

Influence of solvents in obtaining black quinoa extracts: antioxidant capacity and potential use in the synthesis of nanoparticles

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Abstract– The study evaluated the extraction of bioactive compounds from black quinoa by sonication with three different solvents: 96° ethanol black *Chenopodium quinoa* 1 (BCQ1), ethanol:water 1:1 (BCQ2) black *Chenopodium quinoa* 2 and ultrapure water (BCQ3) black *Chenopodium quinoa* 3. The extracts showed variations in the composition of the extracted compounds, suggesting that the solvent used influences their solubilization. The concentration of total phenols in the extracts was determined by the Folin-Ciocalteu assay, obtaining values of 51.677 µg/mL for BCQ1, 47.689 µg/mL for BCQ2 and 57.187 µg/mL for BCQ3. No significant differences were observed between solvents ($p > 0.05$), suggesting that the choice of solvent does not drastically affect the extraction of total phenols. Regarding antioxidant capacity, the DPPH method was used, and it was found that the BCQ2 extract, prepared with the ethanol:water mixture, exhibited the highest antioxidant capacity, which was measured in µMol Trolox Equivalents per Liter (µMol TE/L), reaching 44,152 µmol TE/L, BCQ3 showed an antioxidant capacity of 31.152 µmol TE/L, whereas BCQ1 reached 0.939 µmol TE/L. These results indicate that the combination of ethanol and water optimizes the extraction of phenolic compounds and flavonoids with high free radical neutralization capacity. In addition, the synthesis of silver nanoparticles was evaluated using the extracts, observing a higher efficiency in BCQ2, which showed an absorption peak at 410 nm, indicating the formation of silver nanoparticles. In general, sonication at 25°C for 10 min with the ethanol:water mixture favored the extraction of bioactive compounds and the generation of nanoparticles with potential technological applications.

Keywords-- Black quinoa, phenolic compounds, antioxidant capacity, silver nanoparticles.

I. INTRODUCTION

Black quinoa (BCQ) is a variety of the *Chenopodium quinoa* Wild species, which presents a phytochemical profile abundant in phenols, anthocyanins, flavonoids and other secondary metabolites such as quercetin, kaempferol and rutin,

in addition to important phenolic acids such as gallic acid, ferulic acid and caffeic acid [1], [2] and phenolic compounds bound to sugars such as p-coumaric acid 4-glucoside. These compounds could neutralize free radicals, making BCQ a potential agent for the synthesis of nanoparticles.

In comparison with other varieties, such as red or white quinoa, black quinoa exhibits higher concentrations of phenolic compounds and greater antioxidant capacity, characteristics that highlight its interest in the scientific field [3]. Efficient extraction of these bioactive compounds is essential for their utilization and application as research material for the development of new bioactive materials [3], [4],[5].

Ultrasound-assisted extraction (UAE) has emerged as an innovative and sustainable technique for the recovery of bioactive compounds. This method transmits energy by ultrasonic waves inducing acoustic cavitation [6], [7]. The UAE process is based on the generation of microbubbles that collapse violently, producing strong shear forces and high localized pressures, facilitating the extraction of intact bioactives [8], [9]. UAE presents advantages, such as reduced extraction time, use of less toxic solvents, operation at low temperatures, and preservation of thermolabile compounds, which positions UAE as an optimal technique for sustainable applications [9], [10], [11]. A determining role in the efficiency of UAE processes is the selection of the solvent, as the polarity and chemical properties of the solvent directly influence the extraction of phenolic compounds and flavonoids [12], [13], [14]. Recent studies have shown that polar solvents, such as hydroalcoholic mixtures, maximize the recovery of antioxidants, whereas non-polar solvents have limited efficiency in this context [14], [15], [16].

These extracts rich in bioactive compounds are gaining prominence in the green synthesis of silver nanoparticles (AgNPs) because they allow the use of phenols and flavonoids

as reducing and stabilizing agents, instead of conventional chemical reagents [17], [18]. Biofunctionalized AgNPs obtained by green synthesis exhibit antimicrobial, antitumor, catalytic and antibacterial properties, making them promising materials for biomedical, technological and environmental applications [18].

