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Research Article
Impact of Zeatin and Thidiazuron on Phenols and Flavonoids Accumulation in Callus Cultures of Gardenia (*Gardenia jasminoides*)

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Abstract

Background and Objective: Gardenia (*Gardenia jasminoides*) has many pharmacological actions such as anti-inflammatory, antioxidant and fibrolytic activities and cytotoxic effects, etc. This study was conducted to recognize the effect of zeatin and thidiazuron (TDZ) on callus proliferation, total phenolic content, total flavonoids and DPPH scavenging activity of gardenia callus cultures. **Materials and Methods:** Calli were cultured on Murashige and Skoog (MS) medium supplement with different concentrations (2, 4 or 6 mg L^{-1}) of zeatin or TDZ individually as well as combination of 2 mg L^{-1} zeatin+4 mg L^{-1} TDZ. Cultures contained 4 mg L^{-1} TDZ gave the highest callus fresh weight followed by those contained 2 mg L^{-1} zeatin then that cultured on 4 mg L^{-1} zeatin. Data reported as Mean±Standard Deviation (SD). Data were subjected to one-way analysis of variance ($p< 0.05$). Results were processed by Excel (2010) and SPSS Version 17.0. **Results:** It was found that callus growing on medium supplemented with 4 mg L^{-1} zeatin gave the maximum value (14.93%) of yield extract. Callus cultured on 4 mg L^{-1} zeatin recorded the maximum total phenol (268.33 mg GAE/100 g FW of callus) and total flavonoids (2703.33 µg QE/100 g FW of callus) accumulation. The antioxidant activity of each extract was determined through DPPH radical scavenging activity. Callus cultured on 4 mg L^{-1} TDZ showed the highest antioxidant activity then those cultured on 4 mg L^{-1} zeatin. The HPLC analysis for phenolic acids showed that chlorogenic acid, rosmarinic acid and cinnamic reached their highest contents with callus cultured on 4 mg L^{-1} TDZ (123.24, 322.14 and 278.22 µg g^{-1}, respectively). Regarding flavonoids and using HPLC analysis, rutin, apigenin-7-glucoside and kaempferol were detected. Callus cultured with 4 mg L^{-1} TDZ gave the highest rutin and kaempferol contents (287.76 and 10.38 µg g^{-1}, respectively). However, apigenin-7-glucoside was detected with high content (129.86 µg g^{-1}) in callus culture with 4 mg L^{-1} Zeatin. **Conclusion:** The HPLC analysis recommended that TDZ is more effective in accumulation of individual phenolic and flavonoid than Zeatin. The present study provided a useful system for further study on *in vitro* culture of *G. jasminoides* as alternative and new source for important secondary products.

Key words: *Gardenia jasminoides*, zeatin, thidiazuron, spectrophotometer, HPLC analysis, phenolic and flavonoids

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

All medicinal plants play a great role in industries of drugs around the world. Herbal products play an important role in many health care systems in different countries. Some chemical substances are responsible for the medicinal value of different plants, which can produce a physiological action in the human body. Phenolic compounds, flavonoids, alkaloids and tannins are the most important compounds of the medicinal plants[1].

Gardenia jasminoides is considered as an evergreen shrub. It is native to the tropical and sub-tropical regions of Africa, Southern Asia, Australia and Oceania. It is a member of family *Rubiceae*[2]. Gardenia can be used as hedges, borders, screens, borders or ground covers. It is considered as an important plant in the traditional Chinese medicine and can be effective as drains fire, hemostatic agent and it is also used in curing muscles injuries, tendons and joints[3]. The major components of Gardenia's fruits are iridoid glycosides, which could be converted into blue and red pigments. These derivatives of crocetin have coloring properties and have also particular water-soluble behavior, opposing most plant families of carotenoid[3]. Carotenoids are also the main contributors to the antioxidant content of plants and have pharmacological effects, such as preventing cardiovascular diseases[4]. The TDZ and zeatin are considered as cytokinins which are a class of plant growth substances that promote cell division. They are involved in cell growth and differentiation. There are two types of cytokinins, adenine type like zeatin and phenyl-urea type like TDZ[5].

Gardenia jasminoides is rich in phenolic compounds and anti-inflammatory flavonoids and it could be used for inflammatory diseases treatment and also for pain treatment[6].

