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Determination of Phenolics and Flavonoids with Antioxidant Effect of *Brunfelsia Pauciflora* (Cham. & Schtdl) Benth through *in Vitro* Propagated Cultures

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Abstract
The present study considered the *in vitro* propagation of *Brunfelsia pauciflora* utilizing various cytokinins, as well the estimation of the antioxidant, total phenolics (TPs), and flavonoids (TFs) for shootlets of each treatment. The explants were sterilized with 0.2% mercuric chloride (20 min) and then cultured on Murashige and Skoog medium (MS) supplemented with three cytokinins; benzyl aminopurine (BAP), 6-Furfuryl-aminopurine (Kin), and isopentenyl adenine (2iP) at different concentrations. The highest shootlets number and fresh weight were obtained using 1.0 mg/L BAP, while shootlets length is increased using 2.0 mg/L kin. Additionally, significant numbers of leaves were observed for the plantlets supplemented with kin (1.0 & 3.0 mg/L) and 2iP (2.0 & 3.0 mg/L). The shootlets were rooted one month later with different strengths (full, ¾, ½, or ¼) on MS media without or with 1.0g/L activated charcoal (AC). Shootlet cultured on full-strength MS medium with AC gave the longest root, while the greatest numbers of roots were recorded using ¾ strength without AC. All the rooted shootlets were acclimatized on peat moss and recorded with the longest root length and greatest leaves and roots numbers. Alternatively, the methanolic extract of shootlets supplemented with 2iP (3.0 mg/L) yielded the maximum antioxidant effect (89.89 ± 0.28 mg $_{Trolox}$ /g $_{extract}$), TPs (57.574±0.82 mg $_{Gallic}$/g $_{extract}$), and TFs (46.146 ± 0.31 mg $_{Catechin}$/g $_{extract}$) compared with the control (MS free) and mother plant. In conclusion, the antioxidant potential was shown to be strongly correlated with the TFs and the number of leaves /shootlet.

Keywords: *Brunfelsia pauciflora*, *in vitro* propagation, phenolics, flavonoids, antioxidant

1. Introduction

Brunfelsia L. is a genus of flowering plants belonging to the family Solanaceae. Different *Brunfelsia* species have traditionally been used as an additive in the hallucinogenic drink "ayahuasca" by the indigenous cultures in the Ecuadorean Amazon region, often added to deepen the effects of the psychedelic beverage [1]. Certain *Brunfelsia* species are also used in the treatment of rheumatism, fever, cold, and arthritis, as well as acting as a tonic, laxative, antipyretic, anesthetic, and larvicidal agents [2, 3]. Like many other Solanaceae species, several *Brunfelsia* species contain alkaloids, anthocyanins, coumarins, and phenolics [4, 5].

Brunfelsia pauciflora (Cham. & Schltdl.) Benth. is one of 50 *Brunfelsia* species endemic to Brazil that has been ornamentally cultivated worldwide and become a popular garden and pot plant due to its large coloured flowers and pleasant fragrance [6, 7]. The surprisingly rapid colour changes of *B. pauciflora* flowers from deep purple on the day of opening to lavender and then fades to white within 3 days, suggesting its common name as "Yesterday–Today–Tomorrow" [8]. The colour changes in B. pauciflora flowers are due to the decomposition of anthocyanins and this process depends on the synthesis of novel proteins [9]. Phytochemically, Bertrand et al. [6] noticed that the diversity and quantities of the volatile constituents

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found in the mature white flowers are more than those of the young deep purple flowers of *B. pauciflora*.

Plant tissue cultures are considered as easy and rapid mass multiplication of diseases-free plants, large-scale revegetation, and biodiversity preservation [10]. Moreover, it is a way of augmenting the production of secondary metabolites which can be larger than that of the mother plants [11].