The aim of the study is to evaluate the influence of different solvents on the antioxidant capacity and total phenol content of *Chenopodium quinoa negra*, the application in green synthesis and biofunctionalization of AgNPs using the extracts obtained. The formation of AgNPs was evaluated by means of the chromatic changes generated in their synthesis. This study not only broadens the knowledge on the sustainable use of Andean natural resources but also contributes to the development and evaluation of green nanotechnological procedures with innovative applications.

II. MATERIALS AND METHODS

A. Reagents and equipment

The reagents used were Folin-Ciocalteu (Merck), PA calcium carbonate (Merck), Gallic acid standard (GA) (Merck), Trolox, DPPH (2,2-diphenyl-1-picrylhydrazyl) and 96° ethanol. Ultrasound-assisted extraction was carried out using a Branson® ultrasonic cleaner 40 kHz ultrasonic bath. Spectrophotometric measurements were performed on a microplate reader (Varioskan LUX Multimode Microplate Reader).

B. Sample collection

Chenopodium quinoa wild black (BCQ) grains were collected in Ayaviri district, Melgar province, Puno (latitude: -14.848299, longitude: -70.647408). Samples were stored at room temperature for later analysis.

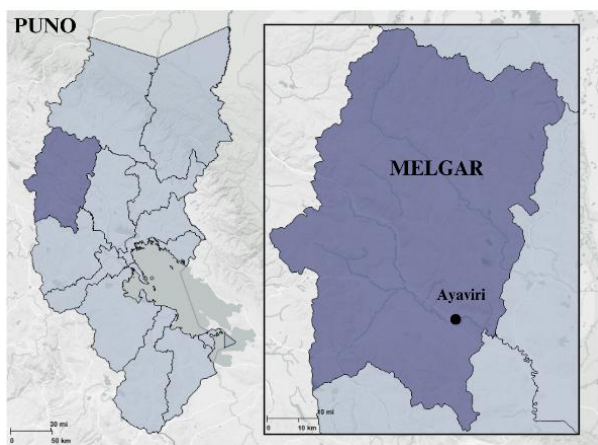


Fig. 1 Geographic map of the province of Melgar - Puno - Peru.

C. Obtaining the extract

To obtain the extracts, 8 g of crushed BCQ were weighed and 40 mL of different solvents (ultrapure water, ethanol:water (1:1) and 96° ethanol) were added. Ultrasonic bath extraction was performed for 10 min at a constant temperature of 25 °C [19], [20].

Subsequently, the extracts were filtered using slow-speed Whatman filter paper and stored in amber glass vials for further analysis. The complete process of extract preparation is detailed in Fig. 2.

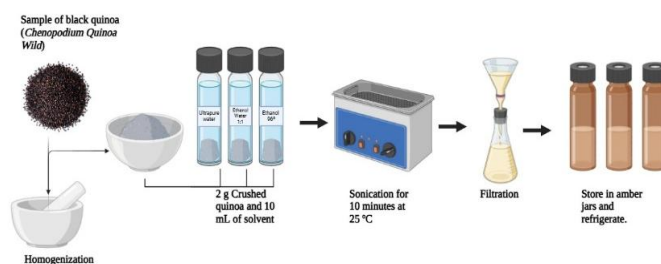


Fig. 2 Preparation process of black quinoa extracts (BCQ).

D. Determination of total phenolic compounds by the Folin-Ciocalteu method.

La Quantification of total phenolic compounds (TPC) in BCQ extracts was carried out following the protocol described by Buitrago D *et al.* [21]. Standard solutions of Galic Acid at concentrations of 20, 30, 40, 40, 50 and 60 µg/mL were prepared from a stock solution of 100 µg/mL. The calibration curve was prepared in triplicate.

