

## S. Materials and methods

### S1. Total anthocyanin contents by pH-differential method

The total anthocyanin content (TAC) of the callus was measured using the pH differential method. The extracts were centrifuged at 15,000 rpm for 20 min, and the optical density of the supernatants was measured at 510 and 700 nm with a spectrophotometer (Bio-Teck, USA). Each sample was calculated according to the following formula:  $TAC (mg/g) = (A \times MW \times DF \times 1000) / (\epsilon \times 1)$  where  $A = (A_{510 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH}1.0} - (A_{510 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH}4.5}$  (The difference in absorbancy),  $\epsilon$  = the molar extinction coefficient for cyanidin-3-glucoside (26,900),  $MW$  = the molecular weight of cyanidin-3-glucoside (449.2 g/mol), and  $DF$  = the dilution factor. The absorbance of the extract was then measured at 510 nm and 700 nm.

### S2. HPLC analysis

HPLC analysis was used by modifying the method of Madhavi et al[1]. 0.2 g of the freeze-dried callus was mixed with 5 ml of ethyl acetate and kept for 3 hours in the shaking incubator. After that the extracts were centrifuged for 30 minutes and the supernatant was moved to a new glass tube. The same method was repeated three times. Samples were filtered with Nylon Syringe Filters, 0.45  $\mu\text{m}$  and kept in the HPLC vial for running the machine. Solvents were 100% Acetonitrile (A) and 1% phosphoric acid (B). Separation was obtained by an isocratic elution at 0.7 ml/ min (% ratio of A : B = 20 : 80). The flow rate was 0.7ml/min and injection volume were 10  $\mu\text{l}$ . Cyanidin-monoglucoside was eluted with an isocratic condition and detected at 200nm ~ 400nm.

### S3. LC-MS analysis method

Each 1g of the fresh callus grown under different lights was homogenized and extracted in 10 ml methanol for the LC-MS analysis method. Chromatographic conditions were as follows: total run time 10 min; Phenomenex Kinetex C18 column (2.1 mm  $\times$  30 mm, 1.7  $\mu\text{m}$  particle size) kept at 35°C; a binary mobile phase consisting of water with 0.1% formic acid (v/v) and acetonitrile with 0.1% formic acid (v/v); gradient elution of 10% B in 0 min, 10% B in 1 min, increasing to 50% B in 5 min, decreasing to 10% B in 5 min and 10% B in 7 min; flow rate 350  $\mu\text{l}/\text{min}$ .; injection volume 5  $\mu\text{l}$ ; autosampler temperature 4°C.

**Table S1:** Analysis of DPPH radical scavaging activity, total phenolic contents, total flavonoids in the callus grown under different hormone concentrations (mg CAT/g dry extracts)

No.	Name	DPPH radical scavenging (%)	Total phenolic (mg /g )	Total flavonoid (mg/g )
1.	MS + NAA 0.5 + BAP 2.0	57.90 ±2.32	0.97±0.03	0.29±0.14
2.	MS + NAA 1.0 + BAP 2.0	47.02 ±3.21	0.93±0.03	0.25±0.11
3.	MS + NAA 3.0 + BAP 2.0	48.55±2.73	0.86±0.02	0.27±0.16
4.	MS + NAA 5.0 + BAP 2.0	34.15±1.79	0.83±0.02	0.23±0.14
5.	MS + NAA 0.5 + Kin 2.0	37.68±2.03	0.89±97.51	0.19±0.00
6.	MS + NAA 1.0 + Kin 2.0	25.64 ±1.56	0.77±0.05	0.18±0.18
7.	MS + NAA 3.0 + Kin 2.0	15.59±1.82	0.84±0.04	0.14±0.18
8.	MS + NAA 5.0 + Kin 2.0	23.04±1.65	0.75±0.04	0.18±0.09
9.	MS + IBA 0.5 + BAP 2.0	40.76±2.52	0.94±0.03	0.29±0.10
10.	MS + IBA 1.0 + BAP 2.0	34.87±2.05	0.89±0.04	0.29±0.10
11.	MS + IBA 3.0 + BAP 2.0	24.71 ±2.73	0.83±0.05	0.28±0.17
12.	MS + IBA 5.0 + BAP 2.0	32.23±1.81	0.95±0.03	0.33±0.20
13.	MS + IBA 0.5 + Kin 2.0	33.33 ±1.62	0.95±0.04	0.27±0.20
14.	MS + IBA 1.0 + Kin 2.0	46.49 ±1.41	1.02±0.04	0.35±0.19
15.	MS + IBA 3.0 + Kin 2.0	29.44±1.76	0.85±0.04	0.23±0.12
16.	MS + IBA 5.0 + Kin 2.0	22.37 ±2.15	0.81±0.05	0.19±0.12

