

EFFECT OF SILVER NANOPARTICLES ON PHENOLIC COMPOUNDS PRODUCTION AND BIOLOGICAL ACTIVITIES IN HAIRY ROOT CULTURES OF *CUCUMIS ANGURIA*

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The present study describes the elicitor effect of silver ion (Ag^+) and biologically synthesized silver nanoparticles (AgNPs) to enhance the biomass accumulation and phenolic compound production as well as biological activities (antioxidant, antimicrobial and anticancer) in genetically transformed root (hairy root) cultures of *Cucumis anguria*. The biomass of hairy root cultures was significantly increased by AgNPs whereas decreased in Ag^+ elicitation at 1 and 2 mg/L. AgNPs-elicited hairy roots produced a significantly higher amount of individual phenolic compounds (flavonols, hydroxycinnamic and hydroxybenzoic acids), total phenolic and flavonoid contents than Ag^+ -elicited hairy roots. Moreover, antioxidant, antimicrobial and anticancer activities were significantly higher following AgNPs-elicitation compared with that in Ag^+ -elicited hairy roots. We suggest that AgNPs could be an efficient elicitor in hairy root cultures to increase the phytochemical production.

Keywords: Biological activity – *Cucumis anguria* – hairy root cultures – silver nanoparticles – phenolic compounds

INTRODUCTION

Cucumis anguria L. is a highly nutritious cucurbit vegetable and also contains many potential phytochemicals which are used in traditional medicine for various diseases [21]. *Agrobacterium*-mediated gene transformation has been commonly studied as a strategy for producing hairy root lines with a high-yield of bioactive compounds. Silver was employed as an abiotic elicitor to stimulate the phytochemicals production in hairy roots of various plants [11, 23]. Nanoparticles (NPs), which are engineered structures with a diameter of less than 100 nm, have been shown great interest nowadays due to their small size per volume ratio in contrast to their bulk states, and they can be manufactured via inorganic synthesis or by exploiting living organisms [8, 17]. Silver nanoparticles (AgNPs) are one of the most common metallic nanoparticles in consumer products as a result of their biocidal action. Nanoparticles are promising to be used as novel effective elicitors in plant biotechnology for the elicitation of sec-

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ondary metabolites production [7, 14, 22]. Elicitation of AgNPs (5.0 ppm) led to the enhancement of taxol production in cell suspension culture of *Corylus avellana* [8]. In our previous study we found that the hairy root line induced by *A. rhizogenes* strain KCTC 2703 on the leaf explants of *C. aunguria*, were able to produce higher amount of phenolic compounds than the non-transformed roots [21]. The present study describes that the elicitation can further enhance the production of phenolic compounds in these hairy roots. We have studied AgNPs as a novel efficient elicitor for the production of phytochemicals in hairy root cultures of *C. aunguria*, aiming to evaluate the influences of Ag⁺ and AgNPs on biomass and phenolic compounds production of hairy root cultures. Furthermore, we evaluated the biological activities (antioxidant, antimicrobial and anticancer) of (AgNO₃ and AgNPs) elicited and non-elicited hairy root cultures in *C. aunguria*.

MATERIALS AND METHODS

Establishment of hairy root cultures

The hairy root cultures of *C. aunguria* were induced by *A. rhizogenes* strain KCTC 2703, and molecular confirmation was carried out by following our earlier report [21]. Hairy roots (500 mg FM) was in 250 mL Erlenmeyer's flasks containing 50 mL of MS [12] medium supplemented with sucrose (30 g/L). The cultures were kept on an orbital shaker (100 rpm) and maintained in a growth chamber at 25 °C, 16/8 h photoperiod. The roots were subcultured every two weeks.

Elicitation of silver (Ag⁺) ions and silver nanoparticles (AgNPs) in hairy root cultures

AgNO₃ (Daejung Chemicals and Metals Co. Ltd, South Korea) was dissolved in distilled water. In a previous study we described biologically synthesized silver nanoparticles (AgNPs), and confirmation using UV-visible spectroscopy, dynamic light scattering and transmission electron microscopy analysis [17]. Different concentrations of AgNO₃ and AgNPs (0.5, 1.0 and 2.0 mg/L) were added on 18 d of culture medium. Non-elicited cultures were considered as control. Cultures were kept under continuous agitation at 110 rpm in an orbital shaker and incubated at under the same temperature and light conditions as above. Root biomass growth, silver content and phenolic compound production were measured at 21 d of culture. The roots were washed thoroughly with deionized water to remove the traces of the medium and their fresh mass (FM). The root samples were oven-dried at 60 °C for 72 h their dry mass (DM) was determined.