Plant growth regulators (PGRs) are important media components in determining the development pathway of plant cells [12]. Cytokinins are types of PGRs generally known to promote the formation of buds and the differentiation of shoots in micropropagation. Cell division mediating factors such as development shoot. The quality and quantity of PGRs play a major role in the ability to produce a particular in-vitro propagation [13]. The optimal growth of the tissues may vary for different plants according to their nutritional requirements, and tissues from diverse parts of plants may have different requirements for satisfactory growth [14]. Activated charcoal (AC) is commonly added to tissue culture media. Its effect may be due to establishing a darkened environment, adsorption of undesirable/inhibitory substances, adsorption of growth regulators and other organic compounds, or the release of growth-promoting substances present in or adsorbed by activated charcoal [15]. Peat moss is one of the most important constituents of mixture media due to its capacity in affecting plant growth either indirectly or directly. Indirectly, it improves the physical conditions of the media by enhancing aggregation, aeration, and water retention, thereby creating a suitable environment for root growth [16].

Recent research describes the *in vitro* propagation of *B. pauciflora* shoot through the effects of gamma irradiation on shooting, rooting, and acclimatization behaviours, chlorophyll, and carotenoid contents, as well as genetic fidelity [7]. Nevertheless, PGRs, especially cytokinins have significant effects on the synthesis of secondary metabolites such as phenolics and flavonoids which could be responsible for the antioxidant potential and help to discover safe antioxidants from natural sources rather than manufactured ones. Consequently, the current research describes the morphogenic responses of B. pauciflora explants to some cytokinins (BAP, Kin, and 2ip). The proliferated shoots were then rooted, and the regenerated plantlets acclimatized ex vitro. Additionally, the total phenolics, total flavonoids, and DPPH assay were evaluated for the aqueous methanol extracts of the *in vitro* propagated shootlets.

2. Experimental

2.1. Chemicals, standards, and instruments

Murashige and Skoog medium was purchased from Dutchiva Co., Germany. Cytokinins: -6-Benzylaminopurine (BAP), 6-Furfuryl-aminopurine (Kinetin; Kin), and isopentenyl adenine (2ip) were obtained from Sigma Scientific, Egypt. Activated charcoal (AC), mercuric chloride (MC), and tween-20 from Nasr lab, Egypt. Peat moss from Syker Agrarberatungs (SAB), Germany. Perlite from Harraz, Egypt, and Rezolix from Sumitomo chemical, India ltd. Methanol (HPLC grade) and ethanol were obtained from Fisher Scientific, UK. Folin-Ciocalteu's phenol reagent, aluminum chloride, and catechin were acquired from Merck Co., Darmstadt, Germany. DPPH (1, 1-diphenyl-2-picryl-hydrazil) and Trolox were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gallic acid from Oxford Lab Chem, India. A water distiller (Hamilton, Westwood Industrial Estate), water bath (Labtech), and rotary evaporator (HEIDOLPH 4000, Germany) have been used in laboratory works. UV-vis spectrophotometer (Jasco, V-730 UV-Vis, Japan) was used to record the absorbance of the TPs, TFs, and the DPPH assay.

2.2. Plant Material

Explants (nodal) of *B. pauciflora* were collected in the year 2020 from the samples grown at El-Zohria Garden, Horticulture Research Institute (HRI), Agricultural Research Center, Giza, Egypt.

2.3. In vitro propagated cultures

2.3.1. Explant source and disinfection

The nodal explants were soaked for 1 min in 70% ethanol solution under an aseptic condition in the laminar air-flow cabinet. After that, they were immersed in MC (0.2% *w/v*) with a few drops of tween-20 for 20 min followed by rinsing three times with sterile distilled water. The sterilized explants were cultured on MS medium-free growth regulator. After three weeks all survival and free-contamination explants were taken for the multiplication stage.

2.3.2. Culture medium and conditions

The culture medium was enriched with 30 g/L sucrose and 7 g/L agar. The medium was adjusted to pH 5.7±0.1 and autoclaved at 121°C and 1.2 kg/cm^2 for 20 min. The culture medium was incubated for one

week before the culture explants. The explants were placed vertically in 200 mL capacity glass containers containing a 25 mL medium. The cultures were incubated at 24±1° C under fluorescent illumination of 2000-2500 lux at 16h (daylight).

