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INFLUENCE OF PLANT GROWTH REGULATORS ON CALLUOGENESIS AND SECONDARY METABOLITES PRODUCTION IN *BOUGAINVILLEA SPECTABILIS* WILLD. CULTURES

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ABSTRACT

The influence of plant growth regulators (PGR) on establishment of callus cultures of *Bougainvillea spectabilis* Willd., from different explants, was investigated. Callus cultures were initiated from shoot tip and flower explants of the field plant. Yellowish green, friable and non-differentiated callus cultures were established on MS basal medium supplemented with different concentrations of 0-11.10µM benzylaminopurine (BAP) in combination with 0-26.90µM naphthalene acetic acid (NAA) in the presence of vitamin E (5mg/l) as antioxidant. The best callus cultures showing 100% survival, were afforded by shoot tip explants on media 15 (MS+2.22µM BAP+2.69 µM NAA) & 24 (MS+4.44µM BAP+26.90 µM NAA) and flower explants on medium 25 (MS+11.10µM BAP). The highest moisture contents were observed in shoot tip cultures on medium 11 (MS+1.11µM BAP+13.43 µM NAA) and flower cultures on medium 23 (MS+4.44µM BAP+13.43µM NAA). The highest polyphenolic content was observed in shoot tip cultures on medium 15 and in flower cultures on medium 23, also they showed highest antioxidative activity, thus, they were chosen for further phytochemical investigation. HPLC/ESI-MS analysis of ethanolic extracts of both callus cultures compared to the field plant showed that they were qualitatively and quantitatively different. This is the first report concerned with the investigation of secondary metabolites in different callus cultures of *Bougainvillea spectabilis* Willd.

KEYWORDS: *Bougainvillea spectabilis* Willd, calluogenesis, Antioxidant, HPLC/ESI-MS, polyphenols.

INTRODUCTION

Bougainvillea is a genus belonging to family Nyctaginaceae (Four-O'Clock family) and comprising 18 species. Members of this genus are native to South America,[1] widely cultivated in tropical and subtropical areas of the world in borders and gardens. Bougainvillas are appraised as decorative plants because of their lovely blossoms, high variation in type of foliage, production of many flowering inflorescence on one plant and continuous blooming of flowers with short production cycle.[2] The most horticulturally important species are *Bougainvillea spectabilis* Willd., *B. glabra* Choisy, and *B. peruviana* Humbo. and Bonapl.[3]

Bougainvillea spectabilis Willd. is a large climber with distinctive thorns and hairs on stems and large ovate leaves. The flowers are small, cream coloured, and having large curved thorns, while the bracts are red, dark pink or purple and appear up and down the branches.[4]

The traditional multiplication of *Bougainvillea spectabilis* Willd. is very difficult as it does not produce seeds, thus tissue culture technique was adopted as a quick and efficient tool for mass propagation. On reviewing the current literature, few reports were traced[5-9] concerned with increasing the propagation through stimulation of rooting and regeneration of the plant via tissue culture techniques. Fewer authors were concerned with the *in vitro* micropropagation through callus induction using shoot tip explants[10,11] and nodal explants[12] Therefore, the present investigation aimed to establish different callus cultures through selection of proper explants and suitable plant growth regulators concentrations. The study also intends to evaluate the antioxidant activity, as well as, assessing their phytochemical constituents of the different callus cultures compared to that of the field plant.

MATERIAL AND METHODS

Plant material

Fresh shoot tips, stems, leaves and flowers of *Bougainvillea spectabilis* Willd. were obtained from 4 years old plants cultivated in the Experimental Station of Medicinal Plants, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Giza, Egypt, during the flowering stage from March to July (2010 - 2011). The plant was kindly authenticated by Mrs. Thérèse Labib, consultant of Orman Botanical Garden, Giza, Egypt and its identity was verified by Dr. Mohamed El Gebaly (Plant Taxonomy and Egyptian Flora Department,

National Research Center, Dokki, Giza). Voucher samples are kept at the Museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Material for tissue culture

Culture medium

Murashige and Skoog (MS) basal medium; available as powdered medium (Duchefa, Netherlands), Sucrose (Adwic, ARE), PGR as BAP and NAA (Sigma chemicals, USA), dilute HCl and/or KOH solutions for pH adjustment, prepared according to EP 2005, Agar (Oxoid Bacteriological Agar No.1, Oxoid ltd, UK), vitamins C and E (Sigma chemicals, USA).