Biotechnologists pay a great attention to medicinal plants all over the world. In the present study, plant cell culture technique was subjected to examine the accumulation of phenols and flavonoids compounds on *Gardenia jasminoides* callus cultures.

MATERIALS AND METHODS

This study was carried out in Department of Plant Biotechnology , National Research Centre, Giza, Egypt, during the period from January-December, 2016.

Plant material: *In vitro* growing Gardenia plantlets were used in this study as a source of plant material. The plantlets were subcultured three times on multiplication medium of MS supplemented with 2 mg L^{-1} BA+0.5 mg L^{-1} NAA.

Callus cultures: *In vitro* derived leaves were excised from shoot segments and used as explants for callus cultures establishment. The *in vitro* derived leaves were cut transversely into two halves, then cultured on MS medium supplemented with 0.5 m L^{-1} BA+0.5 mg L^{-1} Picloram and placed in darkness (40 days) for callus induction. For callus production, the inducted callus was subcultured twice on the same freshly prepared medium for 4 weeks interval.

Effect of Thidiazuron (TDZ) and zeatin on callus response: About 0.5 g of friable callus was transferred to MS medium supplemented with different concentrations of TDZ or zeatin for two subcultures (each subculture is 4 weeks). For control treatment, MS medium supplemented with 2.5 mg L^{-1} TDZ+0.2 mg L^{-1} IAA was used. The different treatments of TDZ and zeatin are as follow (Table 1).

Callus fresh weight (g), growth value and color were recorded at the end of each subculture.

Biochemical analysis

Sample extraction: About 2 g of callus and fresh weight of each treatment was extracted with methanol 80% (10 mL) overnight on a shaker (120 rpm) and at room temperature. Then the extraction procedure was carried out in an ultrasonic water bath for 20 min. Samples were centrifuged for 5 min at 6000 rpm. The supernatants were collected and the pellets were re-extracted twice with 500 µL 80% methanol. The extracts were stored at -20°C until further use.

Determination of callus yield: The percentage of callus yield was obtained using this equation:

$$\frac{W_2\text{-}W_1}{W_0}\times 100^7$$

where, W2 is the weight of the extract and the container, W1 is the weight of the container alone and W0 the weight of the initial dried sample.

Table 1: Composition of media used for callus induction and growth

Treatment abbreviation	Treatments
G1	2 mg L^{-1} TDZ
G2	4 mg L^{-1} TDZ
G3	6 mg L^{-1} TDZ
G4	2 mg L^{-1} Zeatin
G5	4 mg L^{-1} Zeatin
G6	6 mg L^{-1} Zeatin
G7	2 mg L^{-1} Zeatin+4 mg/TDZ
G8 (control)	2.5 mg L^{-1} TDZ+0.2 mg L^{-1} IAA

Determination of total phenols: Total phenols were determined by the Folin-Ciocalteu micro-method[8]. A 20 µL of extract solution was mixed with 1.16 mL of distilled water and 100 µL of Folin-Ciocalteu's reagent followed by 300 µL of 200 g L^{-1} Na_2CO_3 solution. The mixture was incubated in a water bath at 40°C for 30 min and its absorbance at 760 nm was measured. Gallic acid was used as standard for the calibration curve. Total phenolic content as Gallic Acid Equivalent (GAE) was calculated using the following equation[7,9]:

$$A = 0.98C+9.925\times10^{-3} \ (R^2 = 0.9996)$$

where, A is the absorbance and C is the concentration.

DPPH Radical scavenging activity: The DPPH assay according to Gabr *et al.*[9] was used with some modifications. Methanolic extract of different concentrations (0.1 mL of each) were vortexed for 30 sec with 3.9 mL of DPPH solution and left to react for 30 min, after which the absorbance at 515 nm was recorded. A control with no added extract was also analyzed. Scavenging activity[9] was calculated as follow:

$$\text{DPPH radical-scavenging activity (\%)} = \left[\frac{A_{control}-A_{sample}}{A_{control}}\right]\times100$$

where, A is the absorbance at 515 nm.