2.3.3. Shooting behaviour

In the multiplication stage, the culture medium under trials consisted of Murashige and Skoog [17] enriched BAP, Kin, or 2iP at concentrations of 0.0, 1.0, 2.0, or 3.0 mg/L for one month in three subcultures. In this treatment fifteen explants in three replicates were cultured. After the third subculture, the shootlet number per explant, shoot length (cm), number of leaves per shootlet, and fresh weight (g) were recorded.

2.3.4. Rooting behaviour

In rooting treatments, shootlets (1-2 cm length) were cultured on MS medium with different strengths (full, ¾, ½, or ¼) without or with AC (1.0 g/L). Fifteen shootlets in three replicates were used in each treatment for one month. The shootlet length (cm), number of leaves/shootlet, number of roots/ shootlet, and root length (cm) were recorded.

2.3.5. Acclimatization

The rooted shootlets were transferred to plastic pots containing peat moss, peat moss + sand (1:1 *v/v*), or peat moss + perlite (1:1 *v/v*) irrigated with a solution of Rezolix fungicide (0.2g/L) and covered by transparent polyethylene bags. Each treatment contained nine plantlets in three replicates. The acclimatized plantlets were kept in an acclimatized glass house for four weeks before transplanting-of-door, after that plantlet height (cm), leaf number/plantlet, root number/plantlet, and root length (cm) were measured.

2.4. Chemical investigation

2.4.1. Preparation of methanol extracts

The fresh samples of the mother plant, control, and all the treated shootlets were separately dried and crushed into fine powders. The powdered samples (2 g) were exhaustively extracted with 70% methanol (100 mL) three times. Each extract was filtered and evaporated at 40 °C to obtain a crude extract.

2.4.2. DPPH radical scavenging assay (antioxidant evaluation)

The eleven extracts were screened for their free radical scavenging evaluation using the DPPH assay according to the method reported by Hwang and Do Thi [18]. The final concentration was 200 µM for DPPH• and the final reaction volume was 3.0 mL. The absorbance was measured at 517 nm against a blank of pure methanol after 60 min of incubation in a dark condition. Percent inhibition of the DPPH• scavenging activity was calculated by the following equation:

$$\text{Inhibition (\%)} = 100 \times [(A_{control}-A_{sample})/A_{control}],$$

where: $A_{control}$ is the absorbance of the control (containing all reagents except the test sample), A_{sample} is an absorbance with the test sample. The standard curve was prepared using Trolox. Results were expressed as mg Trolox equivalents /g sample). Additional dilution was needed if the DPPH value measured was over the linear range of the standard. The assay was carried out in triplicate and the data were expressed as mean ± SD.

2.4.3. Determination of the total phenolics

The total phenolic contents for elven obtainable extracts were determined using Folin-Ciocalteu reagent following the method of Li et al. [19]. The absorbance was measured at 725 nm against the solvent blank. The total phenolics (TPs) were determined by means of a calibration curve prepared with gallic acid and expressed as mg gallic acid per g of the sample. It was calculated as follows: TPs= $Conc._{(Gallic)} \times V \times m/M$, where "$Conc._{(Gallic)}$" is the concentration of the standard (gallic acid) established from the calibration curves, "V" is the dilution factor, "m" is total extract wt. (g), and "M" is the concentration of dry extract. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

2.4.4. Determination of the total flavonoid contents

The total flavonoid contents for the tested extracts were measured using $AlCl_3$ colorimetric assay according to Žilić, *et al*. [20]. Briefly, 300 µL of 5% sodium nitrite ($NaNO_2$) was mixed with 100 µL of extract. After 6 min, 300 µL of a 10% $AlCl_3$ solution was added, and the volume was adjusted to 2.5 mL using distilled water. After 7 min, 1.5 mL of 1 M NaOH was added, and the mixture was centrifuged at 5000 g for 10 min. The absorbance of the supernatant was measured at 510 nm against the solvent blank. The total flavonoid contents (TFs) were determined by

means of a calibration curve prepared with catechin and were expressed as mg catechin equivalent per g sample and calculated as follows: TFs= Conc. $_{(Catechin)}$×V/ m, where "Conc. $_{(Catechin)}$" is the concentration of the standard (catechin) established from the calibration curve, "V" is the volume of extract in mL, and "m" is total extract wt. (g). Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

2.5. Experimental design and data analysis

The layout of the experiments (shooting, rooting, and acclimatization) was designed in a Completely Randomized Design (CRD) and a test of Least Significant Difference (LSD) at $p \leq 0.05$ was used for comparison among means [21]. The TPs, TFs, and DPPH experiments were performed in triplicates, and the obtained results were expressed as mean ± standard deviation (SD). Test results were calculated in Microsoft Excel software (Microsoft, Redmond, WA, USA).

