-milliliter 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 minutes in each of the solutions and dried at room temperature for 1 hour. Seeds were placed on damp filter paper inside Petri dishes: 10 seeds in each petri dish for 3 trials of each extract. Trials were also performed by 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.

2.7. Statistical analysis.

Data collected were analyzed using the ANOVA procedure and the SPSS 11.0 statistical package. The Duncan's Multiple Range Test at 0.05 significance level was used to compare treatment means for each parameters.

3. Results and Discussion

3.1. Saponin extract preparation and characterization.

This work is focused on Chilean quinoa saponins that were already 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 to classify the saponins in the so-called bidesmosidic family. The reported monosaccharides are consisting 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 LC-MS/MS), as previously reported [14]. The detailed characterization of QE is presented in Table 1.

Mass spectrometry data confirm the presence of fourteen 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 chains 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 it can be seen from Table 1, Saponin **B** (51% weight) is by far the dominant congener within the extract.

Table 1.

3.2. 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 one-week reaction of quinoa extract (QE) at room temperature. In general, the obtained particles exhibit irregular shapes, but some of them shown faceted profiles (truncated triangles and hexagons), as can be observed in figures 1 (b and c). The statistical analysis of the particle size is presented in the frequency histogram of figure 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 that were synthesized by use of *Fenugreek* saponin. Optical images of the main solution after 1 h and 1 week are presented in Figure 1. After one hour, the solution remained transparent. After one week, the solution turned out into pale yellow, indicated that silver nanoparticles had been formed.

Figure 1.

The spectral characterization of QEAgNPs formed by the reduction of silver ions with quinoa extract is shown in figure 2. The UV-visible spectroscopy is a widely used technique to measure the optical properties of nanoparticles. Figure 2(a) shows the UV-visible spectra of samples taken from the reaction vessel after 1h, 1. day, 3. day, 5. day and 7. day from the initial mixture. From 1 h to 3 days, no significant changes are detected in the recorded spectra. After 5 days, an incipient maximum appears at 430 nm in the visible region, which is more pronounced in 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. Since in first 3 days no changes in the coloration were observed and the SPR effect at UV/Vis diagram was not manifested, TEM images were taken only for samples under 1 week reaction and it was decided to stop reaction process at that time. The formation of silver nanoparticles in the presence of quinoa husk extract took a too long time in this work because it was used Ag^+ dissolution approximately 10 times more concentrated, whereas extract was up to 10 times more diluted when compared with data from literature [6]. After one week it was tittered the excess of silver ions in the supernatant, in order to evaluate the yield of formed silver nanoparticles. The obtained results

showed that the excess of silver ions was close to 6% of initial concentration, confirming that a great part of the silver ions (94%) was converted into QEAgNPs.

The chemical composition of quinoa extract and silver nanoparticles was determined by EDS (Figure 2). The EDS diagram of quinoa extract (Figure 2b) displays strong signals around 0.25 keV and 0.55 keV, related to C and O elements, respectively. On the other side, the EDS diagram of QEAgNPs (Figure 2c) reveals two signal 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 which groups from quinoa extract 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 spectrum of the QE and obtained QEAgNPs are shown in Figure 2d. Characteristic bands that correspond to oleanane triterpenoid saponin are 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 to oligosaccharide linkage to sapogenins.

Figure 2.

Considering the spectrum of QEAgNPs, all characteristic bands from quinoa extract have been detected, as well. It is observed a dominant shift of –OH bands to higher frequencies, suggesting that these functional groups are mainly involved in the reduction of silver ions. Moreover, 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 to the ATR spectrum of quinoa extract, which indicated 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 capping agent. These results agree with previously obtained using a natural saponin for obtaining silver nanoparticles [6].

In order to evaluate the surface charge of silver nanoparticles and their stability, 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]. Whereas nanoparticles with zeta potential in the range of -30 mV to +30 mV are considered as slightly charged or neutral (+10 to −10 mV). Silver nanoparticles obtained in this work display a negative surface charge (−36.8 mV), which is considered as sufficient mutual repulsion to 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. Besides, the obtained zeta potential value is higher than previously reported (-18 mV) for QEAgNPs prepared using commercial and naturally obtained saponins from different plant sources, respectively [6, 19]. Hence, the increased negative charge (i.e.) zeta potential of QEAgNPs obtained in this work could be due to the nature of the reducing source (i.e., QE).

3.3 Phytotoxicity assays

The radish seed germination assay is of great importance to evaluate the phytotoxic activity of plant extracts. The method consists of the measurement of the average root length and percentage of seeds germination after seed treatment with water (control sample), and different concentrations of QE and QEAgNPs, as a tested sample. The results of tested samples in the radish seeds phytotoxicity are presented in Table 2. The extract is considered as non-phytotoxic if germination index (GI) is in the range of 66 - 100%, while the GI index above 100% indicates phytonutrient and phytostimulation behavior of extracts.

