BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, August 2014. Vol. **11**(2), 391-399

Production of New Active Phytocompounds by *Achillea millefolium* L. after Elicitation with Silver Nanoparticles and Methyl Jasmonate

Faezeh Ghanati[1]*, Somayeh Bakhtiarian[1], Behrooz Mohammad Parast[2] and Mahboobeh Keyhani Behrooz[2]

[1]Department of Plant Biol., Fac. Biol. Sci., Tarbiat Modares University (TMU), POB 14115-154, Tehran, Iran.
[2]Department of Biol., Fac. Sci., University of Malayer, Malayer, Iran.

doi: http://dx.doi.org/10.13005/bbra/1287

(Received: 06 June 2014; accepted: 03 July 2014)

***Achillea millefolium* L. is a medicinal herb with more than 100 antifungal and antimicrobial active biological compounds. In the present study, *A. millefolium* plants at the flowering stage were treated with different concentrations of methyl jasmonate and silver nanoparticles. Treatments increased lipid peroxidation, flavonoid content, and yield of essential oils (ca. 230 %), but reduced radical scavenging capacity and anthocyanin contents of the plants. Cytotoxicity of the extracts of treated plants against cancer HeLa cells was improved as well. Production of certain antimicrobial isoprenoids, i.e., camphor was significantly increased. Certain precious compounds such as allo-ocimene, germacrene, trans-caryophyllene, and farnesol with antibacterial, antifungal, anti-inflammatory, and anticancer effects were also induced just after elicitation of the plants with silver nanoparticles and methyl jasmonate. The results suggested silver nanoparticles as a novel elicitor in plant biotechnology as effective as methyl jasmonate in order for production of desired secondary metabolites in *A. millefolium*.**

Key words: *Achillea millefolium* L., Essential oil, Methyl jasmonate (MeJA), Redox status, Silver nano particles (AgNPs).

Most biologically active compounds of medicinal plants are defensive metabolites that can be induced by chemical elicitors. Methyl jasmonate (MeJA) and its free-acid jasmonic acid (JA), collectively referred to as jasmonates, are important cellular regulators involved in diverse developmental processes such as seed germination, root growth, fertility, fruit ripening, and senescence. In addition, jasmonates activate plant defense mechanisms in response to insect-driven wounds, various pathogens, and environmental stresses. Exogenous application of JA to a plant cell culture or intact plant simulates the biosynthesis of a wide variety of plant secondary metabolites; different plant species seem to induce different species-specific secondary metabolite biosynthesis pathways[1-2]. Applications of nanoparticles are emerging rapidly. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution, and morphology. One of the potential applications of nanoparticles is the management of plant disease by application of silver nanoparticles (AgNPs). Because silver inhibits the action of microorganisms via multiple modes[3], it may be used to control various plant pathogens in a relatively safe way. Previous researches[4] have shown that the application of low concentrations

* To whom all correspondence should be addressed.
Tel:+98-21-82884403, Fax: +98-21-82884717;
E-mail: ghangia@modares.ac.ir

of AgNPs upregulated the expression of a well-known defense gene, glutathione S-transferase, in *Chironomus riparius*.

It has been accepted that upon treatment of plants with MeJA extensive reprogramming in metabolic pathways takes[5]. This phenomenon may be mediated by change in free radicals status scavenging capacity of the plants. A few studies however examined such a hypothesis on AgNPs and the little available literature has particularly directed their attention on antimicrobial and membrane disruptive effects of these particles[6]. This inspired us to evaluate the potential elicitation effect of AgNPs and MeJA in enhancing the production of specific sets of valuable metabolites of *Achillea millefolium* L. (Asteraceae). Secondary metabolite identified in this plant including essential oils, sesquiterpenes, and phenolic compounds such as flavonoids e.g., apigenin, luteolin, camphor, coumarin, etc[7-8].

MATERIAL AND METHODS

Plant materials, characterization of AgNPs and treatments

A. millefolium L. plants were collected from north of Iran. The plants were identified and authenticated and a voucher specimen was deposited at the Herbarium of the Tarbiat Modares University, Tehran-Iran. Plant specimen was identified by Dr. Shahrokh Kazempour Osaloo from the same institute. The plants were transferred to a nutrient solution containing K_2SO_4 (0.46 mM), KH_2PO_4 (0.1 mM), NH_4NO_3 (0.73 mM), $(NH_4)_2SO_4$ (0.713 mM), H_3BO_3 (0.046 mM), $CuSO_4$ (0.002 mM), Fe-EDTA (0.032 mM), $MgSO_4$ (0.41 mM), $CaCl_2$ (0.5 mM), $ZnSO_4$ (0.0091 mM), Na_2MoO_4 (0.0026 mM), and $MnSO_4$ (0.09 mM), pH 5.8. The nutrient solution was changed every 4 days and the plants were allowed to continue to growth under a 16/8-h photoperiod.

