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The effect of water stress and magnetic water in the production of trignolline in callus of Fenugreek (*Trigonella foenum graecum* L.) plant

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Abstract : This study aimed to study the effect of water stress by using of deferent concentrations of PEG (poly ethylene glycol) which include (0,3,6,9 %) and the effect of combination between magnetic water (0%PEG+1000G, 3% PEG +1000G, 6% PEG +1000G, 9% PEG +1000G) to induce the production of Trigonelline in callus of(*Trigonella foenum graecum* L.) .Callus induced in vitro on MS medium by using different concentrations of 2,4-D (0.5, 1, 1.5 mg/l) and different concentrations of BA (0.5, 1, 1.5 mg/l). The results showed that the combination of (1mg/L BA + 1mg/L 2,4-D) was the best combination to produce the highest callus fresh weight compared with other treatments. Methanol was used to extract of Trigonelline from callus under stresses mentioned above. High performance liquid chromatography (HPLC) technique was used to determination quantity and quality of Trigonelline in methanolic extract of callus. Where it was noted by the results of HPLC that the concentration of Trigonelline was increased with increase of PEG concentration and the highest amount of Trigonelline was in (9%PEG). And where it was noted increasing of Trigonelline concentration with increasing concentration of a combination between PEG and magnetic water and the highest amount of Trigonelline was in a combination of (9% PEG +1000G) and it differed significantly compared with the control treatment and other treatments.
Keywords: Callus, *Trigonella foenum graecum* L., Water stress, Magnetic water, Plant tissue culture, Trigonelline, HPLC.

Introduction:

Introduction

Medicinal plants play an important role in the life of human being is an important source of many medicines and pharmaceuticals, and medical significance lies in its ability to produce many of the same pharmaceutical therapeutic properties of chemical compounds[1]. For these plants the ability to assemble these chemical compounds, called secondary metabolism[2]. Tissue culture technique provides access to important economically and medical compounds of difficulty prepared in the laboratory as well as high cost when manufactured[3]. There are many benefits to used tissue culture technique for compounds production in agriculture technology compared with extracted from full plant, where its provides high purity of products than those extracted material from the full plant, fast and production not dependent on the season for planting and does not need large tracts of land as well as the production of high important strains Production and disease-resistant and difficult circumstances[4].

Fenugreek plant (*Trigonella foenum-graecum L*) of the legume family Fabaceae known for having produced alkaloids, phenols and saponins and therapeutic usefulness and effectiveness biologically, and proven

high susceptibility to lower blood sugar and cholesterol and in ability of inhibition cancer cells[5]. Fenugreek seeds used for centuries to extract important medical compounds including: saponins, diosgenin , yamogenin, gums, volatile oils and alkaloids (choline and trigonelline). [6]

Newly-magnetic technology emerged as an effective way to adapt the properties of water for vegetation and human and industrial production, leading to the improvement of these properties[7]. Magnetic technique works on the forms of concern magnetic field through the tube to reach the water, and contribute to treatment, since the magnetic field works to bring about a change in the water, whether physical ones or chemical because of its influence in the hydrogen bonds of the water property, as the magnetic field works in the breakdown of water molecule and make water more liquid.[8]

The magnetic field also plays a role in biological functions of organisms and all seems clear that role through the water processor magnetically effect on plant tissues, with causes an increase in plant growth and increase productivity[9]. The fenugreek plant of the oldest known for its ability to lower blood sugar and cholesterol in the blood and inhibition of cancer cell growth[5]. Based on the foregoing, the medical importance of the plant and it contains an important secondary compounds involved in the pharmaceutical industry, but produced few compared to the need actual of these compounds. This study aimed to inductive plant cells to increase the production of secondary metabolic compounds have been conducted through using media for callus production at different concentrations of the PEG and the PEG with magnetized water. And detecting the quantity and quality of the products by HPLC technique for methanol extract of fenugreek callus.

Materials and methods

Collection of plant and sterilized seeds

Fenugreek seeds *Trigonella foenum graecum* L. collected from the local market in the province of Babylon, and diagnosed in the lush Faculty of Sciences / University of Babylon. Takes a suitable seed amount in a flask and washed with distilled water three times to remove dust and impurities suspended, then transferred to the laminar air flow cabinate since been sterilized by flooding with 15% sodium hypochlorite with stirring for 7 minutes. Then washed with distilled water sterile one minute for three times and sterilized then with 70% ethanol and requested for 30 seconds , then washed with distilled water sterile one minute for three times to remove ethyl alcohol, and then put the seeds in a Petri dish on a sheet of sterile filtration to remove water[10].

Planting seeds

After preparation of MS medium and sterilized seeds took three seeds and planted in agriculture tubes containing 10 ml of MS medium equipped with different concentrations of plant growth regulators 2,4-D concentrations (0.5,1,1.5) mg / L and BA concentrations (0.5,1 , 1.5) mg / L were put three seeds in each glass tube 15 replica for each combination of 2,4-D and BA for the purpose of inducing callus, and then incubated under the lighting conditions (16 h. a day) and a temperature of 24 ± 2 ◦C.