For the analysis, 7.5% Na₂CO₃ and 10% Folin-Ciocalteu were used. Samples were incubated for 15 min at 40 °C and the absorbances were measured 765 nm in the microplate reader. The extracts were analyzed in triplicate under the same conditions as the standards. The analysis of the calibration curve and the samples were performed with GraphPad Prism 8 software for which the Galic Acid Equivalents (GAE) concentrations were related to the absorbances obtained.

The concentration of total phenolic compounds was calculated using the linear regression equation derived from the calibration curve and expressed in micrograms of Galic Acid Equivalents per milliliter of extract (µg GAE/mL).

E. Determination of antioxidant capacity by the DPPH method

The antioxidant capacity of BCQ extracts was determined using the DPPH method in a 96-well microplate, following the methodology described by Buitrago D *et al* [21].

For the preparation of calibration curves, concentrations of 20, 40, 60, 80, 100 and 120 μM Trolox were used, starting from a 0.1 mM stock solution.

The calibration curve was prepared in triplicate using a DPPH: Trolox ratio of 2:1 and incubated for 30 minutes in the dark at room temperature. Absorbance was measured at 515nm using a microplate reader [21]. The extracts were analyzed in triplicate under the same conditions as the standards.

The analysis of the calibration curve and the samples were performed with GraphPad Prism 8 software for which the Trolox concentrations (μM) were related to the absorbances obtained. The antioxidant capacity of the extracts was expressed as equivalent Micromoles Equivalents of Trolox per liter of extract ($\mu\text{mol ET/L}$).

F. Optimization of silver nanoparticle synthesis.

5 ml of a 1mM solution of silver nitrate (AgNO_3) was prepared and 40 ml of BCQ hydroalcoholic extract was added to it. The phenolic compounds present in the extract acted as reducing agents, reducing silver ions (Ag^+) to silver atoms (Ag), resulting in the formation of nanoparticles. During this process, the solution changed color, which is a visual indication of nanoparticle formation. During the process, the pH of the mixture was adjusted to a slightly alkaline value (around pH 9) using 1M sodium hydroxide (NaOH) solution. Subsequently, the mixture was centrifuged to separate the silver nanoparticles from the excess reagents, and the nanoparticles were washed 6 times with distilled water to remove impurities.

Once the silver nanoparticles were obtained, UV-Visible spectroscopy was used to characterize them. The spectrum showed an absorption maximum at a characteristic wavelength around 420-450 nm, which corresponds to the surface plasmon resonance of the silver nanoparticles.

II. RESULTS AND DISCUSSION

A. Black quinoa extracts

After sonication, the extracts showed variations in their coloration, suggesting differences in the composition of the extracted compounds. The hydroethanolic extracts exhibited orange tones, being more intense in the extract obtained with 96° ethanol (BCQ1) compared to the 1:1 hydroethanolic extract (BCQ2). On the other hand, the aqueous extract (BCQ3) presented a milky appearance with a slight yellow coloration.

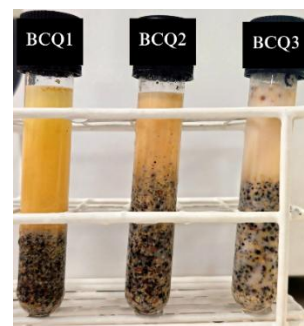


Fig. 3 Extracts of *Chenopodium quinoa wild* black after ultrasound-assisted extraction. BCQ1: ethanolic extract BCQ2: hydroalcoholic extract (1:1) and BCQ3: aqueous extract.

Studies have shown that solvent selection directly influences extraction efficiency, antioxidant activity and the type of compounds extracted. The polarity of the solvent determines its capacity to extract certain compounds, facilitating the solubilization of those to which it has greater affinity. In this regard, solvents with a higher ethanol content favor the extraction of substances such as carotenoids [22]. In addition, it has been reported that carotenoids present a range of colors from orange (carotenes) to yellow (xanthophylls) [22].

