

Antioxidant activity assessment with optimized biomass and elicitor treatment from adventitious root of *Hibiscus hamabo* Siebold & Zucc.

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Introduction

Hibiscus hamabo Siebold & Zucc. (Yellow hibiscus) is a deciduous shrub plant, belonging to the Malvaceae, and inhabits mainly in coastal areas of Jeju island and south Jeonnam. Yellow hibiscus was **newly classified as an observed species** by Ministry of Environment, Korea from 2023. In our previous study, we confirmed the physiologically optimal culture condition of the adventitious root with biomass in air-lift bioreactor vessel and stored in Jeju Endemic Plant Cell Line Bank (JBRI-S20-003). In this study, we investigated **the optimized biomass production and the antioxidant activity of yellow hibiscus adventitious root according to different concentration of methyl jasmonate, as an elicitor treatment method**. These assessment could be useful for **developing the high-quality ingredient of yellow hibiscus adventitious roots to mass-proliferate pilot-scale biomass and bioactive compounds accumulation** such as phenolic acids and flavonoids.

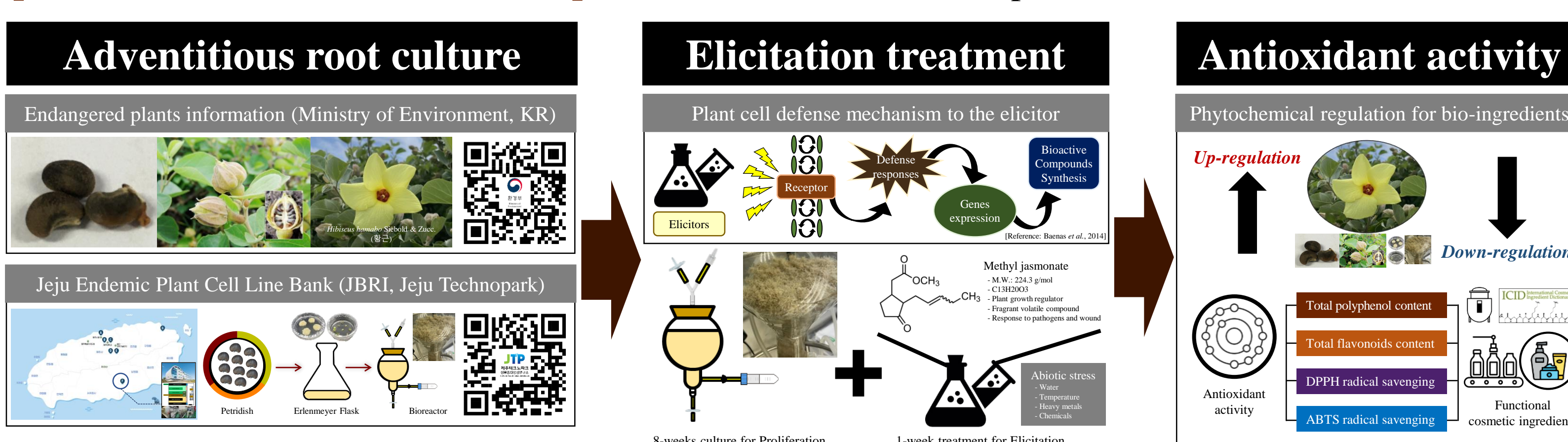


Fig. 1. Experimental scheme of adventitious root culture and screening from *Hibiscus hamabo*.

Materials & Methods

1 Plant materials and cell line induction

Table 1. Plant materials and culture medium composition of *H. hamabo*

Scientific name (Common name)	Collection location site	Adventitious root-derived origination	Culture medium and condition
<i>Hibiscus hamabo</i> Siebold & Zucc. (Yellow hibiscus)	Gujwa, Jeju (N 33.53096, E 126.83750)	Seeds	- 1/2MS, 2 mg·L ⁻¹ IBA, 30 g·L ⁻¹ sucrose - 4 g·L ⁻¹ inoculum density, 25±1°C, 1 vvm

