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Metabolite profiling, terpenoid and kaurenoic acid production of *Adenostemma platyphyllum* at different concentrations of hydroponic solutions in the wick system

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Abstract. *Adenostemma platyphyllum*, a medicinal plant belonging to the Asteraceae family, has gained increasing attention due to its potential as a source of bioactive compounds with diverse therapeutic properties but has not been widely cultivated. This work aims to obtain the optimum concentration of AB-mix solution to produce higher terpenoid and kaurenoic acid, as well as metabolite profiling in cultivating *A. platyphyllum* using a hydroponic wick system. This research uses a one-factor randomized block design of different concentrations of AB-mix nutrient solutions. Total terpenoids were quantified using the UV-Vis spectrophotometric method, total kaurenoic acid was determined using a high-performance liquid chromatography (HPLC) method, and the metabolite profiling was analyzed using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) instrument. Several terpenoid compounds have been identified in *A. platyphyllum*, including Ent-17-Oxo-15-kauren-19-oic acid, andrographolide, cafestol, alpha-Farnesene, curcumene, as well as ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic acid (11αOH-KA) and 11α,15-dihydroxy-16-kauren-19-oic acid (11α,15OH-KA), which belong to the kaurenoic acid group. The plants had the highest total terpenoid and kaurenoic acid found in 1,300 mg L^{-1} nutrient concentrations. On the other hand, the highest terpenoid and kaurenoic acid productivity were found in plants with 900 and 1,300 mg L^{-1} AB-mix solution, respectively. Therefore, the optimum concentration of nutrient solution to produce optimum terpenoid and kaurenoic acid levels in *A. platyphyllum* cultivation by hydroponic wick system was 1,300 mg L^{-1}.

Key words: *Adenostemma platyphyllum*; hydroponic; kaurenoic acid; plant growth; total terpenoid.

INTRODUCTION

Adenostemma genus, a plant of the Asteraceae family, has received much attention due to its traditional and widespread use in herbal medicine. *A. platyphyllum* from this genus contains diverse bioactive compounds associated with therapeutic properties, such as terpenoids (Fauzan et al., 2018). A large and diverse natural product derived part of isoprene, like terpenoids, is known for its various biological activities, including anti-inflammatory, anti-melanogenic, and antioxidant properties (Bisht et al., 2021). Apart from this, the agricultural potential of this genus needs to be explored and studied further.

Kaurenoic acids, a type of diterpenoid, are a group of compounds found in *A. lavenia*, another species from the *Adenostemma* genus. This compound is responsible for 50% of the anti-melanogenic activity in the extract (Hamamoto et al., 2020). On the other hand, there is no investigation of the total terpenoid and kaurenoic acid levels in *A. platyphyllum* species. Understanding the total terpenoid of *A. platyphyllum* is crucial for clarifying the chemical basis of its therapeutic effects and exploring its potential as an herbal medicine.

To conduct a comprehensive analysis of plant content, a substantial sample size is necessary. However, the *A. platyphyllum* species remains uncultivated and thrives in its natural habitat within tropical regions. Therefore, a cultivation method is needed to produce plants with high levels of secondary metabolite, for example hydroponic method. Cultivating plants using hydroponic systems has demonstrated efficiency and sustainability in enhancing plant biomass and boosting secondary metabolite production (Majid et al., 2021).

In hydroponic systems, the composition of the AB-mix nutrient solution plays a vital role in plant growth (Ghatage et al., 2019) and the synthesizing of the phytochemicals responsible for the medicinal properties (Bulawa et al., 2022). According to a study conducted by Attarzadeh et al. (2020), it was found that the utilization of hydroponic systems with nutrient solutions rich in elements like phosphorus demonstrated the potential to enhance the synthesis of secondary metabolites in plants belonging to the Asteraceae family. This research aims to comprehensively examine the effect of different concentrations of the AB-mix solution in a hydroponic wick system on the growth parameters and phytochemical production of *A. platyphyllum*.

MATERIALS AND METHODS

Experimental material: The experiment was carried out from December 2021 to January 2022 in the greenhouse of the Faculty of Agriculture, IPB University, Bogor, Indonesia. The study area lies at -6°55’ 10.8” S, 106°71’ 57.6” E, 159 m above sea level. Seeds of *A. platyphyllum* were collected from forests in Karanganyar, Pekalongan, Indonesia. The plants' identification was conducted by curators affiliated with the Bandungen Herbarium (FIPIA) at the SITH ITB (Nurlela et al., 2022). Specimens verifying the plants were deposited at the Bandungense Herbarium (FIPIA) at SITH ITB, assigned with collection numbers FIPIA-DEP35, FIPIA-DEP36, and FIPIA-DEP37. The stem cutting of *A. platyphyllum* was transferred into a rock wool medium and cultitaved with wick system of hydroponic method that are generally passive aerated system.

