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Biochemical specification of the anticancer and antioxidant potential of green silver nanoparticles synthesized by *brassica oleracea* extract

Alaa Al-Sharqawi[a], Ali Osman [a]*, Abdelaleim Ismail ElSayed[a], Ahmed E. Abdelhamid[b], Mahmoud Sitohy[a]

[a] *Biochemistry Department, Faculty of Agriculture, Zagazig Univerisity, Zagazig, 44519, Egypt*

[b]*Polymers & Pigments Department, National Research Centre, 33 El-Buhouth St. Dokki, Giza, 12622, Egypt*

Abstract

Broccoli seeds and florets are excellent sources of health-promoting phytochemicals, including phenolics and flavonoids. In the current study, aqueous and ethanolic extracts from both broccoli seeds and florets were prepared. The total phenolic content (TPC), total flavonoids (TF), and antioxidant activity of these extracts were assessed. The identification of phenolic and flavonoid compounds in the extracts was carried out using HPLC analysis. Both broccoli floret ethanolic extract (193.6 mg GAE/g extract) and broccoli seeds ethanolic extract (141.49 mg GAE/g extract) have reported the highest levels of total phenolic compounds (TPCs). The results of the DPPH test showed that the broccoli seeds had the highest radical scavenging activity (RSA) compared to the florets, in both ethanolic and aqueous extracts. Gallic acid was present in significant quantities in the aqueous extract of broccoli florets, measuring 32.34 µg/g of dry extract. In the aqueous extract of broccoli seeds, 3.4-Dihydroxybenzoic acid was found in large amounts, at 56.35 µg/g of dry extract. Additionally, syringic acid was also found in notable quantities in the broccoli seeds aqueous extract, at 26.41 µg/g of dry extract. The green synthesis of silver nanoparticles (Ag NPs) has been gaining popularity in recent years for various applications. The current study focused on synthesizing Ag NPs using an aqueous extract from broccoli florets and seeds and evaluating their efficacy against cancer cells (A549 and HCT116). The green synthesized silver nanoparticles were characterized and confirmed using a transmission electron microscope (TEM), UV-VIS absorbance, and particle size analysis. The TEM image exhibits a good distribution of spherical shape Ag-NPs without any aggregation. The particle size distribution curve of Ag-NPs prepared using broccoli floret aqueous extract (BFAE) was 63±5, and prepared by seed extract (BSAE) was 79±5 nm. The efficacy of the treatments in eliminating cancer cells (A549, and HCT116) over a 48-h varied as follows: BFAE-NPs > BSAE > BFAE > Silver-NPs > BSAE-NPs. The mRNA expression of the apoptotic gene caspase 9 was dramatically elevated, but the expression of bcl-2 was significantly downregulated in A549 and HCT-116 cells administered with Ag-NPs, BSAE-NPs, and BFAE-NPs. Therefore, nanoparticles prepared from BSAE, are crucial for formulating alternative therapy techniques for cancer.

Keywords: Broccoli; aqueous extract; MTT; apoptosis; phenolic compounds; Bcl-2.

Introduction

The World Health Organization (WHO) states that cancer is the second-leading cause of death worldwide, surpassed only by cardiovascular diseases. As the global population ages, the incidence of cancer is rising rapidly. Malignant neoplasms are a major source of illness and death, placing a significant burden on modern society [1-3]. Cancer is characterized by the uncontrolled growth of abnormal cells, which poses a serious health risk. These cells can spread to various parts of the body, leading to increased mortality. The complexities of cancer as a neoplastic disease involve several biological features that develop during its multistep progression. These characteristics include the ability to evade growth inhibitors, maintain continuous proliferative signaling, resist cell death, enable their own indefinite replication, and initiate invasion and metastasis [4]. Notwithstanding progress in diagnostic techniques and medicines, drug resistance and metastasis continue to pose significant challenges to effective therapy [5]. The complexity of tumorigenesis in this situation makes it difficult to find effective cancer drugs. Due to the many side effects that come with chemotherapeutics, dietary phytocompounds are seen as good alternatives for stopping and preventing cancer because they work well and have few negative effects [2]. There is an increasing interest in investigating the anti-cancer benefits of raw vegetables abundant in isothiocyanates, polyphenols, vitamins, and trace minerals [6,7]. There is a growing interest in foods that help alleviate the harmful effects of free radicals (FR) in the human body [8]. FR can be produced during regular oxygen metabolism or attributable to external harm [9]. Prooxidants and antioxidants are crucial in maintaining the balance between free radicals and the body's antioxidant system [10]. Antioxidants are substances that inhibit, diminish, or even eradicate the effects of free radicals, safeguarding the organism against oxidative harm [11]. Broccoli is a significant agricultural product and rich source of endogenous antioxidants, such as α-tocopherol, ascorbic acid, carotenoids, and phenolics [12]. Broccoli is recognized for its potential anticancer effect as one of its major bioactive effects [3]. Nanotechnology is swiftly advancing, concentrating on the creation, manipulation, and application of materials sized between 10 and 500 nm for many medical therapies and drug delivery mechanisms [13]. The application of nanoparticles (NPs) in

*Corresponding author e-mail: aomokhalil82@gmail.com; (Ali Osman).
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contemporary technology has increased markedly. The possible risk to human health remains ambiguous due to insufficient evidence. NPs have garnered attention due to their distinctive chemistry, diminutive size, non-biodegradability, and reactive surfaces [14]. A multitude of plant extracts and metallic NPs demonstrate considerable biological activity. Using plant extracts in the green synthesis of metallic nanoparticles is likely to have a synergistic effect, which could lead to more powerful biological activities [15,16]. In recent years, metallic nanoparticles (MNPs) have garnered considerable attention due to their potential as novel agents with antibacterial and anticancer properties [17-19]. MNPs, especially silver nanoparticles (Ag-NPs), have garnered significant interest in recent years due to their beneficial properties in biomedicine and other health-related fields [20]. The chemically synthesized silver nanoparticles (Ag-NPs) and their by-products may pose a threat to humans and the environment [21]. Biological methods for synthesizing Ag-NPs have been reported using plants. These eco-friendly methods can provide alternative approaches to the chemical and physical synthesis of Ag-NPs. These environmentally sustainable technologies offer alternatives to conventional chemical and physical production of Ag-NPs for application in anti-cancer therapies. In the current research, we prepared and characterized water and ethanol extracts of broccoli seeds and florets, and then evaluated their antioxidant activity. Ag-NPs were synthesized using an aqueous extract of broccoli florets and seeds and then characterized using a transmission electron microscope (TEM), UV-VIS absorbance of Ag-NPs, and particle size. The anticancer activity of the prepared particles was evaluated against A549 and HCT116 cancer cell lines. mRNA expression levels of the apoptotic gene caspase 9 and the antiapoptotic gene bcl-2 in both cell lines (A549 and HCT116) were assessed to investigate the mechanism of action.