2 Growth index investigation of adventitious roots

- Culture system apparatus: 5,000 ml balloon type air-lift bioreactor → 8-weeks culture
- Elicitation: methyl jasmonate; MJ (Non-treat, 50 μM, 100 μM, 200 μM) → 1-week treatment
- Inoculum Density (I.D., g), Fresh Weight (F.W., g), Dry Weight (D.W., g)
- % Dry weight = $\frac{D.W.(g)}{F.W.(g)} \times 100$, Growth Index (G.I.) = $\frac{F.W.(g) - I.D.(g)}{I.D.(g)}$, Relative Growth Rate (R.G.R.) = $\frac{F.W.(g) - I.D.(g)}{I.D.(g)}$

3 Extraction, bioactive compounds and antioxidant activity

- Extraction sample concentration: 10 g DW·200 ml⁻¹ (ultrasonic extraction and solvent evaporation)
- Extraction solvent: Hot water, 30% ethyl alcohol, 50% ethyl alcohol, 70% ethyl alcohol
- Bioactive compounds (mg·g⁻¹ DW): Total polyphenol content (725 nm), Total flavonoid content (510 nm)
- Productivity (mg·L⁻¹) = [Content (mg·g⁻¹ DW) × Sample D.W.] / Volum of culture medium (L)
- Antioxidant activity: DPPH radical scavenging (517 nm), ABTS radical scavenging (734 nm)
- Statistical analysis: Duncan's multiple-range test (DMRT), SAS Studio® (SAS Institute Inc. USA)

