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INCREACING AGOENE COMPOUND OF *Allium sativum* L. USING (UV) LIGHT *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 ***Allium sativum*** 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, (UV) light used with exposure (0, 10, 20, 30) min. The results showed that the (10) min exposure to UV light led to high significant in agoene compound.

KEYWORDS: *Allium sativum* L., UV, agoene.

INTRODUCTION

Plants are an important source of food and equipped with a large number of chemicals that include pharmaceuticals, pesticides, vaccinated, perfumes and colors. In spite of advances in production methods, are still a source of compounds are very complex or expensive price when production in other methods (Safadi *et al*., 1989). Provided different applications for the tissue culture access to economically important compounds, Discosmoand Misawa, (1995) including pharmaceutical compounds that are difficult to prepare at the laboratory as well as high cost when manufactured (Matlob *et al*., 1989). There are a lot of benefits that are associated with the production of such vehicles in this way in comparison with whole plant and get these compound with height degree of purity of Textile farms than those extraction from the plant and full production is fast and is not supported on the season and do not need large space for culture (Al-zubaidi *et al*., 1996), (Taiz and Zeiger, 2002). Garlic is considered of vegetable crops grown in the world due to its medical benefits and food (Alkatib, 2000).

Garlic used fresh or dried or pressed to extract juice, to be used in food preparation and seasoning for fish meat because of its flavor desired because it contains volatile substances. It well-known medical use expectorant and antipyretic (Shafei, 2001). And also advises eating garlic in the treatment of sore throat and hoarseness (Al-zubaidi, *et al*., 1996). Garlic cloves containing in structure sulfa compounds that protect the liver cells from the effects of carbon Ttrakloraid substance that helps the accumulation of fat in the liver (McQuestion, 2006). The agoene compound that found in garlic works to kill the fungi that infect the skin (Safadi *et al.*, 1998). Garlic is known as a factor for lowering high blood pressure, and antipyretic of high blood cholesterol (McNeil, 2005). The aim of the research tissue culture technique employing the possibility of increasing secondary metabolites in garlic plant, which is the medical and pharmaceutical materials intervention in many pharmaceutical industries through the use of UV light as physical stimulate to increase these compounds.

Plant materials and Sterilization

Garlic plants ***Allium sativum*** L. were collected on 15/09/2012 from market in Baghdad, Iraq. The gloves 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 (1.2)% for (5) min. 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 (2,4-D), and 1 mg/l of (BA) tabel (1), after that callus exposure to different periods time for UV light (0,10,20,30) min. with short wave length ranging (100-280) nm. At 10 replicate to each duration of exposure of UV light, then incubated in the light condition, the illumination intensity was 1000 lux for 16 hours\ a day at a temperature of 1 ± 25 C° Purohit, 1999).

Measuring fresh and dry weight of callus

After 4 weeks of culture the fresh weight of callus recorded by a sensitive balance 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 *Allium sativum* 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 (Salkind and Ramsey, 2007).

Estimate the increase or decrease in the secondary metabolite 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 *Allium sativum* L. and compare these increases or decreases with the mother plant .The readings were measured at the wave lengths and by the time of the detention of the Rt solutions to the standard samples under study (Lawson and Hughes., 2003). The concentrations of active substances were 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 (Budhiraja, 2003):

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

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 treatments 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 exposure time of UV light on callus fresh and dry weight (mg)

The results in (Table 2) showed the highest callus fresh weight (655.0) mg at (10) min that had high significant than the other treatments except the treatments (30) min which gave (538.0) mg. While the lowest callus fresh weight found at the control treatment which

reached to (492) mg. The results in the same table showed that the highest callus dry weight (149.0) mg at (10) min that had high significant than the other treatments except the treatments (30) min which gave (140.2) mg. while the lowest callus dry weight was found at the control treatment which reached to (128.2) mg which had no significant difference than other treatments.

