MICROPROPAGATION, ANTIOXIDANT ACTIVITY, TOTAL PHENOLICS AND FLAVONOIDS CONTENT OF *GARDENIA JASMINOIDES* ELLIS AS AFFECTED BY GROWTH REGULATORS

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ABSTRACT

This investigation has been carried out to study Micropropagation of *Gardenia jasminoides* Ellis shootlet, induction and growth of callus and enhancement of antioxidant, total phenolics and flavonoids content in shootlet and calli. The results revealed that for shooting stage adding 2 or 3 mg/l BAP gave the highest shootlet number/explant. But adding 3 mg/l 2iP produced the longest shootlet and leaves number. The highest fresh and dry weight was recorded on medium free hormones. Antioxidant activity, total phenolics and flavonoids content were highest in shootlet cultured in all concentrations of cytokinins compared to medium free hormones and mother plants which gave the lowest amount. Results indicated a strong relationship between total phenolics content and antioxidant activity. On rooting behaviour, all strengths of MS-medium with 1g/l AC produced rooted shootlet. Quarter strength of MS-medium produced the highest rooting, leaf number and shootlet length. Rooted shootlets cultured on peatmoss with sand (1:1 v/v) gave the longest plantlet and greatest number of leaves. For induction and growth callus, adding 2 mg/l NAA recorded the highest percentage, fresh and dry weight of callus.

Key words: *Gardenia jasminoides,* Micropropagation, total phenolics content, antioxidant activity

1. INTRODUCTION

Gardenia jasminoides Ellis belongs to family *Rubaiceae*. It is an ornamental woody plant. It has white flowers with sweet fragrance (1). It use as a cut flower and a garden shrub. It is a popular pot plant in the US and many European countries (2). The leaves and fruits possess antibacterial, demulcent and diuretic properties. They are used in treating fever, jaundice, sore throat, bloody stools and dysuria (3). It is also interesting that the major colorant, crocin, has long been used as a food additive and may act as an antioxidant (4). The crude extract of *Gardenia jasminoides* had been also used for pharmaceutical purpose such as choleretic action for liver disease control and relief of type 2 diabetic symptoms (5) and is capable of inhibiting various types of cancer cells and human fibrosarcomol cells (6).

Terminal cutting of *Gardenia jasminoides* results in a low proliferation rate. Micropropagation via *in vitro* organogenesis offers a high proliferation rate per each starting plant (7).

Establishment of protocols for cultivation of medical plants using different growth regulators to enhance the production of bioactive compounds is required for commercial and research application. Bioactive compounds were found to be accumulating in culture cells at higher level than those in natural plants though optimization of culture conditions (8).

Cytokinins are plant growth regulators used for stimulating cell division, as well as for the formation and growth of axillary and shoots. This group consisted of the naturally occurring cytokinins which include zeatin, 2iP and adenine and another type is synthetic cytokinins that consists of substituted purine, 6-benzylamino-purine and kinetin. The effect of cytokinins *in vitro* on shootlet proliferation was studied on *Ruscus hypoglossum* (9), *Deutzia scabra* (10) and *Pyricantha fortuneana* (11).

The concentration of macro-and micro elements of culture medium without or with activated charcoal (AC) remarkably affected rhixogensis behaviour as showed by Sakr et al (12) on *Yucca elephantipes* and Sayed et al(13) on *Cotoneaster horizontalis*.

The callus has potential to show metabolic activity and can be compared in this respect with mother plants. For callus induction and growth, an exogenous auxin supply of regulators is often recommended to initiate callus formation from explant. Particularly, auxins effect growth, callus formation (14). 15) found that naphthalene acetic acid (NAA) and 2,4-D were the most active compounds.

Antioxidants are important in the prevention of human diseases. Antioxidant compounds may function as free radical scavengers, complexing agents for pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation (16). Antioxidants are often used in oils and fatty foods to retard their auto-oxidation. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years (17).

Derived polyphenols from plants are of great importance because of their potential antioxidant and antimicrobial proper ties. Phenolic compounds exhibit a considerable free radical scavenging (antioxidant) activity, which is determined by their reactivity as hydrogen or electron-donating agents, the stability of the resulting antioxidant derived radical, their reactivity with other antioxidants and finally their metal chelating properties (18 - 19).

Flavonoid compounds have antioxidant, antimicrobial, anticancer and anti-inflammatory properties used in preventing cardiovascular diseases and supporting regeneration of human body cells (20).

