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Original Article

Effect of (UV) light on production of medicinal compounds of *Althaea officinalis* L. *in vitro*

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ABSTRACT

Medicinal compounds of *Althaea officinalis* L. were increased *In Vitro* by using UV light (0, 10, 20, 30, 40) min. then analysis by HPLC technique. The results reported that medicinal compounds had high significant in the most of these compounds.

Key words: *Althaea officinalis* L., UV, medicinal compounds

INTRODUCTION

Althaea officinalis L. belongs to family (Malvaceae). It is one of the medicinal plants used therapeutically since ancient time. The leaves of the *A. officinalis* plant as well as the root are used as medicine (1). Leaves, stem and root of *A. officinalis* is a perennial herb 60 to 120 cm high. Stem is erect and have short petioled leaves (2). *A. officinalis* is a medicinal plant consumed in case of lipemia, inflammation of nasal and oral cavities, gastric ulcer, platelet aggregation, cystitis, and irritating coughs (3, 4). The studies shows that *A. officinalis L.* have antibacterial activity, anticomplement activity, antifungal activity, anti-inflammatory activity, antimycobacterial activity, antitussive activity, antiviral activity, antiyeast activity, common cold relief, cytotoxic activity (5).Plant tissue culture medium contains all the nutrients required for the normal growth and development of plants. It is mainly composed of macronutrients, micronutrients, vitamins, other organic components, plant growth regulators, carbon source and some gelling agents in case of solid medium (6). Cell and tissue *in vitro* culture is a useful tool for the production of secondary metabolites (7). Secondary metabolites are organic compounds synthesized by plants however they are not directly essential for photosynthesis, reproduction, respiration or other primary functions. The chemicals have extremely diverse effects. They often play an important role in the plant defense system. Some of them contribute to pollination and serve as protection from drought, salinity and UV radiation (8).The aim of this study to increase the production of secondary metabolites of *A. officinalis* L. which use as medicinal compounds using UV light as elicitor for the production of secondary plant products *In Vitro*.

MATERIAL AND METHODS

Source of Explants: O*fficinal's* were collected on 1st Nov. 2013 from the garden of Al-Mustansriyia University in Baghdad/Iraq.

Sterilization of Explants: Before the culturing in vials, the leaves were rinsed with running tap water for 1 hr. then submerged in (95%) ethanol for one minute, Washed with sterilized DDH2O, after that rinsed with (2%) concentration of sodium hypochlorite for (10) min. followed by washing with DDH2O three times for five minutes, all this process done in sterilize condition at laminar air flow-cabinet.

Medium of callus induction: Table 1. showed the modify MS medium have been used for callus induction, The subculture have been done every 3 weeks. After that callus exposure to UV light (0, 10, 20, 30, 40) min. with short wave length ranging (100-280) nm. which incubated at 16/8 hrs. light/dark photoperiod at the illumination intensity was 1000 lux at a temperature 25 ± 1 °C. (6).

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Table1. The medium of callus induction components.

No.	Components	Concentration (mg/l.)
1	MS	Full strength
2	Sucrose	30000
3	L- Asparagine	150
4	Glycine	10
5	2,4-D	2
6	Agar-Agar	8000

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 (9).

Analysis of medicinal compounds *A.officinalis* callus extract: The main compound were separated on FLC (Fast Liquid Chromatographic) column under the optimum condition.
Column: phenomenex C-18, 3 µm particle size (50 x 2.0 mm I.D) column
Mobile phase: linear gradient of solvent A 0.1 % formic acid, solvent B was (6:3:1, v/v) of acetonitrile; methanol: 0.1% formic acid, gradient program from 0% B to 100% B for 15 min. UV 264 nm. Flow rate 1.3 ml/min. The separation occurred on liquid chromatography shimadzu 10 AV-LC equipped with binary delivery pump model LC-10A shimadzu, the eluted peaks were monitored by UV-Vis 10 A-SPD spectrophotometer. The results were measured by following law, the concentration of each standard was 25µg/ml (10).

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

Extraction medicinal compounds of *A. officinalis:* About 1.0 gm of samples were dissolved into 5 ml of ethanol- water (80:20, v\v) in glass tubes. The suspension was subjected to ultra-sonication (Branson sonifier, USA) at 60% duty cycles for 25 min at 25°C. followed by Centrifugation at 7.500 rpm for 15 min. The clear supernatant of each sample was subjected to charcoal treatment to remove pigments prior to evaporation under vacuum (Bachi Rotavapor Re Type). Dried samples were re-suspended in 1.0 ml HPLC grade methanol by vortexing, the mixture were passed through 2.5 um disposable filter, and stored at 4°C. for further analysis, then 20 ul of sample injected into HPLC system according the optimum condition (11).