The difference in shades observed in BCQ1 and BCQ2 extracts suggests a higher concentration of carotenes in BCQ1, given that its solvent contains 96% ethanol, while BCQ2, with 50% ethanol, would present a lower concentration of these pigments. On the other hand, the slight yellow coloration of BCQ3 could be associated with the presence of xanthophylls, compounds more polar than carotenes that are soluble in aqueous media.

The milky appearance of BCQ3 may be because ultrasound generates cavitation bubbles that upon collapse create large shear forces, disrupting the cell walls, which facilitates the release of proteins [23] and increases their solubility in aqueous media [24], producing a milky suspension. Furthermore, it was observed that foam formation was higher in BCQ3, followed by BCQ2 and BCQ1. This behavior suggests a higher concentration of soluble proteins in BCQ3, which could be related to its foaming capacity [24].

B. Total phenols in *Chenopodium Quinoa* extracts

The calibration curve performed with gallic acid standards presented an R^2 of 0.9934 as shown in Fig. 4. The results of total polyphenol content were interpreted as $\mu\text{g GAE/mL}$.

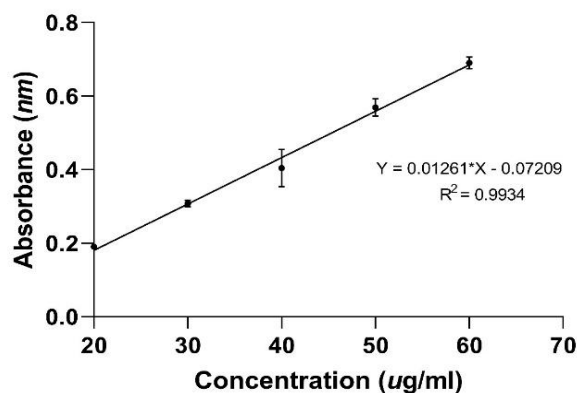


Fig.4 Calibration curve for the determination of total phenolic compounds by Folin-Ciocalteu assay.

The linear regression equation established is shown in equation 1.

$$Y = 0.0126x - 0.0721 \quad (1)$$

The total polyphenol concentration of the extracts was determined by calculating “X” which corresponds to μg gallic acid equivalent in one mL (μg GAE/mL) and considering that “Y” is the absorbance of the extract. Substituting was obtained (equation 2).

$$\text{Total phenols} \left(\frac{\mu\text{g GAE}}{\text{mL}} \right) = \frac{\text{Absorbance} + 0.0721}{0.0126} \quad (2)$$

Table I presents the absorbances recorded in the assay, as well as the equivalent concentration in gallic acid per milliliter of extract.

TABLE I
TOTAL PHENOLS IN EXTRACTS OF CHENOPODIUM QUINOA WILD BLACK BEANS OBTAINED BY ULTRASOUND WITH THREE DIFFERENT SOLVENTS.

Extract	Ethanol 96° (BCQ1)	Ethanol:Water 1:1 (BCQ2)	Ultrapure water (BCQ3)
Absorbance at 765 nm	0.579	0.625	0.470
	0.639	0.449	0.612
	0.685	0.644	0.502
Absorbance Average	0.634 ± 0.053	0.573 ± 0.107	0.528 ± 0.074
$\mu\text{g GAE} / \text{mL}$	51.677 ± 4.22	47.689 ± 8.53	57.187 ± 5.91

The statistical results on μg GAE /mL indicate that there is no statistically significant difference ($p > 0.05$) between the solvents used during the extraction process. Other works such as the solvent optimization reported by Carciochi R et al. report that 80% ethanol solutions presented a higher extraction yield

compared to concentrations of 40%, 20% and water [25] indicating that the type of solvent used is an important factor in the extraction processes. On the other hand, most of the compounds present in black quinoa (BCQ) are bound to sugars or correspond to phenolic acids, such as 3,4-dihydroxybenzoic acid, p-coumaric acid 4-glucoside and ferulic acid 4-glucoside, which are more soluble in water than in ethanol, which may be the reason for finding 57,187 μg GAE/mL in the aqueous extract [26]. Moreover, in the work done by Tsakona et al. it is indicated that gallic acid used in the Folin-Ciocalteu reaction has a higher solubility in water than in ethanol [27].