Thus, this study is provided to establish a protocol for micropropagation of shooting; rooting; acclimatization and induction of callus. Moreover, determining the effect of growth regulators on the antioxidant activity, total phenolics and total flavonoids content on shootlet and calli as compared to mother plants.

2. MATERIALS AND METHODS

This work has been carried out at Tissue Culture and Germplasm Conservation Research Laboratory, Horticulture Research Institute, Agriculture Research Center (ARC) Egypt and National Research center (NRC) Egypt on *Gardenia jasminoides* Ellis during two successive years (2009-2010).

The objective of this study was to establish a protocol for Micropropagation and callus formation to enhance antioxidant activity; total phenolics and flavonoids content in shootlet and calli compared to mother plants.

Plant Materials

Young shoots of *Gardenia jasminoides* were obtained from commercial farm "Egypt Green". The shoots were divided into nodal (explant). The leaf and explant were soaked for 1 min in 70% ethanol solution under aseptic condition in laminar air flow cabinet. After that they were treated with 20% (v/v) colorox with a few drops of tween-20 for 10 min rinsing three times with sterile distilled water. After that they were immersed in 0.1% mercuric chloride (MC) with a few drops of tween-20 for 10 min followed by rinsing three times.

Culture Conditions

In each shootlet proliferation, rooting and callus induction in the used culture medium was enriched with 25 g/l sucrose and 7g/l agar. The medium was adjusted to pH 5.7±0.1 and autoclaved at 121ºc and 1.2 kg/cm^2 for 15 min before using. The shootlet explant and leaf disc were placed in 200 ml glass jar containing 25 ml medium. The cultures were incubated at 25±1ºc under florescent lamps with light intensity of 3 k lux at 16 hours photoperiods.

Shooting Behaviour

The culture medium under this trail was MS (21) supplemented with 6- (γ, γ- dimethylallyamino) purine (2IP); 6-furfurylamino-purine (Kinetin) and 6-benzylamino-purine (BAP) at the concentrations (0, 1, 2 and 3 mg/l). In each treatment twenty five explant with five replicates were cultured for one month and subcultured in the same treatments for three times. Shootlet number per explant, shootlet length (cm), leaf number, fresh and dry weights were calculated. The dry shootlet was extracted to determine antioxidant, total flavonoids and phenolics.

Rooting Response

In the rooting treatments, shootlet (2.0-2.5cm length) were cultured on MS-medium free hormones at (full; half and quarter) salt strength without and with (1 g/l) activated charcoal (AC). Rooting percentage, number of root per shootlet, length of root (cm), Shootlet length (cm) and leaf number were measured.

Acclimatization of *in vitro* plantlets:

Transplants (rooted shootlet) were transferred to plastic pots containing peat moss; peat moss: vermiculite (1:1v/v) peat moss: sand (1:1v/v) and peat moss: vermiculite: sand (1:1:1v/v/v). These treatments were irrigated with solution of 0.2 topsin-70M fungicides and covered by transparent polyethylene bags. The acclimatized transplants out-of door for two months. Survival%, plantlet length (cm) and leaf number /plantlet were measured.

Callus Induction and Growth

The leaf disc (1×1 cm) were horizontally cultured on full MS-medium salt strength supplemented with different kinds of auxins, i.e., indol butric acid (IBA), naphthalene acetic acid (NAA) and 2,4-dichlorophenoxy acetic acid (2,4-D). The leaf disc explants culture were incubated for one month (twenty five leaf disc in five replicates) after that the percentage of leaf disc callusing were calculated. For growth, one gram of calli was put in all jars with treatments of five replicates and incubated for one month. Subculture of these treatments subcultured for three times and lastly, fresh and dry weights of calli were recorded and extraction of dry calli was carried out to determine antioxidant; total phenolics and flavonoids content.

Sample preparation and Extraction

The air dried samples were ground to fine powder. A precisely weighted amount (0.25g) of the powder was extracted with 10 ml of methanol for 24h. The supernatant was recovered and used for the determination of antioxidant capacity, total phenolics and flavonoids.

Determination of antioxidant activity

The free radical scavenging activity using the 1.1-diphenyl-2-picrylhyrazil (DPPH) reagent was determined according to *(*22). 0.75 ml of the extract sample was added to 1.5 ml of freshly prepared methanolic DPPH solution (20 µg ml^{-1}) was added and stirred the decolorizing process was recorded after 30 min of reaction at 517 nm by spectrophotometer and compared with a blank control.