Determination of total flavonoids: Total Flavonoid content was determined according to the method of Ordonez *et al.*[10]. A 0.5 mL of 20 g L^{-1} $AlCl_3$ methanol solution was added to 0.5 mL of extract solution. After 1 h at room temperature the absorbance at 420 nm was measured. Yellow color shows the presence of flavonoids. Total flavonoids content expressed as Quercetin Equivalent (QE) were calculated using the following equation based on the calibration curve:

$$Y = 0.0255X \ (R^2 = 0.9812)$$

where, X is the absorbance and Y is the concentration (mg QE g^{-1} DW).

Determination of phenols and flavonoids content by High Performance Liquid Chromatography (HPLC): The extraction was performed according to Gabr *et al.*[11], flavonoids (100 mg) were extracted from the dried residue with 2 mL of methanol at 40°C for 8 h. The methanol solution was evaporated and concentrated to a dry residue. The extract was dissolved in 1 mL of methanol and kept at 4°C in darkness. The content of flavonoids was determined by HPLC on a UNICAM CRYSTAL 200 Liquid Chromatograph. The mobile phase consisted of methanol and water (both acidified with 0.3% orthophosphoric acid p.a.-w/v). Flavonoids were eluted with linear gradient from water to 50% methanol in 5 min, following by isocratic elution with 50% methanol for 20 min. The flow-rate was 1.4 mL min^{-1}. Substances were detected by absorption at $\lambda = 288$ nm and their identification were carried out by the comparison of retention times and absorption spectra with standards complex of phenols: chlorogenic acid, vanillic, ferulic, rosmarinic and cinnamic. For flavonoids standards: Rutin (quercetin-3-rutinoside), apigenin-7-glucoside and Kaempferol (kaempferol-3-rutinoside). Samples content were expressed as µg g^{-1} dry weight and derived using a known concentration of standard and sample peak areas.

Statistical analysis: All analyses were performed in triplicate and data reported as Mean±Standard Deviation (SD). Data were subjected to analysis of variance (one-way ANOVA) ($p<0.05$). Results were processed by Excel (Microsoft Office 2010) and SPSS Version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Effect of Thidiazuron (TDZ) and zeatin on callus response: Effect of different concentrations of TDZ and zeatin on gardenia callus response at the end of two subcultures was studied (Table 2). It was found that MS medium supplemented

Table 2: Effect of TDZ and zeatin on callus response at the end of two subcultures

	First subculture			Second subculture		
Treatments	Callus fresh weight (g)	Growth value	Callus color	Callus fresh weight (g)	Growth value	Callus color
G1	3.90±0.75	6.80	Creamy	4.30±0.20	7.60	Creamy
G2	5.33±0.35	9.66	Green	5.96±0.54	10.90	Green
G3	1.40±0.20	1.88	Creamy slight green	1.70±0.52	2.40	Creamy green
G4	5.02±0.08	9.04	Creamy green	5.72±0.47	10.40	Creamy green
G5	5.00±0.2	9.00	Green	5.60±0.32	10.20	Green
G6	1.13±0.11	1.26	Creamy	1.16±0.29	1.32	Creamy
G7	4.15±0.32	7.30	Creamy	4.73±0.43	8.46	Creamy
G8 (control)	4.21±0.70	7.42	Creamy green	4.90±0.47	8.80	Creamy green

Each value consist of Mean±Standard Error (n = 3)

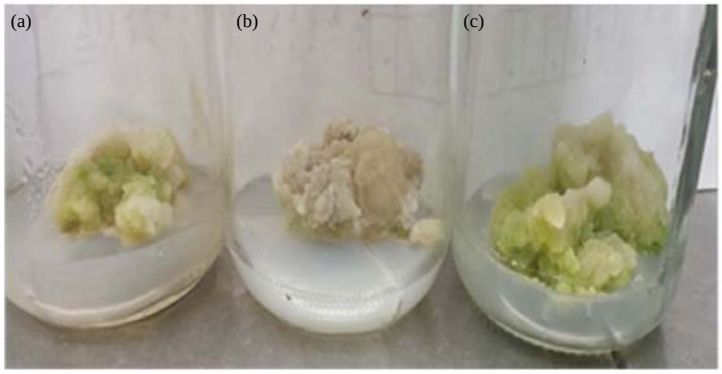

Fig. 1: Callus response on MS supplemented with A: 4 mg L^{-1} Zeatin, B: 2 mg L^{-1} Zeatin and C: 4 mg L^{-1} TDZ after two subcultures