Callus induction phase

Used plant tissue culture technique, according to[11] for the induction of callus from fenugreek seeds, then moved to the newly media for the purpose of stimulating the production of secondary metabolites.

Callus accumulation phase

Cultivation of callus on the PEG media

Taking 250 mg of fresh callus and replaced to cultivation in various PEG concentrations (0,3,6,9%), 15 replica for each of the concentrations of PEG, the culture were under sterile conditions and measuring fresh and dry weight after six weeks of culture.

Cultivation of callus on PEG with magnetized water media

Culture 250 mg of fresh callus and replaced planted on the various PEG concentrations (0%, 3%, 6%, 9%) with magnetized water (1000G) 15 replica for each of the concentrations (PEG + 1000G.) the process of agriculture under sterile conditions and measuring fresh and dry weight after six weeks of culture.

Qualitative and quantitative assessment of compound Trigonelline using HPLC technique

HPLC technique used in the quantification of the compound Trigonelline in fenugreek seeds by (12), and that by injecting 20 micro liters of sample separation column of C18 dimensions type column ((250 cm × 4.6 mm × 5 Mm, and used the stationary phase stationary phase)) the mobile phase mobile phase consisting of ethanol 50% and water,% 50 flow rate = 1ml / min and pH = 5 and a wavelength of 268 nm were separated standard solutions ,to identify the relative size standard model as well as the identification of the detention time, and then was measuring detention time and the relative size of the resulting samples injections for the rest of transactions, compared to packets generated from samples with standard solutions resulting packages under the same conditions mentioned above[12].

Results and discussion

Qualitative and quantitative assessment of Trigonelline by using (HPLC), product under the influence of drought stress caused by the PEG.

We've been depend on the time of detention compound Trigonelline standard solutions in a qualitative assessment of the compound Trigonelline and compare it with the detention of the compounds in the extracts methanolic seeds and callus fenugreek treated with PEG (3%, 6%, 9%) by HPLC. Samples were read at wavelength 250 nm of the compound Trigonelline. The results showed the emergence of a clear summit after 2 minutes and 60 seconds, which represents the time of detention Trigonelline, match the detention of a compound to time in the extracted methanol seeds and callus with a time of detention standard solution of Trigonelline as in the Figures (1), (2), (3), and (4).

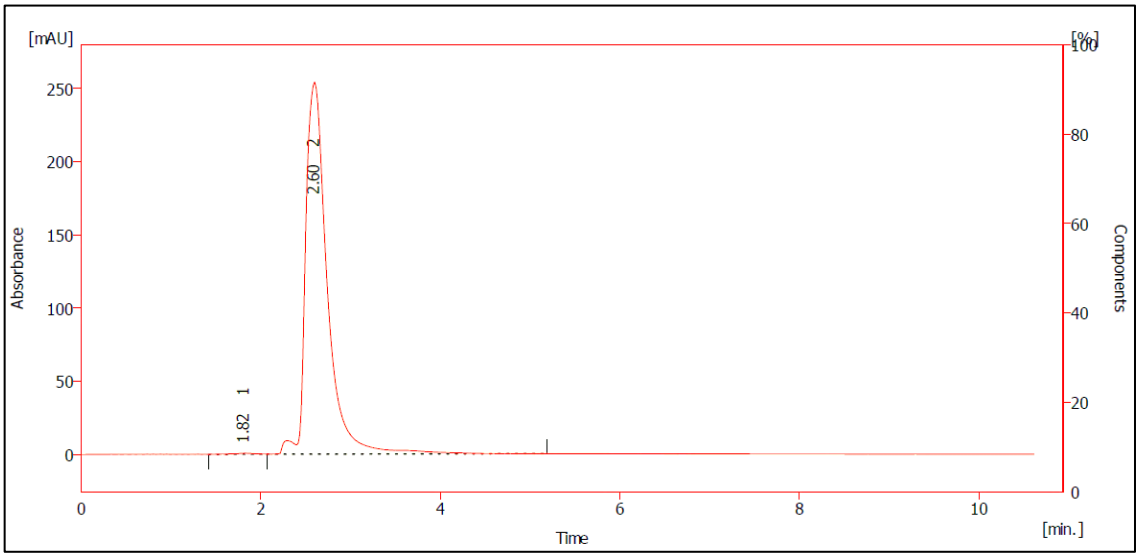

Figure (1):Retention time and standard curve of Trigonelline by using HPLC.

