Research Article

Effect of some Chemical and Physical Elicitors on some Secondary Compound Induction of Ricinus Communis Through Callus Induction

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Abstract

The results of the present study showed that $AgNO_3$ and ABA has a negative effect on fresh and dry weight, where both of weight significantly decreased when treated with ABA and $AgNO_3$ compare to control treatment, except callus treated with $AgNO_3$ at 4mg/l, the rate of fresh and dry weight significantly increased reached to 434,77mg respectively compared to control. Physical treatment also had a significant effect on fresh and dry weight for callus, the highest fresh and dry weight significantly reached to 491,93 mg respectively when callus exposure for 10 minutes to UV ray. The exposure to light for different period had negative effect on the rates of fresh and dry weight, as the control superior significantly for all lighting treatment. The concentration of all the secondary compounds extracted from callus increased significantly compared to their concentration in the seed extract. The addition of chemical treatments to callus lead to the difference in the concentration of secondary compounds. The quercetin compound reached its highest and significantly to 95.17µg/ml at the concentration of 4mg/l of $AgNO_3$, while kaempferol suffer significantly decrease at all concentration of $AgNO_3$, and reached to highest value significantly at control 114.76µg/ml, added concentration at 6 mg/l of $AgNO_3$ had a positive effect in increasing the concentration of Ricinin significantly to 119.90µg/ml.

Keywords: chemical and physical elicitors, Ricinus communis, callus, $AgNO_3$.

الخلاصة

بينت نتائج الدراسة الحالية ان لتراكيز المعاملات الكيمياوية ABA و $AgNo_3$ تأثير سلبي في معدل الوزن الطري والجاف, حيث انخفض معدل الوزنين وبشكل معنوي عند المعاملة ب ABA, $AgNO_3$ مقارنة بمعاملة السيطرة, ماعدا معاملة الكالس بال $AgNO_3$ بتركيز 4 ملغم /لتر الذي ارتفع فيه معدل الوزن الطري والجاف وبشكل معنوي اذ وصل 434, 77مايكروغرام/مل على التوالي مقارنة بمعاملة السيطرة . كذلك كان للمعاملات الفيزيائية تأثير معنوي في الوزن الطري والجاف للكالس ووصل اعلى معدل للوزنين وبشكل معنوي عند تعرض الكالس لمدة 10 دقائق عند التعرض لأشعة UVاذ بلغ 491 ,93 ملغم للوزن الطري والجاف على التوالي , وكان لمعاملة التعرض لفترات ضوئية مختلفة تأثير سلبي في معدلات الوزن الطري والجاف اذ تفوقت معاملة السيطرة معنويا على جميع معاملات الاضاءة. ارتفعت تراكيز جميع المركبات الثانوية المستخلصة من الكالس وبشكل معنوي مقارنة بتراكيزها في مستخلص البذور. وادت اضافة المعاملات الكيمياوية للكالس الى اختلاف في تراكيز المركبات الثانوية, حيث وصل مركب qurecetin الى اعلى معدلاته و بشكل معنوي حيث بلغ 95.17 مايكروغرام /مل عند تركيز 4 ملغم/لتر من $AgNO_3$ في حين عانى مركب Kaempferol انخفاض معنوي في جميع تراكيز $AgNo_3$,ووصل الى اعلى معدلاته و بشكل معنوي عند معاملة السيطرة اذ بلغ 114.76 مايكروغرام /مل ,وكان لتركيز 6 ملغم/لتر $AgNo_3$تأثير ايجابي في زيادة تركيز مركب Ricinin و بشكل معنوي اذ بلغ 119.90 مايكروغرام /مل .

Introduction

Ricinus communis is a flowering plant species and belongs to the Euphorbiaceae family, which contains large numbers of plants [1], Castor evergreen plant and small trees [2] native to north-eastern Africa, now cultivated in several regions Tropics and Subtropics and related areas High temperature [3]. Castor is a non-edible, drought-resistant crop of oilseeds that grows for several years [4]. Castor seeds contain a high percentage of oils ranging from 40-60% which are rich in Triglycerides and mainly Ricinolein. Castor seeds are the

commercial source of Ricinolic acid that used in industrial Lubricants, Dyes and plastics [5]. Castor oil at a high proportion causes Purgative action. Castor oil also contains Ricinine, Toxic Alkaloid, and toxic albumin that named ricin [6]. Effectiveness of the castor plant extract against bacterial infection is due to the presence of Quercetin, Kaempferol, Ricinine [7], Ricinine is present in all parts of the castor plant and is considered a powerful insecticide [8].