A solution of spherical AgNPs was purchased from the US Research Nanomaterial Inc, USA. According to the manufacturer, the diameter of AgNPs was 30–50 nm and the purity was 99.99%. Nanoparticle solution was dispersed by ultra-sonication before adding to the plant hydroponic media. In the flowering onset of the plants they were treated with 0, 50, and 100 µM MeJA and 0.4, 0.8, and 1.2 mM AgNPs for 24 h. The duration of treatments and the applied concentration were decided according to literatures. Presence of AgNPs particles in plant extracts (evidenced for absorption of them by the plants) and their size was evaluated using Zeta Plus particle sizer (Malvern 3000 HSA, France) with Zetasizer software Ver 6.12.

After treatments, the plants were harvested, washed and the roots and aerial parts were separated. The samples were frozen in liquid N_2 and kept at "80°C until they were used for biochemical measurements; the remaining parts were shadow-dried before extraction of essential oils.

Membrane lipid peroxidation and radical scavenging activity assay

The level of peroxidation of membrane lipids was measured using malondialdehyde (MDA) as the final product of lipid peroxidation. Aliquots of frozen samples (0.2 g) were homogenized in tricolor acetic acid (TCA, 10 %). The homogenate was centrifuged at 10,000 × *g* for 15 min and 1 mL thiobarbituric acid (TBA, 0.5%) was added to 1 mL of the supernatant. This mixture was incubated at 100 °C in a water bath for 30 min. Thereafter, the reaction tubes were transferred to an ice-water bath. The absorbance of MDA was read at 532 nm and 600 nm. The amount of MDA–TBA complex was calculated from the extinction coefficient of 155 $mM^{-1} \cdot cm^{-1}$ [9].

Radical scavenging activity was evaluated by diphenylpicrylhydrazyl (DPPH) method[9]. The stock solution was prepared by dissolving 24 mg DPPH in 100 mL MeOH. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance at 517 nm using a spectrophotometer. Each sample extract (250 µL) was allowed to react with 250 µL DPPH solution for 30 min in the dark. Next, the absorbance was read at 517 nm using a spectrophotometer. The percentage of DPPH scavenging activity was calculated as the percentage of DPPH inhibition = (Abs control " Abs sample/Abs control) × 100

Measurement of non-enzyme antioxidants

For the measurement of anthocyanin content, aliquots (0.2 g) of frozen tissue were homogenized completely with acidic methanol (MeOH:HCl, 99:1 v/v) and centrifuged at 12,000 × *g* for 15 min. The supernatant was kept in the dark for 1 night. Anthocyanin content was determined

by measuring the absorbance at 550 nm using a double-beam UV-vis spectrophotometer (Cintra6, GBC, Victoria, Australia) with a extinction coefficient of 33000 $cm^{-1} M^{-1}$ [10].

For the assessment of flavonoids, deionized water (1250 µL) was added to 0.25 mL of the sample extract, followed by the addition of 75 µL of 5% sodium nitrite ($NaNO_2$). Six minutes later, after the addition of 150 µL 10% $AlCl_3$, the mixture was allowed to stand for another 5 min, after which 500 µL 1 M NaOH was added. Final volume was made up to 2500 µL by the addition of deionized water, and the absorbance was measured at 510 nm. Catechin was used as a standard [2].

Extraction of essential oils and their analysis by gas chromatography (GC) and mass spectrometry (GC-MS)

The essential oils were obtained by hydrodistillation of 50 g of dry aerial parts of the plant using a clevenger-type system for 4 h. The yield of each essential oil was determined by averaging three replicates. After extraction, the oil was collected in screw-capped glass vials and dried over anhydrous Na_2SO_4. The oils collected from the samples were kept at 4°C in the dark and were analyzed using GC/MS.

GC analysis was performed using a Shimadzu GC-2010 equipped with a FID and a HP-5 fused silica column with a 5% phenyl-substituted methyl polysiloxane phase. The oven temperature was programmed at 60–250 °C, with a heating ramp of 5 °C min^{-1}. The carrier gas was helium with a linear velocity of 43 cm/s. The essential diluted solution was injected into the GC/MS in the split mode with a split ratio of 1/20. MS analyses were performed using a Shimadzu MS-QP2010 with ionization energy of 70 eV, a scan time of 0.5 s, and a mass range of 33–450 atomic mass unit/Dalton (amu/Da). The components of the oil were identified by comparing their mass spectra with those of the NIST147 mass spectral database and also with the authentic fragmentation patterns and retention index reported in the literature. The percentages of compounds were calculated by the area normalization method without considering response factors to establish abundances. The retention index was found with a standard mixture of C8–C22 compounds under chromatography conditions that were consistent with the chromatography conditions of the analyzed samples. For each essential oil, the RI and peak area percentages were calculated as mean values of the three injections.