Results

1 Adventitious root biomass index and extraction yield

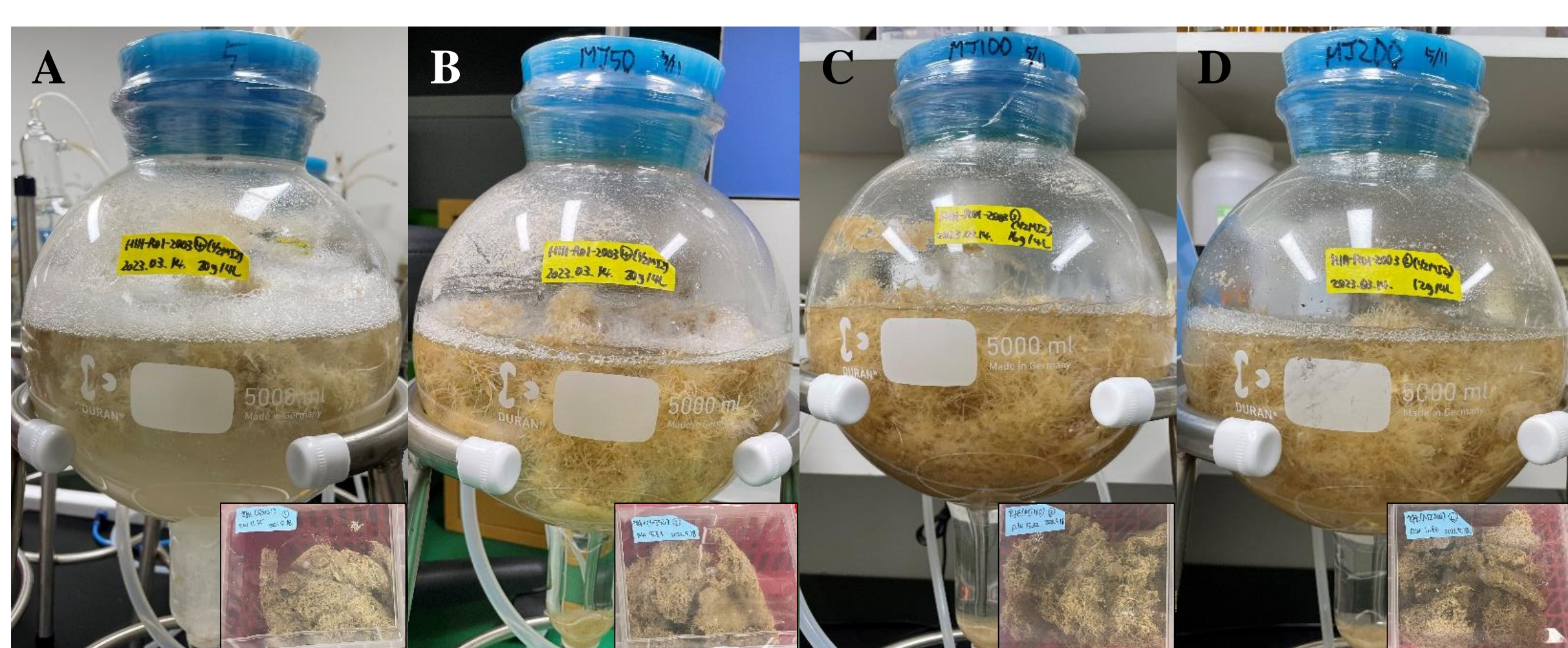


Fig. 2. *In vitro* culture and dry biomass of *Hibiscus hamabo* adventitious root in 5,000 ml bioreactor.
A. Non-treat;
B. MJ 50 (50 μM);
C. MJ 100 (100 μM);
D. MJ 200 (200 μM).

Table 2 Effect of methyl jasmonate concentration on *Hibiscus hamabo* adventitious root biomass growth characteristics in 5,000 ml bioreactor

Methyl jasmonate (MJ) concentration	I. D. (g·L ⁻¹)	F.W. (g)		D.W. (g)		% Dry weight		G. I. ^z		R.G.R. ^y	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Non-treat	4.0	376.10	332.24	13.76	11.35	3.66	3.42	22.51	19.77	3.16	3.03
MJ 50		403.93	423.69	14.45	15.89	3.58	3.75	24.25	25.48	3.23	3.28
MJ 100		341.02	411.49	12.30	15.32	3.61	3.72	20.31	24.72	3.06	3.25
MJ 200		135.94	359.24	4.68	11.80	3.44	3.28	7.50	21.45	7.50	3.11

^yThe relative growth rate (RGR) is calculated as follows: $RGR = [\ln W_2 - \ln W_1] / CP$, where \ln is the natural log, W_1 and W_2 are the initial weight and final weight, respectively, and CP is the culture period (8-week)

^zGrowth index (GI)=[Final fresh weight (g) – Initial fresh weight (g)]/Initial fresh weight (g)

Table 3 Extraction yield according to solvent concentrations on methyl jasmonate 50 μM treatment from *Hibiscus hamabo* adventitious root

Solvent	Extraction Yield (%)
Heat water	19.4
30% EtOH	19.3
50% EtOH	10.5
70% EtOH	12.7