Antioxidant activity = (control absorbance – sample absorbance)/(control absorbance) × 100

Determination of total phenolics

The total phenolics content of methanolic extract was determined according to the method described by Makkar et al (23) by folin-ciocalteu reagent. The absorbance was recorded at 725nm by spectrophotometer against the reagent blank. The amount of total phenolics was calculated as pyrogallol equivalents from a calibration curve.

Determination of total flavonoids

Total flavonoids were estimated using the method of (24) using Aluminum chloride, the absorbance was measured at 420 nm by spectrophotometer. Total flavoniods content were calculated as quercetin equivalent to a calibration curve.

Experimental Design and Data Analysis

The lay-out of the experiment was designed in a completely randomized design and test of Least Significant Difference (L.S.D.) at p≤0.05 was used for comparison among means according to (25).

3. RESULTS

Shooting Behaviour

The effect of cytokinins (2iP, kin and BAP) at various concentrations (0, 1, 2 and 3 mg/l) on the behaviour of shooting *in vitro* (i.e. shootlet number/explant; shootlet length (cm), leaf number/ shootlet and fresh and dry weight) are presented in Table (1).

The data in Table (1) revealed that the number of shootlet/explant gave the highest number (3.90 and 4.0 shootlet/explant) when the medium hold 2, 3 mg/l BAP. On the other, culture explant on free MS-medium (control) gave the lowest number (1.07 shootlet/explant).

Concerning the effect of various concentrations of cytokinins on both shootlet length (cm) and number of leaves/shootlet, the data in Table (1) recorded that adding 3 mg/l 2iP produced the tallest shootlet (2.76 cm) and the largest number of leaves (8.70 leaf/shootlet). However, the explant cultured on MS-medium supplemented with 2.0 and 3.0 mg/l BAP gave the shortest shootlet length (1.28, 1.48 cm) and lowest number of leaves (6.50, 6.96 leaf/shootlet).

Data for fresh and dry weights indicated that MS-medium free hormones gave the highest fresh weight of shootlet (4.12 g) as compared with 3.0 mg/l kin which gave the lowest fresh weight of shootlet (0.73 g). For dry weight, adding 1, 2 and 3 mg/l BAP to MS-medium resulted in the highest dry weight of shootlet (0.38 g). However, high concentration of kin (3 mg/l) caused the lowest value of dry weight (0.13g).

Table 1. Effect of different types and concentrations of cytokinins on shooting behaviour of *Gardenia jasminoides* Ellis *in vitro* culture

Measurements Treatments	Shootlet No.	Shootlet length (cm)	Leaf No.	Fresh weight (g)	Dry weight (g)
MS free (control)	1.07	2.07	8.38	4.12	0.28
1.0 mg/l 2iP	1.16	1.70	7.36	1.36	0.28
2.0 mg/l 2iP	1.37	2.63	8.28	1.90	0.32
3.0 mg/l 2iP	1.14	2.76	8.70	2.05	0.35
1.0 mg/l kin	1.18	1.58	7.32	1.42	0.24
2.0 mg/l kin	1.38	2.08	7.80	1.23	0.23
3.0 mg/l kin	1.28	1.53	7.30	0.73	0.13
1.0 mg/l BAP	2.70	2.17	8.42	1.63	0.38
2.0 mg/l BAP	3.90	1.28	6.50	2.45	0.38
3.0 mg/l BAP	4.00	1.48	6.96	2.26	0.38
L.S.D0.05	**0.495**	**0.574**	**1.309**	**1.770**	**0.115**

Determination of Antioxidant activity, Total phenolics and Total Flavonoids content in shootlet extract

Data in Table (2) revealed that, all shootlet cultured on different concentrations of 2iP, kin and BAP produced significant effect on antioxidant activity as compared to MS-free medium (control) and mother plant. Antioxidant activity increased by (45.40, 47.54 and 47.10%, respectively) when cultured on 2 mg/l 2Ip,2mg/l kin and 3 mg/l BAP more than control. While it increased by (17.52, 19.22 and 17.74%, respectively) with (2mg) 2iP, kin and(3mg) BAP more than the mother plant. For total phenolics content, it increased in all treatments more than control and mother plant. Treatment with 2iP (1 mg/l) increased phenolic cotent by (23.14 and 44.29%) more than the control and mother plant. Treatment of 2 mg/l kin increased phenolics by 23.94, 43.86% exceeding the control and mother plant. Similar trend was obtained when using 3 mg/l BAP. Regarding, total flavonoids content, data in Table (2) showed that culturing the shootlet in all treatments increased the total flavonoids content as compared to the control and mother plant. The highest amounts of flavonoids (0.297 and 0.293mg/g) were accumulated with 3 mg/l of 2iP and BAP, while the control and mother plant recorded (0.217 and 0.210 mg/g, respectively).