Evaluation of cytotoxicity

Cytotoxicity of extracts from flowers of *Achillea millefolium* L. was assessed on HeLa-60 cell line. The ovarian cancer cell line (HeLa-60) was obtained from the Pasteur Institute of Iran and was cultured in RPMI 1640 medium, supplemented with 10 % FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin. The cells were incubated at 37 ºC, 5 % CO_2, and were subcultured every 4 days. The cytotoxic effects of the plant essential oils were investigated by colorimetric bioassay using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) in a 24-well plate (triple holes) [10]. Compounds were dissolved in dimethyl sulfoxide (DMSO) to make a stock solution. The DMSO concentration was kept below 0.05% throughout the cell culture and treatment periods and did not exert any detectable effect on cell growth or cell death. After incubation for 4 h, viability of the cells was measured at 492 nm by ELISA Reader (Anthuos 2020, Australia).

Statistical analysis

All experiments and observations were repeated three times each with at least three samples. All data are expressed as mean ± standard deviation (SD). One way ANOVA from SPSS (version 16, Chicago, IL, USA) was used, and a least significant difference (LSD) test was calculated for multiple means comparisons at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Treatment of *A. millefolium* L. plants with 50 µM MeJA brought no significant change in lipid peroxidation rate of the plants, however, treatment with 100 µM MeJA significantly increased the lipid peroxidation rate (157% of the control plants) (Figure 1A). Treatment with lower concentration of AgNPs (0.4 mM) did not significantly affect membranes lipid peroxidation rate. In 0.8 and 1.2 mM of AgNP however, the level of membrane lipid peroxidation increased and the most pronounced level of it was observed in plants treated with 1.2 mM AgNPs, compared with the control group (Figure 1B).

DPPH scavenging capacity of *A.*

millefolium L. plants in treatment with 50 µM MeJA was identical to that of the control ones (Figure 2A), however it significantly increased by treatment with 100 µm MeJA (Figure 2A). Exposure to AgNPs, particularly at higher concentrations significantly decreased radical scavenging activity of the plants so that the least capacity to remove free radicals was observed in 1.2 mM AgNPs-treated plants, compared with the control ones (Figure 2B).

Treatment of *A. millefolium* L. plants with MeJA and AgNPs decreased anthocyanin content of the plants. This decrement was more pronounced in 50 and 100 µM of MeJA (18% and 30%, respectively) and 0.8 and 1.2 mM AgNPs (14% and 20%, respectively), compared with the control plants (Figure 3A, B).

Treatment of *A. millefolium* L. plants with

Table 1. Chemical composition and contents of essential oil of *Achillea millefolium* L. treated with different concentrations of silver nanoparticles (AgNPs) and Methyl Jasmonate (MeJA). The contents of essential oil components were determined by GC and GC-MS, regarding RI and the mean peak area percentages of three samples and three injections