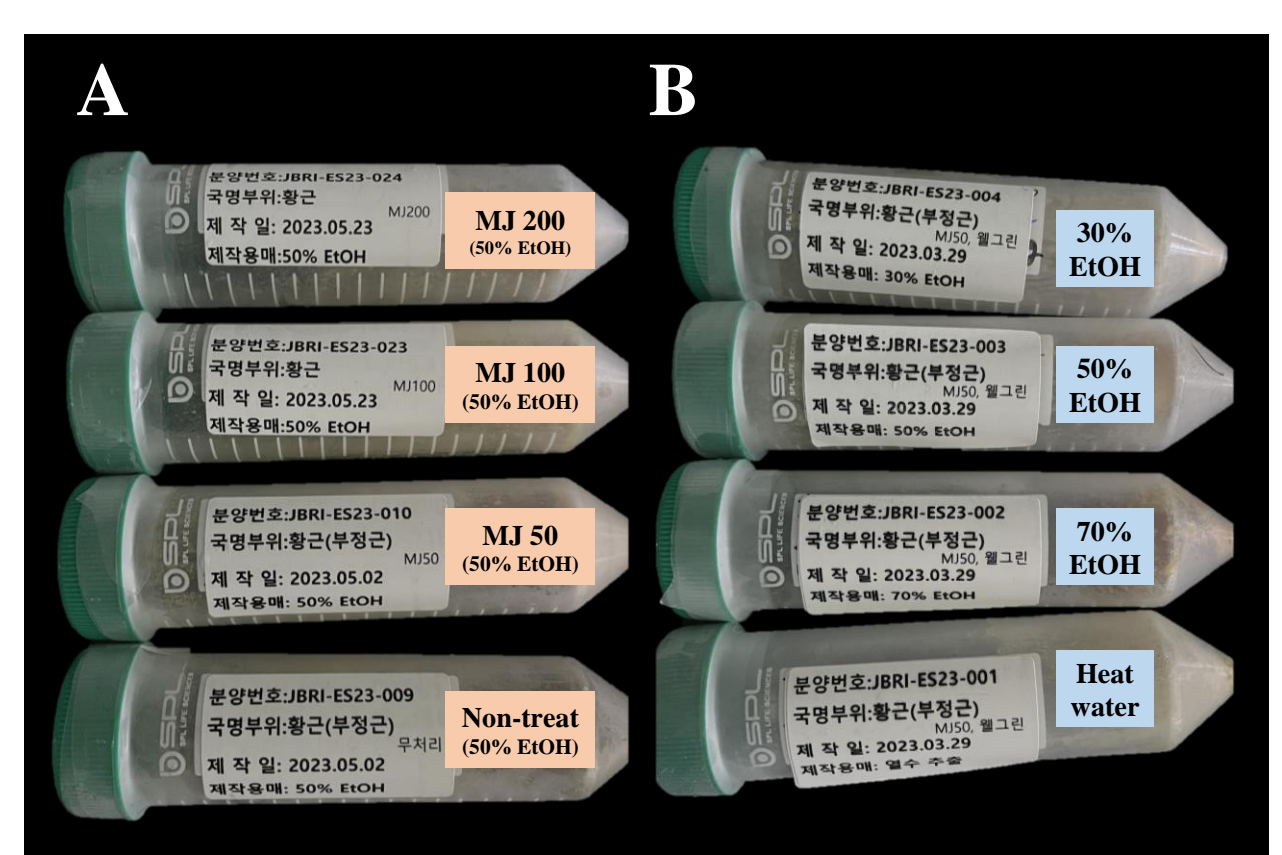


Fig. 3. *In vitro* extraction samples from *Hibiscus hamabo* adventitious root.
A. Effect of methyl jasmonate concentration;
B. Effect of solvent concentration on methyl jasmonate 50 μM treatment.

2 Bioactive compounds and antioxidant activity screening

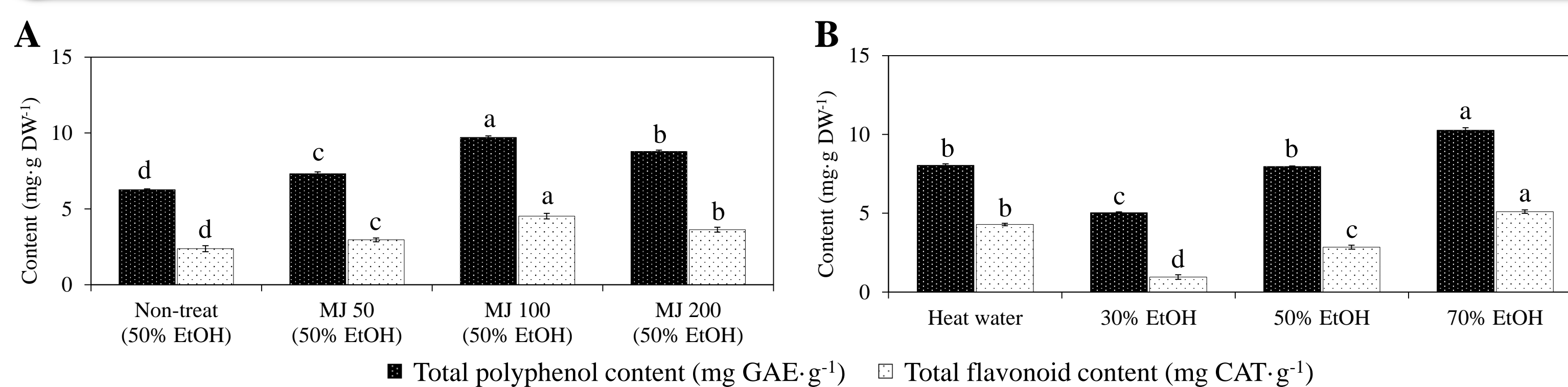


Fig. 4. Total polyphenol content and total flavonoid content on *Hibiscus hamabo* adventitious root analyzed by colorimetric method according to methyl jasmonate concentration (A) and solvent concentration on methyl jasmonate 50 μM treatment (B).