Table 2. Antioxidant Activity, total phenolics and flavonoids content as affected by different types and concentrations of cytokinins on shooting of *Gardenia jasminoides*

Measurements Treatments	Antioxidant activity %	Total phenolics (mg/g)	Total flavonoids (mg/g)
Mother plants	50.74	14.18	0.210
MS free (control)	41.01	16.46	0.217
1.0l mg/l 2iP	59.48	20.27	0.283
2.0 mg/l 2iP	59.63	18.72	0.287
3.0 mg/l 2iP	58.49	18.36	0.297
1.0mg/l Kin	56.63	17.07	0.253
2.0 mg/l Kin	60.49	20.40	0.273
3.0 mg/l Kin	57.90	17.73	0.283
1.0 mg/l BAP	57.75	17.08	0.233
2.0 mg/l BAP	57.50	17.45	0.263
3.0 mg/l BAP	59.74	20.57	0.293
L.S.D0.05	**0.667**	**0.640**	**0.011**

Rooting Behaviour

Data presented in Table (3) demonstrated the effect of different strengths of MS-medium (full, half and quarter strength) without and with (1 g/l) activated charcoal on rooting %, root number/shootlet, root length (cm), shootlet length (cm) and leaf number.

The highest rooting % (83.33 to 100%) was obtained when using full MS-medium with AC, half and quarter MS-medium without and with AC. While using full strength of MS-medium without AC gave the lowest percentage of rooting (8.33%).

It is quite clear from data in Table (3) that quarter strength of MS-medium with (1 g/l) AC gave the highest number of root/ shootlet (14.23). On the other hand, different salt strengths of MS-medium (full, half and quarter) without AC gave the lowest number of root/shootlet (0.67, 3.02 and 3.60, respectively). For root and shootlet length (cm), it was found that using different salt strengths of MS-medium (full, half and quarter) with (1 g/l) AC produced the longest root (3.17, 3.60 and 4.17 cm, respectively) and shootlet (94.93, 4.27 and 4.83 cm, respectively). However, the shortest root (0.67 cm) and shootlet (3.50cm) were recorded with full salt strength of MS-medium without AC. For number of leaves, different salt strengths of MS-medium (full, half and quarter) without and with (1 g/l) AC showed insignificant effect on leaves formed per shootlet

Table 3. Effect of different salt strengths MS-medium without and with activated charcoal on rooting behaviour of *Gardenia jasminoides* Ellis *in vitro* culture

Measurements Treatments	Rooting %	Root No.	Root length (cm)	Shootlet length (cm)	Leaf No.
Full MS	8.33	0.67	0.67	3.50	10.67
Full MS + AC	83.33	9.93	3.17	4.93	13.33
Half MS	100.0	3.02	0.40	4.00	11.63
Half MS + AC	100.0	8.33	3.60	4.27	12.20
Quarter MS	100.0	3.60	0.57	3.87	10.60
Quarter MS + AC	100.0	14.23	4.17	4.83	13.00
L.S.D0.05	**22.99**	**3.149**	**1.561**	**0.737**	**N.S**

Acclimatization

All trasplants(rooted shootlets) were transferred to different culture media(under *ex vitro* conditions). The plantlets in all culture media survived(100%). For the plantlet length and leaf number, the rooted shootlet cultured on peat moss with sand (1:1 v/v) gave the longest plantlet (11.0 cm) with the highest number of leaves (16.80 leaf/plantlet). However peat moss alone gave the shortest plantlet (4.90 cm) and lowest leaf number (9.00 leaf/plantlet).

Table 4. Effect of different culture media on acclimatization of *Gardenia jasminoides* Ellis *in vivo* culture.

Measurements Treatments	Survival (%)	Plantlet length (cm)	Leaf number/plantlet
Peat moss	100	4.90	9.00
Peat moss + Sand	100	11.20	16.80
Peat moss + vermiculite	100	10.70	12.80
Peat moss + Sand + vermiculite	100	9.70	12.20
L.S.D0.05	**NS**	**3.797**	**4.719**

Callus Formation Behavior

The callus formation behaviour of explants such as rate of callus induction %, fresh and dry weight of calli were studied (Table 4) under the effect of different types of auxins (IBA, NAA and 2,4-D) with different concentrations (0, 1, 2 and 3 mg/l).