Treatments: In this study, a one-factor randomized block design was employed, focusing on different concentrations of AB mix fertilizer. The five AB-mix concentrations were replicated three times (in three groups: group 1, group 2, and group 3), resulting in 15 experimental units. Each experimental unit comprised five hydroponic containers, totaling 75 containers. Within each container, nine plants were grown, resulting in a total of 675 *A. platyphyllum* plants across all treatments. The concentration of AB-mix fertilizer was set in 5 concentrations of 0, 700, 900, 1,100, and 1,300 mg L^{-1}. The fertilizer used as the nutrient solution is AB mix fertilizer manufactured by CV. Agrifam. The environmental conditions in the greenhouse have a 14% UV plastic roof, a 50-mesh insect net, and a 50% shaded net.

Preparation of AB-mix Nutrient Solution

The nutrient solution is prepared by dissolving separate stocks of A and B, both produced by CV. Agrifam. Each stock, weighing 500 g, is dissolved in 5 L of water to create concentrated solutions. Subsequently, these concentrated stocks A and B are combined. The resulting nutrient solution is then poured into plastic tubs, with each tub containing a water volume of 7 L.

Observation of Plant Growth

The plants were harvested six weeks after applying treatment at each concentration. During the cultivation period, non-destructive observations were also made, including the leaf and branch number and plant height at once-a-week intervals. Destructive observations included determining leaf area, relative growth rate (RGR), net assimilation rate (NAR), and chlorophyll content. RGR calculation refers to Saharuddin et al. (2018), based on the dry weight of plants per unit time based on the following formula :

$$\text{RGR (g week}^{-1}) = 1\ W\ X\ \Delta W\ \Delta t = \ln W2 - \ln W1\ t2 - t1 \quad (1)$$

where W1: ln dry weight of plants at observation 1; W2: ln dry weight of plants at observation 2; t1: observation time 1; t2: observation time 2.

NAR calculations refer to those carried out by Saharuddin et al. (2018), based on dry weight and plant area per unit time using the following formula:

$$\text{NAR (g cm}^{-2}\text{week}^{-1}) = 1\ A\ X\ \Delta W\ \Delta t = \ln A2 - \ln A1\ A2 - A1\ X\ \ln W2 - \ln W1\ t2 - t1 \quad (2)$$

where W1: ln plant dry weight at observation 1; W2: ln plant dry weight at observation 2; t1: observation time 1; t2: observation time 2; A1: ln leaf area 1; A2: ln leaf area 2.

Sample Preparation

Subsequently, after harvested the leaves were dried, and their wet and dry weights were measured. Finally, the dried leaves were ground using a blender. A 1 g sample of the powder was extracted with 7 mL of methanol using a Sartorius shaker for one hour. The resulting mixture was then filtered through Whatman filter paper number 93. The residue underwent two additional solvent extractions until the solvent volume reached 20 mL. To determine the moisture content of *A. platyphyllum*, the gravimetric method was employed, specifically following the guidelines outlined in AOAC 2012, chapter 4, item 4.1.06, method 930.15.

Determination of Total Terpenoid Content

Determination of chlorophyll content was carried out referring to Sims and Gamon (2003) by weighing 0.02 g of fresh sample, at that point putting the sample in a mortar and including 1 mL of acetone, then grinding until homogeneous. The homogenized sample was transferred into a 2 mL microtube, then the mortar and pestle were washed with acetone solution and after that the wash water was included into the microtube until the volume was full. The sample within the microtube was then isolated using centrifugation at a speed of 18.845 g for 10 seconds. The isolated supernatant was then transferred 1 mL into a test tube and after that homogenized until the precipitate disappeared. The samples were then analyzed using a UV-Vis spectrophotometer at wavelengths of 537, 647 and 663 nm. The equation for deciding chlorophyll content is appeared within the condition below. The coefficients contained within the equation are decided based on the particular retention coefficients recorded in Lichtenthaler (1987).

Determination of Total Terpenoid Content

According to Łukowski et al. (2022), a total of 0.2 g of A. platyphyllum leaf simplicia was combined with 2.8 mL of cold methanol. The mixture was then macerated for 48 hours at low temperature and subsequently centrifuged at 4,000 g for 15 minutes. The resulting supernatant was filtered and transferred to a test tube. Next, 1.5 mL of chloroform and 100 µL of H_2SO was added. The absorbance was analyzed by UV-Vis spectrophotometer at a wavelength of 520 nm. The total terpenoid content was determined based on the standard nerol curve ($y = 0.1817x + 0.0249$; $R^2 = 0.9912$). Terpenoid productivity (mmol nerol plant^{-1}) is determined by multiplying the total terpenoid concentration, mmol nerol equivalent/g dry weight units (mmol NE g^{-1} DW), by the dry weight of the plants at harvest (g plant^{-1}). Average mass of a plant and the dry matter content, as results are given (g plant^{-1}) based on previous research (Tamsin, et al., 2023).