Secondary metabolites in plants are an important source of pharmaceuticals, flavors and a number of important biochemical compounds. These compounds are collected when the plant is placed under a stress or using some biotic and abiotic stimulants. The use of stimulants with tissue culture is an important tool for improving phytochemical production. The production of secondary metabolic compounds by plant tissue culture will show the importance of producing some effective compounds and then isolate them and determine their effective compounds, which may not be produce in adult plants [9].

The cultivation of cells and plant tissues is an important tool in plant technology that leads to the increase of Biomass or the production of biological materials by the use of several techniques, including callus culture or through morphological changes. Among the techniques is the use of elicitation with tissue culture technology, to increase the production of some plant compounds [10]. Callus induced from different plant species using different plant parts, media and different growth regulators. The effective metabolic compounds extracted from callus are of high purity [11] [12] Some of the active compounds extracted *in vitro* determined from the castor plant, which used as antibacterial, antifungal [13], cytotoxicity, antiviral [14], have been identified and these compounds did not match their results when extracted from adult plants.

The exposure of cell suspense callus of Asparagus racemosus to UV rays and salicylic acid concentration of 100, 200µm led to the accumulation of shatavarin and the effect of UV radiation was more effective than the accumulation of this active substance compared to salicylic acid [15]. Notice [16] reduced callus growth with increased UV exposure time and was reflected in Fresh Wight, where it decreased by 5 minutes. The effectiveness of ultraviolet radiation is not limited to increasing the production of secondary compounds, but may also produce new compounds.

[17] Observed two new compounds of phenols had separated from rice callus irradiated by UV, Isoorientin2, Isoorientin, and Brassica oleracea treated with UV rays contained three new alkaloids: Caulilexin A-C [18].

There were also results showing the positive effect of light on the growth of callus [19] and increased Vindoline compound and other alkaloid indol compounds from callus Catharanthus roseus [20]. Light found to have an important effect in increasing fresh weight in callus of Brassica napus [21]. He stated [22] that $AgNO_3$ stimulated the production of two Tropan alkaloid compounds, hyoscyamine and scopolamine in the culture of Brugmasia candida. Use [23] different concentrations of $AgNO_3$ to stimulate the compound capsidol from several varieties of pepper which proved effective in induction reached [24] that ABA acid has been shown to reduce the formation of Rishitin, Lubimin, which is the return of cescoterpenoids and produced in Solanum tuberosum. Showed [25] that all concentration of $CdCl_2$ has significantly reduced fresh and dry weight of callus induced from Nerium oleander, and the concentration of $CdCl_2$ at 2.5mg / l has significantly increased the concentration of oleandrin, Neritaloside compound from callus. The concentration of $CdCl_2$ significantly decreased in fresh and dry weight from callus cordia myxa, while $CdCl_2$ increased significantly in the secondary metabolites Hesperdin, Rubinin [26].

Materials and Methodology

Callus initiation: - Stem explanted of Ricinus communis, cultured in MS media [27]. supplemented with 3mg/l IAA and BA at 0.5mg/l concentration, incubate the cultures in incubator conditions, 16/8hrs.light/dark at temperature 25+1 ^{0}C for 28 days for obtaining callus, used the same combination for

maintenance media for callus induced and repeated the subculture every three weeks, to get the required amount of callus for subsequent experiments.

Elicitation of secondary compounds:

Chemicals treatment:

After obtaining enough amount of callus, weighting 350 mg of callus and cultured in maintenance media plus the concentration of ABA acid at (0, 1, 1.5, 2 mg/l) and second group treated with $AgNO_3$ at (0, 2, 4, 6 mg/l). Fresh and dry weight were measured after 4 weeks from culture, at a rate of 5 replicates per concentration, fresh weight measured by sensitive balance and then placed in an electric oven at 70 ^{0}C for 48 hrs., until the weight was established, to recorded dry weight. All cultures incubated under incubator conditions.