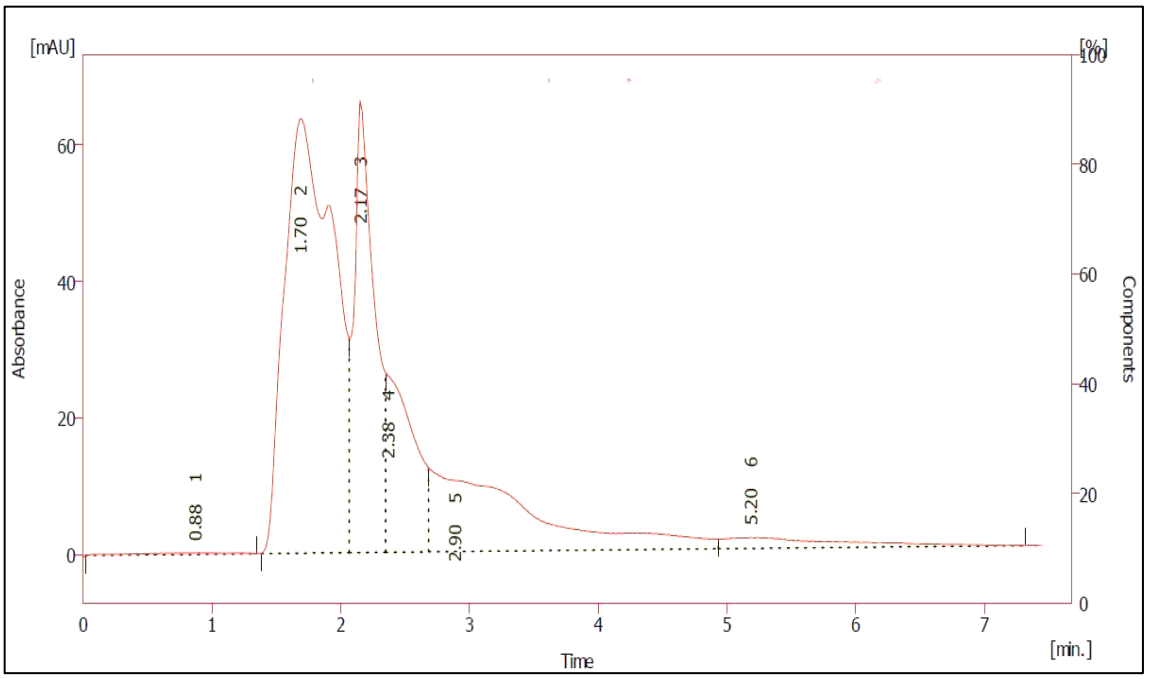

Figure (2):Retention time and curve of control treatment (PEG 0%) (untreated callus) of fenugreek by using HPLC.

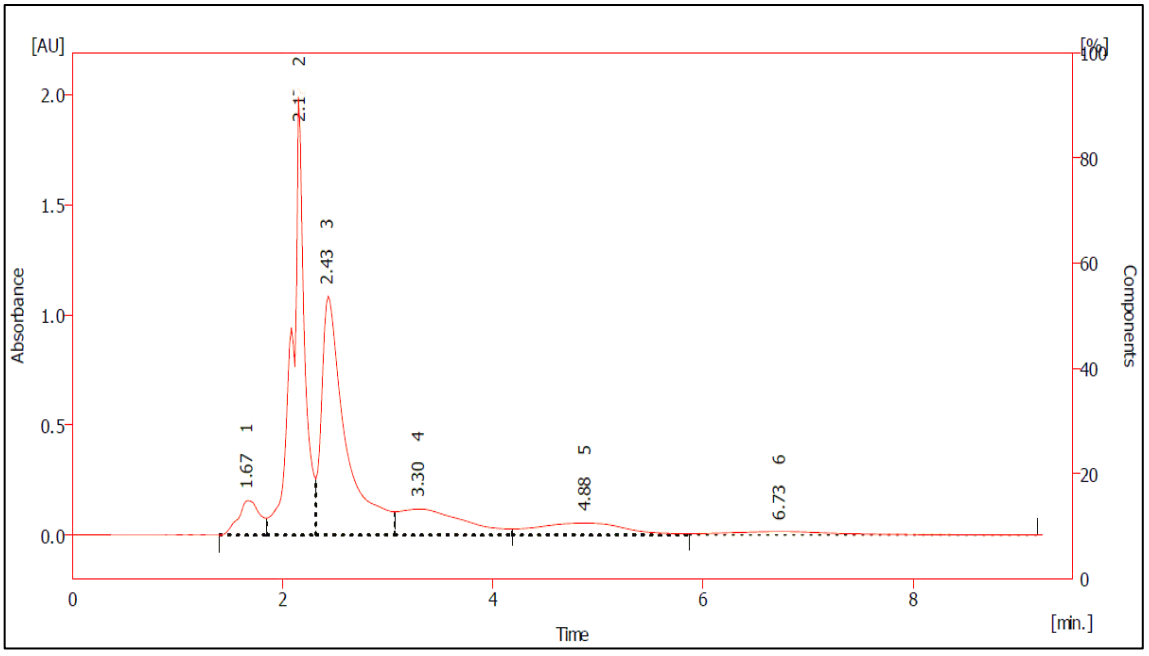

Figure (3) :Retention time and curve of fenugreek callus extract treated with PEG 3% HPLC.