Materials and methods

1.1. Plant materials, chemicals and reagents

Broccoli seeds and florets were obtained from the local market in Zagazig City, Egypt. The fresh florets were washed with deionized water and dehydrated using liquid nitrogen. Seeds and florets were ground to a fine powder and kept at −20 °C until subsequent examination. DPPH (2,2-Diphenyl-1-picrylhydrazyl), quercetin, gallic acid, Na_2CO_3, Folin-Ciocalteu reagent, NaOH, and ethanol were obtained from Merck (Darmstadt, Germany).

1.2. Crude extracts preparation

Crude extracts were prepared by extracting ground broccoli seeds and florets in various solvents. Ten grammes from each sample were combined individually with 70% ethanol and distilled water (100 mL) to achieve a concentration of 10% (w/v). Extractions were conducted using an orbital shaker for 24 h at 20 °C. All extracts were centrifuged at 9000 xg for 15 min, and the supernatants were subsequently filtered using Whatman No. 1 (Merck KGaA, Darmstadt, Germany) filter paper [22]. Filtrates were evaporated to dryness utilizing a rotary vacuum evaporator (BüCHI-water bath-B-480, Czech Republic) at 35–40 °C, followed by freeze-drying (Thermo-electron Corporation–Heto power dry LL 300 Freeze drier). The lyophilized aqueous and ethanolic extracts from seeds and florets were preserved at −20 °C for subsequent examination.

1.3. Total phenolic compounds (TPCs) estimation

The TPCs of aqueous and ethanolic extracts prepared from broccoli seeds and florets were determined using the Folin-Ciocalteu assay [23]. One mL (1000 µg/mL) from each sample was mixed with 4 mL of diluted Folin-Ciocalteu reagent (1-part Folin reagent to 10 parts distilled water, v/v) and 4 mL of sodium carbonate (75 g/L). Mixture was vortex-mixed for 15 s and then allowed to stand for 30 min at 40 °C. Absorbance was measured at 765 nm. A standard curve was prepared using gallic acid. Calibration equation for gallic acid is $y = 0.001x + 0.0563$ ($R^2 = 0.9792$), where y represents absorbance, and x represents the concentration of gallic acid in µg/mL.

1.4. Total flavonoids (TFs) estimation

The total flavonoids (TFs) in aqueous and ethanolic extracts derived from broccoli seeds and florets were quantified following the procedure established by [24]. Two mL of a 2% ethanol solution of $AlCl_3$ were combined with one mL of each extract (1000 µg/mL) and incubated at 25 °C for 60 min, after which absorbance was measured at 420 nm. Quercetin was utilized to establish standard curve, with total flavonoid content represented as quercetin equivalent (QE), computed from calibration curve. Calibration equation for quercetin is $y = 0.0012x + 0.008$ ($R^2 = 0.944$), where y represents absorbance and x is quercetin content in µg/mL.

1.5. HPLC analysis

Aqueous and ethanolic extracts derived from broccoli seeds and florets (20 µL), previously filtered using a 0.45 µm nylon membrane filter, were injected into the HPLC system (Agilent 1100) as described [25]. The chromatographic separation was performed using a Hypersil Gold C18 column (5 µm particle size, 250 × 4.6 mm). The mobile phase comprised methanol (A) and acetic acid in water at a ratio of 1:25 (B). The gradient program commenced and continued for the initial 5 minutes at 100% B, after that, transitioning to 50% eluent A for the ensuing 10 minutes. The concentration of A was elevated to 80% for the following 10 minutes and thereafter decreased to 50% for the final 5 minutes. Phenolic substances were identified by analyzing their chromatographic behavior and observing UV absorption at 320 nm in comparison with legitimate standards and documented data.

1.6. Antioxidant activity (DPPH-assay)

DPPH assay [26] was used to test antioxidant activity of aqueous and ethanolic extracts from broccoli seeds and florets (100–2000 µg/mL). One mL of extract was mixed with 2.5 mL of a 0.06 mM DPPH ethanol solution. Mixture was allowed to react at ambient temperature for 30 min in the absence of light. Absorbance values were measured at 517 nm, and then inhibition of DPPH was calculated using the following formula.

$$Inhibition\ (\%) = [(Abs\ control - Abs\ sample)/Abs\ control]x\ 100$$

1.7. Green synthesis of silver nanoparticles using broccoli seeds and florets aqueous extract

In summary, 1 mM AgNO3 was solubilized in 90 ml of distilled water. Ten mL of extract were included into silver nitrate solution while maintaining continuous agitation for one hour at 85°C. Color changes from light yellow to brown, which shows

that broccoli extract helps change Ag+ to Ag0. This is likely because of surface plasmon resonance (SPR) of silver nanoparticles according to [27].

1.8. Characterization of green Ag-NPs

High-Resolution Transmission Electron Microscopy (HRTEM) JEOL (JEM-2100 TEM) was used to get a good picture of the shape of Ag-NPs. The particle distribution in liquid was analyzed using a computer-controlled particle size analyzer (ZETA sizer Nano series, Malvern instrument Nano Zs). The surface Plasmon resonance of NPs was performed using a UV-VIS (Shimadzu spectrophotometer) with a 300–800 nm spectral range.