**Table S2:** Effects of plant growth regulators response on *G. procumbens* for callus induction

Hormone treatments (mg/L)	Callus response	Color	Texture	Fresh weight(g)	Dry weight(g)
MS + NAA 0.5 + BAP 2.0	+++	Greenish white	Soft	123.36	7.08
MS + NAA 1.0 + BAP 2.0	+++	Greenish white	Soft	119.81	6.94
MS + NAA 3.0 + BAP 2.0	++	Greenish white	Soft	135.4	7.65
MS + NAA 5.0 + BAP 2.0	++	Greenish white	Soft	128.65	7.15
MS + NAA 0.5 + Kin 2.0	+	Greenish white	Soft	113.22	6.95
MS + NAA 1.0 + Kin 2.0	++ (roots formed)	Greenish white	Soft	132.86	8.05
MS + NAA 3.0 + Kin 2.0	++ (roots formed)	Greenish white	Soft	135.78	7.99
MS + NAA 5.0 + Kin 2.0	++ (roots formed)	Greenish white	Soft	123.9	7.24
MS + IBA 0.5 + BAP 2.0	+	Greenish white	Soft	114.94	6.82
MS + IBA 1.0 + BAP 2.0	++	Greenish white	Soft	129.73	7.38
MS + IBA 3.0 + BAP 2.0	++	Greenish white	Soft	126.27	7.25
MS + IBA 5.0 + BAP 2.0	++	Greenish white	Soft	140.32	7.98
MS + IBA 0.5 + Kin 2.0	+	Greenish white	Soft	74.7	5.38
MS + IBA 1.0 + Kin 2.0	+	Greenish white	Soft	82.26	5.57
MS + IBA 3.0 + Kin 2.0	+(roots formed)	Greenish white	Soft	116.51	7.27
MS + IBA 5.0 + Kin 2.0	++ (roots formed)	Greenish white	Soft	110.56	6.52

Footnote: + represents the response of callus for PGRs combination.

**Table S3:** Effects of LED on the accumulation of total phenolic contents, total flavonoid contents, total anthocyanin contents and antioxidant activity in *Gynura procumbens* callus.

Sample	Dark	Blue	White	Red
TPC (mg GAE/g dry extracts)	1.05±0.05 <sup>a</sup>	1.19 ±0.04 <sup>b</sup>	1.04± 0.03 <sup>a</sup>	1.05± 0.06 <sup>a</sup>
TFC (mg CAT/g dry extracts)	0.277±0.12 <sup>a</sup>	0.337±0.07 <sup>b</sup>	0.334± 0.01 <sup>b</sup>	0.334±0.09 <sup>b</sup>
TAC (mg/g)	3.18±0.70 <sup>a</sup>	8.69 ±1.17 <sup>b</sup>	6.86±0.69 <sup>bc</sup>	4.71±2.89 <sup>ac</sup>
DPPH (%)	53.34 ±6.16 <sup>a</sup>	59.85 ±3.89 <sup>a</sup>	59.64±4.45 <sup>a</sup>	54.63±4.16 <sup>a</sup>