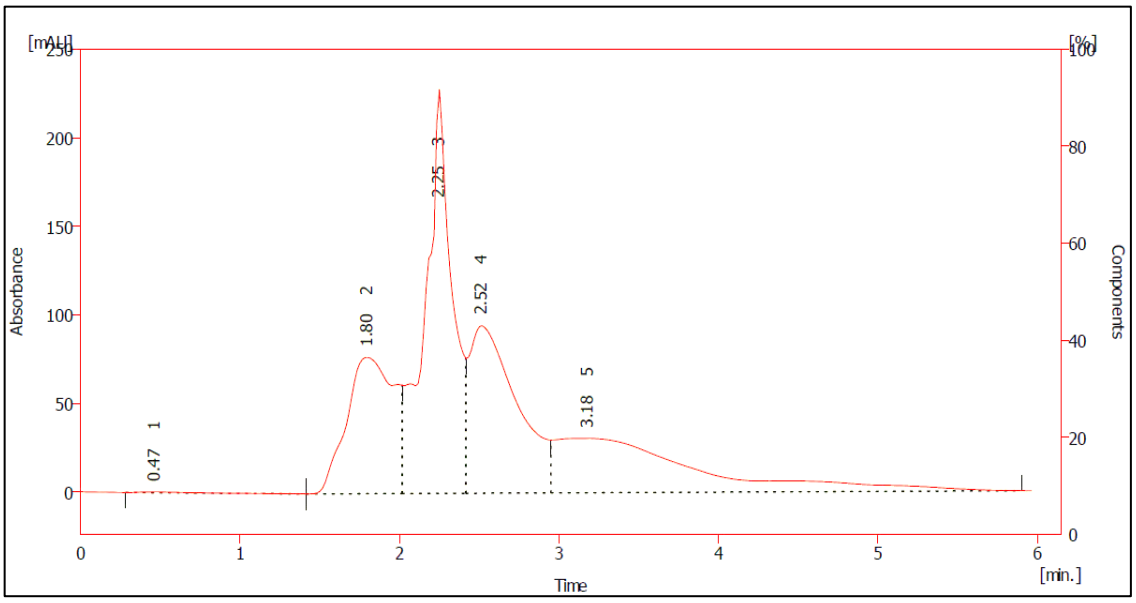

Figure (4): Retention time and curve of fenugreek callus extract treated with PEG 6% by using HPLC.

The results showed in the table (1) the impact of drought stress was significant increase in Trigonelline compound content in callus treated with PEG and in all treatments. As the highest value of the compound Trigonelline reached 469 ppm dry weight in 9% PEG and showed significant differences compared with the control 74 ppm dry weight and with all the treatments of the PEG, followed by 6% PEG ppm 274 dry weight. It was less content Trigonelline among drought stress treatments were in 3% PEG which gave 176 ppm dry weight.

The high content of Trigonelline take the same trend increasing concentrations of PEG in the media to the plants in stress conditions produce large amounts of secondary metabolic compounds that prevent oxidation processes in the plant cells [13-15] . The production of Trigonelline in callus more than produced from the same plant, may be due to the availability of mineral nutrients and vitamins in the media and which are appropriate to produce a larger amount of Trigonelline[16 , 17] refer to increase Trigonelline content in soybean plant in water shortage conditions. Also[18] mention the increase of alkaloids in the *Citronella Java* and *Palmarosa* plant in drought stress conditions. Also[19] mentioned increased Trigonelline content in *Arachis hypogaea* L.) as a case of defense of the plant in response to the shortage of water in the stress conditions.

Table (1): qualitative and quantitative assessment of Trigonelline in fenugreek callus product under the influence of drought stress caused by PEG by using HPLC.

PEG %	Trigonelline content (ppm)
0%	74
3%	176
6%	274
9%	469
L.S.D. (0.05)	11.26

Depending on the time of detention Trigonelline standard solutions in a qualitative assessment of the Trigonelline and compared with the time of the detention of the compounds in the methanolic seeds extracts and fenugreek callus treated with PEG (3%, 6%, 9%) and water magnetized 1000 G. By the HPLC, was samples are read on a wavelength of 250 nm of Trigonelline, results showed the emergence of top peek after 2 minutes and 60 seconds, which represents detention Trigonelline time, match the detention of a compound to time in the

methanol extracted of seeds and fenugreek callus with detention standard solution of Trigonelline time as in the figure (5) , (6) ,(7) ,(8) and (9).