1.9. Anticancer activity

1.9.1. MTT-assay

A549 and HCT-116 cancer cell lines were assessed to evaluate the cytotoxic effect of broccoli seeds and florets aqueous extract, silver-NPs, and green synthesized silver nanoparticles using broccoli seeds and florets aqueous extract by using MTT assay [28]. Cancer cells were cultured in DEME media in 96-well plates and incubated for 24 h. Plates were incubated with treatments at varying concentrations (31.25–1000 µg/mL). All plates were incubated in a 5% CO_2 incubator and maintained at 37 °C for 48 h. After the removal of the medium, cells were rinsed with phosphate-buffered saline (PBS). Subsequently, 50 µl/well of MTT solution (Sigma-Aldrich, 0.5 mg/mL) was introduced to the plates and incubated for 4 h. Subsequently, 50 µl of DMSO solution was added to each well. The absorbance of each well was quantified at 590 nm using an ELISA reader. Cell viability (%) and toxicity were calculated from following equation:

$$\textbf{Cell viability } (\%) = (\textbf{Ab sample/Ab control})\textbf{x } \textbf{100}$$

$$\textbf{Toxicity } (\%) = \textbf{100} - \textbf{cell viability}$$

The cytotoxic effect on A549 and HCT116 cells can be quantified by the concentration required to induce cell death in 50% of the population (IC_{50}).

Alterations in cell morphology were recorded with a standard inverted microscope (Nikon) at 200x magnification in comparison to untreated cells.

1.9.2. Analysis of caspase 9 and Bcl2

Caspase 9 and Bcl2 expression were determined in A549 and HCT-116 control cells, as well as cells treated with silver nanoparticles (Ag-NPs), green silver nanoparticles synthesized using broccoli seed aqueous extract (BSAE-NPs), and broccoli floret aqueous extract (BFAE-NPs). The total RNA from control and treatment cells was extracted using the RNX-Plus reagent kit according to manufacturer's instructions. Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) was selected as a housekeeping gene. Table 1 displays the sequences of the primers employed. The comparative delta-delta CT method ($2^{-\Delta\Delta Ct}$) was utilized to calculate the fold change [29].

1.10. Statistical analysis

Data were analyzed using a factorial totally randomized design in R statistical software version 4.1.1. The distinctions among the examined factors were analyzed using the protected Tukey's HSD test at a significance threshold of $p \leq 0.01$.

Results and discussion

1.11. Crude extracts characterization

Initially, broccoli seeds and florets were extracted using two solvents: 70% ethanol and water. Secondly, TPCs, TFs, and antioxidant activity of obtained extracts were assessed. Identification of various naturally occurring phenolic compounds and flavonoids in extracts was conducted using HPLC analysis.

Broccoli is recognized for its high antioxidant content, primarily phenolic acids. Consequently, TPCs and TFs were assessed to validate the antioxidant activities of various broccoli components comparatively. According to the values in **Figure 1 and Table S1**, broccoli floret ethanolic extract (193.6 mg GAE/g extract) and broccoli seeds ethanolic extract (141.49 mg GAE/g extract) both reported the highest levels of TPCs. The lowest values of TPCs were recorded in broccoli floret aqueous extract (31.46 mg GAE/g extract). Samples extracted with 70% ethanol had a higher TPC than those extracted with water, as illustrated in **Figure 1**. The highest TFs were found in broccoli seed aqueous extract (20.41 mg QE/g extract), followed by florets ethanolic and aqueous extracts (11.42 and 11.4 mg QE/g extract, respectively), with the lowest concentration in seed ethanolic extract (8.13 mg QE/g extract), as illustrated in **Figure 1**. No significant differences were reported between the ethanolic and aqueous extracts of florets for TFs.

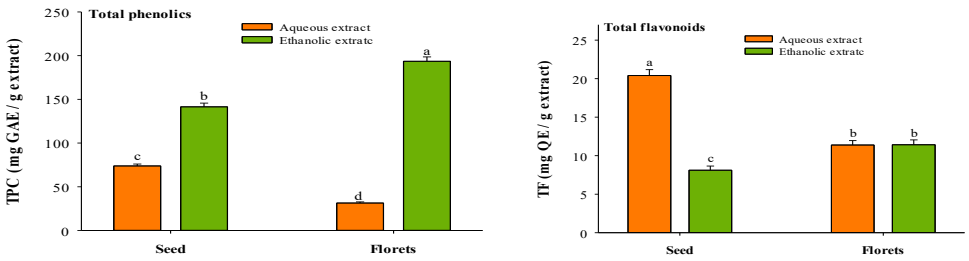

Fig. 1. Total phenolic compounds (TPC) and total flavonoids (TF) in the water and ethanol extracts of broccoli (*Brassica oleracea* var. italica) seeds and florets. Means followed by different letters under each studied factor are significantly different based on Tukey's HSD test (P<0.01).

Aqueous and ethanolic extracts of broccoli florets and seeds were examined for their antioxidant activity utilizing the DPPH assay. **Figure 2** and **Table S2** demonstrate that the DPPH assay findings revealed the strongest radical scavenging activity (RSA) in broccoli seeds of both ethanolic and aqueous extracts, in comparison to the florets. The RSA was increased gradually with increasing concentrations from 100 to 2000 µg/mL in all extracts. The ethanolic extract had significantly more RSA than the aqueous extract, as shown in **Table S2**.