Table 2.

In this study, GI for all tested concentrations of QE and QEAgNPs colloidal suspension is above 66%, confirming that these compounds do not have a phytotoxic effect on radish seed germination. However, it is interesting to note that QE treatment of seeds with QE at 200 µg/mL concentration induces 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. On the contrary, 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. Besides, 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 of the biological activity of obtained silver nanoparticles [20]. The phytotoxic analysis of quinoa husk extract and silver nanoparticles prove that these components have high potential to be used in the agricultural sector at designed concentrations as foliar sprays or as plant growth bio-promoters.

4. Conclusions

A simple and green route is 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 both tested components do not inhibit the germination of reddish seeds. In fact, they promoted the seed germination and growth of roots, by use from 500-800 µg/mL of QEAgNPs. 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 of plant growth and may find potential applications in agriculture.

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Tables and figures capture

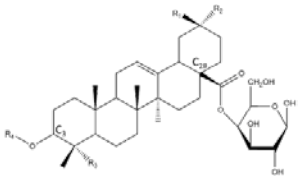
Table 1. Compilation of MALDI- MS and LC-MS(MS) analysis of the quinoa saponin extract and schematic structure of quinoa saponin.

Figure 1. (a) Figure 1. (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 hour and 1 week after mixing silver nitrate with the quinoa saponin extract.

Figure 2. (a) UV-Vis spectra sample after 1 hour and 1, 3, 5 and 7 days after mixing $AgNO_3$ with quinoa extract (QE). (b) EDS analysis of QE and (b) EDS analysis of QEAgNPs. (d) FTIR spectra of QE and QEAgNPs. Both, EDS and FTIR of QEAgNPs is for sample lyophilized after 7 days of initial mixing.

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

Table 1.



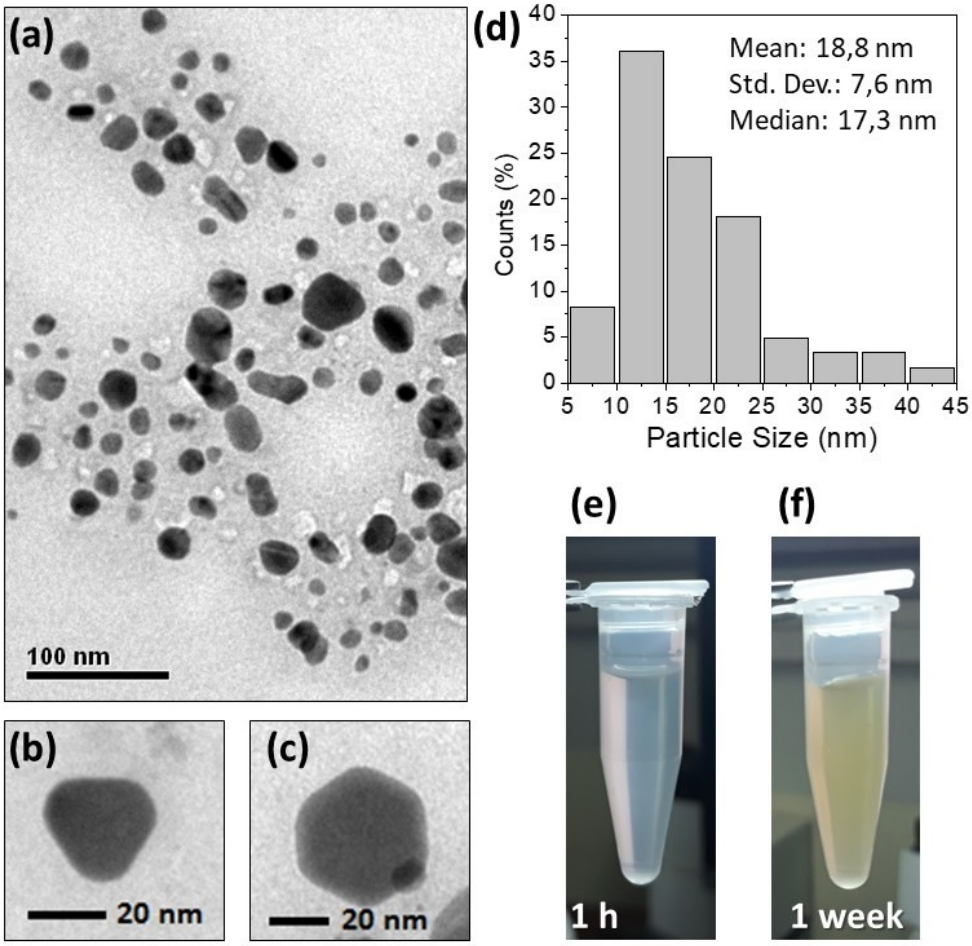
Code	Composition	m/z [M+Na]	ppm	R_1	R_2	R_3	R_4	Tr	Proportion (wt %)
M	$C_{47}H_{76}O_{17}$	935.4980	2.2	- CH_3	- CH_3	- CH_3	Glc – Ara -	8.3	1.2
R	$C_{47}H_{74}O_{18}$	949.4773	4.4	- CH_3	- CH_3	- CH_3	Xyl – GlcA -	5.6	0.7
S				- CH_3	- CH_3	- CH_3	Ara – GlcA -	8	0.6
I	$C_{47}H_{76}O_{18}$	951.4929	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 †	$C_{47}H_{76}O_{19}$	967.4878	4.9	- CH_2OH	- CH_3	CH_2OH	Xyl - Glc -	4.7	2.1
19a †				- 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

