

Growth characteristics and digiferruginol-11-O-β-primeveroside amount of *Damnacanthus major* callus treated with elicitor

Eun Bi Jang[†], Boram Go, Sung Chun Kim, Seon-A Yoon, Hyejin Hyeon, Jong-Du Lee, Yong-Hwan Jung and Young-Min Ham^{††}

Biodiversity Research Institute, Jeju Technopark, Seogwipo, Jeju 63608, Republic of Korea

Introduction

Damnacanthus major is a shrub that grows naturally in the subtropical forests of Jeju Island. *D. major* is difficult to use as a cosmetic ingredient because each plant is small and grows in a limited area, making it difficult to secure biomass. Therefore, we secured biomass through callus culture. Methyl jasmonate is one of the abiotic elicitors that increases plant useful substances in culture. Two culture scales were selected and tested to determine whether similar trends were observed across culture scales. Additionally, previous studies have shown that *D. major* callus extract is effective in anti-oxidant activity and producing hyaluronic acid. Therefore, Digiferruginol-11-O-β-primeveroside, which is presumed to be the active ingredient, was isolated.

Materials & Methods

✓ Plant material and medium composition

- Plant material : *Damnacanthus major* callus
- Medium : MS + NAA 3.0 mg/L + kinetin 0.1mg/L + 3% sucrose
- Culture condition: 3 weeks, dark, 0.1vvm