Chemicals for sterilization

Savlon: antiseptic solution containing 0.3% chlorohexidine gluconate & 3% cetrimide (Johnson & Johnson, Egypt); Clorox: 1.5% sodium hypochlorite (Clorox company, Egypt).

Preparation of culture medium

MS basal medium was supplemented with PGR and 3% sucrose. The pH of the medium was adjusted to 5.7-5.8, then it was solidified with 0.6% agar and autoclaved at 121°C, 1.1 kg/cm^2.

Establishment of callus cultures

Different explants (shoot tips, stems, leaves and flowers) of *Bougainvillea spectabilis* Willd. were excised from the field plant, sterilized using 10% savlon solution followed by 30% clorox solution. Then the explants were rinsed three times with sterile distilled water in a laminar flow to remove sterilants and cultured on sterile MS basal medium supplemented with the combination of BAP (cytokinin) and NAA (auxin) which was reported by Tariqshah *et al*. (2006)[10] and Duhoky and Al-Mizory (2014)[12] to induce callus cultures from *Bougainvillea spectabilis* Willd. explants. The concentrations used from BAP and NAA were: (0-11.1µM) and (0-26.90µM), respectively. In each condition, 32 jars were prepared, each contained 5 explants of about 0.5cm in length. The cultures were incubated at 21˚C ±2˚C, under a 16h light/8h dark with a light intensity of 1500-2000 lux.

Material for phytochemical study

Reference authentic material

Gallic acid (Sigma chemicals, USA) for determination of total polyphenolic activity. Silymarin (Sigma chemicals, USA) for assessing i*n vitro* antioxidant activity.

Chemicals and solvents

Folin-Ciocalteu reagent; 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma chemicals, USA); n-hexane; absolute ethanol; methanol; acetonitrilie and formic acid. (Labscan analytical sciences, Poland).

Preparation of plant extracts for phytochemical study

The dried moderately coarse powdered callus cultures and the field plant (stems and flowers) were separately defatted with n-hexane then exhaustively extracted with ethyl alcohol (70%). The solvents were removed under reduced pressure and finally dried in a desiccator. The dried residues were weighed and kept in amber light tightly closed bottles then stored at low temperature in a refrigerator to be used in the study.

Apparatus

UV spectrophotometer (Unicam, UK)**;** HPLC/ESI-MS system consisted of electrospray ionization (ESI) interfaced Bruker Daltonic Esquire-LC Amazon SL ion trap mass spectrometer (Bremen, Germany) and Dionex ultimate 3000 (Germany) composed of a quaternary pump with an on line degasser, a thermostatted column compartment , a photodiode array detector(DAD), an autosampler and Hystar software.

Determination of survival percentage

The survival percentage of callus cultures was calculated at the end of the incubation period, as the number of the callused explants/100 explants. The values represented were mean values of 3 replicates. Results are recorded in Table (1).

Determination of moisture content

The calli were collected after 3 weeks, at which maximum growth was attained, washed with distilled water to remove the adhering media, dried on filter paper and weighed. The calli were then dried in an oven at 40˚C and reweighed until constant weight was reached. The weights used were mean values of 3 records. Then the moisture percentage (%) was calculated as follows.

$$\text{Moisture \%} = (FW - DW/FW) \times 100$$

Where: FW= fresh weight in grams, DW=dry weight in grams. Results are recorded in Table (2).

Determination of total polyphenolic content

A weight of 50 mg dried callus and field plants (powdered stems and flowers) was separately extracted with 100 ml of 70% ethanolic solution, for the determination of total polyphenolic content using Folin-Ciocalteu reagent (Mruthunjaya and Hukkeri, 2008).[13] From the stock ethanolic extracts, 1 ml of each solution was separately taken into a 10 ml volumetric flask, 2 ml of Folin-Ciocalteu reagent was added then 2 ml of sodium carbonate solution (4%) and the volume was made up to final volume with distilled water, the contents were mixed and allowed to stand for 30 minutes. The absorbance of the solutions was measured at 765 nm using a spectrophotometer (Unicam, UK). The total polyphenols of the different samples were calculated from gallic acid calibration curve plotted (0.4- 4 µg/ml) by using the same procedure and were expressed, as gallic acid equivalents (GAE) in mg/g of dried plant. (Calibration curve equation was calculated as Y= 232.58 X + 0.0762, R^2 = 0.9344). Results are recorded in Table (2).