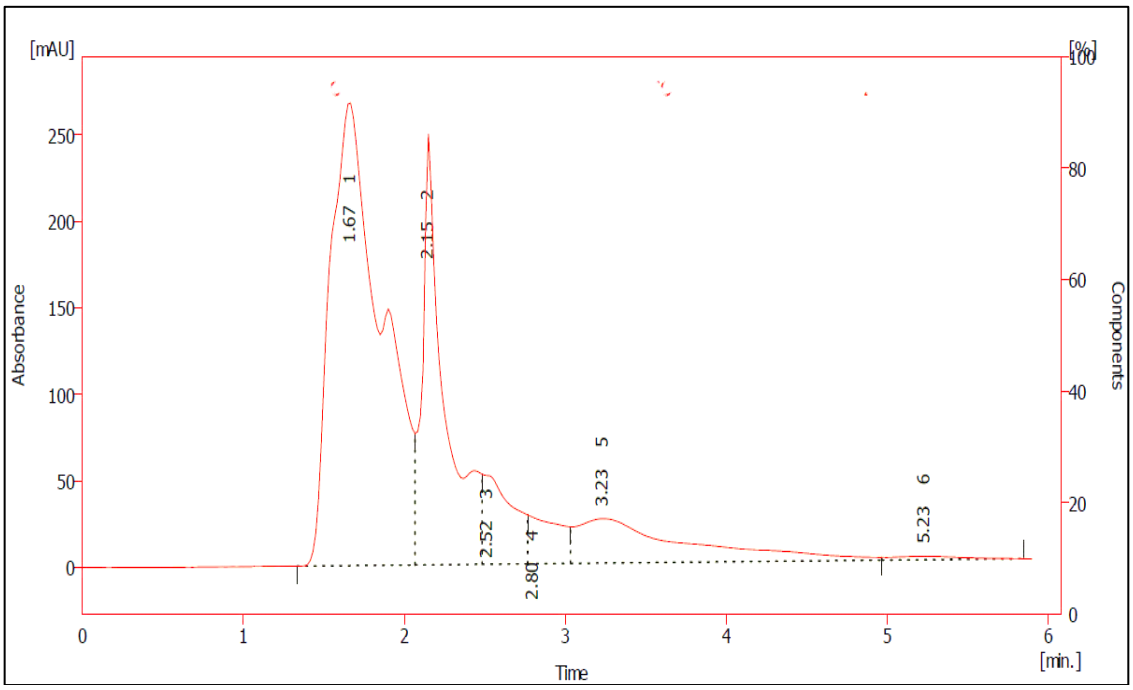

Figure (5): Retention time and curve of fenugreek callus treated with magnetic water (1000 G) by using HPLC.

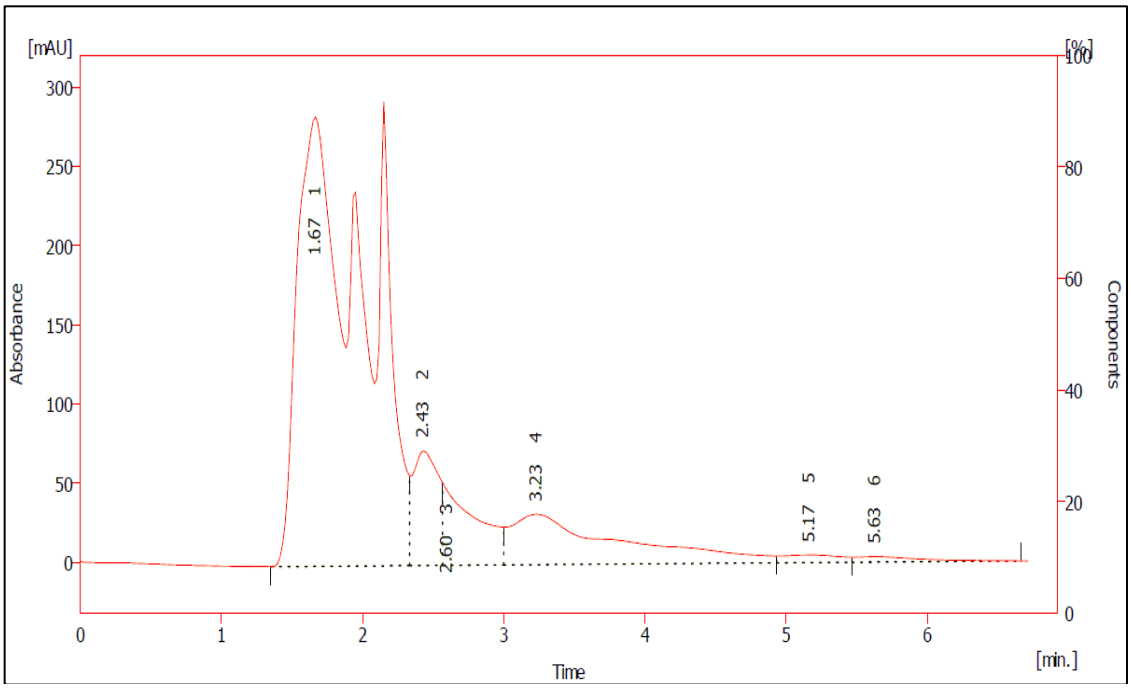

Figure (6): Retention time and curve of fenugreek callus extract treated with (1000G + PEG 3%) by using HPLC.