Statistical analysis and Experimental design: Experiments are designed according to Completely Randomize Design (CRD) and the differences between the test averages compared according to Least Significant Differences (LSD) probability of 5%.% (12).

RESULTS

Effect of different exposure of UV light (min.) on callus fresh and dry weight (mg): The results in table (2) showed that exposure 30 min. of UV light caused. Increasing in fresh and dry weight which gave 414.6, 34.8 mg respectively but this increasing was not significant while the lowest value of fresh weight recorded at treat of 10 min. of UV which gave 352.3 mg , and the lowest values of dry weight recorded at treat of 20 min. of UV which gave 26.3 mg.

Effect of UV light exposure (min.) on the production of medicinal compounds from callus *A. officinalis* by using HPLC: The results in table (3) showed that different exposure of UV light caused increased producing of secondary metabolites. The Salicylic acid gave a highly significant values (28.30) µg/ml at 40 min. than other treatments while the lowest significant(4.26)µg/ml at 30 min. of UV light. The Scopolotein, Caffeic acid and Syringic acid gave high significant at 30 min. of UV light (46.86, 36.13 or 70.93) µg/ml respectively. The lowest significance of Scopolotein and Syringic acid at 10 min. of UV light which gave (20.70, 28.75) µg/ml respectively while the lowest value of Caffeic acid (22.80) µg/ml at 20 min. of UV light.The Quercetin gave highly significant value(51.80) µg/ml at control treatment while the lowest was recorded at 20 min. of UV light which gave (21.10)µg/ml. The Coumarin also gave highly significant value (91.30) µg/ml at 10 min. while the lowest recorded (34.70) µg/ml was at the treatment of control. The P-coumaric acid recorded 69.10 µg/ml at 20 min. of UV light while the lowest was at the control treatment which gave 15.20 µg/ml. The Dosmetin reported highly significant value recorded (31.20) µg/ml at 20 min. while the lowest was (15.50)

µg/ml at 30 min. of UV light. The figures (1), (2), (3), (4), (5) showed the HPLC curves results by using different exposure of UV light and figure (6) shows the callus induction after exposure to UV light..

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 250 mg

Exposure time (min.)	Fresh weight (mg)	Dry weight (mg)
Control	**373.3**	**27.4**
10	**352.3**	**26.8**
20	**366.3**	**26.3**
30	**414.6**	**34.8**
40	**388.0**	**31.3**
LSD(0.05)	**173.o**	**17.35**

Table3: Effect of UV light exposure (min.) on the production of medicinal compounds from callus *A. officinalis* by using HPLC.

Secondary metabolites	UV Light per minute					L.S.D 0.05
	Control	10	20	30	40	
Salicylic acid	9.80	19.60	13.60	4.26	28.30	0.471
Scopolotein	26.70	20.70	28.20	46.86	40.90	0.489
Caffeic acid	28.20	24.30	22.80	36.13	34.60	0.100
Syringic acid	51.11	28.75	50.71	70.93	40.50	17.28
Quercetin	51.80	26.40	21.10	35.40	47.40	4.363
Coumarin	34.70	91.30	60.80	59.90	75.60	0.061
P-coumaric acid	15.20	21.90	69.10	55.30	58.70	0.063
Dosmetin	18.80	21.40	31.20	15.50	25.10	0.045