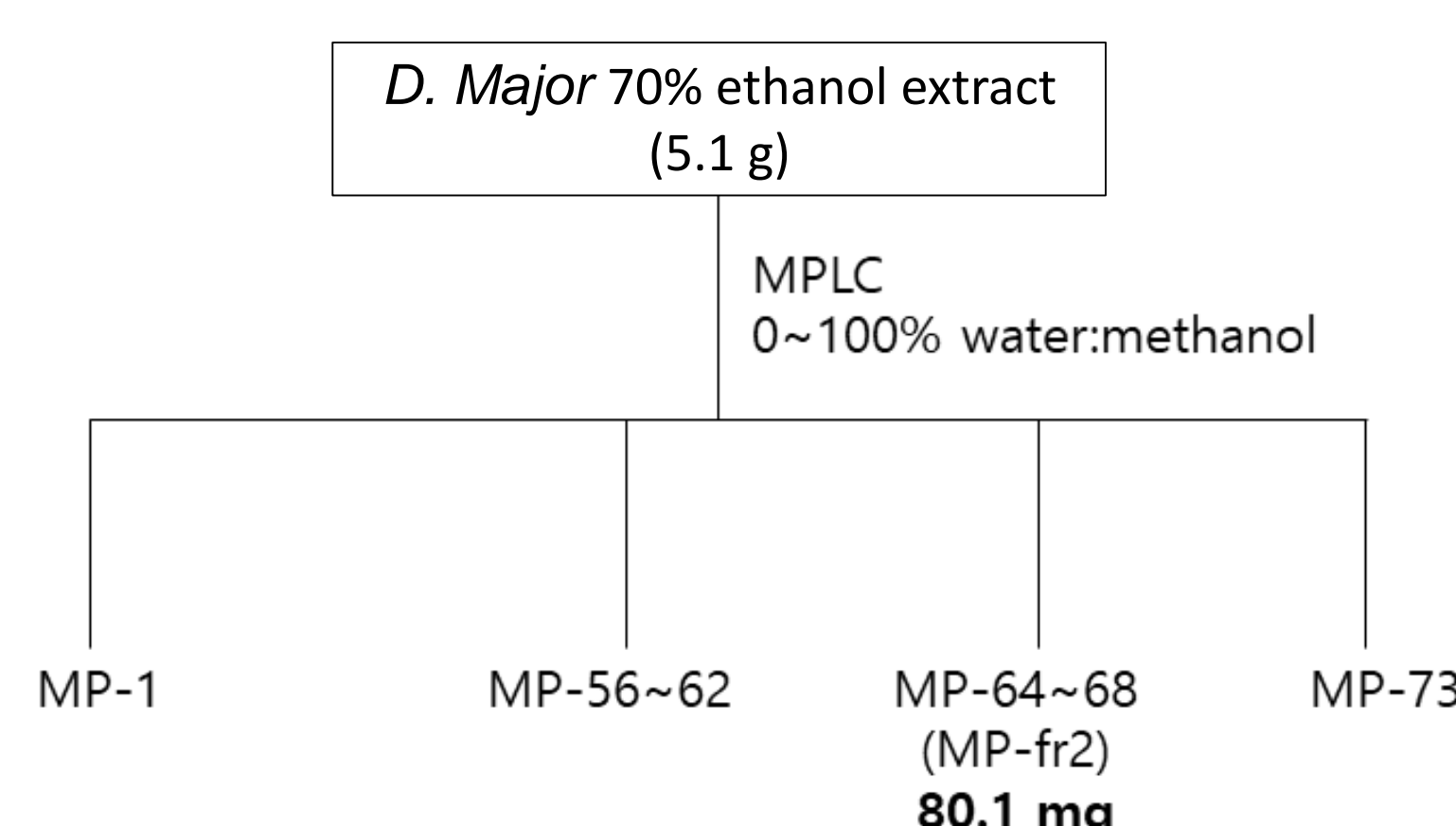
✓ Elicitor treatment

- Added after 2 weeks of cultured, and cultured for 1 week
- Methyl jasmonate (MeJA): 0, 100, 200 μM

✓ Free radical scavenging (ABTS)

✓ Digiferruginol-11-O-β-primeveroside

- MPLC → FT-NMR



■ Amounts: HPLC+ PAD detector (Waters)

- Instrument: HPLC(Waters Co.2998)
- Detector: PDA (Waters Co. e2695)
- Wavelength: 260 nm
- Column: Sunfire C18(4.6 x 150mm)
- Mobile phase

A: 0.1% phosphoric acid in water

B: Acetonitrile

Time (min)	A (%)	B (%)	Flow (mL/min)
0	80	20	1
10	80	20	1
30	0	100	1
40	0	100	1
45	80	20	1
55	80	20	1

- Injection volume 10μL
- Oven temperature: 40°C

Results

❖ Biomass production and characterization

Table 1. Growth characteristics of *D. major* callus treated with methyl jasmonate (MeJA) when cultured in a 250 ml flask

MeJA (uM)	Fresh weight (g)	Dry weight (g)	Dry matter (%)	Growth index ^z
0	52.3±0.1 ^y	2.0±0.0	7.6±0.1	6.5±0.0
100	34.4±1.3	1.8±0.0	10.5±0.2	3.9±0.2
200	28.2±1.0	1.5±0.0	10.7±0.4	3.0±0.1

^z Growth index (GI) = [Final dry weight (g) – initial dry weight (g)] / initial dry weight (g)

^y Mean ± SE.

Table 2. Growth characteristics of *D. major* callus treated with methyl jasmonate (MeJA) when cultured 5L bioreactor

MeJA (uM)	Fresh weight (g)	Dry weight (g)	Dry matter (%)	Growth index ^z
0	864.0±48.5 ^y	32.7±2.1	7.8±0.7	5.1±0.4
100	574.1±48.5	29.3±0.8	9.2±0.4	4.5±0.2
200	497.6±16.9	25.7±0.4	9.2±0.7	3.8±0.1

^z Growth index (GI) = [Final dry weight (g) – initial dry weight (g)] / initial dry weight (g)

^y Mean ± SE.