No	Identification	Formula	Content (%)					
			Control	AgNPs (mM)			MeJA(µM)	
				0.4	0.8	1.2	50	100
1	delta-3-carene	$C_{10}H_{16}$	0.09	0.06	-	0.08	0.1	0.21
2	gama-terpinene	$C_{10}H_{16}$	3.48	3.57	3.84	3.08	2.53	6
3	camphene	$C_{10}H_{16}$	1	0.84	2.18	2.35	0.93	2.03
4	trans-3-carene-2-ol	$C_{10}H_{16}O$	1.1	0.35	0.38	0.27	0.35	0.51
5	cyclohexane-4-(1- *methylethylidene*	$C_{15}H_{24}$	0.51	0.27	0.65	0.65	0.36	0.68
6	carvyl acetate	$C_{12}H_{18}O_2$	1.23	1.56	0.26	0.47	0.89	0.44
7	p-mentha-1,5,8-triene	$C_{10}H_{14}$	3.03	0.46	-	0.15	0.46	0.18
8	cymol	$C_{10}H_{14}$	5.55	4.22	0.44	5.67	4.46	5.08
9	allo-ocimene	$C_{10}H_{16}$	-	2.78	2.88	2.2	2.35	2.88
10	terpinolene	$C_{10}H_{16}$	0.83	1.06	-	1.53	0.2	1.76
11	p-cymene	$C_{10}H_{14}$	0.35	0.5	1.53	0.16	0.35	0.23
12	3-octen-5-yne,27-dimethyl	$C_{10}H_{16}$	0.11	0.16	0.01	0.21	-	0.09
13	2,3-trans-epoxicaran	$C_{15}H_{24}$	0.27	1.48	1.64	2.11	1.54	1.16
14	camphor	$C_{10}H_{16}O$	1.09	2.69	7.26	10.95	3.91	7.0
15	terpinene	$C_{10}H_{16}$	3	4.98	11.74	5.65	2.74	3.77
16	limonene	$C_{10}H_{16}$	1.04	0.41	0.39	0.56	1.85	0.48
17	piperitone	$C_{10}H_{16}O$	0.35	0.55	0.23	0.78	0.33	0.06
18	germacrene - D	$C_{15}H_{24}$	-	-	-	-	18.6	10.33
19	bicyclogermacrene	$C_{15}H_{24}$	-	-	-	-	-	2.89
20	1-methylethenylcyclopropane	C_6H_{10}	0.26	0.21	0.12	0.21	-	0.1
21	junipene	$C_{15}H_{24}$	0.22	0.17	0.26	0.18	4.21	0.17
22	isoledene	$C_{15}H_{24}$	-	14.04	-	-	-	0.34
23	trans-caryophyllene	$C_{15}H_{24}$	-	0.49	-	-	-	17.26
24	valencene	$C_{15}H_{24}$	-	4.06	-	-	-	0.14
25	calarene	$C_{15}H_{24}$	0.66	-	0.59	0.48	-	
26	j-guaiene	$C_{15}H_{24}$	27.84	18.11	32.06	26.2	-	4.98
27	muurolene	$C_{15}H_{24}O$	7.88	4.31	6.34	4.27	4.44	0.3
28	ä-cadinene	$C_{15}H_{24}$	0.96	3.2	0.02	1.02	0.74	0.53
29	isolongifolene	$C_{15}H_{24}$	5.68	2.99	3.6	3.13	-	4.53
30	farnesol	$C_{15}H_{26}O$	-	1.18	-	-	-	0.5
31	cedrene	$C_{15}H_{24}$	-	1.06	-	-	-	0.56
32	aromadendrene VI	$C_{15}H_{24}$	2.7	0.16	2.36	2.58	-	0.33
33	4,5,9,10-dehydro-isolongifolene	$C_{15}H_{20}$	1.75	1.16	2.18	2.52	5.34	1.5
	Yield (v/dry weight)		0.16	0.18	0.21	0.41	0.36	0.14

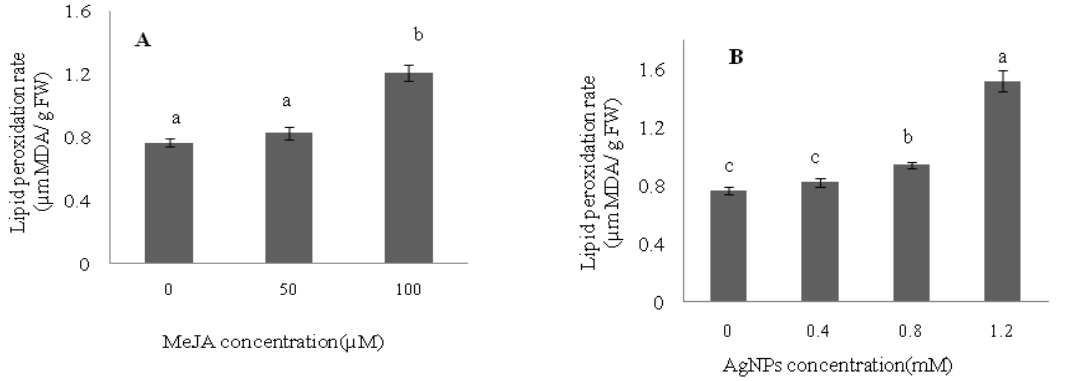

Fig. 1. Membrane lipid peroxidation of *Achillea millefolium* L. treated with different concentrations of MeJA (A), and AgNPs (B). Data are mean ± SD, n = 3, at *P* d" 0.05 according to LSD test