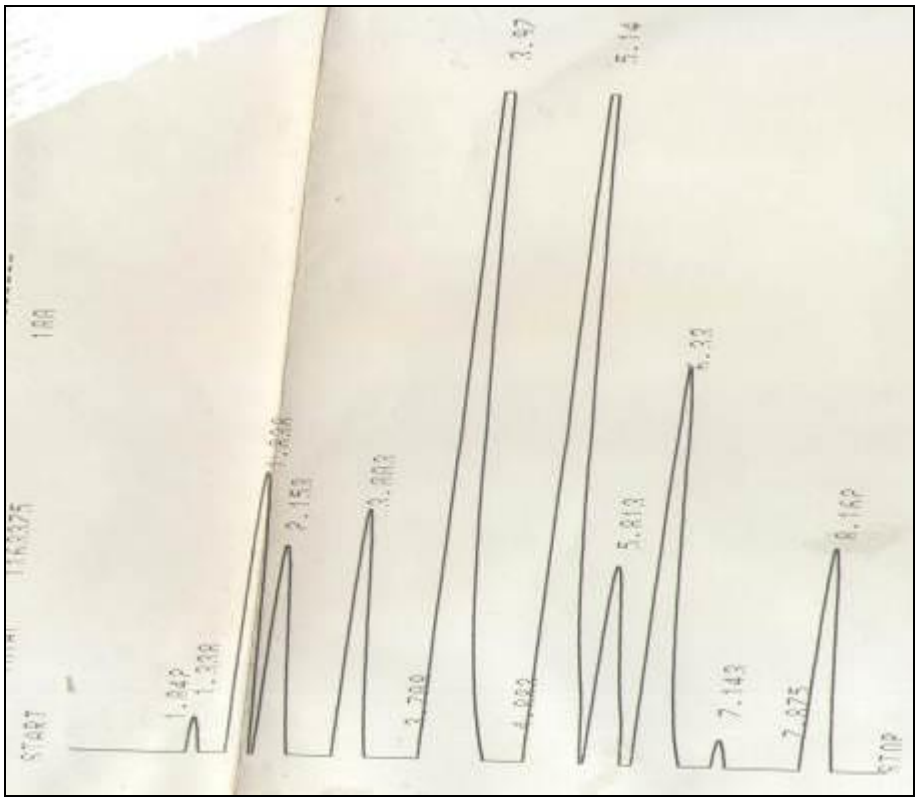

Fig 1. HPLC for control treatment of UV light.

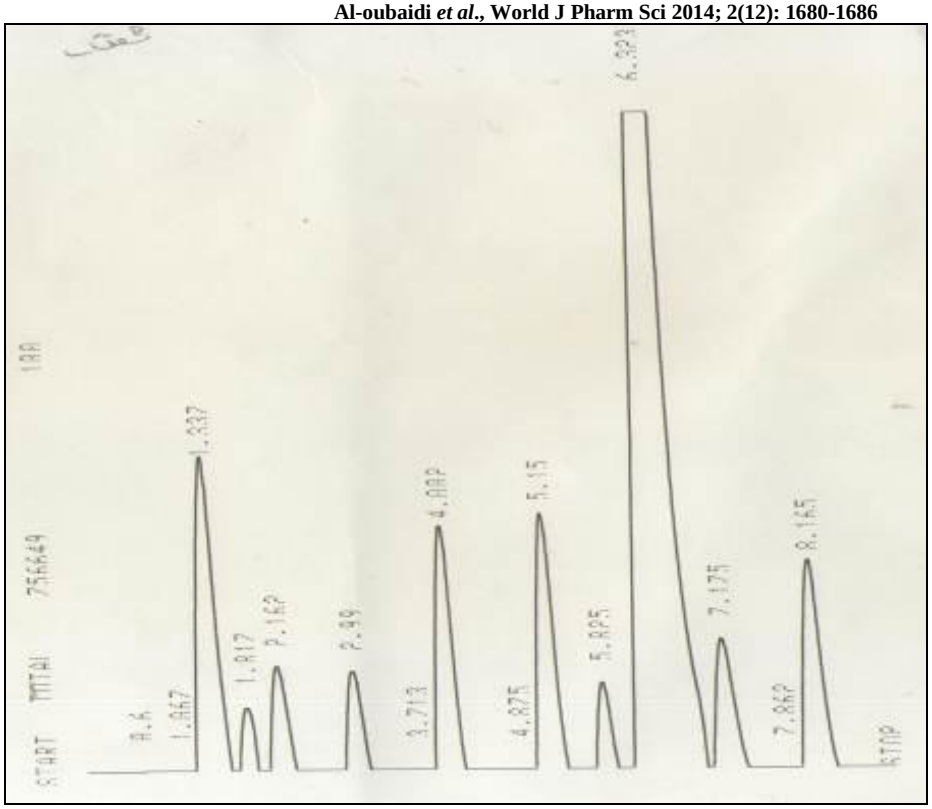

Fig 2. HPLC after 10 min. of UV light.