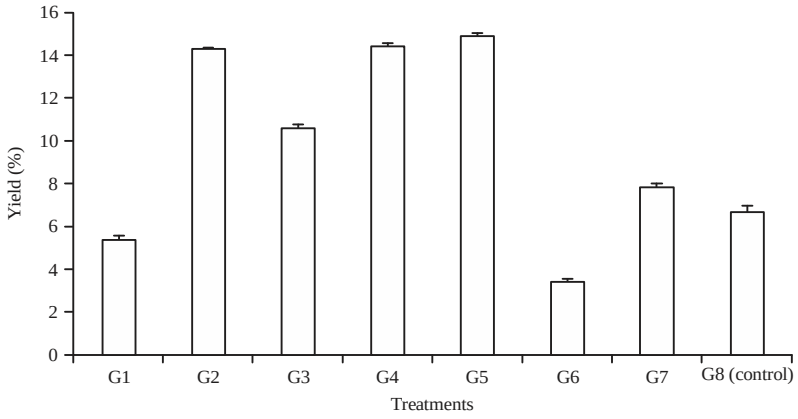

Fig 2: Yield extracts percentage of Gardenia callus grown on different culture media
Each value consist of Mean±Standard Error (n = 3)

with 4 mg L^{-1} TDZ (G2) gave the highest callus fresh weight followed by using 2 mg L^{-1} zeatin (G4) then 4 mg L^{-1} zeatin (G5). Subsequently the highest growth value and friable green or creamy green callus. This trend was observed also in the second subculture (Fig. 1). While, using high concentration (6 mg L^{-1}) from either TDZ or zeatin gave the lowest callus fresh weight and subsequently the lowest growth value with creamy or creamy green friable callus. It is well known that, TDZ and zeatin are considered as cytokinins which are a class of plant growth substances that promote cell division. They are involved in cell growth and differentiation. There are two types of cytokinins, adenine type like zeatin and phenyl-urea type like TDZ[11]. Most adenine-type cytokinins are synthesized in plants while, no phenylurea cytokinins have been found in plants. Gairi and Rashid[12] reported that using a medium containing 10 µM from TDZ or BA resulted in regeneration of somatic embryos and shoots in 30% of the non-responsive caryopses of rice cultures within 10-15 day. On the other hand, Konate *et al.*[13] reported that using The combination of 2,4-D with four cytokinins (Benzyl adenine, Kinetin, TDZ and zeatin) resulted in reduction of both callus formation rate and cell proliferation in *Bambara groundnut* (African crop).

Assessment the yield extracts percentage of callus cultures: Yield extract percentage (w/w) was determined for Gardenia callus grown on MS-media containing different concentrations (2, 4, 6 mg L^{-1}) either of TDZ or zeatin compared with control treatment (G8) as presented in Fig. 2. Callus cultured on media with 4 mg L^{-1} zeatin (G5) recorded the highest yield percentage, followed by media with 2 mg L^{-1} zeatin (G4) then media with 4 mg L^{-1} TDZ (G2), while media containing 6 mg L^{-1} zeatin (G6) gave the minimum value of yield extract percentage. Callus cultures which grown on media fortified with 6 mg L^{-1} TDZ (G3), 2 mg L^{-1} zeatin+4 mg L^{-1} TDZ (G7), 2.5 mg L^{-1} TDZ+0.2 mg L^{-1} IAA (G8 control), 2 mg L^{-1} TDZ (G1) and 6 mg L^{-1} zeatin (G6) showed different values of the yield extract percentage. The current investigations revealed that each of TDZ and zeatin separately appeared variation in terms

Table 3: Total phenolic content for callus extracts of gardenia

Treatments	Total phenolics (mg GAE/100 g FW of callus)
G1	161.13±0.882
G2	249.38±0.882
G3	240.07±0.577
G4	257.22±0.762
G5	268.33±0.934
G6	50.00±1.154
G7	220.00±1.763
G8 (control)	184.25±1.154