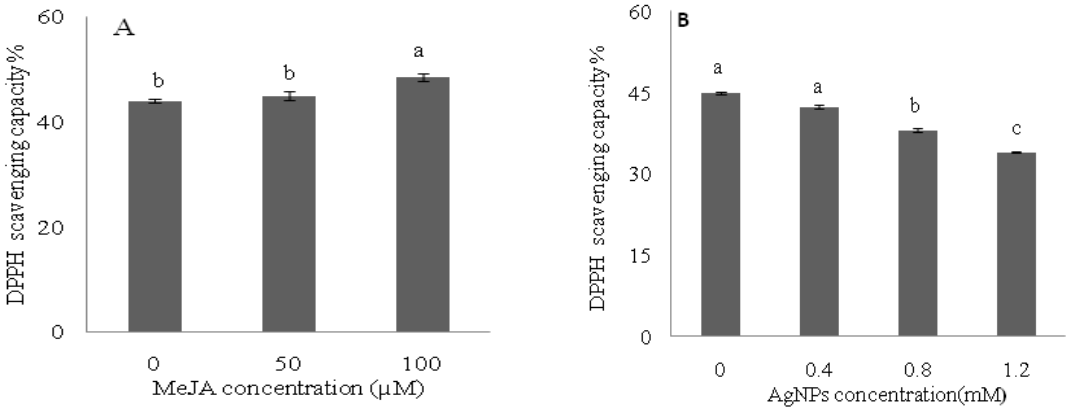

Fig. 2. Radical scavenging activity (% of DPPH) of *Achillea millefolium* L. treated with different concentrations of MeJA (A), and AgNPs (B). Data are presented as mean ± SD with n = 3. Bars with different letters in each graph are significantly different at *p* d" 0.05 according to LSD test

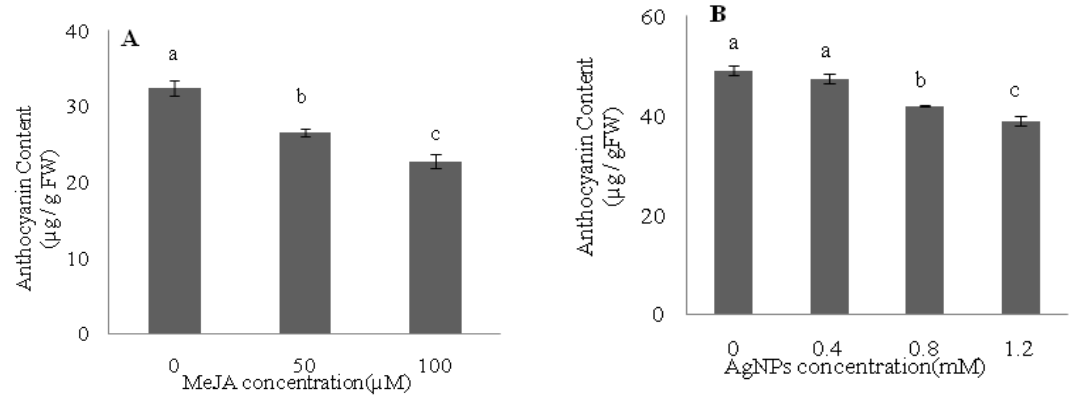

Fig. 3. Anthocyanin content of *Achillea millefolium* L. treated with different concentrations of MeJA (A), and AgNPs (B). Data are presented as mean ± SD with n = 3. Bars with different letters in each graph are significantly different at *p* d" 0.05 according to LSD test

50 and 100 µM MeJA significantly increased the flavonoid content compared with the control plants (Figure 4A). Flavonoid contents of plants treated with 0.8 and 1.2 mM AgNPs were significantly higher than that of the control group (Figure 4B). Chemical composition and contents of essential oil of *Achillea millefolium* L. before and after treatment with different concentrations AgNPs and MeJA are presented in Table 1. As shown, the yield of essential oil of the plant increased by treatment with increasing concentrations of AgNPs, so that the most yields (256% of the control) was observed in plants treated with 1.2 mM AgNPs. Exposure to 50 µM MeJA also remarkably (225%) increased essential oil yield of the plants (Table 1). In 100 µM MeJA treatment, a slight decrease was observed in essential yield, compared to the control plants (Table 1).

Essential oils of *Achillea millefolium* L. before and after treatment with AgNPs and MeJA were rich in sesquiterpens followed by monoterpens. Among different components of