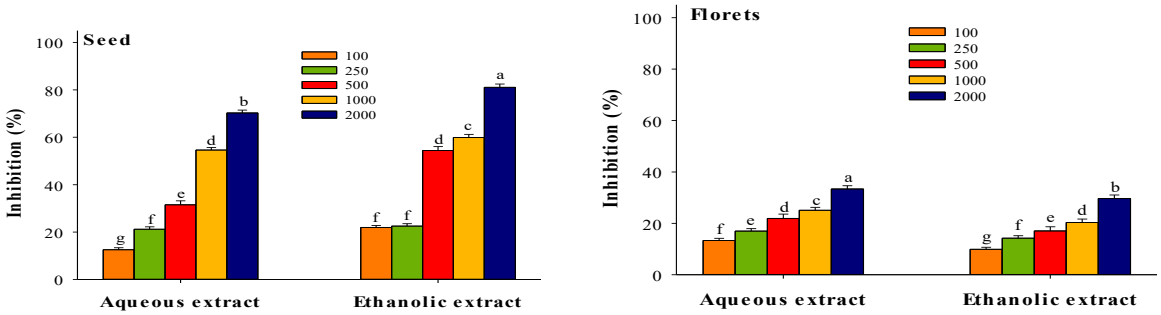

Fig. 2. DPPH scavenging activity (Inhibition %) of different concentrations (100, 250, 500, 1000, and 2000 µg/mL) of the water and ethanol extracts of broccoli seeds and florets. Means followed by different letters under each studied source (seed or florets) are significantly different based on Tukey's HSD test (P<0.01).

HPLC analysis was used to identify phenolic compounds and flavonoids in aqueous and ethanolic extract of broccoli seeds and florets. Ten phenolics compounds, including chlorogenic acid, gallic acid, caffeic acid, coumaric acid, vanillin, ellagic acid, 3.4-Dihydroxybenzoic acid, methyl gallate, ferulic acid, and syringic acid were characterized. **Table 1** and **Figure 3** illustrate that the concentrations of these compounds were accumulated in the aqueous and ethanolic extracts of broccoli seeds and florets, exhibiting substantial variability. Gallic acid was found in large amounts in the broccoli florets aqueous extract (32.34 µg/g dry extract), 3.4-Dihydroxybenzoic acid was found in large amounts in the broccoli seeds aqueous extract (56.35 µg/g dry extract), and syringic acid was found in large amounts in the broccoli seeds aqueous extract (26.41 µg/g dry extract). Chlorogenic acid was absent in the aqueous extract of broccoli florets. Caffeic acid was undetected in the ethanolic extract of broccoli florets. Six flavonoid molecules, namely rutin, naringenin, quercetin, catechin, luteolin, and kaempferol, were characterized (**Table 1** and **Figure 3)**. Rutin, naringenin, quercetin, and kaempferol were identified in the aqueous extract of broccoli seeds, however, catechin and luteolin were not present. Rutin, naringenin, quercetin, luteolin, and kaempferol were detected in the ethanolic extract of broccoli seeds; however, catechin was absent. Rutin, naringenin, quercetin, catechin, and kaempferol were detected in the aqueous extract of broccoli florets; however, luteolin was absent. Rutin and naringenin were found in the ethanolic extract of broccoli florets, but other molecules were absent. Naringenin was identified in significant quantities in the ethanolic extract of broccoli florets and the aqueous extract of broccoli seeds (114.39 µg/g dry extract and 33.88 µg/g dry extract, respectively). Scavenging activity against free radicals generated because of oxidative stress is exhibited by phenolic compounds derived from plant sources [30,31]. Vegetables that are rich in phenolic and flavonoid compounds exhibit a higher level of free RSA [32]. The DPPH radical was suppressed by the extracts in a manner that was highly correlated with the irrespective TPC and TFC values. This is likely the result of the fact that most polyphenolic compounds are effective reducing agents that scavenge FR activity through electron donation [33].

Table 1: Levels of phenolics and flavonoids compound in the dry extracts (µg/g) of broccoli seeds and florets.

Phenolics	Compounds content (µg/g dry extract)			
	Broccoli seeds		Broccoli florets	
	Aqueous	Ethanolic	Aqueous	Ethanolic
Chlorogenic acid	0.46	6.75	ND	85.74
Gallic acid	3.49	0.22	32.34	0.33
Caffeic acid	2.80	3.24	0.70	ND
Coumaric acid	0.21	2.85	0.66	3.21
Vanillin	6.99	12.02	5.32	13.46
Ellagic acid	0.17	0.12	0.12	0.31
3.4-Dihydroxybenzoic acid	56.35	20.67	1.66	1.05
Methyl gallate	0.01	0.00	0.00	0.04
Ferulic acid	4.17	13.61	0.42	17.95
Syringic acid	26.41	5.00	0.42	1.32
Flavonoid				
Rutin	0.01	0.02	0.01	0.01
Naringenin	33.88	8.74	4.01	114.39
Querectin	0.83	0.11	0.02	ND
Catechin	ND	ND	3.23	ND
Luteolin	ND	0.18	ND	ND
Keampferol	21.18	0.08	0.16	ND

ND: Not detected

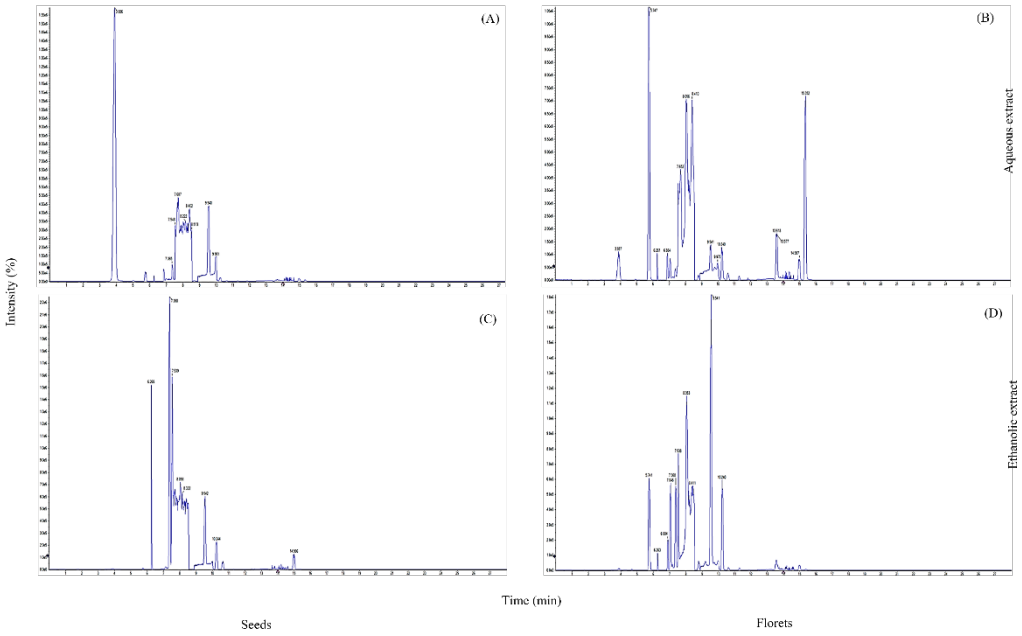

Fig. 3. HPLC chromatograms of broccoli florets aqueous extract (A), broccoli seed aqueous extract (B), broccoli florets ethanolic extract (C), and broccoli seed ethanolic extract (D).