Each value consist of Mean±Standard Error (n = 3)

of the yield extract percentage and the gradually high of TDZ and zeatin concentrations such 2 and 4 mg L^{-1} increased the yield extract percentage, in contrast 6 mg L^{-1} of each decreased the yield extract percentage. Therefore, it could be concluded that the optimum concentration of both of TDZ and zeatin to enhance yield extract percent of Gardenia callus extracts occurred at 4 mg L^{-1} of each substance. It is clearly observed that, both of TDZ and zeatin had frequently enhanced the extraction yield percentage, this achievement comparable agree with the obtained results by those of Huetteman and Preece[14], who proved that TDZ is a potent cytokinin to induce callus in woody explants. Also, Liu *et al.*[15] increased hypericin and clustering shoots in *Hypericum perforatum* on medium supplemented with 2.27 μM TDZ. Likewise, added TDZ to solidified media in culture *Gardenia jasminoides* for helping boost callogenesis[16]. Although, the authors in this study indicated to a considerable attention for addition zeatin in media more than TDZ, where zeatin respond favorable result for yield extraction percentage more than TDZ. Subsequently in order to elevate the percentage of yield extract, zeatin is recommended as the most appropriate cytokinin to promote this percent from callus cultures of Gardenia at 4 mg L^{-1} concentration followed by TDZ at the same concentration[17].

Total phenolics accumulation of Gardenia callus cultures: The content of total phenols were determined in mg/100 g fresh weight of Gardenia callus cultures using Folin-Ciocalteu reagent and were calculated by expression as Gallic Acid Equivalent (GAE). To estimate the phenols of callus, eight extracts were derived from callus cultures which grown on MS-medium supplemented with TDZ and zeatin in different concentrations (2, 4 and 6 mg L^{-1}) compared with control treatment as shown in Table 3. Among the eight extracts, the treatment of 4 mg L^{-1} zeatin (G5) represented a remarkable total phenols accumulation followed by the treatment of 2 mg L^{-1} zeatin (G4). While, it was recorded that the phenols content of the treatments of 2.5 mg/TDZ+0.2 mg L^{-1} IAA (G8 control) and 2 mg L^{-1} TDZ (G1) with recording the lowest value in the treatment of 6 mg L^{-1} zeatin (G6). These investigations verified that, phenols amount decreased with the increment of TDZ and zeatin concentrations more than 4 mg L^{-1} of each. The most used treatments of both of TDZ and zeatin recorded distinguish values in phenols accumulation. These obtained results unveiled the potentiality to assume that Gardenia callus is a promising source of phenols constituents. These results are confirmed by those of Lamien-Meda *et al.*[16], who found 298.50±9.25 and 656.83±10.91 (mg GAE/100 g dw fruit) total phenolic content of *Gardenia erubescens* methanol and acetone extracts, respectively, Sarmah and Baishya[18], who recorded 96.85±1.80 μg total phenols in *G. jasminoides* (leaf) and 89.43±0.33 μg total phenols in *Gardenia jasminoides* (bark), Vindhya *et al.*[19], who estimated phenols content in three different extracts from leaf of from Gardenia latifolia Ait using three solvents, petrolium ether, ethyl acetate and ethanol recording 24.764, 35.595 and 45.856 μg phenols, respectively and Vindhya and Leelavathi[20], who extracted phenols from both of petrolium ether extract (19.813 μg), ethyl acetate extract (32.385 μg) and ethanol extract (39.735 μg) of *G. gummifera* Linn. Hence, it was observed that the results in Fig. 2 are associated with results in Table 2, where the highest percentage of the yield extraction and the highest content of total phenols were occurred at the same treatment (4 mg L^{-1} zeatin). As well as the least percentage of the yield extraction and the least content of total phenols (were achieved at the same treatment (6 mg L^{-1} zeatin). Subsequently, it is noted that all the treatments of zeatin and TDZ gave percentage of the yield extraction proportional to the total phenols content at the same concentrations. These investigations are in accordance with those of Anokwuru *et al.*[21], who reported that leaves of *Acalypha wilkesiana* and bark of *Azadirachta indica* with the highest phenols content also had the highest yield extraction. Overall, it could be advised to use the treatment of 4 mg L^{-1} zeatin in gardenia callus cultures to enhance their yield extraction extend to increase their total phenols content, because of the prominance of phenolic compounds. Polyphenols known as major bioactive phytochemicals that proved effectiveness in the chronic diseases prevention such as cancers, heart diseases and diabetes due to their scavenging of the free radical.