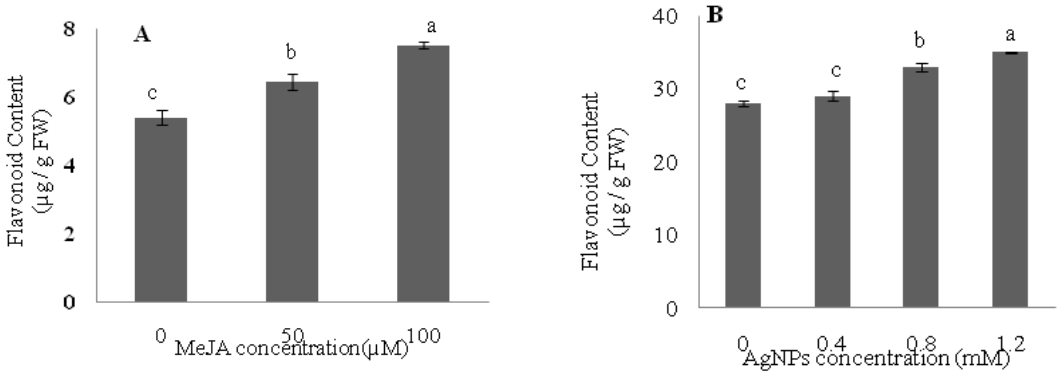

Fig. 4. Flavonoid content of *A. millefolium* L. treated with 0, 50 and 100 µM MeJA (A) and 0, 0.4, 0.8 and 1.2 mM AgNPs (B). Data are presented as mean ± SD with n = 3. Bars with different letters in each graph are significantly different at $p \leq 0.05$ according to LSD test

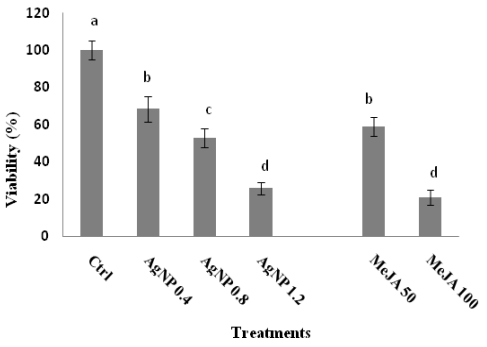

Fig. 5. Evaluation of the viability of HeLa cells by MTT assay. Ctrl: HeLa control cells; AgNPs 0.4, 0.8, 1.2: HeLa cells after exposure to essential oils of the plants treated with 0.4, 0.8, and 1.2 mM AgNP; MeJA 50 and MeJA 100: HeLa cells after exposure to essential oils of the plants treated with 50 and 100 µM MeJA. Data are presented as mean ± SD with n = 3. Bars with different letters are significantly different at $p \leq 0.05$ according to LSD test.

essential oils of *A. millefolium*, j-guaiene had the highest content in control plants (Table 1). Different concentrations of either AgNPs or MeJA resulted in abundance of one or two particular component, most belonging to C10 and C15. Noticeable increments were observed in camphor and terpinen (respectively 6.4 and 4 folds higher than those of the controls). Certain precious compounds e.g., allo-ocimene, germacrene-D, bicyclogermacrene, and farnesol were only produced after elicitation of *A. millefolium* with AgNPs and MeJA (Table 1). Essential oils of *A. millefolium* before and after treatment with different concentrations of AgNPs and MeJA inhibited the growth and viability of HeLa cells (Figure 5). The most pronounced inhibition was observed when the cells were exposed to the extract of 1.2 mM AgNP-treated plants (Figure 5).

Flavonoids, anthocyanins, alkaloids, and essential oils (terpenoids) are the defense secondary metabolites, and their induction by jasmontes has been previously reported[11]. Treatment of A.

millefolium with MeJA and AgNPs in the present study increased the flavonoid content but decreased the anthocyanin content. The role of free radicals in elicitor-induced accumulation of secondary metabolites has been widely observed in the biosynthesis of indole alkaloids in *Catharanthus roseus* cell cultures[12]. Methyl jasmonate-induced anthraquinone accumulation in *Rubia tinctorum* cell suspension culture has been recently reported[13]. External use of MeJA increased the anthocyanin content in strawberry 25 and apple fruit 26 and increased the flavonoid content in blackberries[2]. Decrease in anthocyanin in plants treated with AgNPs and MeJA can be attributed to the shifting of biosynthetic pathways from anthocyanins to flavonoids in these plants.

The essential oils of *A. millefolium* L. have economic importance due to their anti-inflammatory and disinfectant properties[7]. They are also mainly used against cold and influenza. Plants treated with 1.2 mM AgNPs had the highest level of essential oil yield among all control and treated plants.

Several studies indicate that essential oil of *A. millefolium* L. contains several monoterpenes such as α-pinene, α-pinene, 1,8-cineole, camphor, and borneol in addition to some sesquiterpene lactones of germacrene-derivatives [8]. Camphor was observed in both control and treated plants in our study. The highest content of camphor was observed in essential oils of plants treated with 1.2 mM AgNPs. With respect to the well-known antimicrobial potential of camphor, it seems reasonable to conclude that treatment of *A. millefolium* with AgNPs had a positive effect on its medicinal properties.

p-Cymene was detected in all control and treated plants, with no significant changes in its amount. Germacrene-D and bicyclogermacrene were observed only in those plants that were treated with MeJA and were not detected in control plants or in those treated with AgNPs. Germacrene-D has considerable antimicrobial activity[14].

