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THE EFFECT OF ($AgNO_3$) NPS ON INCREASING OF SECONDARY METABOLITES OF CALENDULA OFFICINALIS L. *IN VITRO*

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ABSTRACT

The present study was conducted in order to increase the production of some Secondary metabolic compounds (essential oils) of Marigold plant *Calendula officinalis* L. *In Vitro.* Secondary metabolic compounds quantitative and qualitative analysis using chromatography device with high performance liquid HPLC and compared with the mother plant. In order to increase the production of secondary metabolites, ($AgNO_3$) nanoparticales used with concentrations (0, 0.3, 0.6, 0.9 and 1.2) mg/l. The results showed that the (1.2 mg/l) concentration ($AgNO_3$) NPs led to high significant in all the essential oils of *C. officinalis* L.

Key Words:- *Calendula officinalis* L., essential oils, $AgNO_3$ NPs.

INTRODUCTION

The aim of this study employ technology of tissue culture in the possibility of increasing secondary metabolites of plant marigold is made by developing callus from explants, then treated subcultured callus by some elicitors, which might help to increase the secondary metabolites of *Calendula officinalis* L., which had highly medical importance specially in the pharmaceutical industries, and compared the secondary metabolites resulting from treatment of the callus by elicitors with the secondary metabolites from the mother plant without treatments.*Calendula officinalis* L. (Asteraceae), known as calendula or marigold, is an annual specie widely used around the world as a medicinal plant. It is native to the area surrounding the Mediterranean, it is today and has been historically grown much more widely, throughout many temperate zones (Ao, 2007). *C. officinalis* L. is grown for medicinal herbal (Mohammad and Kashani, 2012), anti-tumor (Matic *et al*., 2012).The marigold inflorescences present essential oils, saponins, flavonoids and carotenoids, among other potentially active chemical constituents (Bilia, *et al*., 2002). The oil of *C. officinalis* is used as an anti-inflammatory, an anti-tumor agent, and a remedy for healing wounds (Okoh, 2008). Plant pharmacological studies have suggested that *Calendula* extracts have anti-viral, anti-genotoxic, and anti-inflammatory properties *in vitro* (Jimenez-Medina *et al*., 2006). Topical application of *C. officinalis* ointment has helped to prevent dermatitis, pain, and missed radiation treatments in randomized trials (McQuestion, 2006). The production of secondary metabolites *in vitro* is possible through plant tissue culture(Barnum, 2003).Essential oils are hydrophobic, are soluble in alcohol, non-polar or weakly polar solvents, waxes and oils, but only slightly soluble in water and most are colourless or pale yellow, With exception of the blue essential oil of chamomile (*Matricaria chamomilla*) and most are liquid and of lower density than water (Martin, *et al*., 2010). The extraction technique have been used for the extraction of essential oils from *Calendula officinalis* L. analyzed by HPLC apparatus (Okoh, 2008).The predominant compounds in the essential oils of *Calendula*

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officinalis L. are α- Pinene, β- Pinene, ρ- cymene, α-thujene, calendulaglycoside, cadinene, cadinol, T-muurolol, 1,8-cineole and limonene (Djilani and Dicko, 2012).In nanoparticales, The prefix"nano," (derived from the Greek "nanos") is becoming increasingly common in scientific literature. Nanotechnology represents one of the reasons behind the intense interest is that permits the controlled synthesis of materials where at least one dimension of the structure is less than 100 nm. This ultra-small size is comparable to naturally occurring proteins and biomolecules in the cell (McNeil, 2005). Silver nanoparticles, have unique optical, electrical, and thermal properties and are being incorporated into products that range from photovoltaics to biological and chemical sensors. Examples include conductive inks, pastes and fillers which utilize silver nanoparticles for their high electrical conductivity, stability, and low sintering temperatures (Li, *et al*., 2010).

MATERIAL AND METHODS

Plant materials and Sterilization

Marigold plants, *C. officinalis* L. (Asteraceae) were collected on 18/09/2013 from the Garden of Al-Mustansiriya University in Baghdad, Iraq. The leaves were cut out, rinsed with running tap water for 1 hr., then transferred to laminar air flow-cabinet where submerged in (99%) ethanol for one minute, Washed with sterilized DDH2O, then rinsed with sodium hypochlorite at the concentrations (1.5%) for (10min). Then washed with DDH2O three times for five minutes and planted in vials of Agriculture (Universal Tubes) (Pierik, 1987).