Physical factors:

Distributed weights of 350 mg from callus, then cultured on maintenance media, and divided into three groups. Each group incubated at incubator conditions with different exposure of light period , it was 24 hour in dark, 24 hour in light and control which includes incubator conditions of 16/8 light/dark with 5 replicates per treatment . transfer the same weight of callus to culture in maintenance media, then exposure to UV light at different time (0, 5, 10 minute) with 5 replicates per treatment ,and incubated the cultures under incubator condition, and measured fresh and dry weight after 4 weeks.

Quantitative and qualitative estimation of secondary products using HPLC technique

High-performance liquid chromatography (HPLC) caused the Spectrophysis / UV visible detector to estimate the quantity and quality of secondary products in the seed extract and callus, due to its ability to separate the chemical components of the Seed and Callus Cells extract and compare them with standard samples. The readings measured on the wavelengths and according to the (RT) rotation time of the standard solutions and samples under study. The concentration of secondary products estimated by equation [28]:

$$Sample\ concentration\ \left(\frac{mg}{ml}\right) = \left(\frac{The\ sample\ area}{Standard\ sample\ area}\right) \cdot (Standard\ samlpe\ concentration$$

The experiments designed at Randomized Completely Block Design (RCBD) to study the effect of different treatment in the studied traits. Mean differences between the averages compared with Duncan test at 5% probability [29].

Results and Discussion

Effect of chemical treatments on fresh and dry weight of callus:

Effect of different concentration of ABA in fresh and dry weight of callus

Increased ABA concentration significantly decreased in fresh and dry weight of callus (Table 1), lower weight and significant reached to 264, 20 mg for both fresh and dry weight at 2mg/l respectively, while control significantly exceeded to 393, 65mg for fresh and dry weight respectively. The addition of ABA in media may increase the total content of phenolic compounds that inhibit the growth of callus and thus reduce its biomass [30], or may have an effect on the growth and vitality of cells by activating the respiratory process and ATP formation leading to reduced cell size or death as well as in tobacco cultures [31].

Table 1: Effect of different concentration of ABA mg/l added to maintenance media in the fresh and dry weight of callus (mg) induced from stem.

Concentration of ABA mg/ l	Fresh weight mg	Dry weight mg
0	393 a	65 a
1	357 b	51 b
1.5	298 c	28 c
2	264 d	20 c

Effect of different concentration of $AgNO_3$ on fresh and dry weight of callus.

From the results of (Table 2) the addition of a different concentration of $AgNo_3$ significantly decreased in fresh weight for callus, except the concentration 4mg/l which lead to increasing the weight significantly 434 mg compared to

control. The lowest rate for fresh weight 276mg at 2 mg/l $AgNO_3$. The dry weight behavior the same as fresh weight, as increasing concentration of $AgNo_3$ lead to the low rate of dry weight and significantly, compared to Control treatment. Except the concentration of 4mg/l which surpassed and significantly on all treatment as it reached 77mg. While the lower rate was significantly 23mg at 2mg/l concentration $AgNO_3$, the addition of heavy metals to culture media may decrease callus weight, due to the toxic effects of these compounds on plant cells [25] [32]. The addition of $HgCl_2$ in culture media for callus Catharan thus roseus led to decrease fresh and dry weight for callus in most of the concentration used [33].

Table 2: Effect of different concentration of $AgNO_3$ mg/l added to maintenance media in fresh and dry weight of callus (mg) induced from stem.

Concentration of $AgNo_3$ mg / l	Fresh weight mg	Dry weight mg
0	393 b	65 b
2	276 d	23 d
4	434 a	77 a
6	302 c	40 c

Effect of physical treatments on the fresh and dry weight of callus:

Effect of light hours on the fresh and dry weight of callus.

The results of (Table 3) indicate that the highest fresh and dry weight of callus and significantly reached at control treatment (incubator conditions of 16 hours light and 8 hours of darkness) at 393,65mg for fresh and dry weight respectively. While the incubation at darkness continuous or continuous light conditions led to a significant decrease in the weight rate and significantly compared to control, although the light treatment significantly surpassed to dark treatment, the lower rate of fresh and dry weight reached significantly to 170, 20, mg respectively at dark treatment. He stated [34] that the balance between Auxin and cytokinin is necessary in the division, and evolution of the plant part, as it affects the metabolism of the plant, through industrialization of proteins. Thus affect the efficiency of enzymes and increase cell division and the growth of callus. Confirmed, that light has a significant effect in increasing cell activity and its division and growth of callus [35].