Both MeJA and AgNPs stimulated the production of essential oils in *A. millefolium* L. plants, showing the defense response of plants against elicitation effects of MeJA and AgNPs. According to the results, j-guaiene (a sesquiterpene) was produced in highest amounts, except in 50 µm MeJA-treated plants.

Junipene content was increased in plants treated with 50 µM MeJA. Junipene is a terpene that is widely used as a natural flavor additive in food, as a fragrance in perfumery, and in aromatherapy with traditional and alternative medicines[15].

One interesting result of elicitation of *A. millefolium* with AgNPs and MeJA was the induction of new defensive compounds. For instance, Allo-ocimene was not observed in essential oils of control plants; however, it was induced by AgNPs and MeJA treatment. Kishimoto and coworkers[16] reported that treatment of *Arabidopsis* with allo-ocimene induced the expression of defense genes against *Botrytis cinerea* in a jasmonate-dependent pathway. They suggested that allo-ocimene could make the plants more resistant against the fungal disease.

The amount of terpinene in essential oils was highest in plants treated with 0.8 mM AgNPs. Gama-terpinene increased significantly in plants treated with 100 µM MeJA compared with that in control plants. Gama-terpinene is a monoterpene with a well-known antibacterial activity. It may cross cell membranes, thus penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity[17].

Some constituents such as isoledene, trans-caryophyllene, valencene, farnesol, and cedrene were observed only in plants treated with 0.4 mM AgNPs and in those treated with 100 µm MeJA.

Farnesol inhibited germ tube formation in *Candida albicans* but did not inhibit the elongation of existing germ tubes[18]. The anticancer effects of farnesol have been demonstrated by the suppression of tumor cell proliferation and induction of tumor cell apoptosis in vitro[19-20].

Increase of the inhibitory effects of essential oils of MeJA- and AgNPs- treated plants on HeLa cells in the present study may be addressed to increase of mono and sesquiterpens compounds, however, further experiments are necessary to evaluate each compound per se.

It is noteworthy also that treatment of *A. millefolium* with MeJA and AgNPs, particularly at high concentrations, shifted terpenoid pathways of the plant toward biosynthesis of certain compounds with well-known anti-inflammatory effects, e.g., trans-caryophyllene which can selectively bind to the cannabinoid receptor type-2 (CB2) and exert

significant cannabimimetic properties[21].

The results presented in this study addressed the elicitation potential of nanosilver particles and MeJA for enhancing plant secondary metabolite production and change of their metabolic pathway. Although the content of free radicals was not measured in this study, reduction of radical scavenging capacity of the plant and change of the amounts of non-enzymatic radical scavengers suggest that elicitation was governed by changing the redox status of *A. millefolium*. It is plausible that AgNPs and MeJA by eliciting certain signaling molecules such as hydrogen peroxide induced defense system of the plants and resulted in biosynthesis of new secondary compounds. This provides us with new insights in the roles of MeJA and AgNPs in reprogramming of metabolic pathways in plants.

ACKNOWLEDGMENTS

This work was supported by grant NO **90000972** from Iran National Science Foundation (INSF) to FGH on "The effects of MeJA and AgNPs on medicinal compounds of plants".