Analysis of silver content in hairy roots

The silver content in the non-elicited and elicited hairy roots of AgNO₃ and AgNPs (1.0 mg/L) was determined using inductively coupled plasma mass spectrometry (ICP-MS; Varian 820-MS, USA). The hairy root samples (25 mg, DM) were digested with concentrated HNO₃ at 115 °C for 1 h. The digests were diluted with deionized water, filtered through 0.2 µm filters. The Ag uptake experiment was repeated three times, and the data were expressed as µg/L.

Extraction and estimation of individual phenolic compounds by ultra-high-performance liquid chromatography (UHPLC)

Non-elicited and elicited (AgNO₃ and AgNPs) hairy roots powder (1 g DM) were extracted following our published protocol [16]. The presence of twenty-four phenolic compounds in the hairy root extracts was measured using UHPLC (Accela UHPLC system, USA) with a reverse phase C₁₈ column (2.1 × 100 mm, 2.6 mm). The details of solvent, standard, and gradient procedure were described earlier [21]. Phenolic compounds in hairy root extracts were identified by previously reported methods [21].

Estimation of total phenolic and flavonoid contents (TPC and TFC)

TPC and TFC were evaluated in non-elicited and elicited (AgNO₃ and AgNPs) dried hairy root extracts of *C. anguria*. TPC was quantified by a spectrophotometric method according to the Folin–Ciocalteu assay as previously reported [16, 21]. TFC of the extracts was determined by using the aluminum chloride spectrophotometric method as described earlier [16, 21].

Screening of biological activities

Biological activities (antioxidant, antibacterial, antifungal and anticancer) potential were determined in non-elicited and elicited (AgNO₃ and AgNPs) hairy root extracts of *C. anguria*.

Antioxidant activities

The DPPH free-radical-scavenging activity, reducing power, phosphomolybdenum, and metal ion-chelating assays were measured using a prior method [2, 16, 21].

Antibacterial and antifungal activities

Pathogenic microorganisms (*Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, *Aspergillus niger* and *Fusarium oxysporum*) were used to test for antibacterial and antifungal activity. Antibacterial and antifungal tests were carried out by the NCCLS disc diffusion method was previously described [16, 21].

Cytotoxic screening of MTT assay

Two human cancer cell lines of colon HT-29 and oestrogen-dependent breast MCF-7 cancer cells were used for cytotoxicity. Cells (5×10^3 cells well⁻¹) were plated in 96 well plates and treated with different concentrations of non-elicited and elicited hairy root extracts (12.5, 25, 50, 100 and 200 µg/mL) for 48 h. The control was used as solvent DMSO treated cells. Cell viability was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] colorimetric method [2].

Data analysis

All experiments were done in triplicate, and each experiment was repeated three times and expressed as a mean \pm standard deviation. One-way ANOVA analysis followed by Duncan's test was used to determine significant differences ($P \leq 0.05$) in the statistical software package.

RESULTS AND DISCUSSION

Determination of silver content in hairy roots

The results show that the silver content was higher at 1.0 mg/L of AgNPs-elicited hairy roots (365.4 ± 2.5 µg/L) than in Ag⁺ ions (215.5 ± 3.8 µg/L) elicited hairy roots of *C. anguria*. Ag was absent in the non-elicited control hairy roots. AgNPs have a greater adhesion to plant tissues than Ag⁺ ions [10]. Consistent with our reports, silver contents were significantly higher in plants which were exposed to AgNPs than Ag⁺ ion exposed *Arabidopsis thaliana* plants [13]. Our results also indicate that the higher amount of Ag in AgNPs-elicited hairy roots, compared to AgNO₃ elicited hairy roots, might be due to the direct uptake of AgNPs by plants, or the AgNPs could be oxidized on the root surface as Ag⁺, which could thereafter enter into the root tissues directly without dissolving in the solution.