Table 3: Effect of different times exposure for light (hour) in fresh and dry weight of callus (mg)

Lighting duration (hours)	Fresh weight mg	Dry weight mg
Control	393 a	65 a
24 light	219 b	44 b
24 dark	170 c	20 c

Effect of different period exposure to ultraviolet ray UV on the fresh and dry weight of callus

The exposure to UV radiation has a significant effect in fresh and dry weight of callus, as UV exposure have significantly increased weight compared to control. The highest weight significantly was 491, 93 mg for fresh and dry weight respectively at 10 min Table 4, while the fresh and dry weight rate was significantly lower in control were 393, 65 mg respectively Table 4.

Table 4: Effect of different time exposure for ultraviolet radiation (min) in fresh and dry weight of callus (mg).

Exposure Time (min)	Fresh weight mg	Dry weight mg
0	393 c	65 c
5	437 b	78 b
10	491 a	93 a

Quantitative and qualitative estimation of some secondary compounds in extract of seed and callus R.communis using HPLC technique

Figure 1 shows the curve of standard secondary compounds, and their comparison with the curves of secondary compounds separated from seed and callus of castor plant Figure 2, which showed the presence of 3 secondary compounds. When comparing the concentration of compounds extracted from seeds with those extracted from callus, there are differences significantly. The concentration of compounds derived from callus all increased (5) the concentration of ricinin, kaempferol significantly increased reached to 105.78 and 114.76 µg / ml respectively. The compound

significantly increased reached to 105.78 and 114.76µg/ml respectively. The compound Querecetin concentration had increased in the callus extract but did not differ significantly from its concentration in the seed extract. The results of this study were agree with the results of [25] which showed an increase in the concentration of secondary metabolism of Nerium oleander from callus extract compared to leaves extract. The difference in the concentration of compounds may be attributed to different factors, plant type, light, plant growth and humidity.

These physiological and environmental differences are due to the growth conditions of the plant parts, as well as the genetic factors that have been affected by the physiological factors mentioned [36].

The reason for the increase in active compounds could explain in Callus extract for the presence of growth regulators added to callus induction, and callus maintenance, which may have stimulated and increased the production of some secondary compounds in callus, and the continuous subculture process may have led to the appearance of Somaclonal variation in the cells resulting in increased production of secondary compounds in callus [35].

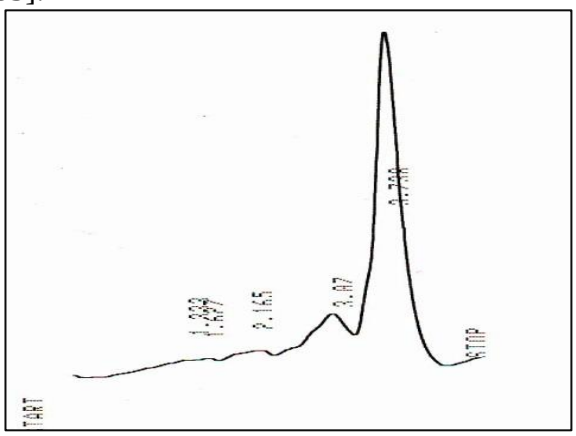
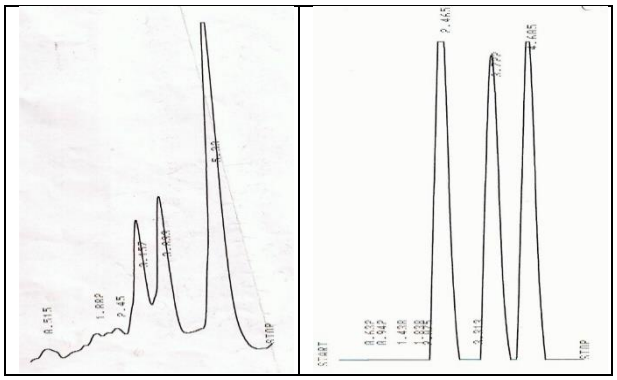


Figure 1: standard curve of secondary compounds of Ricinus communis using HPLC technique.