For callus induction %, the data presented in Table (4) indicated that MS-medium supplemented with either NAA at (2 mg/l) and 2,4-D at (1, 2 and 3 mg/l) resulted in the highest percentage of callus induction (91.67 to 100.0%). While MS-medium free hormones (control) gave the lowest rate of callus induction (8.40%).

Concerning the fresh and dry weights of calli, data in Table (4) demonstrated that the highest fresh weight (10.45, 11.48, 11.55, 9.76, 10.64 and 10.85 g/jar, respectively) and dry weight (0.43, 0.47, 0.47, 0.41, 0.36 and 0.35 g/jar, respectively) of calli were recorded when MS-medium supplemented with IBA or NAA at concentrations of (1, 2 and 3 mg/l). However, Using MS-medium free hormones (control) or supplemented with (1, 2 and 3 mg/l) caused the lowest fresh weight (5.65, 5.15, 5.81and 6.28 g/jar, respectively) and dry weight (0.29, 0.23, 0.27 and 0.29 g/jar, respectively).

Table 5. Effect of different types of auxins and different concentrations on callus formation behaviour of *Gardenia jasminoides* Ellis *in vitro* culture.

Measurements Treatments	Callus induction %	Calli fresh weight (g)	Calli dry weight (g)
MS free (control)	8.40	5.65	0.30
1.0 mg/l IBA	33.33	10.45	0.43
2.0 mg/l IBA	50.00	11.48	0.47
3.0 mg/l IBA	66.67	11.55	0.47
1.0 mg/l NAA	75.00	9.76	0.41
2.0 mg/l NAA	91.67	10.64	0.36
3.0 mg/l NAA	83.33	10.85	0.35
1.0 mg/l 2,4-D	91.67	5.15	0.23
2.0 mg/l 2,4-D	100.0	5.81	0.27
3.0 mg/l 2,4-D	100.0	6.28	0.29
L.S.D0.05	**35.30**	**3.173**	**0.163**

Determination of Antioxidant activity, Total phenolics and Flavonoids content in calli extract

For antioxidiant activity, data in Table (6) revealed that all treatments of calli sample extract increased the antioxidant activity but IBA and NAA resulted in the highest antioxidiant activity that reached 138.00 and 131.85% as relative to control. On the other hand, antioxidant activity of mother plant was higher than control and calli treatments.

While total phenolics, calli cultured on (2, 3 mg/l) NAA and (2 mg/l) IBA have total phenolics (2.23, 2.22 and 2.10 times) higher than control. The calli showed lower amounts of phenolics in both control and auxin treatments than mother plant.

For total flavonoids, calli were cultured on a medium containing (1, 2 and 3 mg/l) NAA recorded the highest accumulation of flavonoids (0.183, 0.180 and 0.187 mg/g, respectively). All treatments increased the flavonoids but with lower amount than mother plant (0.210 mg/g)

Table 6. Antioxidant Activity, total phenolics and flavonoids content as affected by different types and concentrations of auxins on calli of *Gardenia jasminoides* Ellis

Measurements / Treatments	Antioxidant activity %	Total phenolics (mg/g)	Total flavonoids (mg/g)
Mother plants	50.87	14.18	0.210
MS free (control)	32.09	4.62	0.120
1.0 mg/l 2iP	40.78	6.69	0.153
2.0 mg/l 2iP	42.80	9.74	0.167
3.0 mg/l 2iP	44.25	8.03	0.173
1.0 mg/l Kin	41.42	9.78	0.183
2.0 mg/l Kin	42.31	8.23	0.180
3.0 mg/l Kin	42.11	10.28	0.187
1.0 mg/l BAP	36.57	8.59	0.153
2.0 mg/l BAP	35.71	7.28	0.137
3.0 mg/l BAP	36.87	6.41	0.140
L.S.D0.05	**0.543**	**0.528**	**0.013**