Regarding ultrasound-assisted extraction, some authors mention that this method could increase the yield in the extraction of phenolic compounds. This occurs by the formation of bubbles that improve the accessibility of the solvents to the surface of the plant matrix [28]. On the other hand, it has been reported that the efficiency of ultrasound extraction is increased due to the nucleation of bubbles that form between dispersed particles of the plant material [29]. However, the use of very high temperatures reduces the extraction rate due to the mitigation of the cavitation intensity and the increase in the degradation rate of phenolic compounds, finally, it should be noted that the concentration of phenolic compounds tends to decrease when exceeding 20 minutes in ultrasound treatment. All the above was considered to justify the BCQ extraction conditions (25°C ultrasound for 10 minutes).

C. Antioxidant capacity of Chenopodium Quinoa extracts.

Fig. 5 shows the calibration curve developed to determine the antioxidant capacity of BCQ1, BCQ2 and BCQ3 extracts by the DPPH method, and the linear regression equation obtained, which made it possible to express the antioxidant capacity of the extracts in Trolox equivalents ($\mu\text{mol TE/L}$) at 515 nm. The coefficient of determination R^2 obtained was 0.9994.

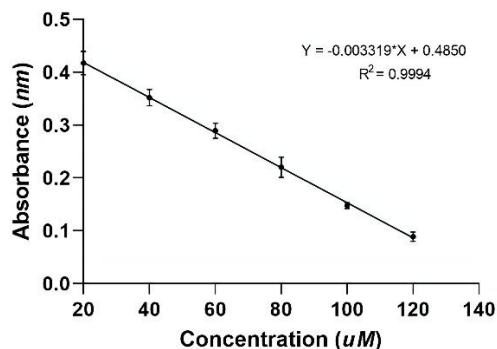


Fig. 5 Calibration curve for the determination of antioxidant capacity by the DPPH method.

The corresponding linear regression equation is shown in (equation 3).

$$Y = -0.003319X + 0.4850 \quad (3)$$

Where “X” and “Y” correspond to the Trolox equivalent antioxidant capacity ($\mu\text{mol TE/L}$) and absorbance at 515 nm (Ab_{515nm}) respectively. From this equation, the antioxidant capacity of the extracts was determined by replacing the average absorbance values of each in (equation 4).

$$\text{Antioxidant capacity} \left(\frac{\mu\text{mol TE}}{\text{L}} \right) = \frac{Ab_{515nm} - 0.4850}{0.003319} \quad (4)$$

Fig. 6 shows the results of antioxidant capacity of BCQ extracts obtained by ultrasound-assisted extraction with different solvents. It was observed that BCQ2 (ethanol:water) extract showed a higher reduction of DPPH color compared to BCQ1 (96° ethanol) and BCQ3 (ultrapure water), suggesting higher antioxidant capacity. This finding was corroborated by calculating the antioxidant capacity using equation 4. Thus, the values obtained were 0.939, 44.152 and 31.152 $\mu\text{mol TE/L}$ for BCQ1, BCQ2 and BCQ3 extract, respectively.

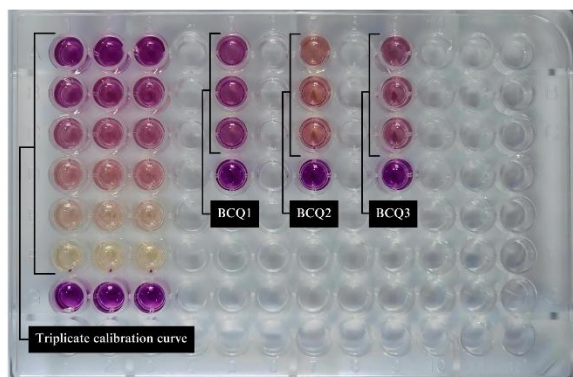


Fig. 6 DPPH - Trolox calibration curve color degradation in 96 micro well plate and results of the process of determining the antioxidant capacity of Black Chenopodium Quinoa extracts.