3. Results and Discussion

3.1. Shooting behaviour

Data presented in **Table 1** and **Fig. 1** described the effects of different types and concentrations of cytokinins on the shooting behaviour of *B. pauciflora*. Results revealed a significant increase in shootlet length by the addition of 2.0 mg/L kin (1.33 cm) and the maximum leaf numbers were recorded through the addition of 1.0, 3.0 mg/L kin or 2.0, 3.0 mg/L 2iP which recorded (7.0, 6.67, 6.67, 6.67 leaf/shootlet, respectively). However, the supplement of BAP (3.0 mg/L) to the MS medium gave the shortest shootlets length (0.33 cm) and lowest leaf number (3.67 leaf/shootlet). On the other hand, MS medium containing 1.0 or 2.0 mg/L BAP gave the greatest shootlets number (8.83 or 8.40 shootlet/explant, respectively) followed by 3.0 mg/L BAP (5.47 shootlet/explant) compared to other treatments. Also, the MS medium supplemented with BAP (1.0 mg/L) recorded the heaviest fresh weight of shootlet (13.50 g) compared to the control (hormone-free) which recorded the lowest weight (2.33 g). Similar observations were previously reported for *Gardinia jasminoides* Ellis [22] by the addition of 2.0 or 3.0 mg/L BAP (increased shootlets number/explant) or the use of 3 mg/L 2iP (increased shootlets and leaves number). Other studies revealed that the maximum number of *Pteris tripartita* Sw. sporophytes were induced by 4 mg/L BAP [23], and the maximum numbers of *Marsilea quadrifolia* L. shootlets were recorded on MS medium supplemented with 0.5 mg/L BAP [24]. Additionally, BAP is the most effective cytokinin for shoot multiplication from the explant of Asiatic Lilium [25]. More recent studies reported that different concentrations of BAP produced significant responses for the shoot length, and/or the number of leaves of *Trigonella foenum-graecum* L. at 2.0 mg/L [26] and *Mammillaria herrerae* Werdermann at 0.1 mg/L [27] as well as of *Salvia officinalis* L. at 0.5 mg/L combined with 0.1 mg/L of the auxin IAA [28].

Table 1. Effect of cytokinins type and concentration for shooting behaviour on *Brunfelsia pauciflora*

Cytokinins	Concentration (mg/L)	Shootlet length (cm)	Shootlet number/ explant	Leaf number/ shootlet	Fresh weight (g)
Control	0.00	0.97 $^{a-c}$	1.00 ^{c}	6.07 ^{ab}	2.33 ^{fg}
BAP	1.0	1.10 ^{ab}	**8.83** ^{a}	6.40 ^{ab}	**13.50** ^{c}
	2.0	0.67 ^{cd}	**8.40** ^{a}	4.80 ^{ab}	8.97 ^{a}
	3.0	0.33 ^{d}	5.47 ^{b}	3.67 ^{c}	10.60 ^{b}
Kin	1.0	1.17 ^{ab}	1.00 ^{c}	**7.00** ^{a}	2.16 ^{g}
	2.0	**1.33** ^{a}	2.06 ^{c}	6.20 ^{ab}	4.78 ^{d}
	3.0	0.93 $^{a-c}$	2.20 ^{c}	**6.67** ^{ab}	3.83 $^{d-f}$
2iP	1.0	0.77 ^{bc}	1.13 ^{c}	6.33 ^{ab}	2.83 $^{e-g}$
	2.0	1.00 $^{a-c}$	1.53 ^{c}	**6.67** ^{ab}	4.18 ^{de}
	3.0	1.17 ^{ab}	1.67 ^{c}	**6.67** ^{ab}	4.17 ^{de}
LSD $_{0.05}$		0.4216	2.668	1.985	1.562

LSD $_{0.05}$ = Least Significant Different at 0.05 level of probability; BAP: benzyl aminopurine; Kin: 6-Furfuryl- aminopurine; 2ip: isopentenyl adenine; Bold numbers designated the highest value among the tested treatment at the same parameter.