Effect of elicitors (AgNO₃ and AgNPs) on biomass accumulation in hairy roots

The biomass (FM and DM) of elicited (AgNO₃ and AgNPs) and non-elicited hairy root cultures were studied in *C. anguria* (Fig. 1A, B). The biomass (97.25 g FM and 9.15 g DM) was reduced at higher concentrations of AgNO₃ (2.0 mg/L) whereas, its growth was slightly increased (98.86 g FM and 10.19 g DM) at a lower concentration of AgNO₃ (0.5 mg/L) compared with that of the non-elicited hairy root cultures (98.55 g FM and 10.15 g DM). Similar to our results, the FM and DM were enhanced the lower concentration at 15 μM whereas decreased at 45 μM Ag⁺-elicited hairy roots of *Salvia castanea* [11]. Ag⁺-elicitor reduced the growth and the water content of hairy roots of *S. miltiorrhiza* [19]. The biomass of hairy roots (99.98 g FM and 10.65 g DM) was significantly enhanced at 1.0 mg/L of AgNPs, while its growth was marginally decreased at a higher concentration of AgNPs (2.0 mg/L). Similarly, the biomass (FM and DM) of hairy roots were higher in nano-silver and AgNO₃ elicited than non-elicited hairy roots of *Datura metel* [14]. Previously, it was demonstrated that enhancement of biomass production in turnip after application of biologically synthesized AgNPs (1.0 mg/L) [17]. These inhibitory or stimulatory effects of AgNO₃ and AgNPs suggest that use of optimal concentration is essential to enhance the biomass accumulation in hairy roots of *C. anguria*.

Effect of elicitors on individual and total phenolic compounds in hairy roots

Silver was considered as an effective elicitor and enhanced the concentration of phenolic compounds (tanshinones, rosmarinic, and salvianolic acids) and also altered the isoprenoid pathways in *S. miltiorrhiza* hairy roots [4, 19, 23]. The present study reports that elicited (AgNPs and AgNO₃) hairy roots could prominently induce the accumulation of the TPC and TFC. Figure 1C shows the TPC was significantly increased the amount of 33.25 mg/g and 31.11 mg/g gallic acid equivalent (GAE) in AgNPs and AgNO₃-elicited hairy root cultures than non-elicited hairy root cultures (29.89 mg/g GAE). TFC of 4.05 mg/g and 3.64 mg/g quercetin equivalent (QE) in AgNPs and AgNO₃-elicited hairy root cultures were higher compared to non-elicited hairy root cultures (2.45 mg/g QE; Fig. 1D). Consistently, the treatment with 15 μM Ag⁺ significantly increased TPC and rosmarinic acid accumulation in hairy roots of *S. miltiorrhiza* [20]. Ag⁺ elicitation significantly promoted the contents of echinacoside and acteoside in cell suspension culture of *Cistanche deserticola* [1]. In contrary, Ag⁺ treatment has not affected the accumulation of TPC in hairy roots of *S. miltiorrhiza* [19]. In our study, AgNPs-elicited hairy roots have significantly increased the contents of TPC and TFC than AgNO₃-elicited hairy roots. Similarly, the contents of TPC and TFC enhanced the elicitation by Ag and AuNPs in callus culture of *Prunella vulgaris* [3]. *Vanilla planifolia* shoots grown in MS medium supplemented with 25, and 50 mg/L AgNPs showed a significant increase in TPC [15].