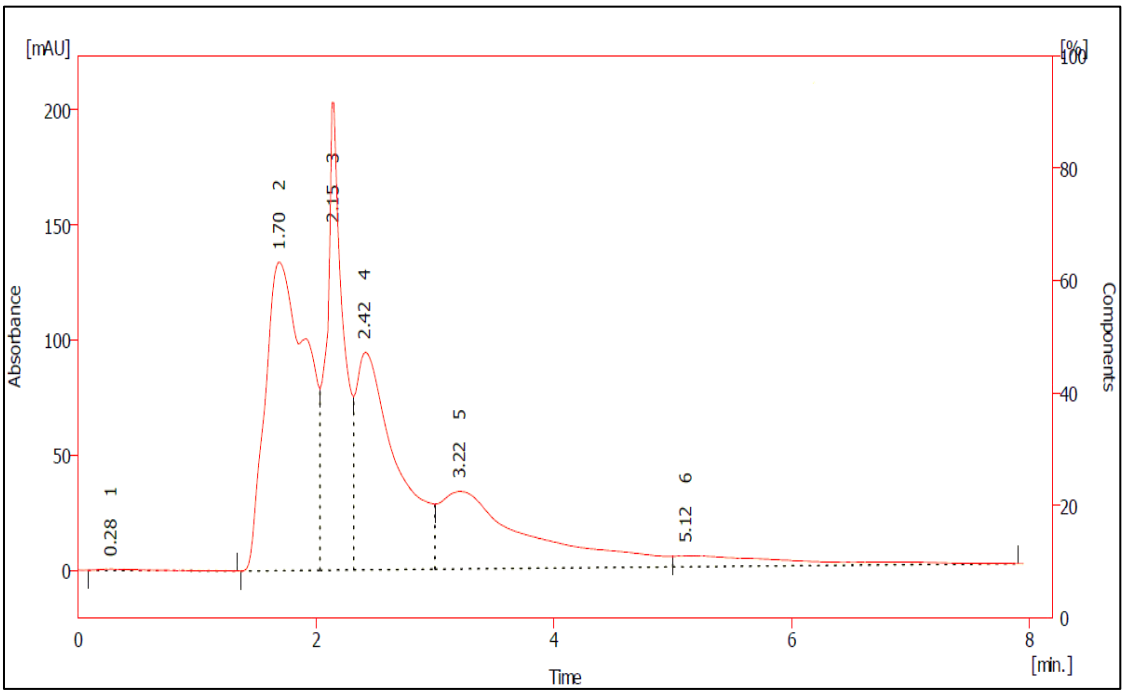

Figure (7): Retention time and curve of fenugreek callus extract treated with (1000 G+PEG 6%) by using HPLC.

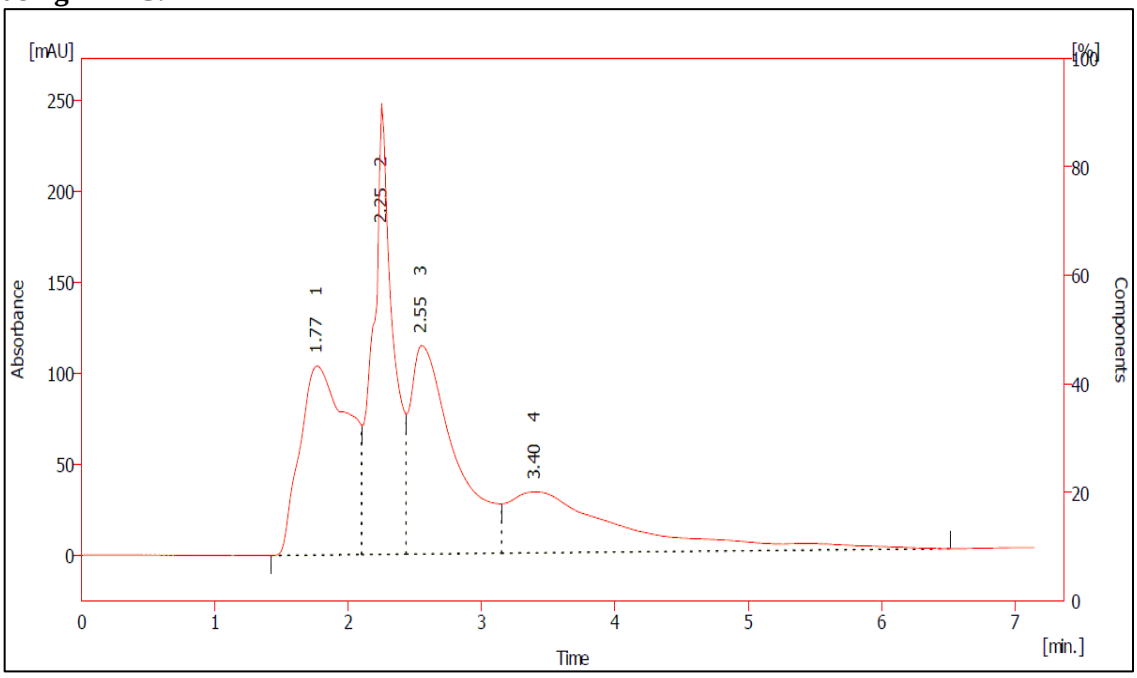

Figure (8): Retention time and curve of fenugreek callus extract treated with (1000 G+PEG 9%) by using HPLC.