The effect of exposure duration to (UV) light (min) on producing secondary metabolites from callus by HPLC technique

The results in (Table 3) showed that exposure to UV light caused increasing the concentrations of secondary metabolites in most treatments than the mother plant but these increases were not signification in all compounds except agoene which scored a significant increase reached (37.61) mg when exposed to UV light for 10 minutes.

Fig 1. Effect different exposure time of UV light (min) on producing secondary metabolites from callus by HPLC technique.

Table 1. Components of media for callus induction

Components	Consternations (mg/l)
MS	Complete power
Sucrose	30000
2-4 D	2
NAA	0.5
BA	1.0
Glycine	100
Myo-inositol	100
Agar	8000

Table 2. The effect of different exposure time of UV light (min) on callus fresh and dry weight (mg) grown on a maintenance medium in light. Initial weight was 300 mg

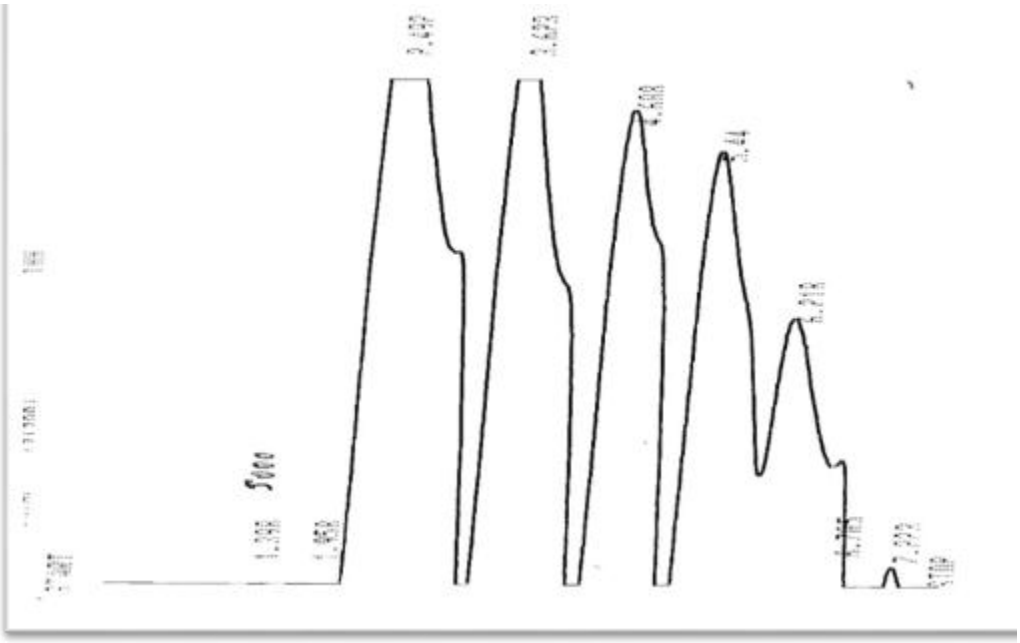
Exposure time (min)	Fresh weight (mg)	Dry weight (mg)
Control	492.0	128.2
10	655.0	149.0
20	449.0	131.5
30	538.0	140.2
L.S.D0.05	131.0	15.26

Table3. The effect of time exposure to UV light (min.) in the production of secondary metabolites from callus and appreciation quantitative and qualitative by use HPLC.

U.V light Secondary metabolites	Control	10 min	20 min	30 Min	Garlic gloves	LSD 0.05
s-allylcystin	47.3	44.3	55.3	35.7	12,5	N.S
y-gltomylcystin	23.0	26.2	22.2	24.7	3.3	N.S
Allicin	18.3	26.0	24.4	29.4	16.7	N.S
Vinyl-[4H]-1, 2dithlin(agoene)	21.0	37.6	27.7	24.1	5.9	26.24
Diallyldisulfide	25.2	27.5	63.6	27.4	3.8	N.S
Diallyltrisulfide	71.2	74.3	22.7	59.0	94.4	N.S