1.12. Characterization of green Ag-NPs

The prepared nanoparticles were characterized using TEM, Zeta Sizer instrument and UV-Vis spectroscopy to assess particle size of Ag-NPs green synthesized using broccoli florets and seeds aqueous extract. The TEM image exhibits a good distribution of spherical shape Ag-NPs without any aggregation as shown in (Figures 4A and B). The particle size distribution curve of Ag-NPs prepared using broccoli florets aqueous extract was 63±5 (Fig.1C), and prepared by seeds extract was 79±5 nm (Figure 4D). We employed UV-Vis analysis, a prevalent method for monitoring Ag^+ ion reduction processes and verifying the synthesis of Ag-NPs. The UV–Vis spectra of Ag-NPs displays a prominent band in the visible range of 300 nm to 800 nm, featuring a distinct surface plasmon resonance peak at 438 nm for Ag-NPs synthesized with broccoli florets aqueous extract (Figure 4E), and at 395 nm for those derived from seeds extract (Figure 4F). This unique peak signifies the presence of silver nanoparticles, hence reinforcing the credibility of the reduction process. Conversion of silver nitrate into Ag-NPs upon exposure to plant extracts is indicated by a progressive color change from clear to yellowish-brown, attributable to the surface plasmon resonance phenomenon. Ag-NPs are recognized for their yellowish-brown hue in aqueous solution, attributed to the activation of SPR of Ag-NPs [34]. It is widely acknowledged that UV-Vis spectroscopic analyses of aqueous suspensions [35]. Prior investigations have shown that Ag-NPs have a yellowish-brown hue in solution as a result of the activation of surface plasmon vibrations [36]. The synthesis of Ag-NPs utilizing a 1 mM $AgNO_3$ solution was validated through UV-visible spectrum spectroscopy. The metal nanoparticles, such as silver (Ag), possess free electrons, resulting in the emergence of the SPR absorption band [37].

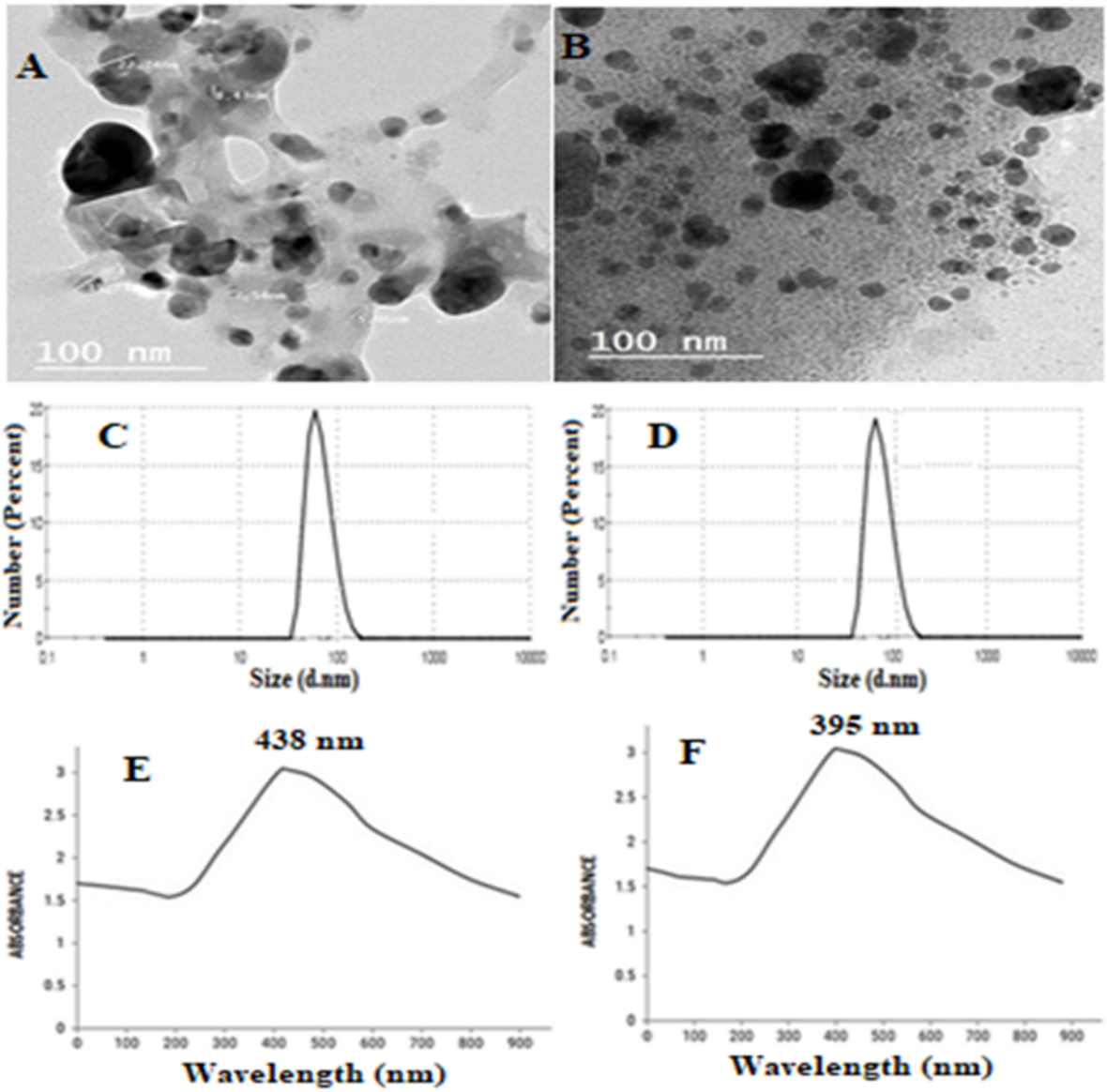

Fig. 4. Transmission electron microscope of Ag-NPs prepared from the aqueous extract of broccoli florets (A) and broccoli seeds (B), particle size of florets (C) and seeds (D) Ag-NPs, and UV-Vis absorbance of florets (E) and seeds (F) extract.