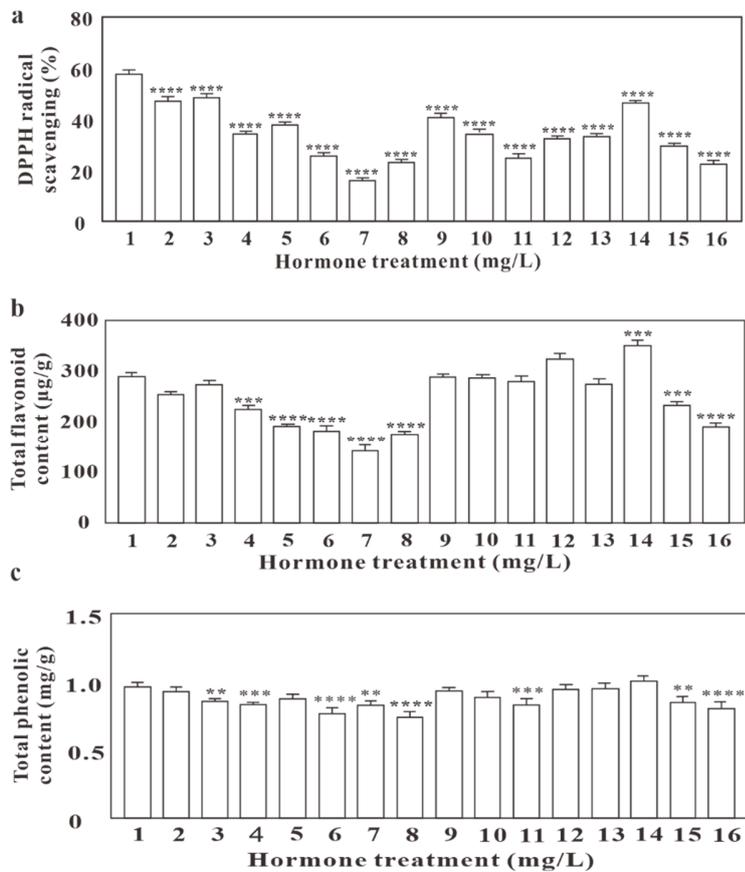
Ordinary one-way ANOVA, Tukey's multiple comparison test was performed with  $p > 0.05$  as significant and the different letters (a,b,c) denote for their significance with each other

**Table S4:** HPLC conditions for the analysis of cyanidin-monoglucosides in *Gynura procumbens*

Instrument	Shimadzu HPLC System (CBM-20A, LC-20A, SPD-20AD, and CTO-20A, Japan)
Column	C18
Column temperature	40 °C
Final concentration of mobile phase (isocratic)	A : acetonitrile 20% B : phosphoric acid 0.8% in water
Flow rate	0.7ml/min
Injection volume	10 µl
UV wavelength	200 nm ~ 400 nm
Run time	25 mins

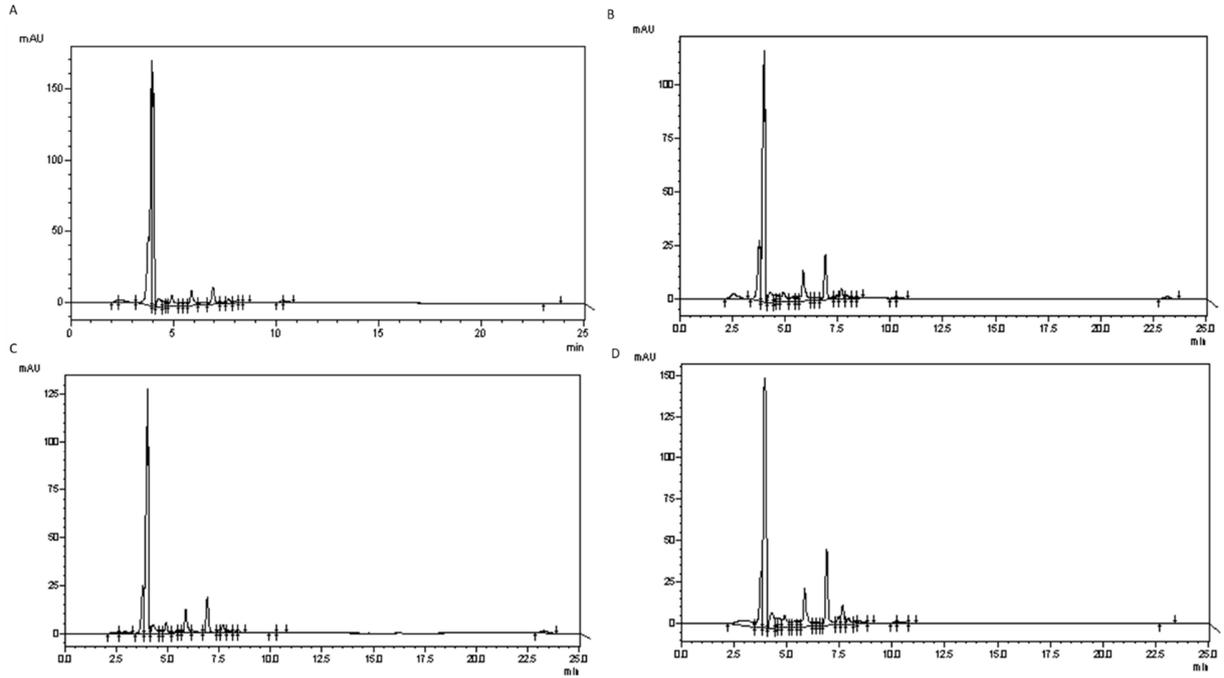
**Table S5:** LC-MS condition for the analysis of cyanidin-monoglucosides in *Gynura procumbens*