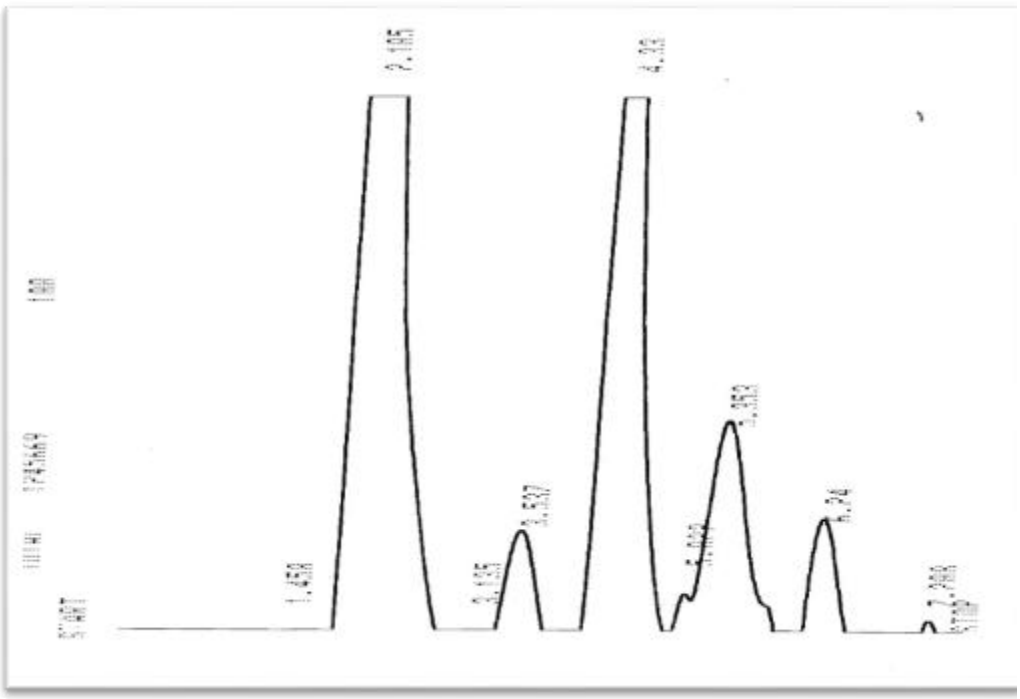
DISCUSSION

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 exposure time to UV light on producing secondary metabolites from callus by HPLC technique, the results showed there were agreed with Alqudsi, (2004) stated the UV light stimulate increasing in the mass of most secondary metabolites of ***Allium sativum*** L. because UV light act damage in The genetic material in the cell, Therefore, plant lose the stored genetic codes that regulate its vital operations Moreover, those rays break chlorophyll which without it plant cannot receive the sun's energy that necessary to complete the process of photosynthesis, And thus affect the growth of plant cells, To reduce the devastating impact of UV light some plant act to produce large amount from secondary metabolites that act Protection plant from UV light (Alqudsi, 2004).