Free radical scavenging activity

The antioxidant activity was evaluated adopting the radical scavenging DPPH method of Adib *et al*. (2010)[14] with slight modifications. The ethanolic extracts (70%) of the different cultures and the field plant (stems and flowers) were separately prepared yielding a concentration of 5 mg/ml and 0.3 mg/ml, respectively and kept for the analysis of the antioxidant activity. Aliquots (0.5, 1 &1.5 ml) of each extract were separately mixed with 3 ml of a freshly prepared methanolic solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, 20mg/l), completed to 5 ml by methanol and left to stand for 30 minutes in the dark. The absorbance of samples was determined using a spectrophotomer at 517 nm and a control solution containing all reagents except the test sample was prepared. Silymarin, a potential antioxidant, was used as a positive control. All the experiments were carried out in triplicate. The radical-scavenging activity was calculated as a percentage of DPPH discoloration (I%) using the following equation.

$$I\ (\%) = (Abs_{control} - Abs_{sample}/Abs_{control}) \times 100$$

Where: Abs_{sample} = absorbance of tested extracts/reference material, $Abs_{control}$ = absorbance of control solution.

Finally, the inhibition percentages were plotted versus the respective concentrations (µg/ml) used and IC_{50} (the concentration needed to cause 50% inhibition) was calculated from the graph.

HPLC/ESI-MS analysis

Total ethanolic extracts of shoot tip and flower callus cultures together with the field plant (stems and flowers) of *Bougainvillea spectabilis* Willd. were analyzed through HPLC/ESI-MS system. HPLC was achieved using a Dionex ultimate 3000 equiped with Dionex bounded silica C_{18} column (150 mm x4.6 i.d., 3 µm) and a photodiode array detector; scanning from 200 to 400nm and electrospray ionization (ESI) interfaced Bruker Daltonic Esquire-LC Amazon SL ion trap mass spectrometer (ion spray voltage was 45 V and spray temperature was 350˚C). All analyses were performed at 30˚C with a flow rate of 0.5 ml/min. The sample volume injected was 5 µl. The mobile phase consisted of solution A (water) and solution B (acetonitrile with 1% formic acid), in a ratio 12% solution A: 88% solution B (isocratic elution). Nitrogen gas was used as nebulizing gas at a pressure of 65 PSI and the flow was adjusted to 11 l/min. The heated capillary was maintained at 350˚C. Analysis was done at both positive and negative ion modes in the range *m/z* 200 - 2000. Firstly, a full-scan mass spectrum to acquire data on ions in the *m/z* range, then an MS/MS experiment was performed in which data-dependent scanning was carried out on deprotonated molecules of the compounds. The data of the identified compounds was compiled in Table (4).

RESULTS AND DISCUSSION

Effect of different concentrations and combinations of BAP and NAA on growth and morphological characters of *Bougainvillea spectabilis* Willd. callus cultures. was investigated A major obstacle that hindered growth in all studied callus cultures was the browning and necrosis of callus tissues (Fig. 1a), that could be referred to the production of excessive polyphenols as reported by Naz *et al*. (2008)[15], while Hyser & Moft (1980)[16] emphasized the relationship of phenolic content with the rate of browning of the cultures. Therefore, addition of antioxidants like vitamin C[17] and vitamin E,[18] were separately added to the media, at different concentrations, to overcome the browning effect ((10-100 mg/l) and (1-5 mg/l), respectively). Vitamin C was shown to reduce significantly the browning effect of the initiated callus as reported by Habibi *et al.* (2009)[19] but at the same time the cultures did not survive (Fig. 1b), so vitamin C was excluded from the experiment. On the other hand,

supplementation of vitamin E (5mg/l) optimized callus growth without browning (Fig.1c), thus supporting the use of vitamin E as antioxidant in our study.