TABLE II
RESULTS OF ANTIOXIDANT ACTIVITY IN EXTRACTS OF CHENOPODIUM QUINOA WILD BLACK QUINOA GRAINS OBTAINED BY ULTRASOUND AND BY THE ACTION OF THREE DIFFERENT SOLVENTS.

Extracts	Ethanol 96° (BCQ1)	Ethanol:Water 1:1 (BCQ2)	Ultrapure water (BCQ3)
Absorbance at 515 nm	0.482	0.329	0.367
	0.482	0.343	0.380
	0.481	0.347	0.400
Absorbance Average	0.482 ± 0.001	0.339 ± 0.009	0.382 ± 0.016
$\mu\text{mol TE/L}$	0.939 ± 0.17	44.152 ± 2.89	31.152 ± 4.95

The higher antioxidant activity observed in BCQ2 may be attributed to the fact that the ethanol:water mixture (1:1) optimizes the solubilization of polyphenols, flavonoids and compounds with high antioxidant capacity, on the other hand, it is known that water favors the extraction of more polar flavonoids [30], while ethanol facilitates the solubilization of less polar polyphenols so the synergy between both solvents could explain the good antioxidant capacity obtained in BCQ2.

Previous studies have shown that solvent polarity influences the solubilization of specific antioxidants [30], [31], and in this case, the ethanol:water mixture (1:1) seems to have favored the extraction of compounds with a higher free radical neutralizing capacity. On the other hand, the lower antioxidant capacity of the 96° ethanolic extract (BCQ1) could be since some phenolic compounds extracted in this medium do not have a strong reactivity with the DPPH radical [31] or could be in glycosylated or polymerized forms, which reduces their electron-donating capacity [32].

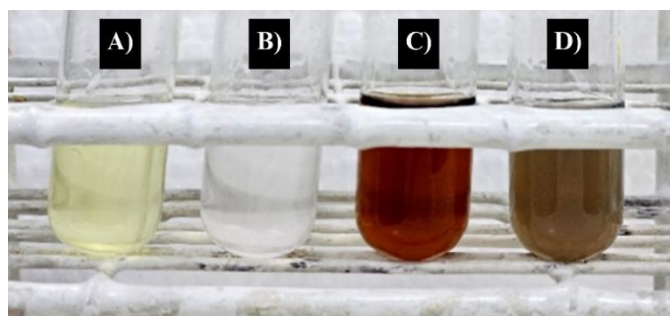


Fig. 7 nanoparticle synthesis: (A) Distilled water, extract and NaOH, (B) Silver nitrate, (C) Silver nitrate, extract and NaOH, (D) Extract and silver nitrate.

Fig. 7 shows the modifications detected in the mixtures prepared under various conditions of AgNPs synthesis employing BCQ extracts, likewise, Fig. 8 presents the spectrophotometric scans from 350 to 750 nm of the solutions in Fig. 7. Fluctuations in the composition and addition sequence of the reagents exerted a significant influence on the formation of the nanoparticles. In Fig. 7-A (1 mL extract, 1 drop NaOH and 5 ml distilled water): This sample plays the role of control, registering a pale yellow coloration, indicating that no significant reaction was observed with the addition of sodium hydroxide. This finding confirms that any chromatic modification in the other tubes can be attributed to interaction with AgNO_3 , and not merely to the influence of the alkaline environment. Nikolaeva et al. postulated that oxidative coupling reactions involving phenol substitution in an alkaline environment require the presence of specific catalysts to generate detectable chromatic modifications [33]. In Fig. 8A

the spectrophotometric scan is shown where the spectrum of the extract is mainly observed which presents higher light absorption around 400 nm. On the other hand, in Fig. 7B (1 mL distilled water together with 5 mL AgNO₃): the solution maintained its transparency, without the generation of visually perceptible precipitates.