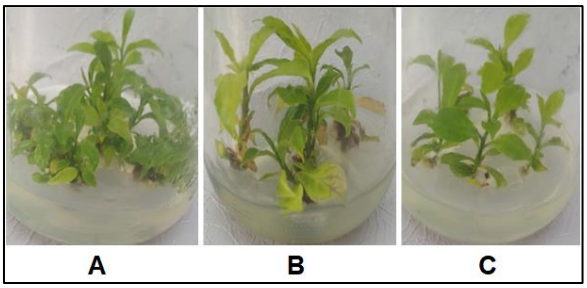

Fig.1. The significant types and concentrations of cytokinins on shooting behavior of *Brunfelsia pauciflora*; A: 1.0 mg/L BAP (The highest shootlets number), B: 2.0 mg/L Kin (The longest shootlets length), C; 3.0 mg/L 2iP (The maximum number of leaves)

3.2. Rooting behaviour

Table 2 and **Fig. 2** illustrated that the shootlet produced from shooting behavior cultured on different strengths of MS medium without or with AC. The use of ¼ strength MS medium with AC recorded the longest shootlet (5.17 cm), while the use of full-strength MS medium without AC recorded the shortest shootlet length (3.47cm). When the MS power was

decreased to ¾ strength, the shootlet recorded the highest leaf number (8.17 leaf/shootlet) while ¼ MS strength without AC produced the lowest leaf number (6.67 leaf/shootlet). Shootlet culture on ¾ strength MS medium without AC recorded the largest root number (3.40 root/shoot). While the same strength with AC reduced the number of roots to 1.77 root/shootlet. Shootlet cultured on full-strength MS medium with AC produced the longest root length (7.50 cm). While ¾ MS without AC, ½ MS, or ¼ MS with AC reduced the root length to the minimum (4.23, 4.50, or 4.33 cm, respectively).

A previous study on *Bacopa monnieri* (L.) Pennell [29] revealed that the addition of AC at concentrations ranging from 0.5 to 2.0 g/L, enhanced root formation. Medium supplemented with AC was important not only for promoting the rooting frequency but also for improving overall root quality, essential for subsequent *ex vitro* survival [30, 31]. The use of different MS strengths was discussed by Woldeyes *et al.* [32] for rooting of *Abelmoschus esculentus* (L.) Moench (Okra). They found that full-strength MS medium recorded the highest shootlet length and leaf number. While ½ MS strength recorded the maximum root length and number.

Table 2. Effect of MS salt strength fortified without or with activated charcoal on rooting behaviour for *Brunfelsia pauciflora*

MS strength without or with AC	Shootlet length (cm)	Leaf number/ shootlet	Root number/ shootlet	Root length (cm)
Full MS	3.47 ^{e}	7.80 ^{ab}	2.33 ^{d}	5.23 ^{b}
¾ MS	4.03 ^{cd}	8.03 ^{ab}	**3.40** ^{a}	4.23 ^{c}
½ MS	3.73 ^{de}	7.03 ^{ab}	2.73 ^{c}	5.20 ^{b}
¼ MS	3.73 ^{de}	6.67 ^{b}	3.00 ^{bc}	5.50 ^{b}
Full MS + AC	4.50 ^{bc}	6.87 ^{b}	2.40 ^{d}	**7.50** ^{a}
¾ MS + AC	4.60 ^{b}	**8.30** ^{b}	1.77 ^{e}	4.87 ^{bc}
½ MS + AC	4.80 ^{ab}	6.90 ^{b}	3.07 ^{b}	4.50 ^{c}
¼ MS + AC	**5.17** ^{a}	7.00 ^{ab}	3.20 ^{ab}	4.33 ^{c}
LSD $_{0.05}$	0.5283	1.397	0.3181	0.6872

LSD $_{0.05}$ = Least Significant Different at 0.05 level of probability; MS: Murashige and Skoog medium; AC: activated charcoal; Bold numbers designated the highest value among the tested samples at the same parameter.