Determination of Kaurenoic Acid Content

Determination of kaurenoic acid content by high-performance liquid chromatography (HPLC) refers to the method used by Hamamoto et al. (2020). A 10 mg sample of the extract was combined with 1,000 µL of 50% methanol. After homogenization for 1 hour, the mixture underwent centrifugation at 14,500 g for 5 minutes. The resulting supernatant was transferred to a microtube, from which 10 µL was drawn using a syringe and injected into the HPLC injector port. The column specifications included a C18 ODS column (4.6 mm × 50 mm: RP-18 GP, Kanto Kagaku, Tokyo, Japan) with acid A solvent (0.1% formate in distilled water) and solvent B (100% acetonitrile). The gradient elution profile consisted of 10% solvent B at 0.0–0.1 minutes, followed by 10.0–35.0% at 1.0–7.0 minutes, 35.0% – 47.5% solvent B at 7.0–10.0 minutes, and 47.5% – 100% solvent B at 10.0–12.0 minutes. The system was then maintained at 100% solvent B for an additional 5 minutes. Detection of the 11αOH-KA compound occurred via UV absorption at a wavelength of 245 nm. The concentration of the 11αOH-KA compound in A. platyphyllum was determined using a standard curve based on 11αOH-KA isolated from A. lavenia plant ($y = 3953.2x + 64465$; $R^2 = 0.9982$). Kaurenoic acid productivity (mmol/plant) is determined by multiplying the total kaurenoic acid concentration (mmol g^{-1} DW) by the dry weight of the plants at harvest (g plant^{-1}).

Metabolite Profiling with LC-MS/MS

Metabolite profiling from methanol extract of *A. platyphyllum* leaves prepared before was investigated through UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS ThermoScientific system according to Rafi et al. (2020), with Accucore™ Vanquish C18 column (100×2.1 mm, 1.5 µm) was used for separation. The positive scan mode of an MS ES (electrospray ionization) was used to acquire accurate masses in the 200−1,500 m/z range. The injection volume used was 2 µL with a flow rate of 0.2 mL min^{-1} and a column temperature of 30 °C. The gradient separation used 0.1% formic acid in H_2O (phase A) and 0.1% formic acid in acetonitrile (phase B) as a mobile phase. The eluent composition was 5% phase B at 0–1 min, 90-5% phase A, and 10–95% phase B at 1–17 min, 100% phase B at 17–18.5 min, and 5% B at 18.5 min. Three sample replicates were blank-corrected.

The UHPLC-Q-Orbitrap HRMS analysis involved several additional conditions. Fragmentation was achieved using collision energies of 18, 35, and 53 eV. The spray voltage was set at approximately 3.9 kV, and the capillary temperature was maintained at 320 °C. Sheath gas and auxiliary gas flow rates were set to 15 and 3 mL min^{-1}, respectively. We used scan type full MS/dd MS^2 for positive ion mode. The potential identification of detected metabolites in *A. platyphyllum* extracts was made by analyzing the mass spectrum obtained from UHPLC-Q-Orbitrap HRMS. The data was processed using Compound Discoverer version 2.2. Furthermore, the obtained MS/MS spectra were subjected to another compound identification and characterization by utilizing various databases, including PubChem, ChemSpider, HMDB (Human Metabolome Database), and CFM-ID (Compound Fragmentation Mass Spectrometry Identification).

Statistical analysis

The data were subjected to an ANOVA test. Duncan's multiple range test (DMRT) was used for mean separation. Data were analyzed using SAS on Demand for academic programs. LC-MS/MS data were processed using Thermo Scientific Xcalibur software. Afterward, Origin software was employed to generate chromatograms and perform data visualization.

RESULTS

Plant Growth

The growth observations were carried out 1 to 6 weeks after planting (WAP). Parameters observed during the study were plant height (Fig. 1, a), leaf number (Fig. 1, b), branch number (Fig. 1, c), and leaf area (Fig. 1, d), aimed to know a plant's response during the cultivation period to the treatment applied. Increasing the concentration of the hydroponic nutrient solution in *A. platyphyllum* plants tends to improve their growth. AB-mi concentrations significantly increased the plant height, branch number, leaf number, and leaf area.

The growth of *A. platyphyllum* plants without adding AB-mix nutrient solution did not increase every week, resulting in significantly lower plant height, leaf number, branch number, and leaf area. In contrast, the growth of plants with concentrations of 700, 900, 1,100, and 1,300 mg L^{-1} AB-mix increased constantly. However, there was no significant difference between the four treatments on growth parameters at the end of the cultivation period.