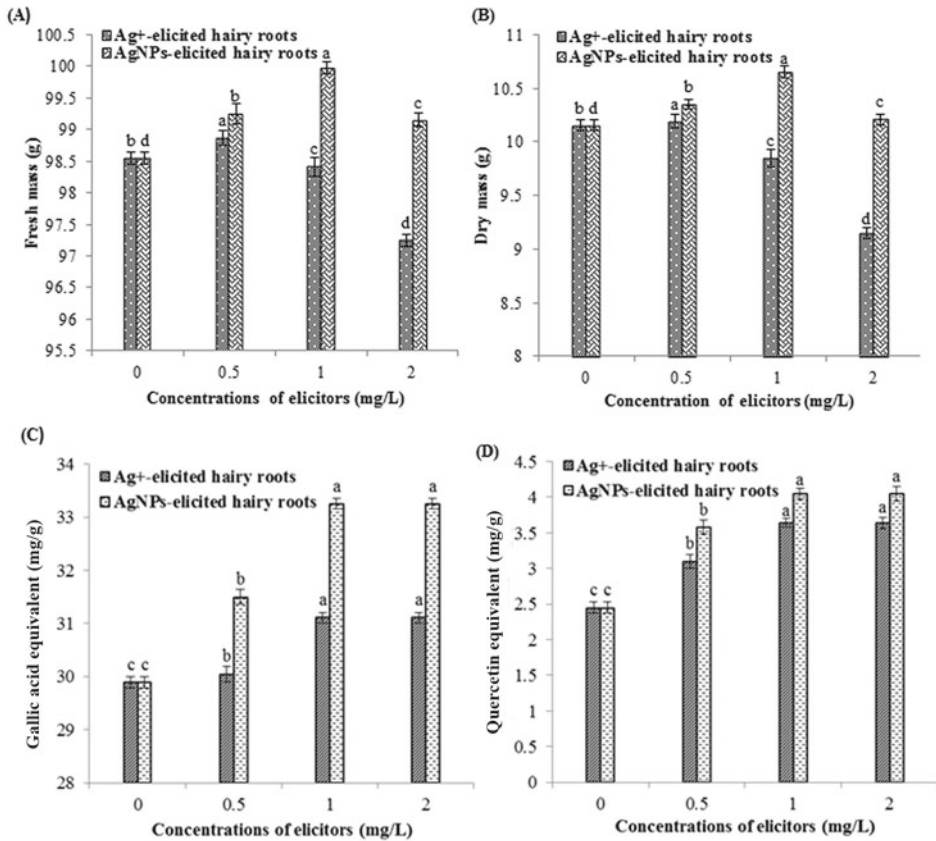


Fig. 1. Effect of different concentrations of elicitors (Ag^+ and AgNPs) on biomass accumulation and phenolic compound production in hairy root cultures of *C. anguria*. A. Fresh mass; B. Dry mass; C. Total phenolic compounds; D. Total flavonoid contents. Means \pm SD of three replicates followed by the same letters are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

AgNPs plays a significant role in the production of anthocyanins, TPC and TFC in *B. rapa* ssp. *rapa* [17] and *Artemisia annua* [5]. The yields of atropine content were significantly increased by nanosilver-elicited hairy roots in *Datura metel* [14]. TPC and TFC were prominently improved at higher concentrations of AgNO_3 and AgNPs (1.0 and 2.0 mg/L), while it was slightly increased at a lower concentration of AgNO_3 and AgNPs (0.5 mg/L). The flavonoid contents of plants treated with 0.8 and 1.2 mM AgNPs were substantially higher than that of the control plants in *Achillea millefolium* [6].

An enhancement of phenolic acid production using Ag^+ possibly induced the phenolic compound biosynthesis pathway and gene expressions were up-regulated [19]. Both non-elicited and elicited (AgNPs and AgNO_3) hairy roots contained flavonols, hydroxybenzoic and hydroxycinnamic acids (Table 1). AgNPs and AgNO_3 -elicited

Table 1
Ultra-HPLC analysis of the phenolic compounds from non-elicited and elicited (Ag⁺ and AgNPs) hairy roots of *C. anguria*