A B

Figure 2: .HPLC analysis of secondary compounds. (A) Seed extract of Ricinus communis (B) extract of callus induced from stem.

Table 5: Concentration of secondary compounds µg/ml in seed and callus extract using HPLC technique.

secondary compounds µg/ml	Seed	Callus
Qurecetin	67.49 a	71.41 a
Kaempferol	76.71 b	114.76 a
Ricinin	30.81 b	105.78 a

Effect of chemical treatment on the production of some secondary compounds: Effect of various concentrations ABA in the production of secondary compounds from callus of Ricinus communis.

The concentration of ABA had a significant effect on the production of secondary compounds from callus of castor plant; the concentration of ABA had a significant Effect on the production of secondary compounds from castor plant, Figure 3 showed the differences in curves of secondary compound of treated with ABA.

The results of Table 6 indicate that the concentration of kaempferol compound had decreased significantly at all ABA concentration compared to control treatment, and its lowest concentration significantly reached to 14.05µg/ml at 1mg/l from ABA. The concentration of Ricinin significantly increased to a maximum 194.34µg/ml at 2 mg/l concentration compared with the other concentration of ABA and control.

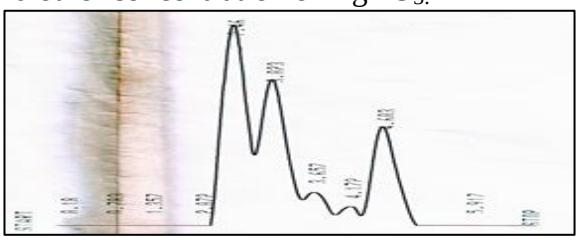
Quercetin concentration increased at a concentration of 1mg/l of ABA to78.64µg/ml, but this increase was not significant compared

with Control, but it was significantly to the rest of the other concentration of ABA.

The increased concentration of some compounds may be due to increased ABA concentration, because high concentration may cause increased stress and thus stimulate the production of some secondary metabolites [37], ABA stimulates biosynthesis and increases the phenolic content of the Vitisrotum difolia [38]. The plant that exposed to water stress stimulates the accumulation of ABA, which leads to an increase in H_2O_2, which leads to the activation of the enzyme oxidation and production of secondary metabolites as in zea mays [39].

Effect of various concentration of $AgNO_3$ in the production of secondary compounds on callus Ricinus communis.

Figure 4 illustrates the curve of secondary compounds from callus cultured in media treated with a different concentration of $AgNO_3$. Differences concentration of secondary compounds was obtained depending on the $AgNO_3$ concentration added to the maintenance media Table 7. The results of the table indicate a high concentration of some compounds, when adding $AgNO_3$ compared to control. The highest concentration of qurecetin significantly reached to 95.17µg/ml at 4mg/l concentration of $AgNo_3$ compared with control and other concentration of $AgNO_3$.



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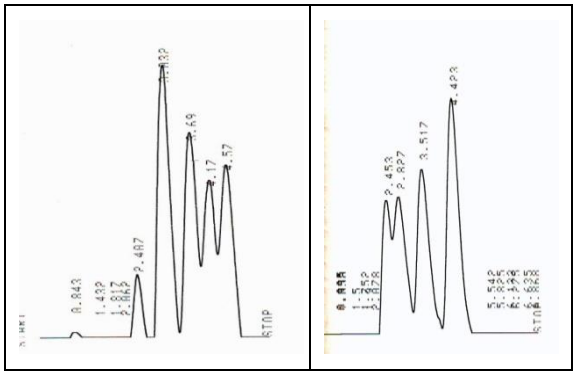
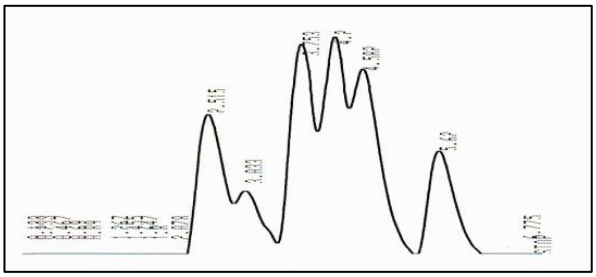


B C

Figure 3: HPLC analysis of callus treated with various concentration of ABA. (A) 1mg/l (B) 1.5mg/ l (C) 2mg/l.