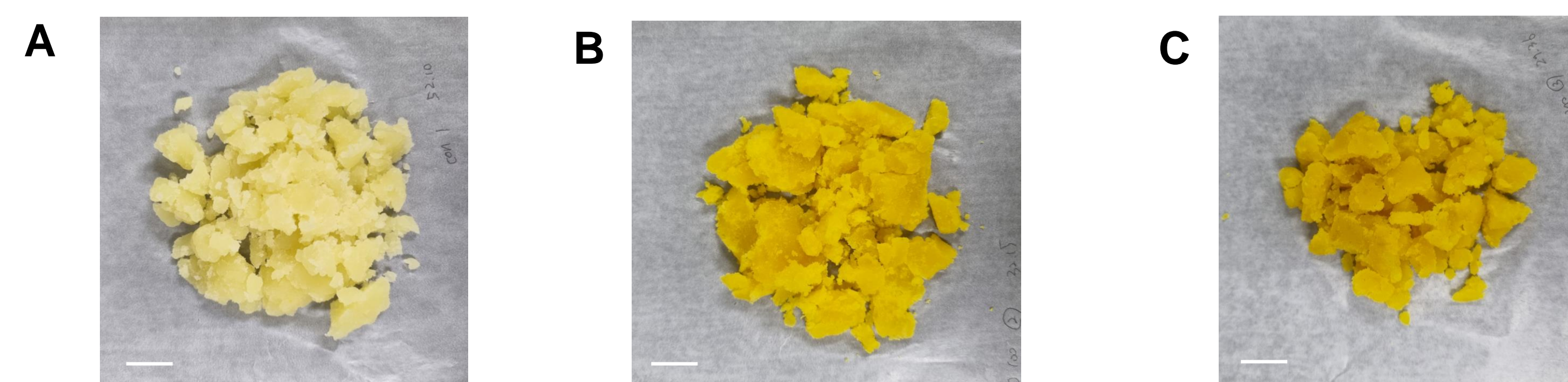


Fig. 1. Effect of MJ concentration on callus proliferation in *D. major* cultured after 3-week. A: MeJA untreated (control), B: 100 μM, C: 200 μM (Scale bar = 1 cm).

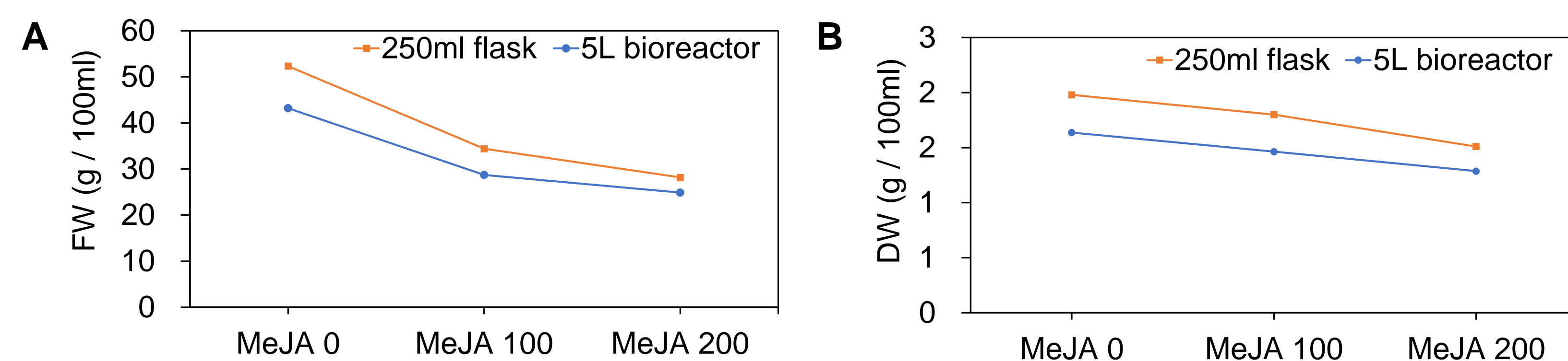


Fig. 2. Production per 0.1 liter according to culture scale fresh weight (A), and dry weight (B) of callus in *D. major*.

