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PHENOLIC AND FLAVONOID PRODUCTION, PHYTOCHEMICAL PROFILE, AND ANTIOXIDANT CAPACITY OF *Adenostemma platyphyllum* AT DIFFERENT CONCENTRATIONS OF HYDROPONIC SOLUTIONS

Produksi Fenolik dan Flavonoid, Profil Fitokimia, dan Kapasitas Antioksidan Adenostemma platyphyllum pada Konsentrasi Larutan Hara Hidroponik Berbeda

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ABSTRACT

Adenostemma platyphyllum is considered a weed with various benefits but has not been widely cultivated. This work aims to obtain the optimum concentration of a nutrient solution (AB-mix) to produce optimum phenolic and flavonoid levels, antioxidant capacity and metabolite profiling in A. platyphyllum using a hydroponic wick system. Different AB-mix nutrient solution concentrations were employed in a one-factor randomized block design. The highest total phenolic, flavonoid concentrations and antioxidant capacity were found in plants without additional nutrient solutions. However, the highest phenolic and flavonoid productivity was found in plants with a nutrient solution concentration of 1100 mg/L. The phenolic/flavonoid group compounds were successfully identified by separating the components using thin-layer chromatography. The intensity of the bands produced from each treatment was quite varied. The untreated plant produced thicker phenolic/flavonoid bands than the other treatments. This was supported by the heatmap pattern produced by the untreated ones, which had high color intensity. Therefore, the optimum concentration of nutrient solution to produce optimum phenolic, flavonoid levels, and antioxidant capacity in A. platyphyllum cultivation by hydroponic wick system was the concentration of 1100 mg/L.

Keywords**:** *Adenostemma platyphyllum, Antioxidant capacity, Flavonoid, Hydroponic, Phenolic*

ABSTRAK

Adenostemma platyphyllum merupakan gulma yang memiliki berbagai macam manfaat, namun belum banyak dibudidayakan. Penelitian ini bertujuan untuk mendapatkan konsentrasi larutan nutrisi (AB-mix) yang optimum untuk menghasilkan kadar fenolik, flavonoid, dan kapasitas antioksidan yang optimum pada *A. platyphyllum* menggunakan hidroponik sistem sumbu. Rancangan acak kelompok satu faktor berupa konsentrasi yang berbeda dari larutan nutrisi campuran AB digunakan pada penelitian ini. Total fenolik, kadar flavonoid total, dan kapasitas antioksidan tertinggi ditemukan pada tanaman tanpa penambahan larutan AB-mix, namun produktivitas fenolik dan flavonoid tertinggi terdapat pada tanaman dengan konsentrasi larutan AB-mix 1100 mg/L. Senyawa golongan fenolik/flavonoid berhasil teridentifikasi melalui pemisahan komponen menggunakan kromatografi lapis tipis. Intensitas pita yang dihasilkan dari masing-masing perlakuan cukup beragam, tanpa perlakuan AB-mix menghasilkan pita fenolik/flavonoid yang lebih tebal dibandingkan perlakuan lain, hal ini didukung dengan pola *heatmap* yang dihasilkan oleh

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tanaman tanpa perlakuan memiliki intensitas warna yang tinggi. Oleh karena itu, konsentrasi larutan nutrisi AB-mix optimum untuk menghasilkan kadar fenolik, flavonoid, dan kapasitas antioksidan optimum pada budidaya *A. platyphyllum* dengan hidroponik sistem sumbu adalah konsentrasi 1100 mg/L.

Kata kunci: *Adenostemma platyphyllum*, Fenolik, Flavonoid, Hidroponik, Kapasitas antioksidan

INTRODUCTION

The *Adenostemma* genus is a group of flowering plants that belongs to the Asteraceae family (Jeong *et al*., 2017). This genus has 23 species, widely spread in tropical areas, and considered a weed or nuisance plant. However, it has many benefits, such as curing coughs, relieving inflammation, and being an antidote to snake venom in some areas (Moncayo *et al*., 2021). *A. platyphyllum* contains large amounts of secondary metabolites such as alkaloids, phenols and terpenoids. According to Fauzan *et al*., (2018), *A. platyphyllum* contains several compounds from the phenolic group. Phenolic is the primary substance produced by the secondary metabolism of plants. Most medicinal plant's properties are usually influenced by these secondary metabolites, especially their antioxidant capacity. This biological activity protects cells against free radical damage, which is essential in health and the development of many chronic diseases**.** So, further efforts must be carried out to produce *A. platyphyllum* plants with optimum secondary metabolites, primarily related to phenolic and flavonoids.