Table 6: Effect of different concentration of ABA mg/l in the production of secondary compounds µg/ml on callus of Ricinus communis

secondary compound µg/ml	concentration of ABAmg/l			
	0	1	1.5	2
Qurecetin	71.41 a	78.64 a	16.81 c	34.57 b
Kaempferol	114.76 a	14.05 d	42.10 c	65.54 b
Ricinin	105.78 b	36.65 c	43.89 c	194.34 a



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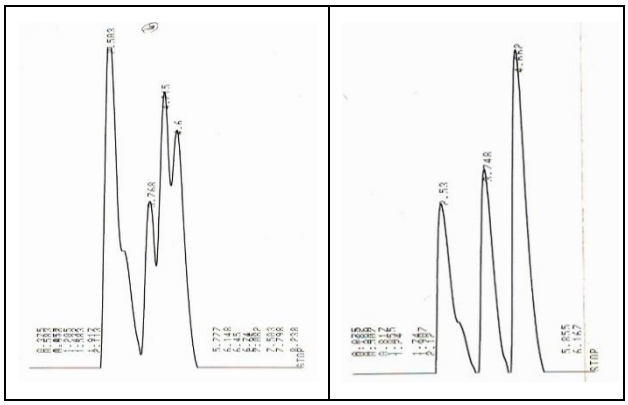


B C

Figure 4: HPLC analysis of callus treated with various concentration of $AgNo_3$ (A) 2mg/l (B) 4mg/l (C) 6mg/l.

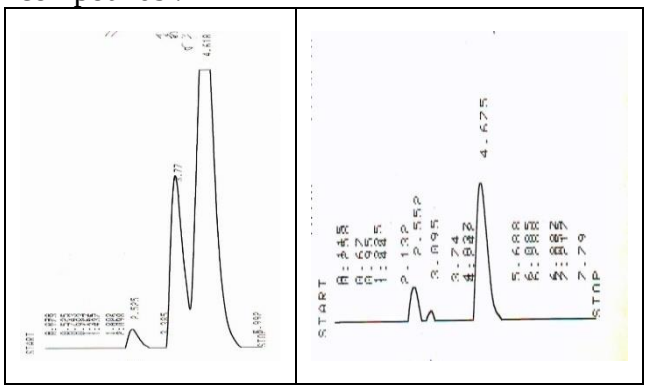
Table 7: Effect of different concentration of $AgNo_3$ (mg/l) in the production of secondary compounds on callus of Ricinus communis.

secondary compound µg/ml	concentration of $AgNO_3$(mg/l)			
	0	2	4	6
Qurecetin	71.41 b	32.67 c	95.17 a	70.34 b
Kaempferol	114.76 a	48.55 c	36.58 d	72.97 b
Ricinin	105.78 b	52.73 c	64.80 c	119.90 a

While control treatment significantly exceeded concentration of the kaempferol compound to 114.76 µg/ml compared at all $AgNo_3$ concentration. While the concentration of $AgNO_3$ had a negative effect on this compound which lead a significant decrease. The concentration of ricinin was significantly increasing to 119.90µg/ml at 4mg/l $AgNO_3$ concentration compared to control and others concentration of $AgNO_3$. The increase in the concentration of some compounds may be due to the role of heavy elements in stimulating the active compounds because they causes stress to the tissues as they are cytotoxic, showed [40] silver nitrates were the most effective among many stimuli used to increase the concentration of hypophyllanthin and Phyllanthin of phyllanthus amarus.