No.	Phenolic compounds	Concentration ($\mu\text{g/g DM}$)		
		Non-elicited hairy roots	Ag ⁺ -elicited hairy roots	AgNPs-elicited hairy roots
	Hydroxybenzoic acid			
1	<i>p</i> -Hydroxybenzoic acid	155.50 \pm 1.50 ^{m,z}	179.45 \pm 1.00 ^{m,y}	193.15 \pm 2.00 ^{m,x}
2	Gallic acid	370.25 \pm 2.00 ^{i,z}	411.05 \pm 1.00 ^{i,y}	455.50 \pm 1.50 ^{i,x}
3	Protocatechuic acid	48.00 \pm 2.20 ^{p,z}	65.55 \pm 1.00 ^{n,y}	77.10 \pm 1.00 ^{n,x}
4	Syringic acid	65.46 \pm 4.20 ^{o,z}	87.50 \pm 1.00 ^{m,y}	95.16 \pm 1.20 ^{n,x}
5	Gentisic acid	758.15 \pm 3.25 ^{b,z}	845.00 \pm 2.00 ^{b,y}	895.00 \pm 3.00 ^{b,x}
6	Salicylic acid	999.00 \pm 5.00 ^{a,z}	1062.50 \pm 2.00 ^{a,y}	1112.85 \pm 1.50 ^{a,x}
7	Vanillic acid	20.00 \pm 1.50 ^{r,z}	31.10 \pm 1.00 ^{q,y}	44.50 \pm 1.00 ^{q,x}
8	β -Resorecylic acid	30.25 \pm 1.11 ^{q,z}	39.25 \pm 1.00 ^{p,y}	46.25 \pm 1.00 ^{q,x}
	Total	2446.61 ^z	2721.40 ^y	2919.51 ^x
	Hydroxycinnamic acid			
9	Caffeic acid	661.50 \pm 2.00 ^{d,z}	695.72 \pm 1.50 ^{d,y}	715.10 \pm 1.50 ^{d,x}
10	<i>p</i> -Coumaric acid	215.02 \pm 1.55 ^{l,z}	252.10 \pm 2.00 ^{k,y}	280.55 \pm 1.00 ^{k,x}
11	<i>o</i> -Coumaric acid	18.00 \pm 2.00 ^{r,z}	24.50 \pm 0.75 ^{r,y}	31.10 \pm 1.00 ^{r,x}
12	Ferulic acid	149.05 \pm 1.10 ^{m,z}	175.35 \pm 1.65 ^{k,y}	205.05 \pm 1.00 ^{k,x}
12	Chlorogenic acid	455.00 \pm 2.21 ^{f,z}	525.50 \pm 2.00 ^{g,y}	541.00 \pm 1.00 ^{g,x}
13	<i>t</i> -Cinnamic acid	9.12 \pm 0.21 ^{s,z}	12.05 \pm 0.50 ^{s,y}	15.00 \pm 0.75 ^{s,x}
	Total	1507.69 ^z	1685.22 ^y	1787.80 ^x
	Flavonols			
14	Myricetin	725.45 \pm 5.10 ^{c,z}	779.00 \pm 2.00 ^{c,y}	805.00 \pm 2.00 ^{c,x}
15	Quercetin	645.12 \pm 4.00 ^{e,z}	675.50 \pm 1.50 ^{e,y}	715.00 \pm 2.50 ^{e,x}
16	Kaempferol	400.25 \pm 2.50 ^{h,z}	452.45 \pm 2.00 ^{h,y}	510.05 \pm 1.00 ^{h,x}
17	Catechin	425.12 \pm 4.45 ^{g,z}	545.15 \pm 1.70 ^{f,y}	610.55 \pm 2.00 ^{f,x}
18	Rutin	245.10 \pm 2.50 ^{i,z}	294.45 \pm 2.00 ^{i,y}	335.10 \pm 2.00 ^{i,x}
19	Naringenin	110.32 \pm 2.25 ^{n,z}	125.50 \pm 1.00 ^{l,y}	135.00 \pm 1.00 ^{l,x}
20	Biochanin A	20.17 \pm 0.51 ^{r,z}	22.00 \pm 1.00 ^{r,y}	25.50 \pm 1.00 ^{r,x}
	Total	2571.53 ^z	2894.05 ^y	3136.20 ^x
	Other phenolic constituents			
21	Vanillin	62.25 \pm 2.21 ^{o,z}	62.40 \pm 1.00 ^{n,y}	70.00 \pm 1.00 ^{n,x}
22	Veratric acid	226.14 \pm 4.00 ^{k,z}	244.25 \pm 1.00 ^{l,y}	255.00 \pm 1.00 ^{l,x}
23	Homogentisic acid	33.75 \pm 1.10 ^{q,z}	42.10 \pm 0.50 ^{p,y}	50.00 \pm 1.00 ^{p,x}
24	Hesperidin	44.58 \pm 1.00 ^{p,z}	49.00 \pm 0.72 ^{o,y}	54.10 \pm 1.00 ^{p,x}
	Total	366.72 ^z	397.75 ^y	429.10 ^x

Mean \pm SD of three replicates within a column^{a-s} or row^{x-z} followed by the same letters are not significantly different according to Duncan's multiple range test at $P\leq 0.05$.