One of the best ways to produce plants with optimum secondary metabolites and antioxidant capacity is by selecting appropriate cultivation methods, such as hydroponic, that are starting to develop rapidly. Hydroponics is a modern farming technique without using soil media but using highly nutritious water containing nutrients in plant cultivation (Jan *et al*., 2020). Hydroponic cultivation developed on the basis of plant growth can reach its maximum point when placed under optimum growth conditions. This optimum condition is supported by nutrient solutions containing nutrients for good growth (Dani, 2020). Attarzadeh *et al*., (2020) reported that using nutrient solutions containing elements such as phosphorus in a hydroponic system could increase the production of secondary metabolites, especially phenolics and flavonoids, in the Asteraceae family.

The optimal environmental condition can produce better quantity and quality plants than conventional cultivation methods (Nurbaity *et al*., 2019). Optimal growth conditions will undoubtedly affect plant growth. One indicator of plant growth is leaf thickness which is generally related to plant transpiration rate. The factor that affects leaf thickness is exposure to sunlight, so greenhouses in hydroponic cultivation methods are expected to have a positive role in leaf thickness. Hydroponic techniques, especially the wick system for cultivating medicinal plants/herbs of the Asteraceae family, give good results. This technique increases the productivity of herbal plants in producing secondary metabolite compounds such as phenolics and flavonoids when cultivated using an optimum nutrient composition (F. Ahmed, 2018). Therefore, this study will describe phenolic, flavonoid concentration, and antioxidant capacity from *A. platyphyllum* plants at different concentrations of hydroponic nutrient solution in a wick system which is expected to find the most optimum nutrient concentration solution.

METHODS

Experimental material

Experiments were performed in the Department of Agronomy and Horticulture greenhouse, IPB University, Bogor, Indonesia, from December 2021 – January 2022. The region of study falls within the latitudes -6°55' 10.8" S, longitudes 106°71' 57.6" E, at the altitude of 159 m above sea level. The seed of *Adenostemma platyphyllum* was obtained from Pekalongan, Indonesia. These plants were identified by curators at the Bandungen Herbarium (FIPIA) SITH ITB (Nurlela *et al*., 2022). *A. platyphyllum* seeds were sown into the ground, and the roots were grown for three weeks. Then, stem cutting of *A. platyphyllum* was transferred into a rock wool medium.

Treatments

The plants were treated with the concentration of AB-mix fertilizer (CV. Agrifam) which consisted of five levels, namely 0, 700, 900, 1100, and 1300 (mg/L). For the temperature condition, the greenhouse comprises a 14% UV plastic roof, the partition made of 50 mesh insect net, and covered with 50% shading net. Randomized block design one factorial, namely the different concentrations of AB mix fertilizer were used in this study. A randomized block design is frequently employed to reduce the impact of variability when it is linked to distinct entities such as plants. The five concentrations of AB-mix were repeated three times (three groups; group 1, group 2, and group 3) to obtain 15 experimental units. Each experimental unit consisted of 5 hydroponic containers, with the total hydroponic containers was 75. In 1 container, there were nine plants, so each treatment contains 45 plants with the total of *A. platyphyllum* planted was 675 (Fig.1).

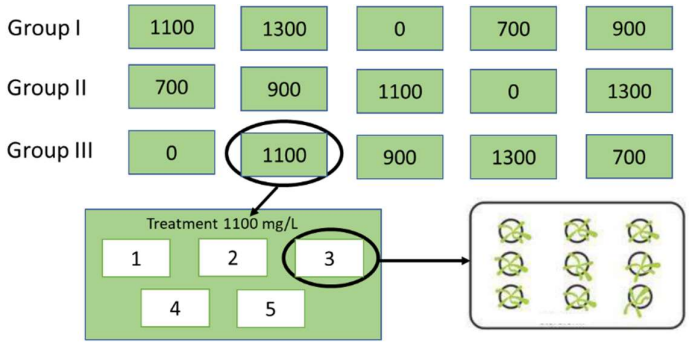

Figure 1. Experimental layout

Sample Preparation

The plants were harvested six weeks after applying treatment at each concentration. Leaf thickness was determined using the destructive method based on Dacosta & Daningsih's (2022) method. The leaves were then dried; the wet and dry weights of the leaves were measured. Dried leaves were then ground using a blender.

Sample Extraction

The powder weighed as much as 1 g, then extracted with 7 mL of methanol using a Sartorius shaker for an hour. The mixture was filtered using Whatman filter paper number 93. The residue was then re-extracted using solvent two times until the solvent volume reached 20 mL. Determination of the moisture content of *A. platyphyllum* used the gravimetric method. The method used was based on AOAC 2012-chapter 4 item 4.1.06. method 930.15.