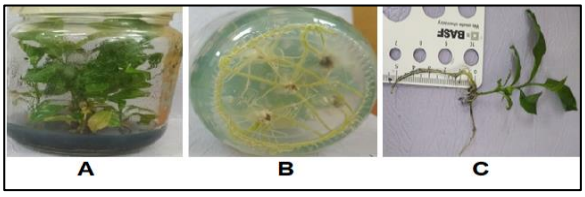

Fig.2. The significant strength MS medium without or with activated charcoal (AC) on rooting behaviour of *Brunfelsia pauciflora*; A: ¼ MS (The longest shootlets length), B: ¾ MS (The highest roots number), C; Full MS (The longest roots length)

3.3. Acclimatization

All rooted shoots were transferred to different culture media of peat moss, peat moss + sand (1:1 *v/v*), or peat moss +perlite (1:1 *v/v*) under *ex vitro* conditions were recorded in **Fig. 3** & **4**. The media investigated gave significant differences in acclimatization on plantlet behaviour. Cultured plantlets in peat moss alone recorded the longest plantlet, greatest leaves number, greatest root number, and longest root (7.83 cm, 13.17 leaf/plantlet, 13.5 cm, and 5.00 root/plantlet). These results are possibly attributed to the high nutrition value of peat moss which can permit the *in vitro* plantlet to grow up in longer length compared to the poor nutrition value of perlite or sand. In this regard, when Cactus (*Cereus peruvianus* Mill.) plantlets were potted on peat moss alone or in combination with sand at (1:1 *v: v*), the stems of the *in vitro* acclimatized plants were allowed to grow up to the highest value [33]. Also, Labrousse *et al.* [34] reported that the use of peat moss as a substrate *in vitro* culture enhanced rooting and increased the number and length of roots.

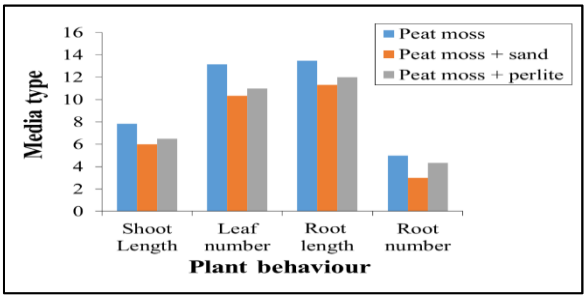

Fig. 3. Effect of media type on acclimatization for plant behaviour on *B. pauciflora*

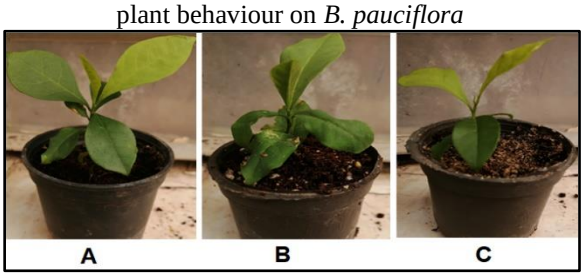

Fig. 4. Effect of media substrate for acclimatization on *Brunfelsia pauciflora*; A: Peat moss, B: Peat moss + Perlit, C; Peat moss + Sand

3.4. Total phenolics, total flavonoids, and antioxidant evaluation

The data summarized in **Table 3** indicated a direct relationship between TFs, and antioxidant activity suggesting that flavonoid compounds are responsible

for the antioxidant potential in *B. pauciflora*. This result is consistent with several studies, while flavonoids are very important plant components that can scavenge free radicals due to their free hydroxyl groups [31, 35]. Also, a significant relationship was observed between the TFs (**Table 3**) and the leaves number/shootlet (**Table 1**), supporting the evidence indicating the abundance of flavonoids in the leaves [36].