Callus induction

MS medium of callus induction was prepared (Murashing, and Skoog, 1962). That had 2 mg/l of the auxin (2,4-D) , and 0.2 mg/l of the cytokinin (kinetin), and different concentrations of $AgNO_3$ NPs (0, 0.3,0.6,0.9 and1.2) mg/l (Table 1). At 10 replicate to each concentration of $AgNO_3$ NPs, then incubated in the light condition, the illumination intensity was 1000 lux for 16 hours a day at a temperature of 1 ± 25 C° (Ghanati and Bakhtiarian, 2013).

Measuring fresh and dry weight of callus

After 4 weeks of culture the fresh weight of callus recorded by a sensitive balance in Laminar Air flow cabinet later placed in an electric oven at a temperature of 70 C° for 24 hrs to calculate the rate of the dry weight of callus (Pacheco, 2013).

Extraction and analysis of violate oil from *C. officinalis* L.

The wet callus (2 g) were crushed and extracted with petroleum ether for 4hrs in a Soxhlet apparatus. The extract was concentrated under reduced pressure. 1 ml concentrated extract was dissolved in 20 ml petroleum ether, 2 ml methanol acid and 2 ml of KOH added. The mixture was shaken for 2 min and allowed to stand for 10 min. The upper layer was removed and washed with water. This oil was analyzed by HPLC according the optimum condition as given above **(**Budhiraja, 2004).

Estimate the increase or decrease in the secondary metabolites compounds by device (HPLC)

High-performance liquid chromatography (HPLC) was used, the samples was performed with the HPLC system equipped with two shimadzu reciprocating pumps, a variable UV-VIS detector shimazeu data processors, to estimate the increase or the decrease in the secondary metabolites compounds of *C. officinalis* L. and compare these increases or decreases with the mother plant (Okoh, et al., 2007) The readings were measured at the wavelengths and by the time of the detention of the Rt solutions to the standard samples under study. The concentrations of active substances was quantified by comparing the area of package material standard package with an area of the model under the same conditions by using the following law:

$$\text{Concentration Of sample (mg/l)} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{conc. of standard} \times \text{dilution Factor}$$

Statistical analysis and Experimental design

Experiments are designed according to the design of full randomization Completely Randomize Design (CRD) to study the effect of various transactions in the studied traits, and compared the differences between the test averages according to Least Significant Differences (LSD) probability of 5% (Salkind and Ramsey, 2007)

RESULTS

The effect of different concentrations of $AgNO_3$ NPs on callus fresh and dry weight (mg)

The results in (Table 2) showed the highest callus fresh weight (958.0 mg) at concentration 0.3 mg/l of silver nanoparticles ($AgNO_3$) that had high significant than the other treatments except the treatments 0.6, 1.2 mg/l of $AgNO_3$ NPs. which gave (801.0, 750.0 mg) respectively. While the lowest callus fresh weight found at the control treatment which reached to (676.5 mg).The results in the same table showed that the highest callus dry weight (73.4 mg) at concentration 0.3 mg/l of silver nanoparticles

($AgNO_3$) that had high significant than other treatments while the lowest callus dry weight was found at the control treatment which reached to (48.0 mg) which had no significant difference than other treatments except the treatment of 0.3 mg/l silver nanoparticles ($AgNO_3$) (Fig.1).