Instrument	XEVO-TQS#WAA250					
Column	C18					
Sample Temperature:	12 °C					
Column temperature	35 °C					
Mobile phase	Solvent name A: 0.1% formic acid in water Solvent name B: 0.1% formic acid in acetonitrile					
Gradient	No	Time(min)	Flow rate	%A	%B	Curve
	1	Initial (0)	0.500	95.0	5.0	Initial (0)
	2	0.50	0.500	95.0	5.0	6
	3	4.00	0.500	0.0	100.0	6
	4	4.50	0.500	0.0	100.0	6
	5	5.00	0.500	95.0	5.0	6
	6	10.00	0.500	95.0	5.0	6
Flow rate	1ml/min					
Injection volume	5 µl					
UV wavelength	530 nm					
Polarity, scan type	-					
Ionization source	ES+					
Mass scan range	-					
Run time	10 mins					

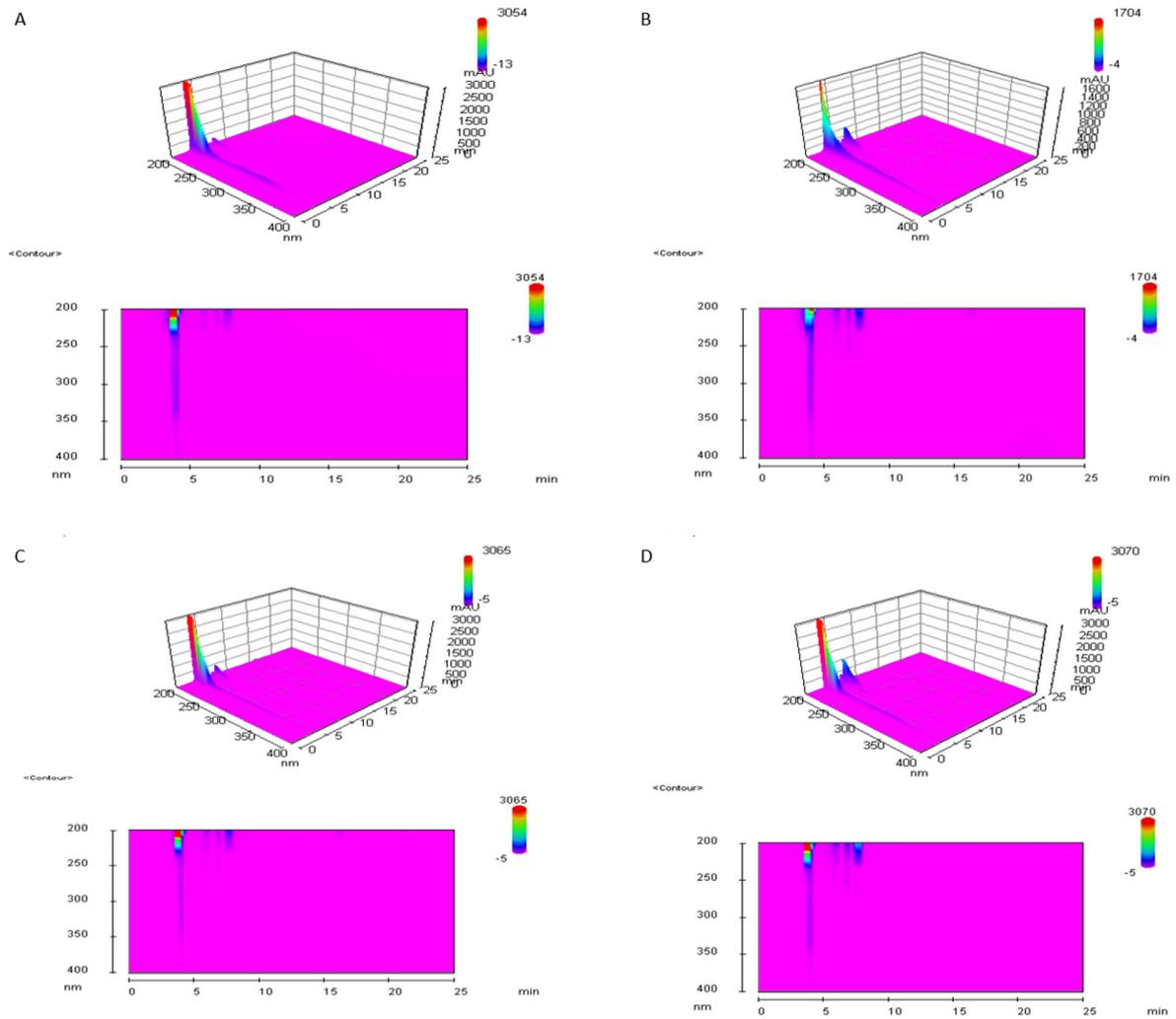


**Figure S1:** Effect of various plant growth regulator combinations on callus culture for (a) DPPH free radical scavenging activity (b) TFC and (c) TPC. Values are shown in mean  $\pm$  standard deviation (SD) from three replicates ( $n=3$ ). Ordinary one-way ANOVA Dunnett's multiple comparisons test was performed where  $P<0.01$ ,  $P<0.001$ ,  $P<0.0001$ , are represented as \*\*, \*\*\* and \*\*\*\*, respectively.