Figure 1.a. Browning of explants during the induction of the callus cultures

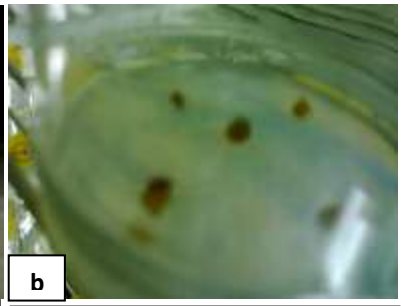


Figure 1.b. Inhibition of browning and growth in callus cultures after addition of vitamin C

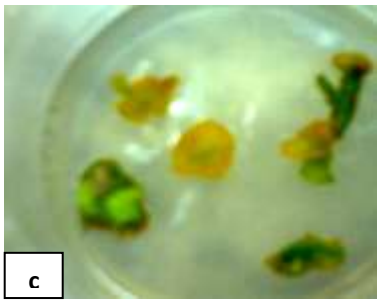


Figure 1. c. Optimized callus growth without browning after addition of vitamin E.

The callus formation and survival percentage of *Bougainvillea spectabilis* Willd. cultures varied with the different concentrations of BAP and NAA supplemented to MS basal medium. Table 1 revealed that the successful callus cultures were produced by shoot tip explants on media number: 11, 15, 22, 23, 24 &26 and flower explants on media number:13, 23, 24, and 25 (Table 1, Fig. 2 (a-c) while, other explants as stems and leaves showed no callus induction. All produced calli were yellowish green in colour, friable and non-differentiated (Fig. 2a-c). The callus cultures reached maximum growth after 3 weeks and some of them showed regeneration (Fig. 2d) after 4 weeks. Table (1) illustrated that the best results (100% survival percentage) were afforded by shoot tip explants on media number 15 & 24 and flower explants on medium number 25. The remaining successful callus cultures (on media no. 11, 13, 22, 23&26), showed less survival percentages varied from 20-80%. Our results are in accordance with those obtained by Tariqshah *et al* (2006)[10] who reported that the shoot tip explants produced profuse callus on medium 15 and showed no callus initiation on medium 19. Similarly, positive results of callus induction on medium 26 were in agreement with Duhoky and Al-Mizory (2014)[12] except for their use of nodal explants. That was the first time to establish callus cultures of *Bougainvillea spectabilis* Willd. from the flower explants.

Table (1): Effect of different concentrations and combinations of BAP and NAA on the survival percentage and callus formation of *Bougainvillea spectabilis* Willd. cultures.

Media No.	BAP (µM)	NAA (µM)	Callus cultures					
			Shoot tip		Flower		Stem	Leaf
			Callus formation	Survival %	Callus formation	Survival %	Callus formation	Callus formation
1	0	0						
2	0	1.34	-	-	-	-	-	-
3	0	2.69	-	-	-	-	-	-
4	0	5.37	-	-	-	-	-	-
5	0	13.43	-	-	-	-	-	-
6	0	26.90	-	-	-	-	-	-
7	1.11	0	-	-	-	-	-	-
8	1.11	1.34	-	-	-	-	-	-
9	1.11	2.69	-	-	-	-	-	-
10	1.11	5.37	-	-	-	-	-	-
11	1.11	13.43	++	63.5±3.50	-	-	-	-
12	1.11	26.90	-	-	-	-	-	-
13	2.22	0	-	-	++	60±1.00	-	-
14	2.22	1.34	-	-	-	-	-	-
15	2.22	2.69	+++	100	-	-	-	-
16	2.22	5.37	-	-	-	-	-	-
17	2.22	13.43	-	-	-	-	-	-
18	2.22	26.90	-	-	-	-	-	-
19	4.44	0	-	-	-	-	-	-
20	4.44	1.34	-	-	-	-	-	-
21	4.44	2.69	-	-	-	-	-	-
22	4.44	5.37	++	60±2.00	-	-	-	-
23	4.44	13.43	+	40±1.00	++	80±1.00	-	-
24	4.44	26.90	+++	100	++	60	-	-
25	11.10	0	-	-	+++	100	-	-
26	11.10	1.34	±	20±1.00	-	-	-	-