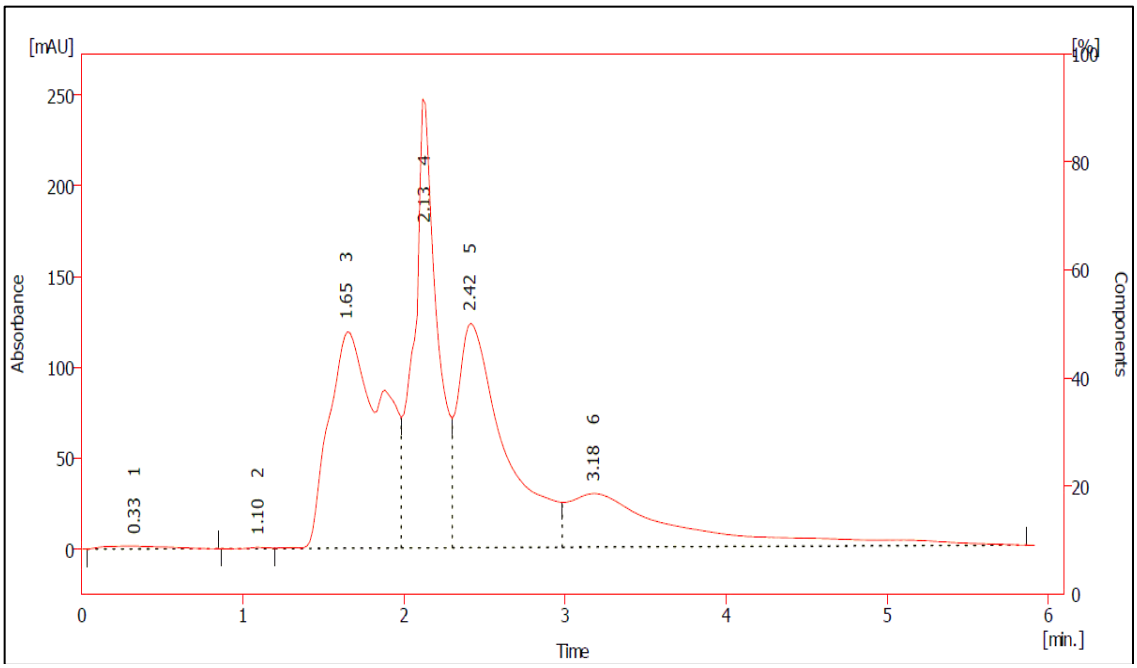

Figure (9): Retention time and curve of fenugreek seeds extract by using HPLC.

Results shown in the table (2) to drought stress and water magnetized significant effect in increasing Trigonelline content in callus treated with PEG and magnetized water and in all treatments. As the highest value of the Trigonelline valued at 282 ppm dry weight when treatment PEG% 9 + 1000G and showed significant differences compared with the control treatment PEG% 0 + 1000G, which amounted to 70 ppm dry weight with all other treatments, and there was a significant decrease in Trigonelline content in PEG% 3 + 1000G. , Which stood at 92 ppm dry weight compared with the control treatment, and others.

Table(2): Qualitative and quantitative assessment of Trigonelline in fenugreek callus by using HPLC product under the influence of drought stress magnetic water.

PEG %+ Magnetic water	Trigonelline content (ppm)
1000 G.	70
3% +1000G.	92
6%+1000G.	237
9%+1000G.	282
L.S.D. (0.05)	10.48

[20]mentioned the exposed Alfafa plant (*Medicago sativa*) to stress conditions lead to increased two-fold concentration in Trigonelline. And its production and accumulation in the soybean plant response to drought stress and salinity [21]. When exposed plants to severe stress conditions (such as drought, salinity and magnetic field) begins the accumulation of materials such as osmoregulaters like Trigonelline prevent water loss [22]. Also [23] mentioned that stress increases the various secondary metabolites in plants.[24] fixed the increase the concentration of alkaloids in the stress conditions in the two types of lemongrass.[25.26]

References

1- Taylor, V. E.; Brady, L. R.; and Robbers, J. E. (1988). Pharmacognosy. Economic Bot., 50(1): 3_9.
2- Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry. 64(1):3-19.
3- Purohit, S. S. (1999). Agriculture Biotechnology. Agro Botanical. J. N. V. Yas Naggrr, Bikaner, India, P: 833.
4- Discosmo, F. and Misawa, M. (1995). Plant cell and tissue culture: alternatives for metabolite production. Biotechnology Advances, 13(3):425-453.