The effect of different concentrations of ($AgNO_3$) NPs (mg/l) on producing secondary metabolites from callus by HPLC technique

The results in (Table 3) showed that adding $AgNO_3$ NPs caused increasing the concentrations of secondary metabolites in all concentrations than the mother plant. The α-pinene, α-thujene, Calendula-glycoside, α-cadinene, cadinol, t-muurolol, 1,8-cineol had high significant at the 1.2 mg/l concentration of $AgNO_3$ NPs. which gave (20.06, 162.99, 232.60, 247.78, 122.71, 326.02, 412.51 mg\l) respectively, while the lowest significant found at the control treatment which reached to (6.44, 51.98, 45.76, 56.49, 22.34, 96.57, 56.04 mg/l) respectively.The β-pinene, ρ-cymene, limonene had high significant at the 1.2 mg/l concentration of $AgNO_3$ NPs which gave (171.00, 52.43, 259.81 mg/l) respectively,while the lowest significant found at the 0.3 mg/l concentration of $AgNO_3$ NPs. which reached to (27.10, 5.88, 27.38 mg/l) respectively.

Table 1. Components of media which used of Stimulation secondary metabolites compounds by adding $AgNO_3$ Nanoparticles

Material	Consternations (mg/l.)
MS	Complete power
Sucrose	30000
L-Asparagine	150
Glycine	100
Kinetine	0.2
2,4-D	2
$AgNO_3$	0,0.3,0.6,0.9,1.2
Agar-Agar	8000

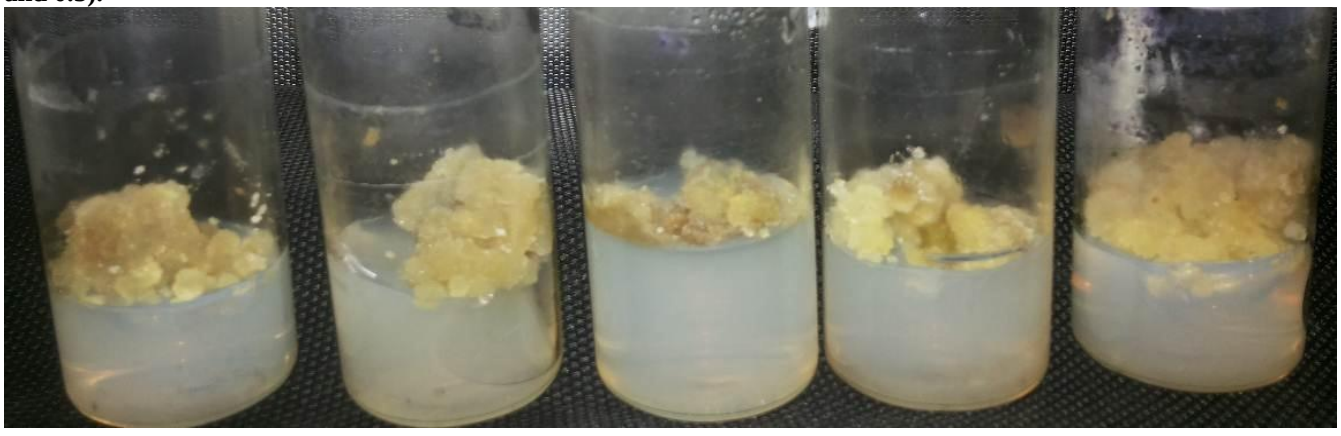
Table 2. The effect of different concentrations of $AgNO_3$ NPs on callus fresh and dry weight (mg) grown on a maintenance medium in light. Initial weight was 350 mg

Concentration of $AgNO_3$ NPs (mg/l)	Fresh Weight (mg)	Dry Weight (mg)
Cont.	676.5	48.0
0.3	958.0	73.4
0.6	801.0	52.7
0.9	693.2	52.4
1.2	750.0	51.0
L.S.D 0.05	227.1	15.91

Table 3. The effect of different concentrations of Silver nanoparticles ($AgNO_3$) (mg/l) on producing secondary metabolites from callus by HPLC technique

Secondary Metabolites	Concentration of $AgNO_3$ NPs (mg/l)					*C.officinalis*	L.S.D 0.05
	Cont.	0.3	0.6	0.9	1.2		
α-pinene	6..44	10.34	15.09	10.85	20.06	0.31	1.44
β-pinene	50.00	27.10	67.10	56.40	171.00	2.76	9.60
ρ-cymene	10.57	5.88	16.81	16.98	52.43	0.64	1.66
α-thujene	51.98	115.60	111.80	120.77	162.99	0.63	3.38
Calendula- -glycoside	45.76	104.83	97.67	106.75	232.60	3.18	3.66
α-cadinene	56.49	98.91	118.04	123.25	247.78	3.47	2.23
cadinol	22.34	26.86	49.44	67.89	122.71	0.70	1.32
t-muurolol	96.57	101.03	217.11	296.39	326.02	4.88	4.55
1,8-cineol	56.04	58.55	156.28	197.12	412.51	4.11	2.88
limonene	47.47	27.38	66.92	84.45	259.81	3.37	1.74