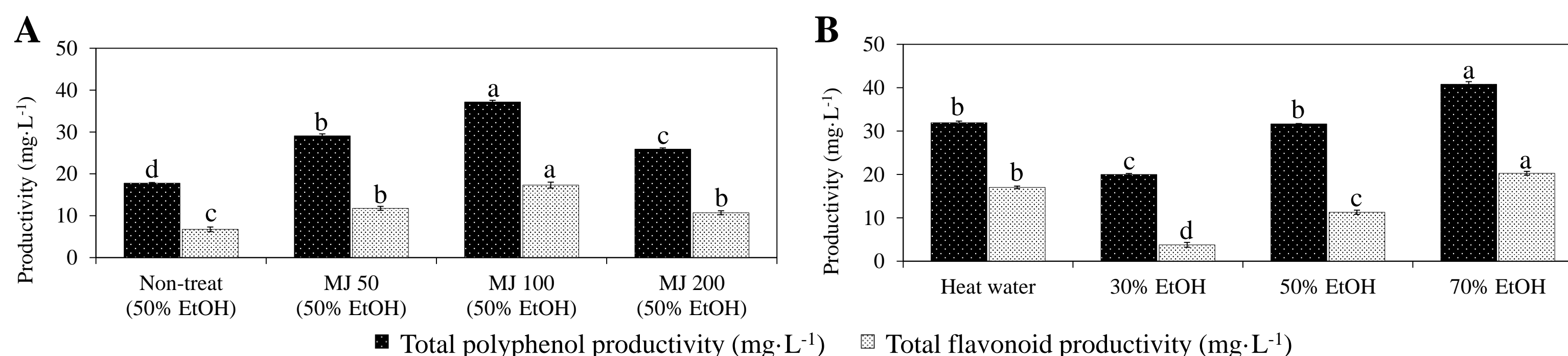


Fig. 5. Total polyphenol productivity and total flavonoid productivity on *Hibiscus hamabo* adventitious root cultured in 5,000 ml bioreactor according to methyl jasmonate concentration (A) and solvent concentration on methyl jasmonate 50 μM treatment (B).

Table 4 DPPH and ABTS radical scavenging activity on *Hibiscus hamabo* adventitious root analyzed by colorimetric method according to methyl jasmonate concentration (A) and solvent concentration on methyl jasmonate 50 μM treatment (B).

Extraction solvent	DPPH IC ₅₀ (μg·g ⁻¹)				Extraction solvent	DPPH IC ₅₀ (μg·g ⁻¹)			
	Non-treat	MJ 50	MJ 100	MJ 200		Heat water	30% EtOH	50% EtOH	70% EtOH
Heat water	>1,000	229.9	577.0	>1,000	ABTS IC ₅₀ (μg·g ⁻¹)	>1,000	>1,000	>1,000	685.4
70% EtOH	>1,000	522.9	545.6	652.6		>1,000	>1,000	>1,000	685.4
Extraction solvent	ABTS IC ₅₀ (μg·g ⁻¹)				Extraction solvent	ABTS IC ₅₀ (μg·g ⁻¹)			
	Non-treat	MJ 50	MJ 100	MJ 200		Heat water	30% EtOH	50% EtOH	70% EtOH
Heat water	>1,000	123.1	111.6	168.4	ABTS IC ₅₀ (μg·g ⁻¹)	168.3	333.2	132.7	73.3
70% EtOH	>1,000	102.5	109.1	117.8		168.3	333.2	132.7	73.3

Conclusion

- Identified optimal concentration of **elicitor treatment (MJ, methyl jasmonate → 50 μM)** and **extraction solvent (ethyl alcohol → 50% EtOH)** from *Hibiscus hamabo* adventitious root in 5,000 ml bioreactor for enhancing bioactive compounds (polyphenols, flavonoids) and antioxidant activities (DPPH and ABTS scavenging activities)
- ✖ **Considered economical bio-ingredient production for saving manufacturing cost**
- The cosmetic products from natural adventitious root cultures of yellow hibiscus will be one of **the ecofriendly manufacture procedures under in vitro controlled culture system without natural habitat destruction**

Acknowledgement

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