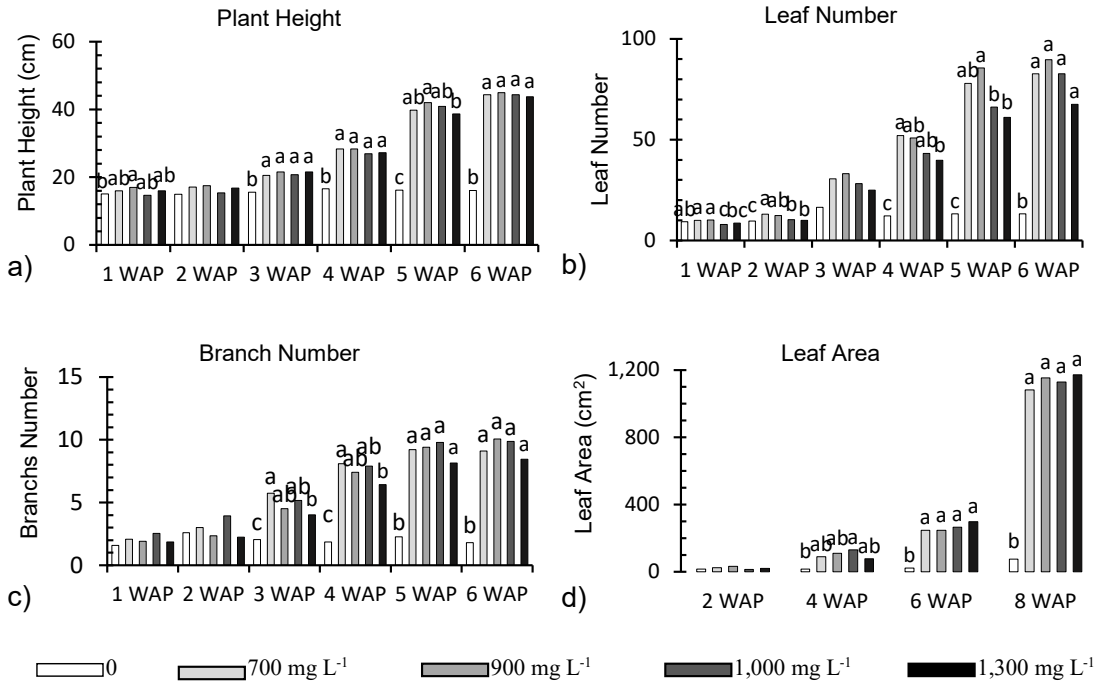

Figure 1. Plant height (a); leaf number (b); branch number (c); and leaf area (d) of *A. platyphyllum*. Different bar charts followed by different letters are significantly different based on DMRT 5%

Relative Growth Rate

The relative growth rate (RGR) or the relative increase in plant biomass per unit of time from *A. platyphyllum* is illustrated in Table 1. Comparing RGR values between treatments allows for identifying factors influencing plant growth. Based on plant growth observation, different concentrations of AB-mix solution did not significantly affect *A. platyphyllum* RGR.

Table 1. The relative growth rate of *A. platyphyllum*

Treatment	Relative Growth Rate ± SD (g $g^{-1}day^{-1}$)		
	Weeks 2 & 4	Weeks 4 & 6	Weeks 6 & 8
0	0.0118 ± 0.0095^{b}	0.0235 ± 0.0149	0.0653 ± 0.0349
700	0.0806 ± 0.0699^{ab}	0.0220 ± 0.0149	0.1128 ± 0.0066
900	0.0793 ± 0.0581^{ab}	0.0258 ± 0.0174	0.1308 ± 0.0748
1,100	0.1278 ± 0.0617^{a}	0.0425 ± 0.0136	0.1170 ± 0.0331
1,300	0.0311 ± 0.0197^{b}	0.0516 ± 0.0538	0.1039 ± 0.0894

Different numbers followed by letters differ significantly with DMRT 5%.

Net Assimilation Rate

In contrast to RGR, the net assimilation rate (NAR) measures plants' photosynthetic ability to produce dry matter (biomass). Based on the data shown in Table 2, the net assimilation rate of *A. platyphyllum* at 6−8 WAP, the plant experienced a significant difference.

Table 2. Net assimilation rate of *A. platyphyllum*

Treatment	Net Assimilation Rate ± SD (g cm^{-2} day^{-1})		
	Weeks 2 & 4	Weeks 4 & 6	Weeks 6 & 8
0	0.0002 ± 0.0001^{b}	0.0003 ± 0.0002	0.0007 ± 0.0002^{a}
700	0.0006 ± 0.0005^{ab}	0.0001 ± 0.0001	0.0004 ± 0.0001^{b}
900	0.0005 ± 0.0004^{ab}	0.0001 ± 0.0001	0.0005 ± 0.0003^{ab}
1,100	0.0009 ± 0.0005^{a}	0.0002 ± 0.0001	0.0005 ± 0.0002^{ab}
1,300	0.0004 ± 0.0003^{ab}	0.0003 ± 0.0004	0.0004 ± 0.0004^{b}

Different numbers followed by letters differ significantly with DMRT 5%.