hairy roots contained a higher level of hydroxybenzoic acid (2919.51 and 2721.40 $\mu\text{g/g}$) than non-elicited hairy roots (2446.61 $\mu\text{g/g}$). Salicylic, gentisic, gallic, *p*-hydroxybenzoic, protocatechuic, syringic, β -resorcylic and vanillic acid contents were significantly higher in AgNPs and AgNO₃-elicited hairy root cultures than that in non-elicited hairy root cultures ($P \leq 0.05$; Table 1). AgNPs and AgNO₃-elicited hairy roots contained a higher concentration of hydroxycinnamic acid (1787.80 and 1685.22 $\mu\text{g/g}$) than non-elicited hairy roots (1507.69 $\mu\text{g/g}$). The level of caffeic, chlorogenic, *p*-coumaric, ferulic, *o*-coumaric and *t*-cinnamic acids was significantly increased in AgNPs and AgNO₃-elicited hairy roots compared to non-elicited hairy roots ($P \leq 0.05$; Table 1). It was possible because that metabolic flux among various phenolic acids was influenced by silver ion. Similarly, Ag⁺ induced accumulations of rosmarinic, caffeic and ferulic acids, but decreased contents of salvianolic and cinnamic acids [19]. AgNPs can increase the activation of enzymatic pathways that contribute to the production of secondary metabolites [22]. Similarly, the elicitation Ag-SiO₂ core-shell NPs improved the content of artemisinin in hairy root cultures of *A. annua* [22]. Consistently, elicitation of cobalt NPs increased the content of artemisinin in cell suspension culture of *A. annua* [7]. AgNPs and AgNO₃-elicited hairy roots contained a higher amount of flavonols (3136.20 and 2894.05 $\mu\text{g/g}$) than non-elicited hairy roots (2571.53 $\mu\text{g/g}$). The amount of myricetin, quercetin, catechin, kaempferol, rutin, naringenin and biochanin A was significantly higher in AgNPs and AgNO₃-elicited hairy roots than that in non-elicited hairy roots ($P \leq 0.05$; Table 1). These results suggest that biologically synthesized AgNPs can be used as a potent new elicitor to enhance the yield of phytochemicals in plant biotechnology. Our results suggest that AgNPs elicitation was increased the level of individual and total phenolic compounds in hairy root cultures of *C. anguria*.

Effect of elicitors on antioxidant activity in hairy roots

The antioxidant capacity of AgNPs and AgNO₃-elicited hairy root cultures were assessed using free radicals scavenging, reducing potential, phosphomolybdenum assay and metal chelating activity. DPPH activity is a proper indicator for investigating the free radical scavenging activities of phenolic compounds. Figure 2A displays the high antioxidant activity in AgNPs (70.45%), and AgNO₃ (67.25%) elicited hairy roots compared with non-elicited hairy roots (61.50%). The phenolic and flavonoid level was higher in the AgNPs and AgNO₃-elicited hairy root cultures, which directly influenced their antioxidant potential. Figure 2B shows the reducing capacity of *C. anguria* hairy root extracts, suggesting that AgNPs and AgNO₃-elicited had a more antioxidant potential than non-elicited hairy roots. The antioxidant capability of the elicited (AgNPs and AgNO₃) hairy roots was higher when compared to non-elicited hairy root extracts (Fig. 2C). The chelating agent may inhibit radical formation by stabilizing transition metals, thus decreasing free radical damage. Ferrozine can make complexes with ferrous ions. In the presence of a chelating agent complex formation is interrupted and as a result, the red color of the compound decreases. Figure 2D

shows the percentage of metal scavenging capacity exhibited in AgNPs (72.55%) and AgNO₃ (69.50%) was high compared with that of non-elicited hairy root extracts (64.00%). Elicitation of AgNPs was higher inhibition than AgNO₃ elicited hairy roots. Consistently, elicitation of AgNPs was stimulation of antioxidant defense system, production of taxol at the highest capacity of cells in *Corylus avellana* [8]. Similarly, the higher activity of enzyme peroxidase was reported in biologically synthesized AgNPs- and AgNO₃-treated seedlings in *Bacopa monnieri* [9]. Antioxidant enzymes (CAT, POD, and GST) gene expression was significantly up-regulated in biologically synthesized AgNPs treated seedlings of turnip [17].