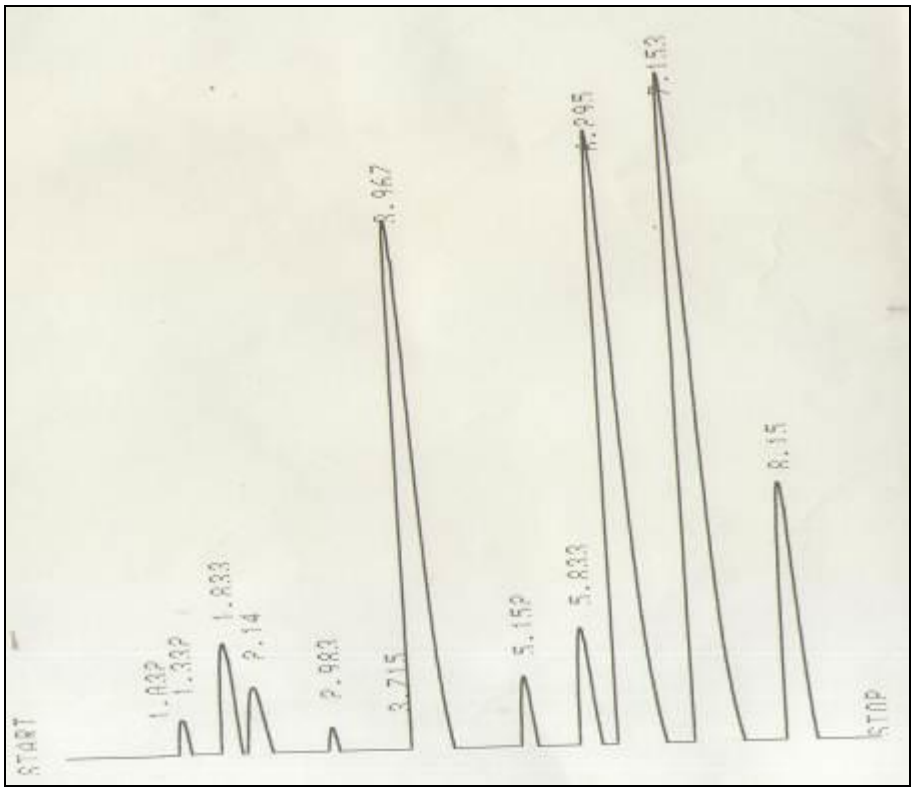

Fig 3. HPLC after 20 min of UV light.

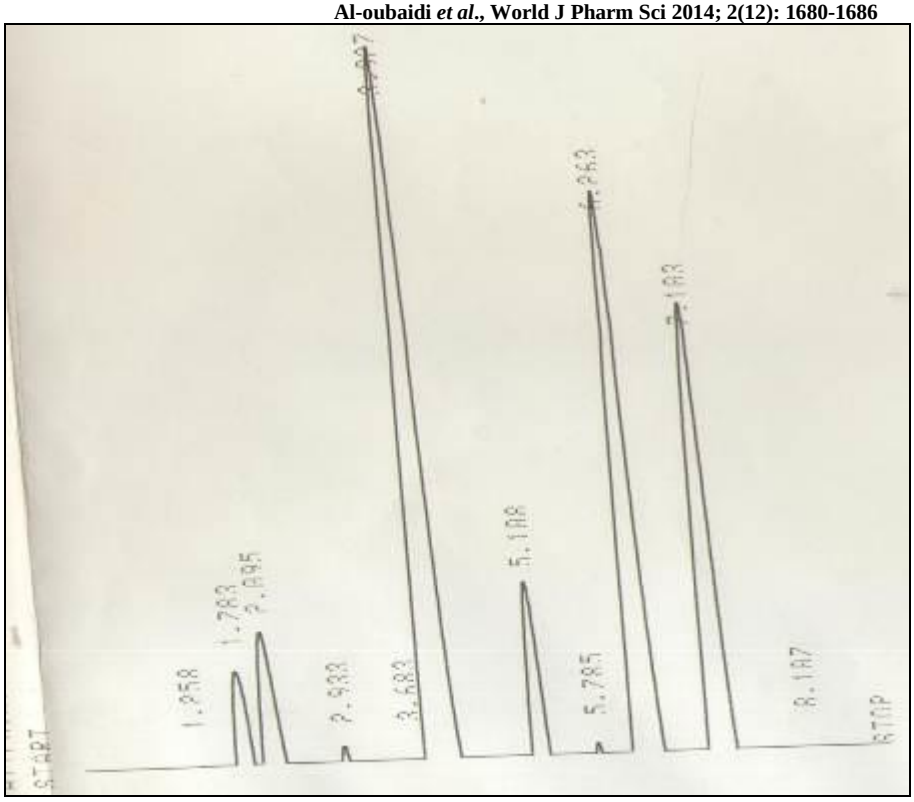

Fig 4. HPLC after 30 min of UV light.