The results are mean values ± SD (standard deviation); (+++): Profuse callus; (++): Moderate callus; (+): Little callus; (±): very few; (-): unsuccessful cultures,

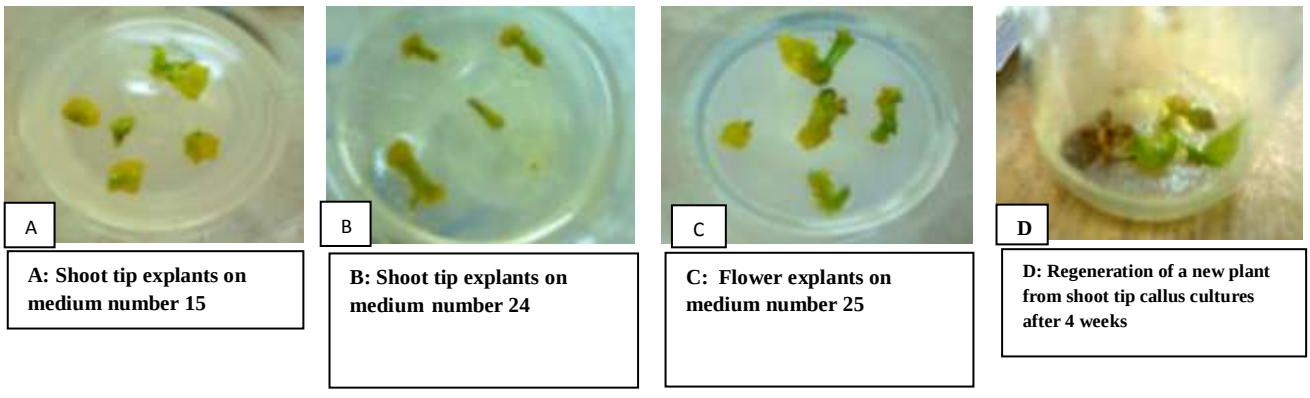


Figure 2. (A-D) The callus induction of different explants (100%)

Determination of moisture content

From Table (2), it is obvious that the moisture contents were comparable to each other in the shoot tip callus cultures and ranging from 87.56 - 94.32%. Likewise, the flower callus cultures varied from 86.77 - 92.72%.

Effect of different concentrations and combinations of BAP and NAA on production of polyphenolic content in *Bougainvillea spectabilis* Willd. callus cultures.

The total polyphenolic content of the different callus cultures were estimated as GAE (mg/g on dry weight basis) and the calibration curve indicated good linearity (R^2 = 0.9344, Y= 232.58 X + 0.0762). Table (2) revealed that the highest polyphenolic content, among shoot tip callus cultures, was observed with that on medium number 15 (63.60 ± 0.22 mg/g) and compared to the stems of the field plant (36.2± 0.05 mg/g), while the culture on medium 24 showed polyphenolic content (34.10 ± 0.84 mg/g) nearly similar to that of the stems. Likewise, the flower callus cultures on media 23 and 24 showed higher polyphenolic content (19.7 ± 0.34 and 12.4 ± 0.74 mg/g, respectively) than that of the flower of the field plant (11.5 ± 0.11 mg/g), while the culture on medium 25 was slightly lower (9.5 ± 0.2 mg/g).

Table (2): Moisture content and total polyphenolic content of different callus cultures of *Bougainvillea spectabilis* Willd.

Media No.	Moisture content (%)		Polyphenolic content (GAE mg/g)	
	Callus cultures		Callus cultures	
	Shoot tip	**Flower**	**Shoot tip**	**Flower**
11	**94.32 ± 0.05**	-	17.80 ± 0.01	-
13	-	86.77±0.10	-	2.92 ± 0.06
15	90.64 ± 0.18	-	**63.60 ± 0.22**	-
22	87.56 ± 0.05	-	3.20 ± 0.12	-
23	90.97 ± 0.12	**92.72 ± 0.07**	5.70 ± 0.33	**19.70 ± 0.34**
24	91.31± 0.08	87.50 ± 0.09	34.10 ± 0.84	12.40 ± 0.74
25	-	88.19 ± 0.05	-	9.50 ± 0.20
26	92.22 ± 0.05	-	15.20 ± 0.54	-
	Field plant		Field plant	
	Stem	**Flower**	**Stem**	**Flower**
	15±2.00	20±3.00	36.20 ± 0.05	11.50 ± 0.11