Table 3. Total phenolics, total flavonoids, and antioxidant evaluation of the extracts on *B. pauciflora*

Cytokinins	Conc. (mg/L)	TPs (mg $_{Gallic}$/g $_{extract}$)	TFs (mg $_{Catechin}$ /g $_{extract}$)	Antioxidant (mg $_{Trolox}$/g $_{extract}$)
Mother plant		40.721±0.57	20.396±0.04	30.68±0.76
Control	0.00	32.279±1.09	27.417±0.49	57.375±1.60
BAP	1.0	49.767±0.51	37.230±0.19	75.96±2.26
	2.0	29.217±1.20	14.037±0.86	25.26±0.27
	3.0	39.767±1.05	26.037±0.86	43.49±0.83
Kin	1.0	32.534±0.74	29.720±0.85	60.89±2.25
	2.0	39.747±1.28	26.250±0.46	49.50±0.49
	3.0	43.917±1.32	30.663±0.27	59.60±1.05
2iP	1.0	40.592±0.78	29.677±0.60	64.75±1.97
	2.0	44.870±1.19	35.761±1.47	64.54±0.60
	3.0	**57.574±0.82**	**46.146±0.31**	**89.98±0.27**

Bold numbers designated the maximum value among the tested samples at the same parameter

Except for the cultured shootlet on medium supplemented with BAP (2.0 mg/L) which recorded the lowest effect, the control group (MS free) and all cytokinins treatments produced significant impacts on TPs, TFs, and the antioxidant as compared to those of the mother plant. The supplement with all treated concentrations of 2iP, the lowest concentration of BAP (1.0 mg/L), and the highest concentration of Kin (3.0 mg/L) produced significant parameters in comparison with the control and the mother plant. The highest TPs (57.574±0.822 mg $_{Gallic}$/g $_{extract}$), TFs (46.146±0.315 mg $_{Catechin}$/g $_{extract}$), and antioxidant effect (89.98±0.277 mg $_{Trolox}$ /g $_{extract}$) were found in the cultured shootlet on MS medium with 2iP (3.0 mg/L), followed by that of 1.0 mg/L BAP (75.96±2.258, 49.767±0.518, and 37.230±0.187 mg $_{Standard}$/g $_{extract}$, respectively), then Kin at 3.0mg/L, and the other two concentrations of 2iP.

Similarly, the highest examined concentration of 2ip (0.6 mg/L) improved the biosynthesis of phenolics, and flavonoids as well as induced the antioxidant activity in shootlet multiplication of *Antigonon leptopus* Hook. & Arn [13]. Likewise, the significant effect of 2iP on TPs and TFs has been reported before in other ornamental and medicinal species such as *Garcinia brasiliensis* Mart [37]. Moreover, TFs were found to be high in the callus obtained from MS medium treated with Kin (2.0 mg/L) combined with NAA (1.0 mg/L) of *Canscora decussata* (Roxb.) Roem. &Schult. [38]. Also, the highest TFs were accumulated with 2iP and BAP at 3.0 mg/L of *G Gardenia jasminoides* Ellis [22].

4. Conclusion

The current study magnificently recognized the optimal requirements for the *in vitro* propagation of *B. pauciflora* with prospective production of phenolic and flavonoid metabolites. Investigation of cytokinins concentrations, strength MS medium without or with AC, and acclimatization media on *in vitro* culture of *B. pauciflora* revealed that the shootlet length increased with the addition of 2.0 mg/L kin to MS medium while using 1.0 mg/L BAP produced the maximum shootlet numbers and fresh weights. On the other hand, medium supplemented with 3.0 mg/L 2iP recorded a significant leaves number, higher content in total phenolics and total flavonoids, as well as the greatest antioxidant effect. For rooting, decreased MS strength medium with AC enhanced rooting behaviour. Additionally, culture shootlets in peat moss recorded good growth of the plantlet through the acclimatization process. The reachable results will be helpful for future easy, rapid, and scale-up productions of antioxidant components as well as can be used in further transformation studies. More extensive phytochemical analysis and biological investigation for the significant cultured plantlets are required.

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