Effect of physical treatment on the production of some secondary compounds:

The effect of different exposure periods for lighting in the production of secondary compounds from callus of Ricinus communis can be explained as: The length of lighting period effect in callus to stimulated increase the production of secondary compound. Figure 5 shows the difference in the curves of the secondary compounds according to the period of the exposure to the lighting. When calculating the concentration of the secondary compounds depending on the period of the light, and show the result of Table 8 increasing significantly to for all compound when exposing the callus to light for 24hour compared control treatment (incubator conditions) and dark treatment. The concentration of secondary compounds reached the highest and significantly, to 87.87,148.2,132.91µg/ml for qurecetin, kaempferol, ricinin respectively. The callus was subjected to dark condition for 24 hour had a negative and significant effect for all secondary compounds concentration, as the treatment significantly decreased in the concentration of all secondary compounds and reached the lowest levels compared to control treatment. The results of this study showed that exposure to lighting for 24hour was the best treatment, in stimulating the increase in the production of secondary compound, [41] showed the importance of lighting in stimulating the production of secondary compounds [35] notice that the light has a significant effect on the growth of cell activity for growth and production of secondary metabolites, while [42] reached that light affects the gene expression of genes responsible for the formation of secondary compounds .



A B

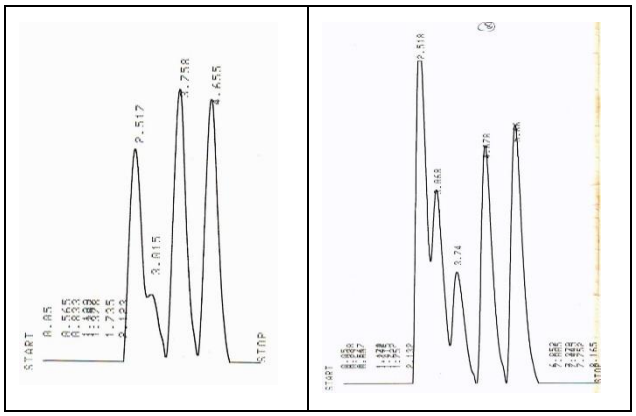
Figure 5: HPLC analysis of callus exposure to various period of light.(A) 24hrs.light.(B) 24 hrs. dark.

Effect of exposure period of UV in the production of secondary compounds from callus of Ricinus communus

The results in Figure 6 show the curves of secondary compounds from callus samples after exposure to different periods of ultraviolet radiation.

Table 8: Effect of the exposure period (hour) in the production of secondary compounds µg/ml from callus of Ricinus communis

secondary compound µg/ml	period of light(hours)		
	control	24 h light	24 h dark
Qurecetin	71.41 b	87.87 a	5.42 c
Kaempferol	114.76 b	148.26 a	44.03 c
Ricinin	105.78 b	132.91 a	19.03 c



A B

Figure 6: HPLC analysis of callus exposure to various period of UV light. (A) 5 minute (B) 10 minute.

Differences in compounds rates had obtained depending on periods of callus exposure to UV radiation (Table 9). Where the results of this Table are showed a high concentration of qurecetin when exposed callus to UV rays for 10 min at 68.58µg/ml, which did not differ significantly from control treatment.

When callus exposure to ultraviolet radiation led to decrease the concentration of kaempferol significantly for both period of exposure. This compound was significantly higher at control treatment reached to 114.70µg/ml. The concentration of Ricinin was significantly lower when callus was exposed, to UV radiation for 10 min to 62.07µg/ml. while the concentration of this compound increased significantly at control treatment, was 105.78µg/ml, which did not differ significantly from the concentration in the treatment of callus exposure for 5 minutes.

When comparing all the results, we observe that control treatment was significantly superior for all concentration of secondary compounds, compared with treatments that exposure callus to UV ray for 5 and 10 minute. This indicate that UV ray was not the highest efficient in increasing the concentration of secondary compound of Ricinus communis.

Table 9: Effect of exposure period of UV ray (min) in the production of secondary compounds µg/ml in callus of Ricinus communis

Secondary compound µg/ml	**period of UV exposure (min)**		
	control	5	10
Qurecetin	71.41 a	3.30 b	68.58 a
Kaempferol	114.76 a	37.20 c	51.20 b
Ricinin	105.78 a	96.79 a	62.07 b

Conclusions

The addition of $AgNO_3$ increased the concentration of some secondary compounds in the callus extraction compared to control. Physical stimuli and exposure to light had a significant effect in increasing the concentration of some secondary compounds, while UV ray did not increase the concentration of secondary compounds.

There was a significant increase in the concentration of all secondary compounds in the callus extraction compared to the concentration of compounds in seeds extraction.

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