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|-----------------------|-----------------------|-----------------------|-----------------------|
| 1. NAA 0.5 + BAP 2.0  | 2. NAA 1.0 + BAP 2.0  | 3. NAA 3.0 + BAP 2.0  | 4. NAA 5.0 + BAP 2.0  |
| 5. NAA 0.5 + Kin 2.0  | 6. NAA 1.0 + Kin 2.0  | 7. NAA 3.0 + Kin 2.0  | 8. NAA 5.0 + Kin 2.0  |
| 9. IBA 0.5 + BAP 2.0  | 10. IBA 1.0 + BAP 2.0 | 11. IBA 3.0 + BAP 2.0 | 12. IBA 5.0 + BAP 2.0 |
| 13. IBA 0.5 + Kin 2.0 | 14. IBA 1.0 + Kin 2.0 | 15. IBA 3.0 + Kin 2.0 | 16. IBA 5.0 + Kin 2.0 |



**Figure S2:** HPLC peaks for *Gynura procumbens* callus grown in dark, blue, (C) white and red



**Figure S3:** HPLC analysis on 3D capture of cyanidin-monoglucosides content in *Gynura procumbens* callus culture in Dark, Blue, (C) White and Red

## Reference

1. Madhavi, D., M. Smith, and M.D. BERBER-JIMÉNEZ, *Expression of anthocyanins in callus cultures of cranberry (Vaccinium macrocarpon Ait)*. Journal of food science, 1995. **60**(2): p. 351-355.