❖ Digiferruginol-11-O-β-primeveroside

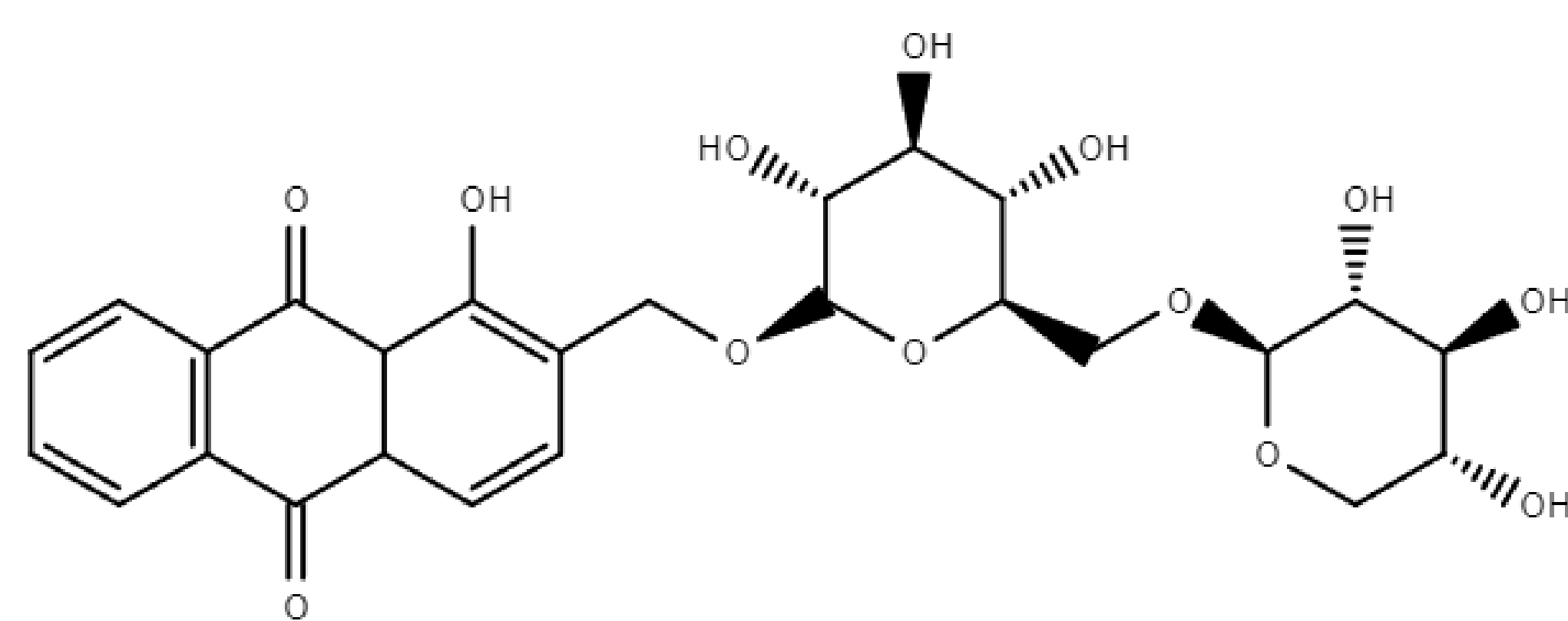


Fig. 3. Compound structure of digiferruginol-11-O-β-primeveroside.

Table 3. Digiferruginol-11-O-β-primeveroside amount of *Damnacanthus major* callus extract (DMC) according to methyl jasmonate (MeJA) treatment concentration

Sample	Amounts (mg/g)
DMC_MeJA_0	0.04
DMC_MeJA_100	1.84
DMC_MeJA_200	0.79
Wild plants leaf	None