†Double bond between C_{21} and C_{22}

Table 2.

Bioproduct Concentration (µg/mL)	Germination (%)		Root Length (mm)		GI (%)	
	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

*The different letter means a significant difference according to the Duncan test ($P<0.05$).



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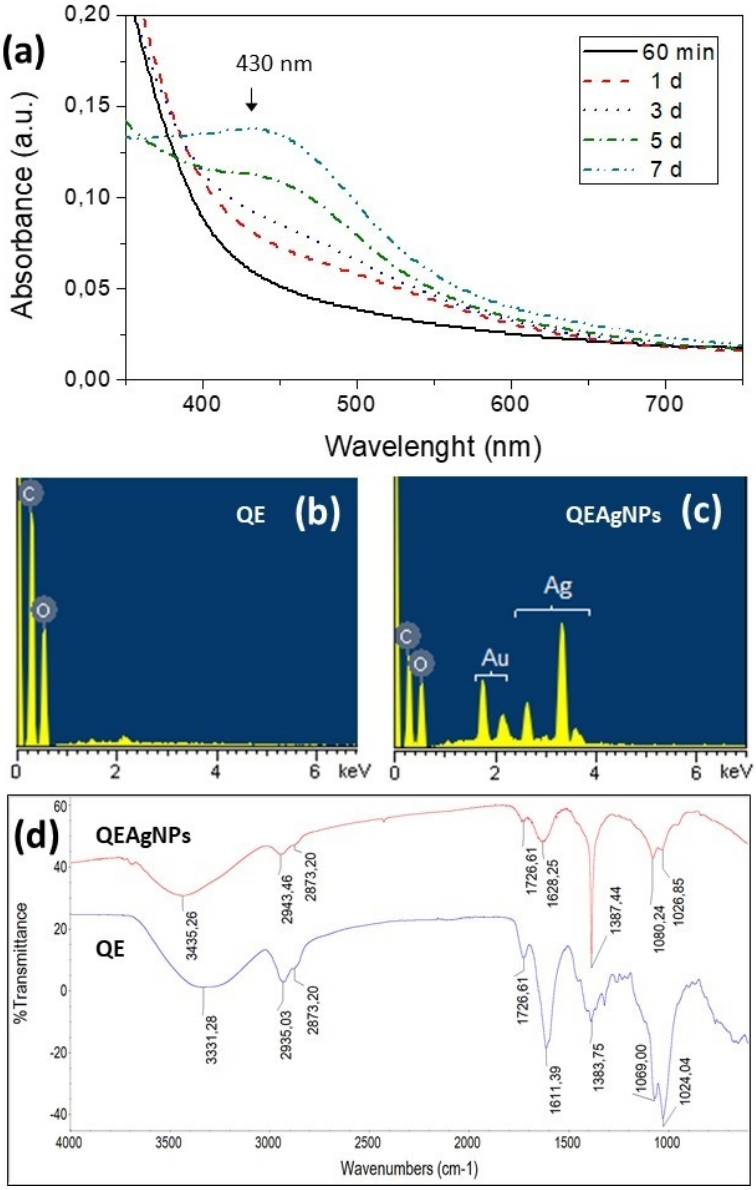

Figure 2. (a) UV-Vis spectra sample after 1 hour and 1, 3, 5 and 7 days after mixing AgNO3 with quinoa extract (QE). (b) EDS analysis of QE and (b) EDS analysis of QEAgNPs. (d) FTIR spectra of QE and QEAgNPs. Both, EDS and FTIR of QEAgNPs is for sample lyophilized after 7 days of initial mixing.