Chlorophyll Content

The chlorophyll content obtained from the *A. platyphyllum* plant is shown in Table 3, the highest value was obtained from the *A. platyphyllum* with 1,100 mg L^{-1} AB-mix solution. Meanwhile, plants treated with concentrations of 700, 900, and 1,300 mg L^{-1} did not significantly differ. On the other hand, plants without AB-mix solution produced the lowest chlorophyll content.

Table 3. Chrolophyll content of *A. platyphyllum*

Treatment	Chrolophyll a ± SD (µmol cm^{-2})	Chrolophyll b ± SD (µmol cm^{-2})	Total Chrolophyll ± SD (µmol cm^{-2})
0	0.0055 ± 0.00087^{c}	0.0025 ± 0.00039^{c}	0.008 ± 0.00131^{c}
700	0.0079 ± 0.00043^{b}	0.0036 ± 0.00019^{b}	0.0114 ± 0.00048^{b}
900	0.0087 ± 0.0004^{ab}	0.0039 ± 0.00018^{ab}	0.0126 ± 0.0002^{ab}
1,100	0.0094 ± 0.00085^{a}	0.0042 ± 0.00038^{a}	0.0136 ± 0.00094^{a}
1,300	0.0084 ± 0.00073^{ab}	0.0034 ± 0.00032^{ab}	0.0123 ± 0.00032^{ab}

Different numbers followed by letters differ significantly with DMRT 5%.

Total Terpenoid Concentration and Productivity

A. platyphyllum without AB-mix solution treatment resulted in a significant minimum total terpenoid concentration, 3.46 mmol NE g^{-1} DW (Fig. 2, a). Total terpenoids increased significantly with increased AB-mix concentration. The highest total terpenoid concentration was found in plants with 1,300 mg L^{-1} of AB-mix treatment (9.65 mmol NE g^{-1} DW). Moreover, the highest terpenoid productivity was found in plants treated with 900 mg L^{-1} AB-Mix solution (24.38 mmol NE $plant^{-1}$).

Total Kaurenoic Acid Concentration and Productivity

The total kaurenoic acid contained in *A. platyphyllum* was significantly affected by the concentration of the AB-mix nutrient solution (Fig. 2, b). The results of the analysis using the HPLC instrument stated that AB-mix concentration of 1,300 mg L^{-1} produced the highest total kaurenoic acid and kaurenoid acid productivity in *A. platyphyllum,* 2.74 mM g^{-1} DW and 5.91 mM $plant^{-1}$ respectively. On the other hand, plants without AB-mix solution generate the lowest total kaurenoid acid and productivity, 1.85 mM g^{-1} DW and 0.46 mM $plant^{-1}$, respectively.

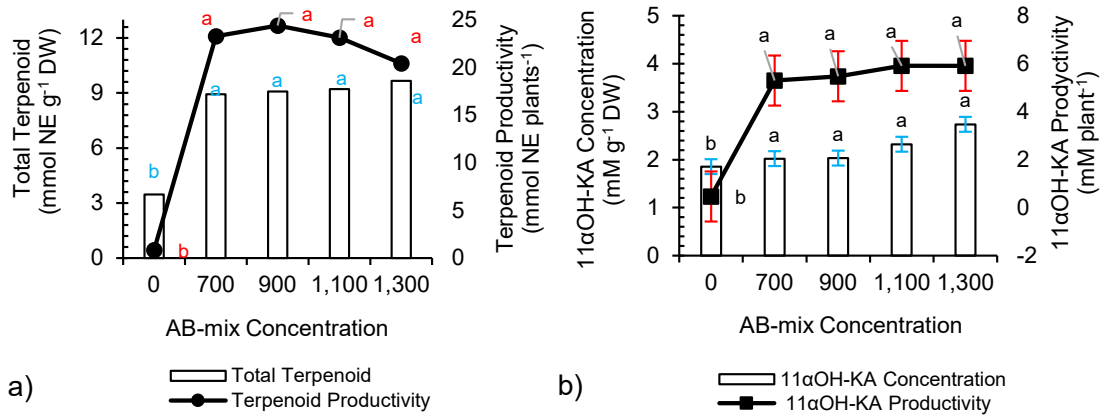

Figure 2. Total terpenoid and terpenoid productivity (a); Total kaurenoic acid and kaurenoic acid productivity (b) of *A. platyphyllum.* Different bar/line charts followed by different letters are significantly different based on DMRT 5%.

Metabolite Analysis by LC-MS/MS

A total of 17 metabolites from six prominent compounds were putatively identified (Table 3) in the treatment methanol extract of *A platyphyllum* using UHPLC-Q-Orbitrap-HRMS (Fig. 3, a). A cluster analysis was conducted on the metabolites identified in *A. platyphyllum*. The results were visualized using a heatmap format (Fig. 3, b).