1.13. Anticancer activity

Ag-NPs were synthesized utilizing an aqueous extract of broccoli florets and seeds. Antitumor efficacy of synthesized particles was assessed against A549 and HCT116 cancer cell lines. Following 48 h of incubation, concentration-dependent inhibition of A549 lung cancer cells was found in the cytotoxicity experiment using broccoli seeds aqueous extract (BSAE), broccoli floret aqueous extract (BFAE), silver nanoparticles (NPs), BSAE-NPs, and BFAE-NPs **(Figure 5)**.

Compound dose values needed to achieve a 50% reduction in survival (IC_{50}) served as an indicator of its cytotoxic activity. The measured IC_{50} values after 48 h of treatment indicated that all the treatments exhibited variability within the cytotoxicity range. The efficacy of the treatments in eliminating cancer cells (A549) over a 48-hour incubation period varied as follows: BFAE-NPs (IC_{50}=36.25 µg/mL) > BSAE (IC_{50}=106.63 µg/mL) > BFAE (IC_{50}=191.87 µg/mL) > Silver-NPs (IC_{50}=222.07 µg/mL) > BSAE-NPs (IC_{50}=443.27 µg/mL). **Figure 6** illustrates the viability and toxicity of HCT 116 cells following exposure to different concentrations of BSAE, BFAE, Ag-NPs, BSAE-NPs, and BFAE-NPs. The efficacy of the treatments in eliminating cancer cells (A549) over a 48-hour incubation period varied as follows: BFAE-NPs (IC_{50}=38.99 µg/mL) > BSAE (IC_{50}=113.03 µg/mL) > BFAE (IC_{50}=185.72 µg/mL) > Silver-NPs (IC_{50}=345.82 µg/mL) > BSAE-NPs (IC_{50}=464.72 µg/mL). The MTT assay findings demonstrated that all treatments exhibited toxicity to the A549 and HCT 116 cells. **Figure 7** illustrates the effects of BSAE, BFAE, Ag-NPs, BSAE-NPs, and BFAE-NPs on the morphological structures of A549 and HCT116 cells. The control groups exhibited no notable alterations. In the treated groups, a concentration-dependent drop in cell counts was found. The cytotoxicity of Ag-NPs has been documented in various cancer cell lines [38]. Ag-NPs have been documented to exhibit more toxicity towards cancer cells than to normal cells [39]. The cytotoxicity of nanoparticles is contingent upon their size and morphology,

which are influenced by the fabrication process employed. The variation is contingent upon the specific types of cancer cells exhibiting aberrant metabolism and morphology [40]. qRT- PCR was employed to assess mRNA expression of Caspase 9 and bcl-2 in A549 and HCT 116 subjected to IC_{50} concentrations of Ag-NPs, BSAE-NPs, and BFAE-NPs for 24 h, relative to the control group. The results indicated that Ag-NPs, BSAE-NPs, and BFAE-NPs significantly modified mRNA expression of Caspase 9 and bcl-2 in A549 and HCT116 **(Figure 8)**. mRNA expression of the apoptotic gene caspase 9 was dramatically elevated, but the expression of the antiapoptotic gene bcl-2 was significantly downregulated in A549 and HCT-116 cells treated with Ag-NPs, BSAE-NPs, and BFAE-NPs. The RT-PCR results demonstrated differential expression levels of Caspase 9 and bcl-2 in A549 and HCT116, underscoring anticancer efficacy of our biosynthesized Ag-NPs, due to the targeted apoptotic response in cancer cells.