DPPH radical scavenging assay for gardenia callus cultures extracts: The DPPH test is a good *in vitro* method depend on the antioxidant efficiency assessment of plant crude extracts, based on conversion the free radical 2,2-diphenyl-1-picrylhydrazyl to stable diamagnetic molecule

Table 4: Effect of different concentrations of TDZ and zeatin on free radical scavenging capacity of DPPH (%) for Gardenia callus extracts

	Scavenging activity (%)	
Treatments	0 time	1 h
G1	46.05±0.017291	63.11±0.006006
G2	91.03±0.002358	93.08±0.001311
G3	82.94±0.002001	91.86±0.003092
G4	87.78±0.004361	89.67±0.00173
G5	89.67±0.002358	91.15±0.00196
G6	53.23±0.002367	76.16±0.001311
G7	62.13±0.003364	85.85±0.01037
G8 (control)	82.67±0.002733	91.56±0.003607

Each value consist of Mean±Standard Error (n = 3)

Table 5: Total flavonoids content for callus extracts of gardenia grown on different culture media

Treatments	Total flavonoids (µg QE/100 g FW of callus)
G1	1049.63±0.0088
G2	2016.00±0.1202
G3	1120.50±0.0033
G4	2222.22±0.1155
G5	2703.33±0.0882
G6	1141.18±0.0058
G7	1073.33±0.0058
G8 (control)	1356.30±0.0882

Each value consist of Mean±Standard Error (n = 3)

diphenyl picryl hydrazine by reduction using an antioxidant compound or various plant extracts as hydrogen donors, through change the color from purple to yellow in a short time[22]. Recently experiments were conducted to assess the antioxidant potential of several callus extracts of Gardenia plant, antioxidant activity were analyzed by the ability of Gardenia callus extracts on reduction the stable, purple-colored radical; DPPH· into the yellow-colored; DPPH·-H. Results in Table 4 show an increment magnitude in reduction of DPPH from time 0-1 h of reaction, indicated to the high antioxidant capacity donated by these particular extracts as the following increased order: 2 mg L^{-1} TDZ (G1) <6 mg L^{-1} Zeatin (G6) <2 mg L^{-1} Zeatin+4 mg L^{-1} TDZ (G7) <2 mg L^{-1} Zeatin (G4) <4 mg L^{-1} Zeatin (G5) <2.5 mg L^{-1} TDZ+0.2 mg L^{-1} IAA (G8 control) <6 mg L^{-1} TDZ (G3) <4 mg L^{-1} TDZ (G2) recording at 1 h from reaction. It is clear that the extracts from callus grown on medium containing 4 and 6 mg L^{-1} TDZ were more efficiency to reduce the stable, purple-colored radical; DPPH· into the yellow-colored; DPPH rather than the extracts from callus grown on medium supplemented with 2 mg L^{-1} TDZ (G1) which had poor performance. From these results it could be concluded that TDZ was more influential than zeatin as inducer the efficiency of the extracts for donating hydrogen atom to improve their antioxidant capacity. The DPPH experiment was indicated to the ability of extracts for donating hydrogen atom[8]. The present work in this part of study recommended adding TDZ to MS-medium for enhancement the efficiency of the extracts as antioxidant effective. These obtained findings are in harmony with data published earlier[23], who reported that TDZ has an efficient role in plant cell and tissue culture, response of TDZ applications in various plant species exhibited vast array of physiological and biochemical responses, TDZ modify directly or indirectly the endogenous plant growth regulators to produce reactions in cell or tissue for its division or regeneration, other modifications may be induced in energy levels, transport and assimilation, cell membrane and nutrient absorption.

Determination of total flavonoids: Total flavonoids content of methanolic extract from *Gardenia* callus with different concentrations of TDZ and zeatin are shown in Table 5. Methanolic extract from *Gardenia* callus cultured on 4 mg L^{-1} zeatin (G5) had the highest total flavonoids and followed by 2 mg L^{-1} zeatin (G4) compared with other TDZ concentrations alone or in combination with zeatin. On other hand, TDZ at 4 mg L^{-1} (G2) showed the highest flavonoids content compared with other TDZ concentrations alone or in combination with zeatin. Miliauskas *et al.*[24] observed that the amount of flavonoids in 12 medicinal and aromatic plant extracts showed only low correlation with the total amount of phenolics. Chen *et al.*[25] found that *Camellia sinensis* (GABA tea) had a low level of flavonoids.