Fig. 1. The effect of different concentrations of $AgNO_3$ NPs on callus fresh weight (mg) from left (1.2, 0.6, cont., 0.9 and 0.3).



Figures below showed the effect of different concentrations of ($AgNO_3$) NPs (mg/l) on producing secondary metabolites from callus by HPLC technique

Fig. 2. HPLC for control treatment of ($AgNO_3$) NPs

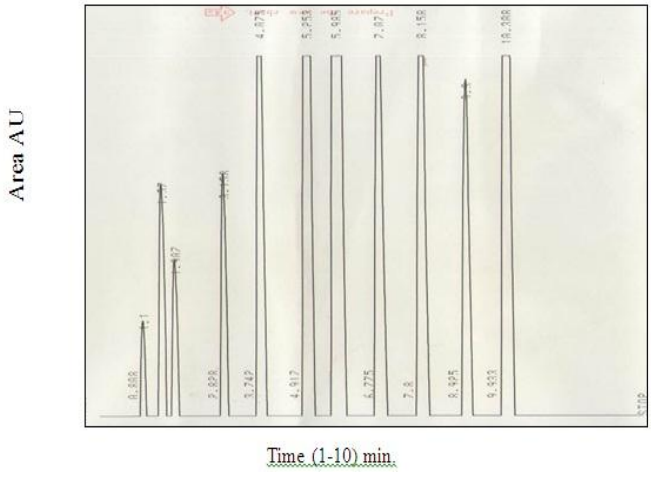


Fig. 3. HPLC for 0.3 mg/l treatment of ($AgNO_3$) NPs

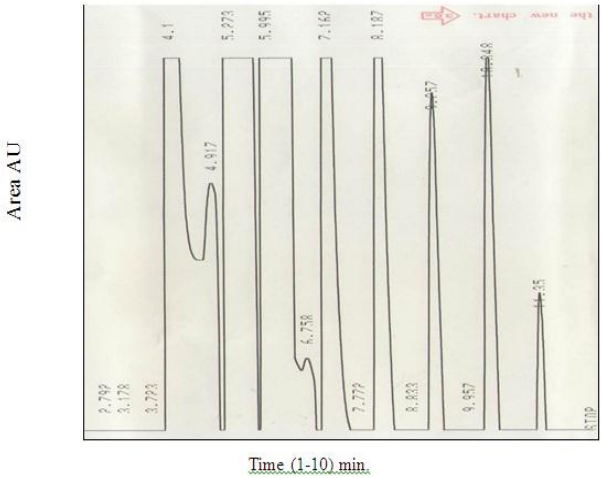


Fig. 4. HPLC for 0.6 mg/l treatment of ($AgNO_3$) NPs

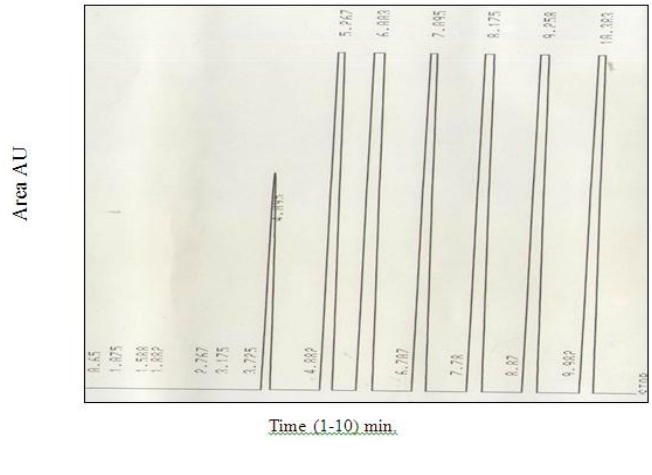


Fig. 5. HPLC for 0.9 mg/l treatment of ($AgNO_3$) NPs

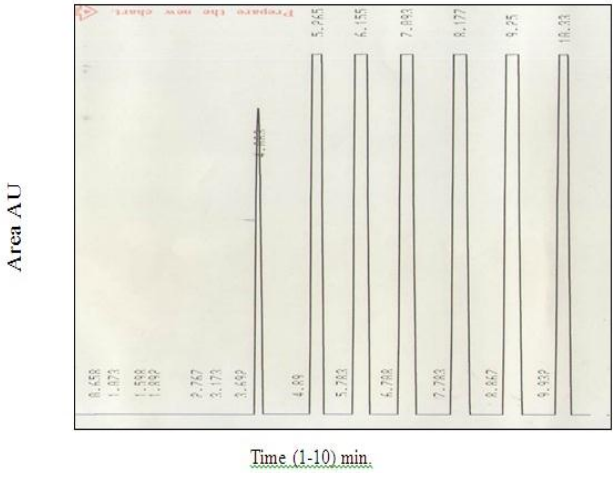
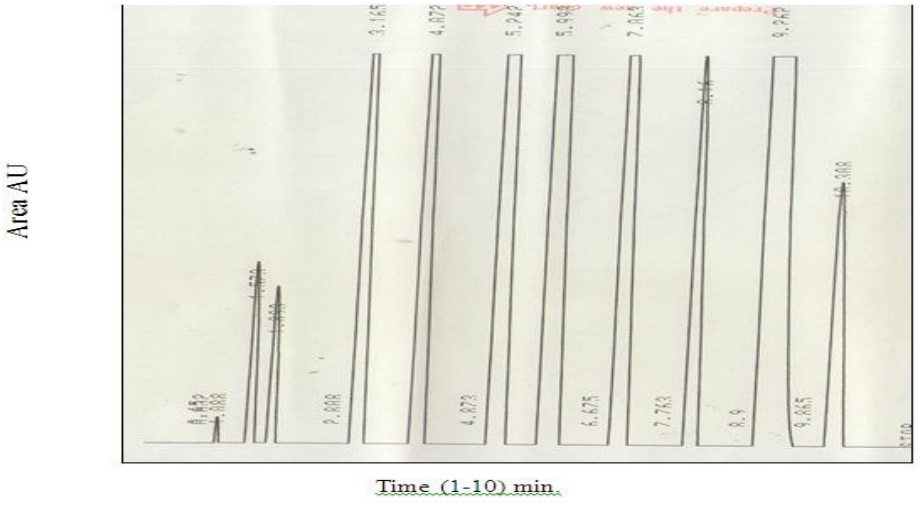


Fig. 6. HPLC for 1.2 mg/l treatment of ($AgNO_3$) NPs



DISCUSSION

The results showed that there were high significant on callus fresh and dry weight (mg) grown on a maintenance medium in light. The effect of different concentrations of ($AgNO_3$) NPs on producing secondary metabolites from callus by HPLC technique, the results showed there were agreed with Ghanati and Bakhtiarian stated that increasing the concentration of silver nanoparticles ($AgNO_3$) led to increasing the essential oil of secondary metabolites in *C. officinalis* L. and treatment with Ag NPs damaged membranes of plants and the level of membrane lipid peroxidation increased along with the increase of $AgNO_3$ NPs concentration, so that the most pronounced level of lipid peroxidation was observed in plants treated with 1.2 mg/l $AgNO_3$ NPs, in comparison with the control plant. Treatment of the plants with $AgNO_3$ NPs can be suggested as good strategies in order to increase of secondary components and improve of medicinal properties of the plant (Jimenez-Medina et al., 2006). The secondary metabolites of *Calendula officinalis* showed a potent in vitro inhibition of tumor cell proliferation. This potential increased after treatment of the plants with $AgNO_3$ NPs. Anticancer, antibacterial, fungicidal, and antioxidant activities of *C. officinalis* L. (Savithramma *et al*., 2011).