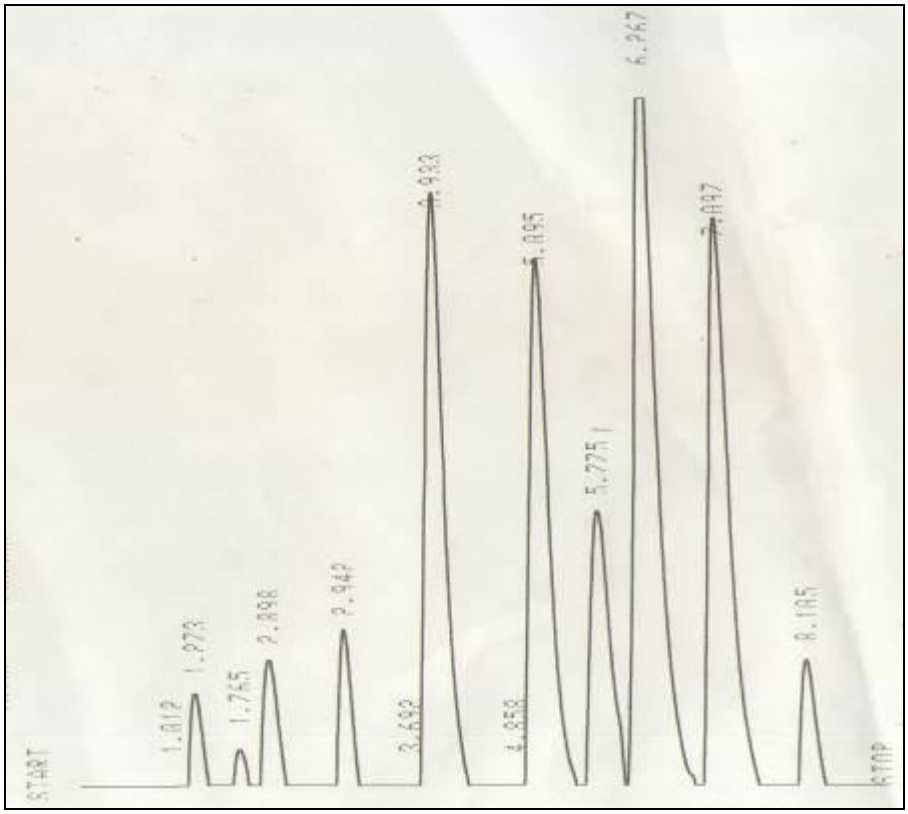

Fig 5. HPLC after 40 min of UV light.

Fig 6. Callus induction after exposure to UV light.

DISCUSSION

Ultraviolet (UV) light were used in this research as elicitor on *A. officinalis* callus culture. The results showed that some secondary metabolites of *A. officinalis* had high significant when exposed to UV light for 10 - 40 min. , this results agree with other studies, (13) who stated that UV light can be acts as abiotic stress which triggering plant to perform defense mechanism of this particular stress. Plants responded UV stress to overcome it by producing secondary metabolites also (14) stated that UV light radiation induced gene transcription process of enzymes which involved in secondary metabolites biosynthesis. Stimulation of the synthesis by UV radiation, including secondary metabolites might be explained by the fact that they acts as an absorbent in the UV region of the spectrum and therefore capable of protecting plant cells from the harmful effects of UV (15). Their results showed that the secondary metabolite biosynthetic capacity of *A. officinails* can be enhanced by UV irradiation.

CONCLUSION

The results showed that some secondary metabolites of *A. officinalis* had high significant when exposed to UV light for 30 min. like Scopolotein , Caffeic acid and Syringic acid while Salicylic acid significant increased at 40 min. of UV light, also coumarin significant increased at 10 min. finally P-coumaric acid and Dosmetin significant increased at 20 min. of UV light

Recommendation: Using another elicitors to increase secondary metabolites of *A. officinalis* L.

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