The results are mean values ± SD (standard deviation); (-) : unsuccessful cultures

Free radical scavenging activity

The antioxidant activities of *Bougainvillea spectabilis* Willd. callus cultures (expressed as inhibition percentages) and IC_{50} (µg/ml) values are listed in Table (3). It was clear that the

DPPH scavenging activity was dose dependant.The shoot tip callus cultures on medium 15 (IC_{50}=254.9 µg/ml) and flower callus cultures on medium 23 (IC_{50}=385.9 µg/ml) showed the highest antioxidant activity between all callus cultures under investigation. On the other hand, the stems of the field plant showed a higher activity (IC_{50}=27.5 µg/ml) than that of the standard drug; silymarin (IC_{50} = 31 µg/ml) and the flowers (IC_{50}=33.3 µg/ml). These results agreed with Jain *et al* (2012)[20] that reported that plant growth regulators play a vital role in secondary metabolites accumulation and the concentration of auxin and cytokinin individually or in combination significantly alters both the growth and secondary metabolites accumulation in culture cells.

Therefore, it could be concluded that shoot tip callus cultures on medium 15 and that of the flower on medium 23 showed high survival percentage, highest polyphenolic content and antioxidative activity compared to that of the corresponding field plant. Therefore, both callus cultures were chosen for further phytochemical study using HPLC/ESI-MS analysis to identify the compounds which may be responsible for the antioxidant activity.

Correlation between free radical scavenging activity and polyphenolic content

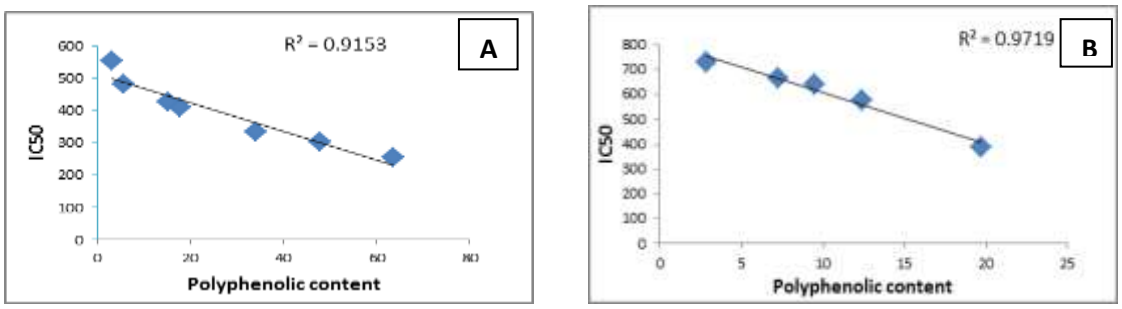
In our study, the correlation between the polyphenolic content (GAE mg/g) and the free radical scavenging activity (µg/ml) in both shoot tip (R^2= 0.9153 Fig. 3A) and flower callus cultures (R^2= 0.9719, Fig. 3B) was found to be high. These results confirm the positive correlation between the polyphenols present in *Bougainvillea spectabilis* Willd. callus cultures and their free radical activity.

Such Results are in agreement with those of Venkatachalam *et al* (2012)[21] who stated that the amount of phytochemical constituents and antioxidant activity exhibited a significant linear relationship in *Bougainvillea spectabilis* Willd. leaves and Pintathong *et al* (2012)[22] who reported that phenolic compounds represented in *Bougainvillea* spp. flowers had a strong contribution towards their antioxidant capacity.

Table (3): Free radical scavenging activity of *Bougainvillea spectabilis* Willd. callus cultures compared to that of field plant.