CONCLUSION

Adding 1.2 mg/l of $AgNO_3$ NPs led to high significant in all of the secondary metabolites (essential oils) of *C. officinalis* L.

RECOMMENDATION

Examination of $AgNO_3$ NPs with high concentration higher than 1.2 mg/l. on the *C. officinalis* L. and with another medical plant.

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REFERENCES

Ao CQ. Comparative anatomy of bisexual and female florets, embryology in Calendula officinalis (Asteraceae), a naturalized horticultural plant. *Scientia Horticulturae*, 114 (3), 2007, 214-219.

Barnum SR. Biotechnology, An Introduction. 2nd ed. Visa Publishing House, 2003, pp. 888-893.

Bilia AR, Bergonzi MC, Gallori S, Mazzi G and Vincieri FF. Stability of the constituents of calendula, milk-thistle and passionflower tinctures by LC-DAD and LC-MS. *J. Pharm. Biomed. Anal*, 30, 2002, 613-624.

Budhiraja RP. Separation Chemistry. New Age International Ltd, Publishers, New Delhi, 2004, pp.171.239.

Djilani A and Dicko A. The Therapeutic Benefits of Essential Oils, Nutrition, Well-Being and Health, Dr. Jaouad Bouayed (Ed.), 2012, ISBN: 978-953-51- 0125-3.

Ghanati F and Bakhtiarian S. Effect of methyl jasmonate and silver nanoparticles on secondary metabolites of *Calendula officinalis* L. *Advances in Environmental Biology*, 7(9), 2013, 2251-2258.

Jimenez-Medina E, Garcia-Lora, A, Paco L, Algarra I, Collado A and Garrido F. A new extract of the plant *Calendula officinalis* produces a dual in vitro effect: cytotoxic anti-tumor activity and lymphocyte activation. *BMC Cancer*, 6, 2006, 119.

Li WR, Xie XB, Shi QS, Zeng HY, Ou-Yang YS and Chen YB. Size- dependent antibacterial activities of silver nanoparticales against oral anaerobic pathogenic bacteria. *Appl Microbiol Biotechnol*, 85(4), 2010, 1115-22.

Martin A, Varona S, Navarrete A and Cocero MJ. Encapsulation and Co-Precipitation Processes with Supercritical Fluids: Applications with Essential Oils. *The Open Chemical Engineering Journal*, 4, 2010, 31-41.

Matic IZ, Juranic Z, Savikin K, Zdunic G, Nadvinski N and Godevac D. Chamomile and Marigold Tea: Chemical Characterization and Evaluation of Anticancer Activity. *Phytotherapy research*, 2012.

McNeil SE. Nanotechnology for the biologist. *J. Leukoc. Biol*., 78, 2005, 585–94.

McQuestion M. Evidence-based skin care management in radiation therapy. *Semin Oncol Nurs*, 22, 2006, 163-73.

Mohammad S and Kashani H. Pot marigold (Calendula officinalis) medicinal usage and cultivation. *Scientific Research and Essays*, 7 (14), 2012, 1468-1472.

Murashing T and Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*, 15, 1962, 473-497.

Okoh O, Sadimenko AA and Afolayan AG. The effect of age on the yield and composition of the essential oils of Calendula officinalis L. *J applied, Sci*, 7 (23), 2007, 3806-3810

Okoh OO. The effects of drying on the chemical components of essential oils of *Calendula officinalis* L. *African Journal of Biotechnology*, 7(10), 2008, 1500-02.

Pacheco AC, Cabral DS, Fermino ÉS and Aleman CC. Salicylic acid-induced changes to growth, flowering and flavonoids production in marigold plants, Journal of Medicinal Plant Research. Academic Journals, São Paulo, Brasil, 2013.

Pierik RLM. *In vitro* Culture of Higher Plants. 3rd ed. Martinus Nijhoff Publishers, Dordrecht, the Netherlands, 1987, pp. 471-507.

Salkind NJ and Ramsey PH. Encyclopedia of Measurement and Statistics, Sage research methods, 2007, ISBN: 9781412916110.

Savithramma N, Rao ML, Rukmini K and Devi PS. Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. *Int J ChemTech Res*, 3, 2011, 1394-1402.