5- Suboh, S. M.; Bilto, Y.Y. and Aburjiai, T. A.(2004). Protective effects of selected medicinal plants against protein degradation, lipid peroxidation and deformability of oxidatively stressed human erthocytes. Phytothapy, Res., 18(4):280-284.
6- Aasim, M.; Hussain, N.; Muhammed, U. E.; Zubair, M.; Bilal, S. H.; Saeed, S.; Rafique, T. S. and Sancak, C. (2010). In vitro Shoot using different cytokinins. Afric. J. Biotech., 9(42):7174-7179.
7- Fahd, A,; Koutaiba M, H, ;Adnan F, H, and Tariq L,R,. (2005). Adaptation to magnetic properties of salt water for crop irrigation. 2- Maize and wheat. Iraqi Journal of Agricultural Sciences. 34-29: (1) 36.
8- Al Jawdhira, Hayawi Attia .. (2006). The effect of the magnetic adjustment of irrigation water and fertilizer potassium in some of the chemical characteristics of the soil and the growth of maize holds. M.Sc. Baghdad univesity, Faculty of Agriculture, Department of Soil Science and Water Resources.
9- Ahmet, E.,(2003). Effects of magnetic fields on yield and growth in strawberry, Camarosa. J. Hort. Sci. Biotec., 78, 145–147.
10- Awika, J. M.; and Rooney, L. W. (2004). Sorghum Phytochemical and their potential aspects on human health.Photochemistry., 65(9):1199-1221.
11- Hirata, K.; Yamanaka, A.; Karano, N.; Miyamoto, K. and Miura, Y. (1990). Production of indol alkaloids in multiple shoot culture of Catharanthus roseus. Agric. Biol. Chem. 51:1311-1317.
12- Illiana, I.; Witte, L.; Alfermann, A.W. (1989). Production of alkaloid by transformed root cultures of Datura innoxia. Planta Medica., 55: 229-230.
13- Omidbaigi, R.; Hadjiakhoondi, A. and Saharkhiz, M.(2003). Changes in content and chemical composition of PimpinellaanisumL. oil at various harvest time. J. Esse. Oil Bearing Plants., 6 (1): 46-50.
14- Orav, A.; Raal, A. and Arak, E. (2008). Essential oil composition of Pimpinella anisum L. fruits from various European countries. Nat. Prod. Res., 22(3): 227-232.
15- Acimovic, M. G.; Korac, J.; Jacimovic, G.; Oljaca, S.; Djukanovic, L. and Vuga-Janjatov, V. (2014). Influence of ecological conditions on seeds traits and essential oil contents in anise (Pimpinella anisum L.). Not. Bot. Horti. Agrobo, 42(1):232-238.
16- Radwan, S. S.; Kokate, C. K. (1980). Production of higher levels of trigonelline by cell cultures of Trigonella foenum-graecum than by the differentiated plant.Planta., 147 (4) 340-344.
17- Cho,Y.; Njiti,V.N.; Chen, X.; Lightfoot, D.A.and Wood, A.J. (2003). Trigonelline concentration in field-grown soybean in response to irrigation. Biologia Plantarum.,46(3) :405-410.
18- Fatima, S.; Farooqi, A.H.A. and Sangwan, R.S. (2006). Water stress mediated modulation in essential oil, proline and polypeptide profile in palmarosa and citronella java. Physiology and Molecular Biology of Plants. Central Institue of Medic.,11: 256-261.
19- Cho, Y.; Kodjoe, E.; Puppala, N. and Wood, A.J. (2011).Reduced trigonelline accumulation due to rhizobial activity improves grain yield in peanut (Arachis hypogaea L.). Acta Agriculturae Scandinavica, Section B - Soil & Plant Sci. 61(5):395-403.
20- Tramontano, W. A. and Jouve, D.(1997).Trigonelline accumulation in salt-stressed legumes and the role of other osmoregulators as cell cycle control agents.Phytochemistry., 44 (6) 1037–1040.
21- Wood, A. J.; Cho, Y.; Chen, X. B.(2000). Trigonelline (Nicotinic Acid Betaine) biosynthesis in plants: NaCl- and drought-stress induced accumulation in cultivated soybean (Glycine max). J.Rec. Res. Develop. Phytochem., 4: 89-98.
22- Cho, H.Y.; Rhee, H.S.; Yoon, S.Y. and Park, J.M (2008). Differential induction of protein expression and benzophenanthridine alkaloid accumulation in Eschscholtzia californica suspension cultures by methyl jasmonate and yeast extract. J. Microbiol. Biotechnol.,18(2): 255-62.
23- Akula, R. and Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants . Plant Signaling and Behavior, 6 (11) : 1720-1730.
24- Singh-Sangwan, N.; Farooqi, A. H. A. and Singh-Sangwan, R. (2006). Effect of drought stress on growth and essential oil metabolism in lemon grasses. New Phytol., 128(1): 173 – 179.
25- Salman JM, Abdul-Adel E, Alkaim AF. Effect of pesticide glyphosate on some biochemical features in cyanophyta algae oscillatoria limnetica. International Journal of PharmTech Research. 2016; 9(8): 355-365.
26- Omran AR, Baiee MA, Juda SA, Salman JM, AlKaim AF. Removal of congo red dye from aqueous solution using a new adsorbent surface developed from aquatic plant (Phragmites australis). International Journal of ChemTech Research. 2016; 9(4): 334-342.

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