Fig. 8B shows the spectrum of the AgNO₃ solution, which shows higher light absorption near 350 nm. In Fig. 7C (5 mL AgNO₃, 1 mL extract and 5 drops of NaOH): Intense red pigmentation was observed, indicating the generation of silver nanoparticles. In alkaline environments, depletion of Ag⁺ ions can promote the nucleation and development of nanoparticles, as documented in investigations of green synthesis of metal nanoparticles. Pirtarighat et al. reported the biosynthesis of silver nanoparticles using *Salvia spinosa* extract, where the manifestation of an analogous coloration signaled the generation of these nanoparticles [34]. Fig. 8C shows the absorption spectrum of the silver nanoparticles formed by interaction of AgNO₃ and the extract in alkaline medium, in this spectrum a Gaussian absorption spectrum with maximum absorption peak at 410 nm is observed, which is characteristic of the formation of silver nanoparticles.

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Finally, in Fig. 8D (5 mL of AgNO₃, one drop of NaOH and 1 mL of extract): A brownish hue was recorded, suggesting the potential generation of silver nanoparticles in a more moderate grade reduction process. The percentage of NaOH in this reaction is lower compared to tube C, which could affect the rate and magnitude of nanoparticles generated. Khatun et al. performed the synthesis of silver nanoparticles employing plant

extracts, finding that the amount of added base plays a crucial role in the morphology and stability of these nanoparticles [35]. However, a spectrum that is not characteristic of silver nanoparticle formation is observed in Figure 8D. This finding suggests that the reaction between the compounds present in the extract and the Ag⁺ ions did not materialize, or that the concentration of the reactive groups in the sample was insufficient to cause a detectable alteration. However, the pH level could exert an important impact on the reactivity of the compounds present in the extract. Previous research has shown that the stability of complexes composed between Ag⁺ ions and phenolic compounds is determined by the environmental pH, affecting the solubility and the generation of precipitates Khatun et al [35].

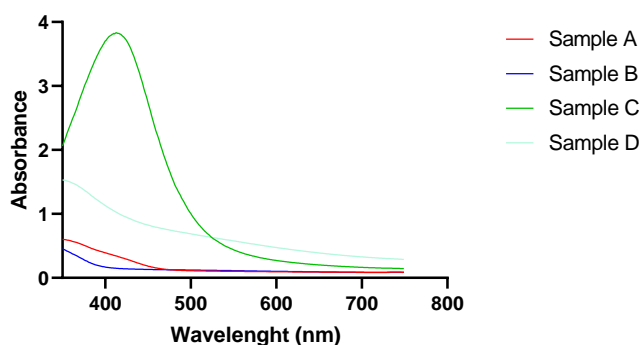


Fig. 8 Spectrophotometric sweeps of nanoparticle synthesis: (A) Distilled water, extract and NaOH, (B) Silver nitrate, (C) Silver nitrate, extract and NaOH, (D) Extract and silver nitrate

IV. CONCLUSIONS

The results of the study show that the extraction of bioactive compounds from black quinoa by sonication is influenced by the solvent used, although no significant differences were observed in the concentration of total phenols among the three extracts. Despite this, the extract obtained with the ethanol:water mixture (BCQ2) presented the highest antioxidant capacity, reaching 44,152 μmol TE/L, suggesting that this combination favors the extraction of compounds with a greater capacity to neutralize free radicals. On the other hand, the efficiency in the synthesis of silver nanoparticles was also higher with BCQ2, evidenced by an absorption peak at 410 nm. These findings highlight the importance of the ethanol-water mixture in the extraction of bioactives and in the generation of nanoparticles, which opens new possibilities for their application in technological and pharmacological areas.

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