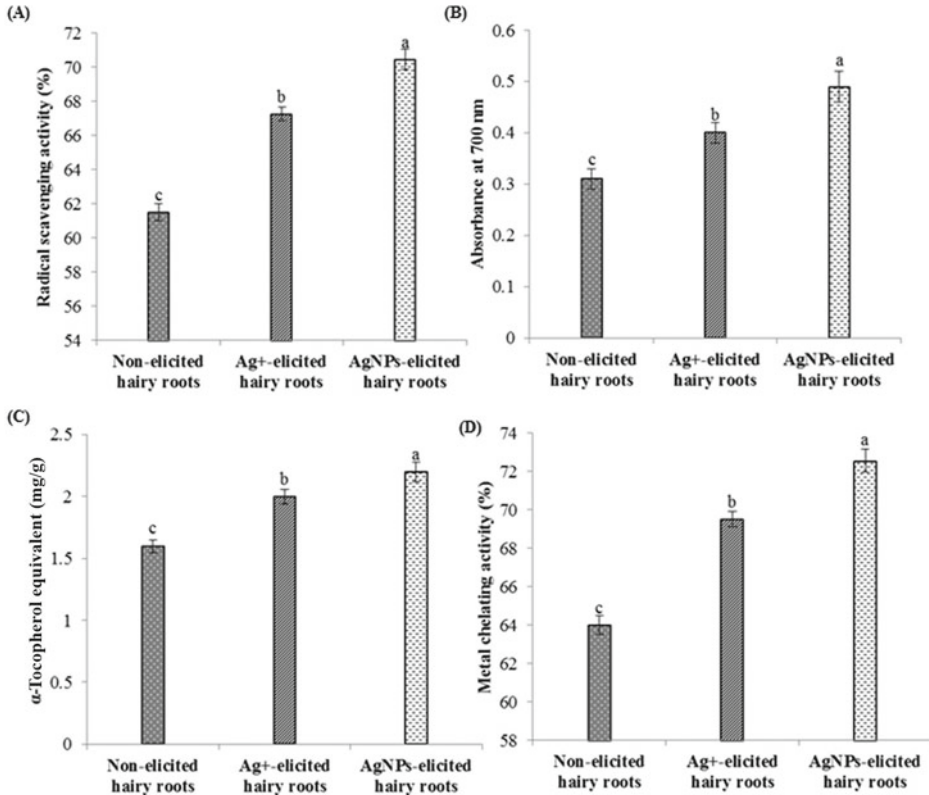


Fig. 2. Effect of elicitors (Ag⁺ and AgNPs) on levels of antioxidant activities in hairy root cultures (100 μL/1 mL of methanolic extracts) of *C. anguria*. A. Free radical-scavenging activity by DPPH method; B. Total Fe³⁺–Fe²⁺ reductive potential reference antioxidants of butylated hydroxytoluene (BHT); C. Total antioxidant capacity (TAC) by phosphomolybdenum method (TAC was expressed as equivalents of α-tocopherol (μg/g of extract)); D. Metal chelating activity. Means ± SD of three replicates followed by the same letters are not significantly different according to Duncan's multiple range test at P < 0.05

Effect of elicitors on antimicrobial activity in hairy roots

The disc diffusion results showed that elicited (AgNPs and AgNO₃) and non-elicited hairy root extracts had anti-bacterial (Gram-positive and Gram-negative bacteria) and anti-fungal activities. Figure 3A shows the elicited (AgNPs and AgNO₃) hairy root extracts exhibited higher activity against antibacterial and antifungal compared to control hairy root extracts. However, antibacterial and antifungal activities were higher in AgNPs-elicited than in AgNO₃-elicited hairy root extracts. Similarly,