REFERENCES

1. Maksymiec, W., Krupa, Z. The in vivo and in vitro influence of methyl jasmonate on oxidative processes in *Arabidopsis thaliana* leaves. *Acta Physiol. Plant*., 2002; **24**: 351-7.
2. Wang, S. Y., Bowman, L. Ding, M. Methyl jasmonate enhances antioxidant activity and flavonoid content in blackberries (Rubus sp.) and promotes antiproliferation of human cancer cells. *Food Chem*., 2008; **107**: 1261-9.
3. Oukarroum, A., Bras, S., Perreault, F., Popovic R. Inhibitory effects of silver nanoparticles in two greenalgae, *Chlorella vulgaris* and *Dunaliella tertiolecta*, *Ecotoxic. Environ. Safe*., 2012; **78**: 80-5.
4. Nair, P. M., Choi, J. Identification, characterization and expression profiles of *Chironomus riparius* glutathione S-transferase (GST) genes in response to cadmium and silver nanoparticles exposure. *Aquat. Toxicol*., 2011; **101**: 550-60.
5. De Geyter, N., Gholami, A. Goormachtig, S., Goossens, A. Transcriptional machineries in jasmonate-elicited plant secondary metabolism. *Trends Plant Sci*., 2012; **17**: 349-59.
6. Zhang, B., Zheng, L. P., Yi Li, W., Wen Wang, J. Stimulation of artemisinin production in *Artemisia annua* hairy roots by Ag-SiO_2 core-shell nanoparticles. *Curr. Nanosci*., 2013; **9**: 363-70.
7. Benedek, B., Koopand, B., Melzig, M. F. *Achillea millefolium* L. s.l. – is the anti inflammatory activity mediated by protease inhibition? *J. Ethnopharmacol*., 2007; **113**: 312-17.
8. Potricha, F. B., Allemand, A., Da Silva, L. M., Dos Santos, C., Baggio, C. H., Freitas, C. S., Mendes, D. A., Andre, E., Werner, M. F., Marques, M. C. Antiulcerogenic activity of hydroalcoholic extract of *Achillea millefolium* L.: Involvement of the antioxidant system. *J. Ethnopharmacol*., 2010; **130**: 85-92.
9. Payez, A., Ghanati, F., Seyedi, S. M. Aluminum affects the antioxidant activity of tobacco cells and the expression of salicylate-induced protein kinase (SIPK). *Not. Bot. Horti. Agrobo*., 2011; **39**: 77-81.
10. Bemani, E., Ghanati, F., Yousefzadeh Boroujeni, L., Khatami, F. Antioxidant activity, total phenolics and taxol contents response of hazel (*Corylus avellana* L.) cells to benzoic acid and cinnamic acid. *Not. Bot. Horti. Agrobo*., 2012; **40**: 69-73.
11. Kondo, S., Tsukada, N., Niimi, Y., Seto, H. Interactions between jasmonates and abscisic acid in apple fruit, and stimulative effect of jasmonates on anthocyanin accumulation. *J. Jpn. Soc. Hortic. Sci.,* 2001; **70**: 546-52.
12. Zhao, J., Hu, Q., Guo, Y. Q., Zhu, W.H. Elicitor-induced indole alkaloid biosynthesis in *Catharanthus roseus* cell cultures is related to Ca? influx and the oxidative burst. *Plant Sci*., 2001; **161**: 423-31.
13. Perassolo, M., Quevedo, C. V., Busto, V. D., Giulietti, A. M., Talou J. R. Role of reactive oxygen species and proline cycle in anthraquinone accumulation in *Rubia tinctorum* cell suspension cultures subjected to methyl jasmonate elicitation. *Plant Physiol. Biochem*., 2011; **49**: 758-63.
14. Duarte, M. C. T., Figueira, G. M., Sartoratto, A., Rehder, V. L. G., Delarmelina, C. Anti-Candida activity of Brazilian medicinal plants. *J. Ethnopharmacol*., 2005; **97**: 305-11.
15. Greer, D., Pfahl, L., Rieck, J., Daniels, T., Garza, O. Comparison of a novel distillation method versus a traditional distillation method in a model gin system using liquid/liquid extraction. *J . Agric. Food Chem*., 2008; **56**: 9030-6.
16. Kishimoto, K., Matsui, K., Ozawa, R., Takabayashi, J. Volatile C6-aldehydes and allo-ocimene activate defense genes and induce resistance against *Botrytis cinerea* in *Arabidopsis*

thaliana. *Plant Cell Physiol*., 2005; **46**: 1093-102.

17. Athikomkulchai, S., Watthanachaiyingcharoen, R., Tunvichien, S., Vayumhasuwan, P., Karnsomkiet, P., Sae-Jong, P., Ruangrungsi, N. The development of anticancer products from *Eucalyptus blobulus* and *Psidium guajava* oil. *J. Health Res.*, 2008; **22**: 109-13.
18. Mosel, D. D., Dumitru, R., Hornby, J. M., Atkin, A. L., Nickerson, K. W. Farnesol concentrations required to block germ tube formation in *Candida albicans* in the presence and absence of serum. *Appl. Environ. Microbiol*., 2005; **71**: 4938-40.
19. Rao, C., Newmark, H., Reddy, B. Chemopreventive effect of farnesol and lanosterol on colon carcinogenesis. *Cancer Detect. Prev*., 2002; **26**: 419-25.
20. Burke, Y., Ayoubi, A., Werner, S., McFarland, B., Heilman, D., Ruggeri, B., Crowell, P. Effects of the isoprenoids perillyl alcohol and farnesol on apoptosis biomarkers in pancreatic cancer chemoprevention. *Anticancer Res*., 2002; **22**: 3127-34.
21. Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J. Z., Xie, X. Q., Altmann, K. H., Karsak, M., Zimmer, A. Beta-caryophyllene is a dietary cannabinoid, *P. Natl. Acad. Sci. USA*., 2008; **105**: 9099-9104.