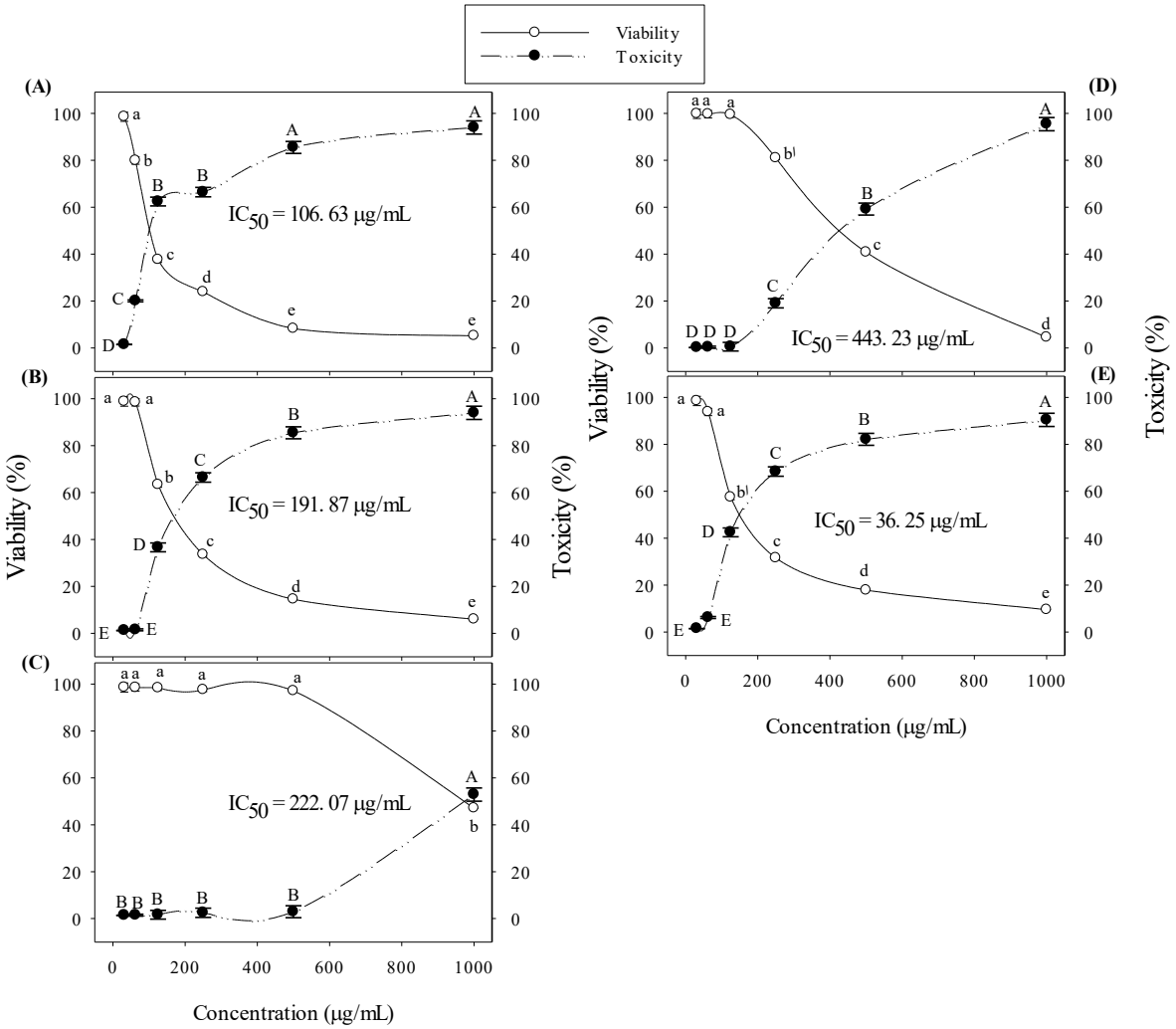

Fig. 5. Toxicity (%) and cell viability (%) of A549 cell line (A) treated with aqueous extract of broccoli seeds [BSAE] or (B) broccoli floret aqueous extract [BFAE], (C) Silver-NPs, (D) Green Silver-NPs synthesized from broccoli seed aqueous extract [BSAE-NPs], (E) green silver nanoparticles synthesized from broccoli florets aqueous extract [BFAE-NP_s]. Means followed by different letters under each studied factor (toxicity or viability) are significantly different based on Tukey's HSD test (P<0.01).