DISCUSSION

Plant growth observations are crucial for understanding plant development and assessing crop performance. Plant growth can be explained as increased plant volume and mass, with or without constructing new structures such as organs, tissues, cells, and organelles. Growth is usually associated with development and reproduction (Brukhin & Morozova, 2011). Observations of plant growth provide valuable insights into plant strength, biomass accumulation, and productivity.

According to Wang et al. (2020), nitrogen in the AB-mix nutrient solution is a factor affecting plant height, which is required in large quantities for plant growth because it functions in the formation of cells, tissues, and plant organs. In addition, nitrogen also plays a role in various metabolic processes, including photosynthesis, that trigger vegetative growth. Increasing the available nitrogen will produce large amounts of protein, so plant tissue growth will also increase. A lack of nitrogen elements will inhibit plant growth because it cannot form cells or tissues (Jiaying et al., 2022), causing the plant height of *A. platyphyllum* at a concentration of 0 mg L^{-1} to become stunted.

Another parameter of plant growth is relative growth rate (RGR) and net assimilation rate (NAR). RGR measures a plant's average growth rate over time by quantifying the increase in plant biomass per unit of time (Rajput, 2017). Determining the RGR provides insight into resource utilization efficiency, overall growth potential, and the impact of different processing and environmental conditions. The RGR during the 2−4-week period after planting exhibited a statistically significant variation, primarily attributed to the ongoing adaptive responses of plants to the environmental conditions.