❖ Anti-oxidant activity

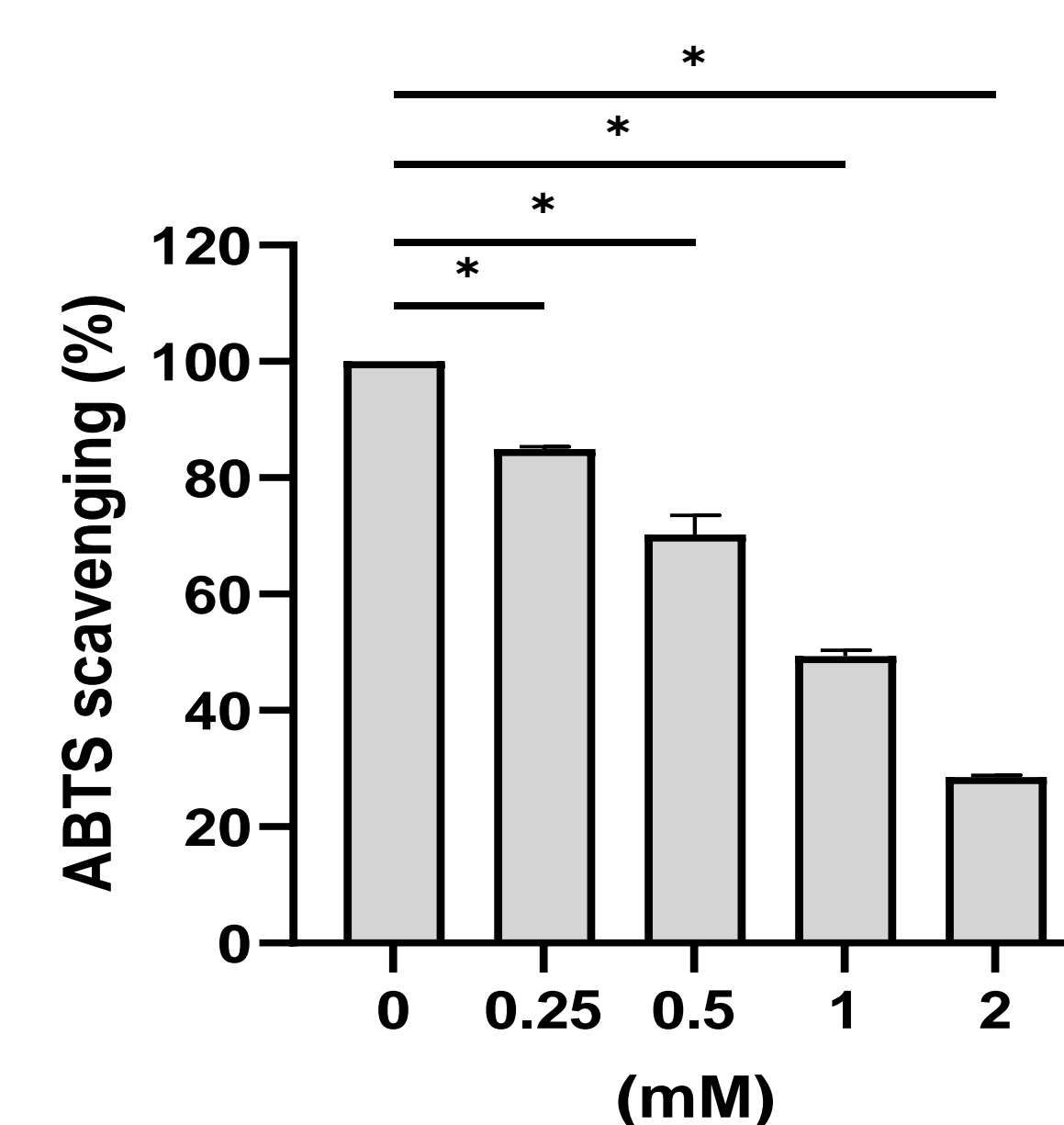


Fig. 4. ABTS scavenging activity of digiferruginol-11-O-β-primeveroside. **p*<0.05

Conclusions

- Damnacanthus major* callus cultured elicitor will be capable of pilot-scale culture and and by further experiment the hyaluronic acid production of digiferruginol-11-O-β-primeveroside, it can be used as a cosmetic ingredient.

Acknowledgement

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