Media no.	Shoot tip callus cultures			Flower callus cultures		
	Concentration (µg/ml)	Inhibition (%)	IC_{50} (µg/ml)	Concentration (µg/ml)	Inhibition (%)	IC_{50} (µg/ml)
11	500	55	410.30	-	-	-
	1000	70				
	1500	94				
13	-	-	-	500	30	732.20
				1000	78	
				1500	91	
15	500	61	**254.90**	-	-	-
	1000	70				
	1500	95				
22	500	48	555.50	-	-	-
	1000	62				
	1500	72				
23	500	50	481.50	500	52	**385.90**
	1000	70		1000	78	
	1500	86		1500	90	
24	500	56	333.30	500	46	578.40
	1000	72		1000	67	
	1500	90		1500	80	
25	-	-	-	500	46	641.90
				1000	60	
				1500	73	
26	500	51	425.90	-	-	-
	1000	74				
	1500	87				
Field plant	**Stem**			**Flower**		
	30	50	**27.50**	30	48	**33.30**
	60	75		60	63	
	90	90		90	75	
Silymarin	10	34	31.00	10	34	31.00
	50	65		50	65	
	70	79		70	79	
	100	85		100	85	

(-): unsuccessful cultures



(A): shoot tip callus cultures **(B): flower callus cultures**

Figure 3. Correlation between free radical scavenging activity (IC_{50} µg/ml) and polyphenolic content (GAE mg/g dry weight basis)

HPLC/ESI-MS analysis

From the HPLC/ESI-MS analyses of the total ethanolic extracts of shoot tip and flower callus cultures, compared to that of the stems and flowers of the field plant, twenty six compounds were identified (Table 4). Identification was based on comparing the mass fragmentation pattern to that of the published data.

The analyses of callus cultures extracts showed that they were qualitatively and quantitatively different than that of the field plant. Sinapic acid, chrysoeriol, betanidine, dihydromyricetin, kaempferol and gomphrenin I were identified in all extracts of callus cultures and field plant in reasonable amounts, however their relative intensities were different in each extract.

In both shoot tip callus cultures and stems, myricetin was additionaly detected. Meanwhile, genestein rutinoside was detected in total ethanolic extract of shoot tip callus cultures while, catechin, isorhamnetin and hesperidin were identified in total ethanolic extract of stems.

This could explain the high radical scavenging activity of stems over shoot tip callus cultures due to the the presence of strong antioxidative polyphenols as myricetin (in slightly higher amounts)[37] due to its *ortho*-dihydroxy substitution in the B ring with the existence of 2,3-unsaturation which conjugates with the 4-keto group[38] and hesperidin which would enrich their radical scavenging activity over the shoot tip callus cultures due to the presence of its hydroxyl group on C-3' and a 4-8 double bond in conjugation with a C-4 ketone function together with the formation of hydrogen bonds between the ketonic oxygen and the hydroxyls at C-3 and C-5.[39]

Moreover, the detection of some polyphenols in trace amounts viz. catechin, isorhamentin, quercetin, 7-hydroxy-3'-methoxyflavone and kaempferol-7-O-diglucoside could produce synergistic interaction between antioxidants in the phenolic fraction in the stems.[40]

On the other hand, the total ethanolic extracts of flower callus cultures and flowers were characterized by the presence of different polyphenolic compounds in addition to the forementioned common compounds viz. catechin, isorhamentin, apigenin, and hesperidin.

Whereas, myricetin was detected in total ethanolic extract of flowers and not existing in the total ethanolic extract of flower callus cultures that supported its antioxidant activity over flower callus cultures. In addition to some minor polyphenols that were detected in the total ethanolic extract of flowers viz. quercetin, dihydromyricetin diglucoside, genestein rutinoside, eupatilin 7-O-β-D-galactopyranoside, kaempferol 3-O-hydroxyferuloyl diglucoside-7-O-glucoside and quercetin 3-O-feruloyldiglucoside 7-O-glucoside that played an important role in increasing its radical scavenging activity remarkably than that of flower callus cultures due to their synergistic activity.

Additionally, the flower extracts contained betalain pigments viz. betanidine, bougainvillein-V, gomphrenin I in higher amounts which are defined by Cai (2003)[41] and Wybraniec (2011)[42] for their strong antioxidant activity.