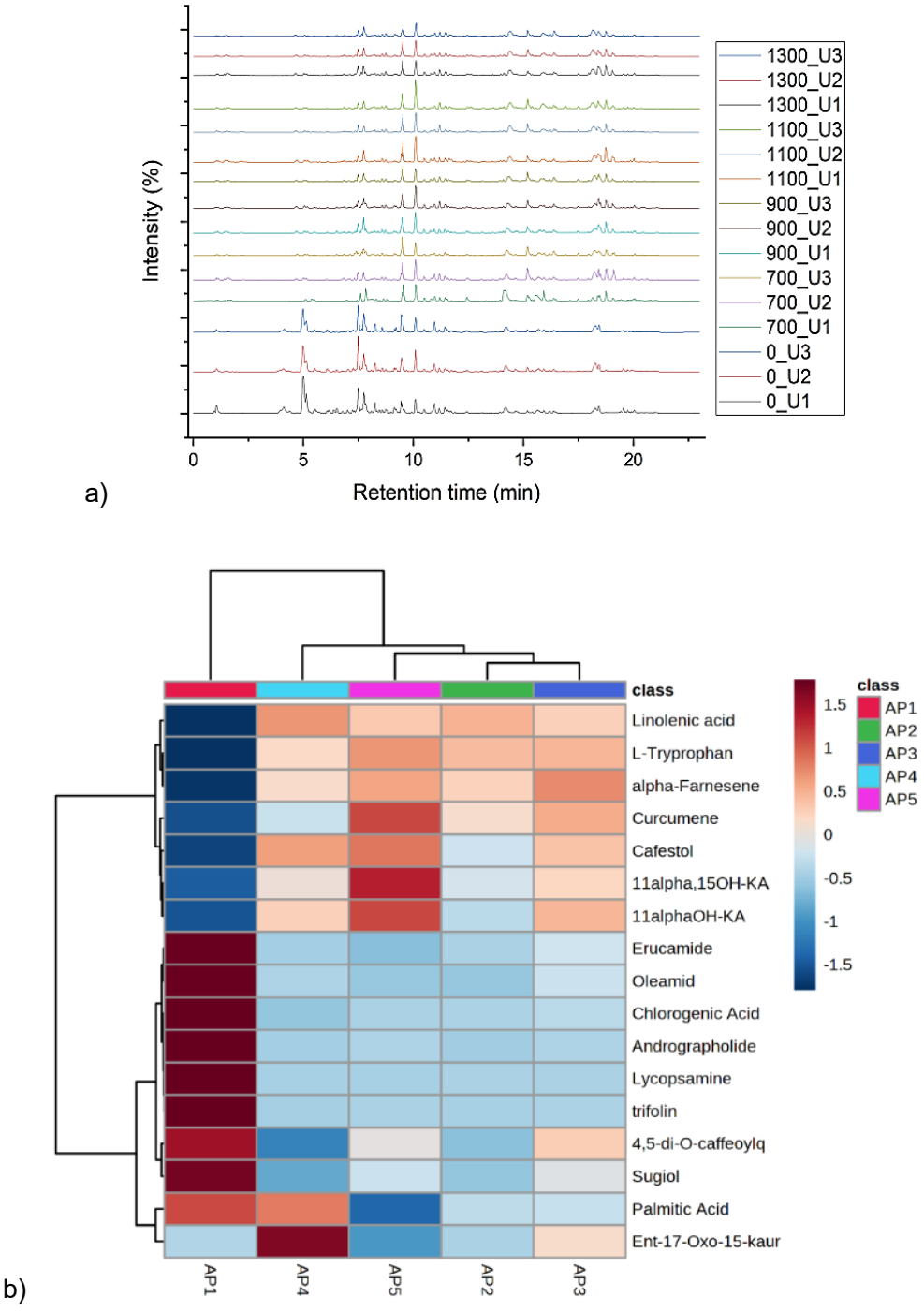

Figure 3. The base peak of the LC-MS/MS chromatogram in the positive ionization mode of *A. platyphyllum* (a); Heatmap analysis of identified compound in *A. platyphyllum* (b). K0 is 0 mg L^{-1}, K1 is 700 mg L^{-1}, K2 is 900 mg L^{-1}, K3 is 1,100 mg L^{-1}, and K4 is 1,300 mg L^{-1}.

The environmental adaptation process induces stunted growth in plants (Li et al., 2021), manifested by a wilted appearance and reduced turgidity in their morphological characteristics. Moreover, during 4−6 WAP and 6−8 WAP, the growth of *A. platyphyllum* exhibited a recovery phase, with the relative growth rate showing an upward trend compared to the preceding weeks. However, the analysis of variance revealed no significant influence of varying concentrations of AB mix fertilizer on the RGR values during the 4−6 and 6−8 WAP time intervals.

The NAR value expresses how plants convert assimilated carbon into new biomass (Safitri et al., 2018). The NAR exhibited significant variations between 2−4 and 6−8 weeks after planting, indicating dynamic physiological responses. Conversely, at the 4−6 weeks marker, the NAR values showed no significant distinctions, implying comparable photosynthetic capacities across the five treatments assessed. Statistical analyses revealed that plants without AB-mix solution exhibited significantly higher NAR values than the 700 and 1,300 mg L^{-1} treatments.

Notably, leaf area is among the influential factors affecting NAR, wherein plants with broader leaves typically exhibit higher NAR due to increased photosynthetic surface area and subsequent biomass production (Lewar & Hasan, 2022). However, an inverse relationship between leaf area and NAR was observed in the 700 and 1,300 mg L^{-1} treatments, whereby a higher leaf area resulted in reduced NAR and could be attributed to shading effects caused by lush leaves, hindering efficient photosynthesis in the shaded portions of leaves (Safitri et al., 2018).

Chlorophyll content determination in plant leaves is crucial in evaluating photosynthetic efficiency and plant health. Chlorophyll content in plants can be influenced by various factors, including intrinsic factors, which pertain to plant-specific characteristics, and extrinsic factors, which refer to environmental influences (Li et al., 2018). One factor that influences chlorophyll content is nutrient availability.

Plants without AB-mix solution exhibit nutrient deficiency, resulting in an insufficient energy supply to support growth and photosynthesis. Nitrogen is an essential component of the chlorophyll molecule, especially in the form of the magnesium porphyrin complex (Borah & Bhuyan, 2017). The presence of sufficient nitrogen promotes chlorophyll synthesis and increases chlorophyll content. Limited nitrogen levels can limit chlorophyll production, lower chlorophyll levels, and cause chlorosis or leaf yellowing (Sakuraba, 2022).

Determining phytochemicals, like total terpenoid and kaurenoic acid content in *A. platyphyllum*, is essential in evaluating the plant's potential medicinal and pharmacological properties. One factor that affects the production of phytochemical content is nutrient availability. Plants treated with AB-mix solution tended to have higher total terpenoids caused by several mechanisms. The availability of adequate nutrients, particularly nitrogen (N), phosphorus (P), and potassium (K), provides the necessary for plants' building blocks (Xu et al., 2020) and energy for secondary metabolite biosynthesis, as well as terpenoid synthesis.

These macronutrients are essential to biosynthesis precursors, enzymes, coenzymes, and energy carriers. For example, sufficient phosphorus promotes some precursors for terpenoid production. The terpenoid precursors IPP (isopentenyl diphosphate), DMAPP (dimethylallyl pyrophosphate), GDP (geranyl diphosphate), and FDP (farnesyl diphosphate) possess high-energy phosphate bonds. In addition, phosphorus is part of ATP and NADPH molecules needed for terpenoid synthesis via the mevalonate (MVA)

and methylerythritol phosphate (MEP) pathways, hence phosphorus may be an essential element for terpenoid biosynthesis (Bustamante et al., 2020).