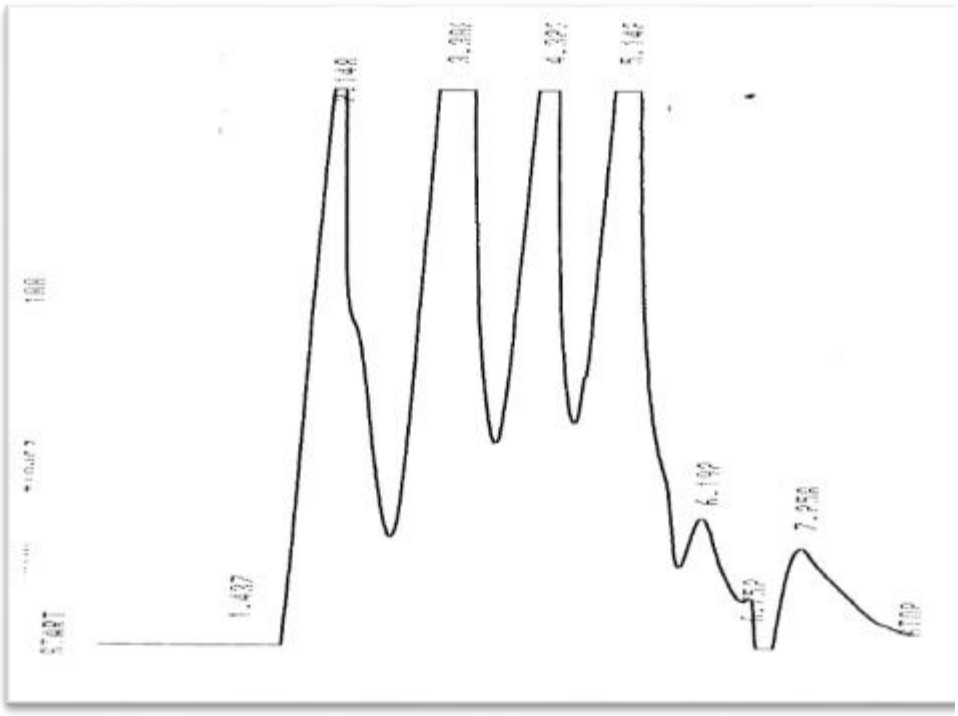
Time (1-10) min.

Fig. (2): HPLC for control treatment of UV light



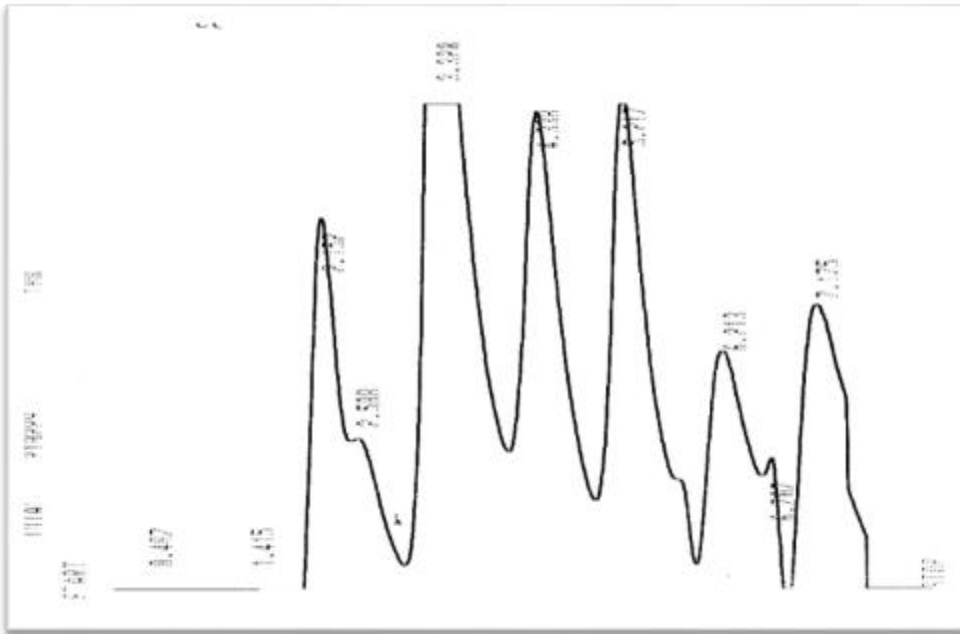
Time (1-10) min.

Fig. (3): HPLC after 10 min. of UV light.



Time (1-10) min.

Fig. (4): HPLC after 20 min of UV light



Time (1-10) min.

Fig. (5): HPLC after 30 min of UV light

CONCLUSION

Exposure to (10) min. for UV light led to high significant in agoene compound (essential oils) of *Allium sativum* L. comparing with other treatments.

RECOMMENDATION

Use another stimulate to increase (essential oil) of *Allium sativum* L.

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