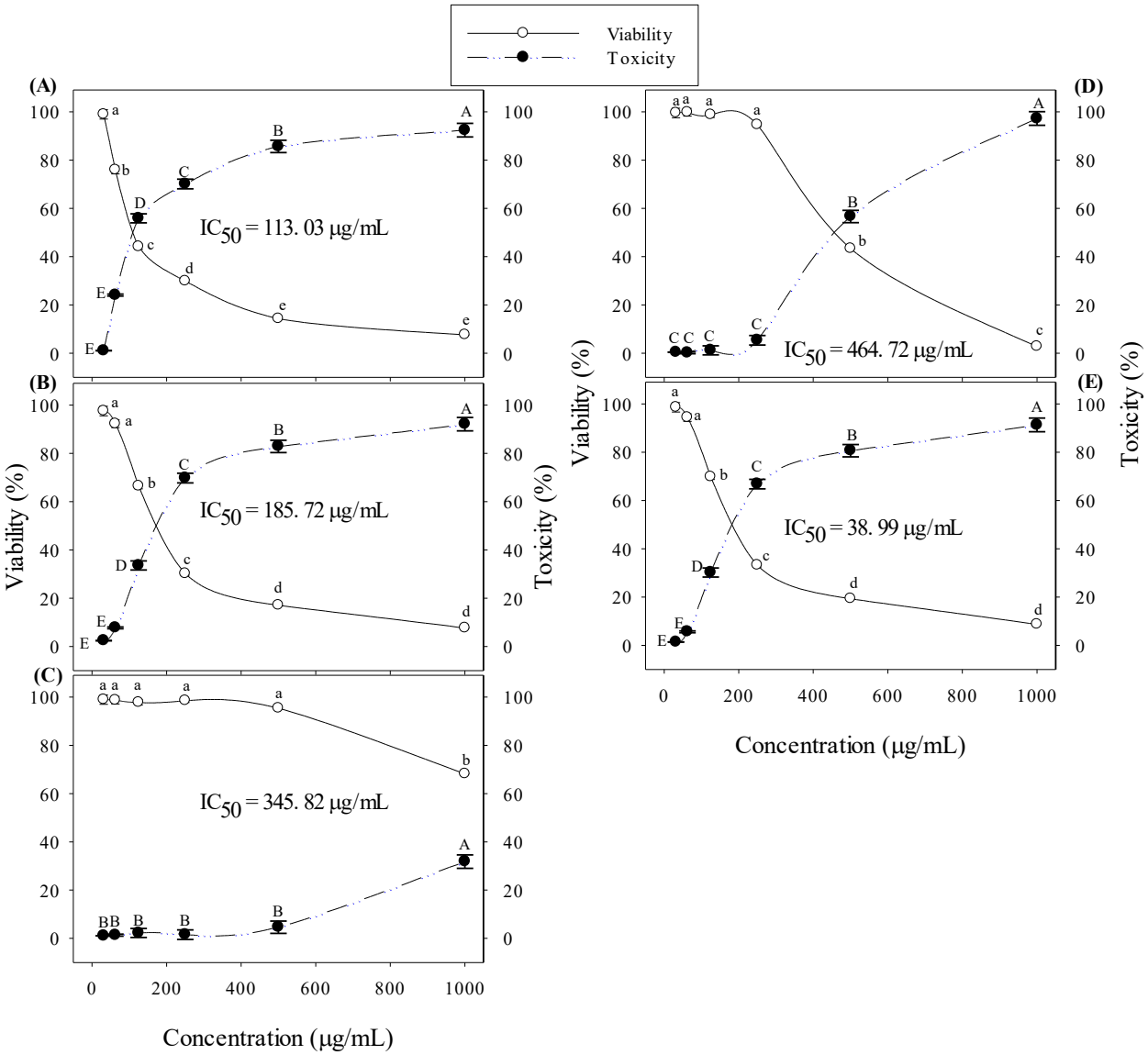

Fig. 6. Toxicity (%) and cell viability (%) of HCT116 cell (A) treated with aqueous extract of broccoli seeds [BSAE] or (B) broccoli floret aqueous extract [BFAE], (C) Silver-NPs, (D) Green Silver-NPs synthesized from broccoli seed aqueous extract [BSAE-NPs], (E) green silver nanoparticles synthesized from broccoli florets aqueous extract [BFAE-NP$_s$]. Means followed by different letters under each studied factor (toxicity or viability) are significantly different based on Tukey's HSD test (P<0.01).

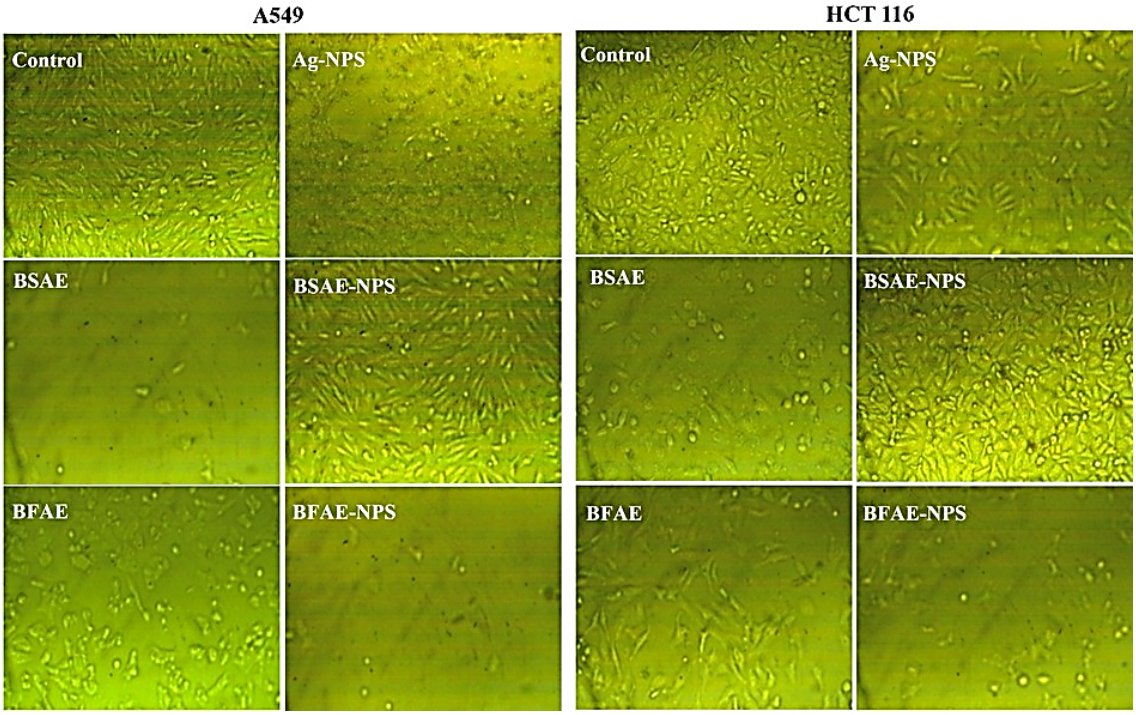

Fig. 7. Morphological changes in A549 and HCT 116 cell lines treated with 250 µg/mL from aqueous extract for broccoli; BSAE: seed aqueous extract, BFAE: floret aqueous extract, Silver-NP$_s$, BSAE-NP$_s$: green silver nanoparticles synthesized using broccoli seed aqueous extract, BFAE-NP$_s$: green silver nanoparticles synthesized using broccoli florets aqueous extract. (200X).

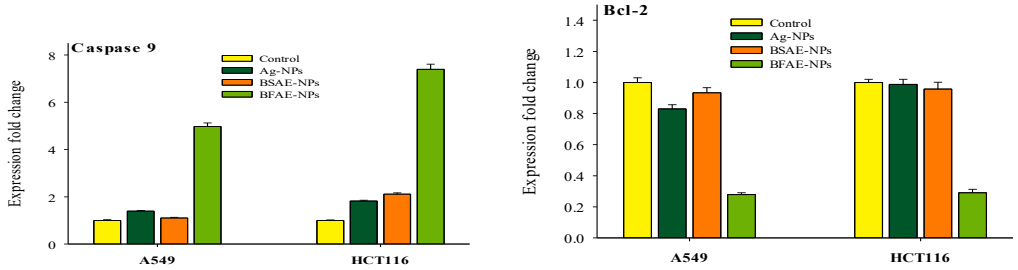

Fig. 8. Caspase 9 and Bcl-2 expression analysis in A549 and HCT116 cell lines treated with silver nanoparticles (Ag-NPs), green silver nanoparticles synthesized using broccoli seed aqueous extract (BSAE-NPs), and broccoli floret aqueous extract (BFAE-NPs) compared to control (untreated cell lines).

Conclusions

The comparison of seeds and florets from various botanical sources is crucial due to their differing polyphenolic compositions and antioxidant activities. Broccoli seeds and florets were extracted with water and ethanol, and their TPC, TF, and antioxidant activities were tested. Seeds and florets extracted with 70% ethanol had a greater total phenolic content than those extracted with water. The highest TFs were found in BSAE, followed by BFEE, and BFAE. This study identifies the unique effects of green Ag-NPs on the viability of A549 and HCT-116. Our molecular data showed that both cell lines treated with green Ag-NPs either up- or down-regulated the levels of caspase 9 and Bcl2, respectively. This suggests that a tumor-suppression mechanism is at play.

Conflicts of interest

There are no conflicts to declare.

Formatting of funding sources

Not applicable.

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