4. DISCUSSION

In this investigation, BAP was superior to 2iP and kin in giving more shootlets per explant. The increment on shootlet proliferation was (0.65 to 0.72%) for number of shootlet/explant in case of using (1and 2 mg/l BAP) than the corresponding values of 2iP and kin. The shootlet length and leaf number inversely decreased with the same concentrations of BAP (1and 2 mg/l) to lowest values and increased to (0.154 and 0.46%) for shootlet elongation and (0.25 and 0.20%) for leaf number when 2 and 3 mg/l 2iP were used. The rate of culture fresh weight using (1, 2 and 3 mg/l) for 2IP, kin and BA were lowest to (0.40 to 0.70%) as comparing with that by control (free hormones) treatment. Whereas, using (1, 2 or 3 mg/l BAP increased dry weight to (0.66%) than that of 3 mg/l kin which gave the lowest value. Similar results were obtained by (26-27) and (11). In general, the results could be attributed to the important physiological effect of cytokinins as they stimulate cell division as well as elongation to active RNA synthesis and to stimulate protein synthesis and enzyme activity as were reviewed by Kulaeva(28).

The reduction in the salt strengths of MS medium to 50 or 25% did not affect rooting behaviour. However, adding activated charcoal (AC) to MS-medium clearly promoted all rooting parameters. This stimulatory effect of activated charcoal on rooting behaviour might be attributed to AC that had a high ability to accelerate the root cell division and elongation rates. Similar results were found by Youssef et al and Sayed et al.(29&30) .

Moreover, callus induction was affected by different types of auxins and different concentrations. 2,4-D was the auxin choice for callus induction but it gave the lowest values of both fresh and dry weight of callus. These results were in agreement with (31). Using IBA or NAA in culture medium promoted fresh and dry weights values. The promotive effect of auxins (IBA, NAA and 2,4-D) on callus induction and growth might be attributed to auxin as it promotes the biosynthesis of ethylene by increasing the activity of 1-aminocylopropane-1-carboxylic acid (ACC) syntheses (32).

In present study there was a strong relationship among total phenolic and antioxidant activity indicated that phenolic compounds were a major contributer of antioxidant activity in *Gardinia Jasminoides* . Our data are in agreement with (33 and 19) who reported the same relationship, as phenolics are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolics content of plants may be contributed directly to their antioxidant action, phenylpropanoid and flavonoid biosynthesis. The great enhancement of total phenolics and flavonoids content in both shootlet and calli may be due to the presence of growth regulators. This data are in agreement with (34) whom found that MS liquid medium supplemented with 2 mg/l (BAP) and 0.1 mg/l (NAA) revealed to be the best *in vitro* condition to produce shoot material with highest phenolic compound contents and stronger antioxidant potential. Also (35) reported that BAP and NAA play an important role in the biosynthesis of secondary metabolic in *in vitro* grown culture.

Mother plant

Callus induction and growth

The highest induction% with highest fresh and dry weight with 2 mg/l NAA

Shooting stage

The highest shootlet number with 3 mg/l BAP

Rooting stage

The highest root number with quarter strength MS + 1g/l AC

Acclimatization stage

The longest plantlet with the largest leaves number on peat moss with sand

Determination of antioxidant activity, total phenols and flavonoids

For Calli	For Shootlet
All concentrations of auxin(1,2 and 3mg/l gave highest amount compared to MS-free hormones but lowest to mother plant	All concentrations of cytokinins (1,2 and 3mg/l) gave the highest amount in compared to MS-free medium and mother plant

Our results indicated that antioxidant activity, total phenolics and flavonoids content of mother plants were higher than control and calli treatments. Similar enhancement effect was reported in *Ocimum sanctum* callus induction studied by (36), who found that the total flavonoids content of the *in vivo* leaf tissues were higher than the total flavonoids content of calli. Also (37) found that an assay of the antioxidant potential of the *in vitro*-grown tissues using BA, NAA, IBA, 2,4-D

and GA revealed that the antioxidant activity of the regenerated shoots was significantly higher than that of callus and the regenerated plantlets,

On the other hand (38) indicated that *in vivo* tobacco leaf tissues consists of differentiated cells and they are able to syntheses, like chalcone synthese which contributes to the flavonoids biosynthesis pathway. However, callus generated from the leaf tissues, which contains partially de-differentiated cells and de-differentiated cells, is unable to produce much enzymes like the *in vivo* leaf tissue. Furthermore, the phase of cell growth in callus also may influence the yield of flavonoids.

4. CONCLUSION

The present study clearly indicates that the use of growth regulators and micropropagation technique is very important to increase the antioxidant activity,total phenolics and total flavonoids production in *Gardinia Jasminoids* as a medicinal plant.

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