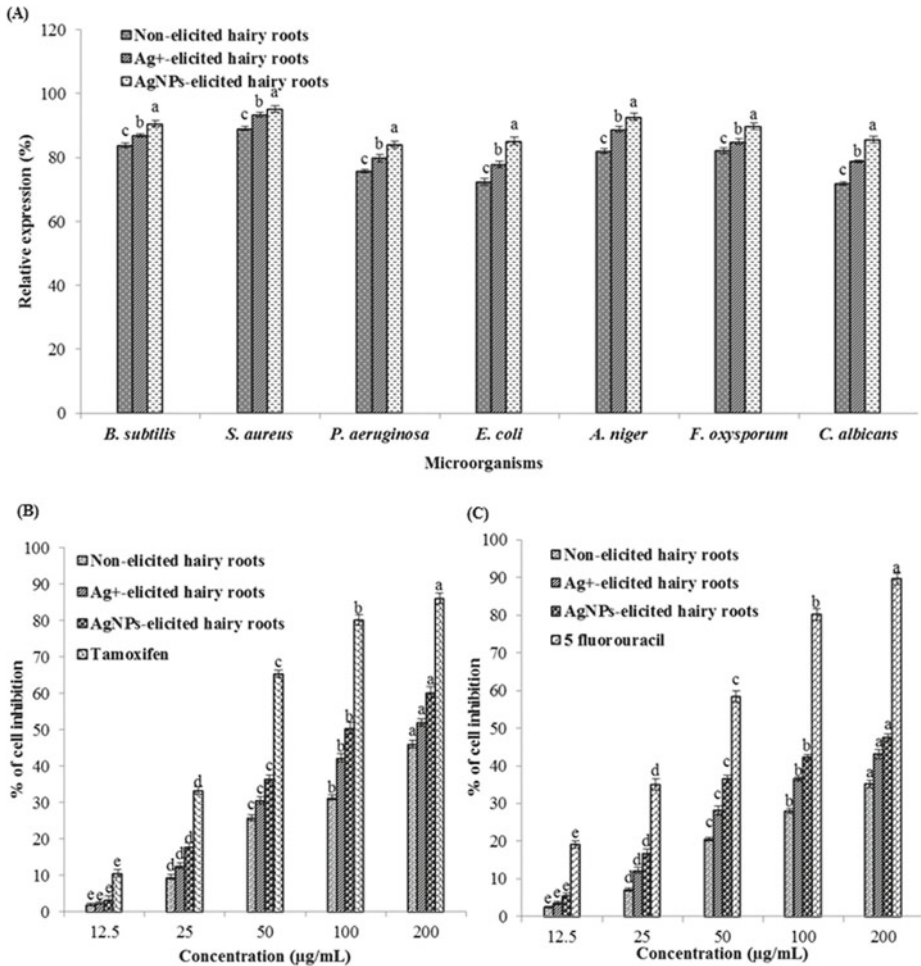


Fig. 3. Effect of elicitors (Ag⁺ and AgNPs) on levels of antimicrobial and anticancer activities in hairy root cultures of *C. anguria*. A. Antimicrobial activity using disc diffusion method; B. MCF-7 cell line; C. HT-29 cell line. Means \pm SD of three replicates followed by the same letters are not significantly different according to Duncan's multiple range test at $P \leq 0.05$

AgNPs-elicited plants increased the antibacterial and antifungal activities in *A. millefolium* [6]. The results demonstrate that hairy root extracts of *C. anguria* could be used for the treatment of bacterial and fungal diseases.

Effect of elicitors on anticancer activity in hairy roots

There are many studies reporting on the phenolic compounds against *herpes simplex virus 1* (HSV-1), *human immunodeficiency virus* (HIV-1) and *Helicobacter pylori* [18]. Anticancer activities against MCF-7 and HT-29 cell lines in elicited (AgNPs and AgNO₃) and non-elicited hairy root extracts were evaluated. The inhibition of these extracts is compared with standard tamoxifen for MCF-7 cell line and 5-fluorouracil for HT-29 cell line (Fig. 3B, C), respectively. The percentage of cancer cell inhibition profiles was found to be concentration-dependent, and the greater inhibition was found at a higher concentration of 200 µg/mL. MCF-7 cell lines grown in DMEM, when exposed to different concentrations of AgNPs and AgNO₃-elicited hairy root extracts resulted in 60.25% and 51.98% inhibition of MCF-7 cell, whereas non-elicited hairy roots displayed weak inhibition of 46.05%. On the other hand, comparison with tamoxifen showed 86.16% of MCF-7 cell line inhibition at the same tested dose (200 µg/mL). AgNPs and AgNO₃-elicited and non-elicited hairy root extracts tested against HT-29 cell line resulted in stronger anticancer activity, than the standard 5-fluorouracil. A maximum of 47.51 and 43.19% inhibition was observed with AgNPs and AgNO₃-elicited hairy root extracts at a tested dose of 200 µg/mL. Estimation with 5-fluorouracil exhibited 89.62% of HT-29 cell line inhibition at the same tested dose (200 µg/mL). MCF-7 cell line displayed a higher inhibition profile than HT-29 cell line (Fig. 3B, C). Similar to our results, AgNPs-elicited plants had an increased inhibitory effects on HeLa cells [6]. This study showed the MTT assay resulted in hairy root cultures of *C. anguria* the inhibition of the growth of breast and colon cancer cell lines. This inhibition of cell growth might be a result of its higher content of phenolic compounds.

CONCLUSIONS

Higher amounts of phytochemicals (phenolic and flavonoids compounds) and biological (antioxidant, antimicrobial and anticancer) activities were produced in Ag⁺ and AgNPs-elicited hairy roots compared with that in non-elicited hairy roots. AgNPs-elicited hairy roots have significantly increased the yield of biomass and phenolic compounds accumulation, compared to Ag⁺-elicited hairy root cultures. Our results suggest that AgNPs elicited hairy root cultures can be efficiently used for the antioxidant, antimicrobial and anticancer treatments, and proved that use of AgNPs elicitation was an effective method for enhancement of phenolic compounds in hairy root cultures of *C. anguria*.

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