Determination of phenols content by High Performance Liquid Chromatography (HPLC): The accumulation of phenols was higher in the callus obtained on TDZ than callus obtained on Zeatin (Table 6). Treatment G2 (4 mg L^{-1} TDZ) was observed the highest accumulation of chlorogenic, rosmarinic and cinnamic as well as total phenols. The levels of total phenols were observed to be higher in the callus obtained in 4 or 6 mg L^{-1} TDZ followed by callus obtained in zeatin (2 mg L^{-1}) plus TDZ (4 mg L^{-1}) (G7) treatment. Zeatin treatments were observed to accumulate vanillic and ferulic acids, which didn't accumulated with TDZ treatments. These results are in conformity with Nitnaware *et al.*[26]. They found that *Phyllanthus amarus* callus culture on higher concentrations of TDZ was inhibitory for accumulation of lignan.

Determination of flavonoids content by High Performance Liquid Chromatography (HPLC): Three flavonoids standards, rutin, apigenin-7-glucoside and kaempferol, were detected in different callus cultured obtained from the different treatments (Table 7). The highest rutin content was observed with G2 treatment (4 mg L^{-1} TDZ). Also, the same

Table 6: Determination of phenols content in gardenia callus cultured on different concentrations of zeatin and TDZ by HPLC

Treatments	Chlorogenic (μg g^{-1})	Vanillic (μg g^{-1})	Ferulic (μg g^{-1})	Rosmarinic (μg g^{-1})	Cinnamic (μg g^{-1})	Total phenols (μg g^{-1})
G1	0	0	0	0	12.96	12.96
G2	123.24	0	0	322.14	278.22	723.6
G3	0	0	0	89.7	215.55	305.25
G4	93.57	7.29	14.64	105.39	0	220.89
G5	23.7	21.09	7.05	39.69	0	91.53
G6	27.45	18.05	6.86	0	0	52.36
G7	108.66	28.01	21.04	0	63.54	221.25
G8 (Control)	27.45	0	0	23.34	84.27	135.06

Each value consist of Mean±Standard Error (n = 3)

Table 7: Flavonoids determination in gardenia callus cultured under different concentrations of zeatin and TDZ by HPLC

Treatments	Rutin (μg g^{-1})	Apigenin-7-glucoside (μg g^{-1})	kaempferol (μg g^{-1})	Total flavonoids (μg g^{-1})
G1	14.88	0.00	0.00	14.88
G2	287.76	110.49	10.38	408.63
G3	89.49	0.00	4.53	94.02
G4	18.03	107.70	0.00	125.73
G5	21.60	129.86	0.00	151.46
G6	26.80	0.00	0.00	26.80
G7	40.59	0.00	0.00	40.59
G8 (Control)	136.80	0.00	0.00	136.80

Each value consist of Mean±Standard Error (n = 3)

treatment showed the highest total flavonoids contents. Apigenin-7-glucoside was accumulated only with 4 mg L^{-1} zeatin, 2 and 4 mg L^{-1} TDZ. Interestingly, kaempferol was detected with 4 and 6 mg L^{-1} zeatin, whereas, it doesn't detected in any other treatments. In this regards, Smetanska[27] investigate that change in the type and concentration of growth regulator may drastically reduce or increase product accumulation in *in vitro* cell culture.

CONCLUSION

This study was successed to establish a suitable and effective protocol for callus proliferation of *Gardenia jasminoides* in purpose to accumulate an important phenolic and flavonoids. HPLC analysis recommended that TDZ is more effective in accumulation of individual phenolic and flavonoid than Zeatin.

SIGNIFICANCE STATEMENTS

This study discovered the possibility for production of flavonoids and phenolics from *in vitro* callus culture of *Gardenia jasminoides*. That can be useful for using callus culture of *Gardenia jasminoides* as an alternative source to study and produce these compounds.

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