Table (4): HPLC/ESI-MS analysis of callus cultures extract compared to that of the field plant of *Bougainvillea spectabilis* Willd

No.	Rt (min)	λ_{max}	M-H	Adduct ($M+HCOO^-$)	Major MS^2 fragments	Identified compound	Relative intensity (%)				Ref.
							Sh. CC	Stem	Fl. CC	Flower	
1	0.20	240, 298, 328	223	269	207,208,214,220, 224	Sinapic acid	54	6	47	32	[23]
2	0.25	280	289	334	205, 232, 249, 273, 288	Catechin	-	5	60	49	[24]
3	0.28	-	359	406	200,299, 359, 360	Unknown	-	-	53	58	-
4	0.30	344	299	345	225,265, 266, 283	Chrysoeriol	63	9	59	100	[25]
5	0.34	520	432	342	258, 278, 325, 347,376	Betanidine	6	6	5	61	[26]
6	1.30	-	315	361	269,270,285, 299, 300	Isorhamnetin	-	8	53	58	[27]
7	2.00	250, 370	317	363	227, 245, 299, 302, 315	Myricetin	40	47	-	54	[28]
8	2.50	292	319	365	263, 302, 318, 320	Dihydromyricetin	5	4	61	42	[29]
9	3.20	269, 336	269	316	225, 253, 271	Apigenin	-	-	64	41	[24]
10	6.00	256, 372	301	347	229, 245, 257, 275, 285	Quercetin	-	1	-	4	[24]
11	7.10	-	267	313	226, 237, 254	7-hydroxy, 3' methoxyflavone	-	7	-	-	[27]
12	8.10	267, 366	285	331	213, 231, 241, 259, 284	Kaempferol	57	47	64	30	[24]
13	8.3	265, 345	593	639	286, 594	Kaempferol-3-O-rutinoside	2	-	3	14	[30]
14	8.7	538	549	595	389, 551	Gomphrenin I	85	1	18	12	[31]
15	9.6	336	643	690	301,320, 355, 482	Dihydromyricetin diglucoside	-	-	-	13	[32]
16	10.00	255, 345	609	654	271, 301, 373, 477	Rutin	12	1	12	1	[33]
17	10.20	540	712	757	388, 550, 712	Bougainvillein-V	-	-	-	5	
18	11.00	264, 366	609	655	286, 448	Kaempferol 7-O-diglucoside	-	2	-	-	[23]
19	12.00	344	607	653	299,444	Chrysoeriol-7-O-rutinoside	12	1	2	8	[25]
20	13.50	-	577	623	224, 251, 270, 431	Genestein rutinoside	11	-	-	12	[34]
21	13.80	284, 324	579	625	253, 271, 313, 459	Naringin	4	4	-	-	[30]
22	14.80	284, 326	609	654	301, 371	Hesperidin	-	31	13	1	[35]
23	15.10	-	797	843	343, 505, 651	Eupatilin-7-O-β-Dgalactopyranoside	-	-	-	6	[36]
24	17.1	268, 332	963	1008	285, 447, 609, 801	Kaempferol 3-O-hydroxyferuloyl diglucoside-7-O-glucoside	7	1	-	5	[23]
25	18.2	-	963	1008	301, 625, 801	Quercetin 3-O-feruloyldiglucoside 7-O-glucoside	5	-	-	4	[23]
26	19.5	338	663	708	302, 315, 320, 333, 477, 495	Methyl-dihydromyeicetin diglucoside	7	6	-	-	[32]
27	20.30	255, 266, 353	625	670	301, 463	Quercetin 3,7 diglucoside	1	3	-	-	[30]

Sh. CC: Shoot tip callus cultures; F CC: flower callus cultures; Ref. Reference; (-): not detected

CONCLUSION

Plant tissue cultures of *Bougainvillea spectabilis* Willd. could be useful as a novel and potential source of useful secondary metabolites mainly polyphenolic compounds. These polyphenolic compounds were present in higher amounts in the callus cultures than the corresponding field plant, especially the shoot tip callus cultures, that could be helpful in health promoting due to their antioxidant activity. Therefore, this technique affords a reliable, simple, rapid and efficient way for the production of these bioactive compounds and also ease their extraction and isolation when compared with extraction from complex whole plant. Also, the protocol described in this report is expected to contribute to the future studies in this plant species for large scale production of these biologically active plant metabolites from *in vitro* callus cultures.

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