A. platyphyllum, a wild species, has been introduced into cultivation for the production of terpenoids. Research conducted on cultivated sunflower (*Helianthus annuus*) has indicated significant compositional diversity of volatiles among the studied lines of *H. annuus*. This diversity includes a notable reduction in the total abundance of volatiles compared to wild *H. annuus* (Bahmani et al., 2023). The findings of this study suggest that the possibility of volatile terpenoids diversity for breeding the *A. platyphyllum*.

One of the terpenoid compounds found in *Adenostemma* is kaurenoic acid. Previous studies by Maeda et al. (2022) have reported the presence of at least three different types of kaurenoic acid in *A. lavenia,* namely ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic acid (11αOH-KA), 9,11α-dehydroxy-15-oxo-kauren-16-en-19-oic acid (9,11αOH-KA), and 11α,15-dihydroxy-16-kauren-19-oic acid (11α,15OH-KA). Additionally, Batubara et al. (2020) have researched *A. lavenia* and highlighted that the terpenoid compound 11αOH-KA, successfully extracted from this plant, exhibits significant biological activities such as antitumor, anti-melanogenesis, and anti-inflammatory effects. This compound belongs to the diterpenoid classification, which has four repeated isoprene units, as described by (Hamamoto et al., 2020).

Plant secondary metabolite productivity refers to the capacity of plants to produce and accumulate secondary metabolites. The quantification of secondary metabolite productivity can be assessed by multiplying the biomass of the harvested plants with the total concentrations or levels of the secondary metabolite (Abbasi et al., 2019). Plants without AB mix nutrient solution exhibited the lowest terpenoid and kaurenoic acid productivity due to their significantly lower biomass.

Insufficient nutrient availability negatively impacts plant biomass, leading to reduced terpenoid production. The observed low secondary metabolite productivity reflects the poor ability of plants to synthesize secondary metabolites due to suboptimal nutrition conditions (Bahmani et al., 2020). In contrast, the four other treatments demonstrated comparable productivity levels for total terpenoids and kaurenoic acid. Statistical analysis indicated no significant differences among these treatments, demonstrating similar performance in secondary metabolite production.

The chemical content from the methanol extract of *A. platyphyllum* analyzed using UHPLC-Q-Orbitrap-HRMS resulted in 17 compounds. These metabolites were identified based on their mass spectra and fragmentation patterns that belonged to various compound groups, specifically, the primary metabolite represented by L-tryptophan, which belonged to the amino acid group. The fatty acid group was represented by four compounds, erucamide, oleamide, palmitic acid, and α-Linolenic acid. The secondary metabolites belonging to the alkaloid group were represented by lycopsamine. The flavonoid group was expressed by trifolin, and the phenolic group included chlorogenic acid, 4,5-O-caffeoylquinic acid, and sugiol.

Additionally, seven metabolites were identified from the terpenoid group, represented by ent-17-Oxo-15-kauren-19-oic acid, 11αOH-KA, 11α,15OH-KA, andrographolide, cafestol, α-farnesene, and curcumene. The dominant compounds in *A. platyphyllum* were phenolic compounds, originated lipids (fatty acid), and terpenoids, as reported by Fauzan et al. (2018). Furthermore, the comprehensive metabolite profiling verified the high total terpenoid content in *A. platyphyllum*, confirming numerous

compounds derived from the terpenoid group found in the *A. platyphyllum* plant. This concurrence between the quantitative analysis and secondary metabolite profile reinforces the rich terpenoid diversity within the *A. platyphyllum* plant.

Hierarchical cluster analysis visually represents the variations in metabolite content among *A. platyphyllum*, which is cultivated using different concentrations of AB-mix solution types through a heatmap. Upon the heatmap pattern displayed in Figure 3, the color intensity corresponding to secondary metabolites derived from the terpenoid group, present in *A. platyphyllum* treated with the AB-mix solution (AP2, AP3, AP4, and AP5), exhibited a significant increase compared to the plants without AB-mix solution (AP1). Specifically, the color pattern exhibited by the *A. platyphyllum* plant treated with a concentration of 1,300 mg L^{-1} AB-mix resulted in the highest intensity of red color for the metabolites 11αOH-KA and 11α,15OH-KA, which both belonged to the kaurenoic acid group. These findings corroborate the results obtained from the HPLC analysis, which demonstrated that *A. platyphyllum* treated with a 1,300 mg L^{-1} AB-mix solution displayed the highest total kaurenoic acid content.

CONCLUSION

A. platyphyllum possesses various beneficial properties and bioactive compounds. The plant has been found to contain metabolites from diverse classes, including flavonoids, phenolics, and terpenoids. The highest terpenoid and kaurenoic acid productivity were found in plants with 900 and 1,300 mg L^{-1} AB-mix solution, respectively. Secondary metabolite compounds from the kaurenoic acid group were identified in *A. platyphyllum*, namely 11αOH-KA and 11α,15OH-KA, which have a wide range of biological activities and have potential, especially in the health and pharmaceutical industries.

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