Determination of Phenolic and Flavonoid Concentration

The total phenolic concentration (TPC) was determined by the Folin-Ciocalteu reagent; meanwhile, the total flavonoid concentration (TFC) was determined using aluminum chloride according to Batubara *et al*., (2020). The standard for determining TPC is gallic acid, and the TPC value was expressed as the gallic acid equivalent (µmol GAE/g DW). Moreover, quercetin was used as the standard to determine TFC (µmol QE/g DW). This means the number of phenolic compounds assumed is proportional to the gallic acid compounds, and the estimated quantity of flavonoid compounds is proportionate to the quercetin compounds contained in a gram of dry leaf weight.

Determination of Phenolic and Flavonoid Productivity

The value of phenolic and flavonoid productivity can be obtained by multiplying the dry weight of the plants at harvest (g/plant) with the concentration of phenolics or flavonoids (µmol GAE/g dry weight and µmol QE/g dry weight) to produce values in units of µmol GAE/plant and µmol QE/plant.

Determination of Antioxidant Capacity

Antioxidant activity was assessed by modifying the DPPH radical scavenger activity method based on Batubara *et al*., (2020). The standard for antioxidant capacity is ascorbic acid (y=1,5362x + 0,0638, R^2 = 0,9943), and its value is reported as ascorbic acid equivalent (µmol AAE/g DW). Unit conversion for antioxidant productivity is carried out by multiplying the dry weight of the plants at harvest (g/plant) with the antioxidant capacity (µmol AAE/g dry weight) to produce values in units of µmol AAE/plant.

Component Separation using Thin-layer Chromatography

The separation of the components contained in the methanol extract of *A. platyphyllum* was carried out by the thin-layer chromatography method (Nadiah *et al*., 2018). TLC silica gel G60 F254 (20 cm × 10 cm) was used to perform chromatography. Then, a 10 µL sample was spotted in bands width 6 mm with a 100 µL CAMAG sample syringe using CAMAG Linomat 5 instrument. The silica plate with the sample spot was transferred to a TLC chamber with 20 µL saturated chloroform. The plate then developed until elution reached the final line. Sample code *A. platyphyllum* 1 (AP1) is an extract without application of AB-mix fertilizer, *A. platyphyllum* 2 (AP2) with 700 mg/L treatment, *A. platyphyllum* 3 (AP3) with 900 mg/L treatment, *A. platyphyllum* 4 (AP4) with 1100 mg/L treatment, and *A. platyphyllum* 5 (AP5) with 1300 mg/L treatment.

The TLC plate was then observed under a UV lamp at 254 nm and 366 nm. After that, the eluted plate was dipped with 10% H_2SO_4 in cold methanol. The plate was then observed again under a UV lamp with a wavelength of 366 nm. The separation of compounds using the TLC method was repeated three times so that 15 chromatogram images were produced. Spot intensities were determined by Image J to find the trend of each retention factor (Rf) and area under the curve (AUC) value at different concentrations of AB-mix solution. The AUC value is the area under the peak curve of the densitogram. Using the ImageJ software, the concentration of various components in the sample can be determined by the AUC value on the densitogram.

Statistical analysis

A randomized block design was used in this study as a design experiment. The advantage of randomized block design is high precision, flexible, easy and simple statistical analysis and can be use when the experimental material is heterogeneous. Outcome data were subjected to an ANOVA test. Duncan's multiple range test was used for mean separation. Data were analyzed using SAS. Correlation analysis from thin-layer chromatography data was performed using heatmap illustration.

RESULT AND DISCUSSION

Leaf Thickness

The results of the analysis using the SAS application described the effect of different concentrations of AB-mix solution and leaf thickness of *A. platyphyllum* plants were obtained. Based on the analysis of variance in a randomized block design, the concentration of the AB-mix nutrient solution (Figure 1A) had a very significant effect (p=0.0001) on leaf thickness.

Healthy plants generally exhibited an upright growth pattern with green leaves. Plants need air, sunlight, warmth, water, and nutrients to maintain their health. Insufficient fulfilment of these requirements can impede growth and even lead to death. The external factors mentioned earlier can affect the transpiration rate of leaves and various aspects of leaf development, including leaf thickness, which indicates a plant's responsiveness to water conditions (Kennard *et al*., 2020). This study's main factor affecting leaf thickness, particularly sun exposure, remained constant, as all plants were cultivated in a 50% shading net.

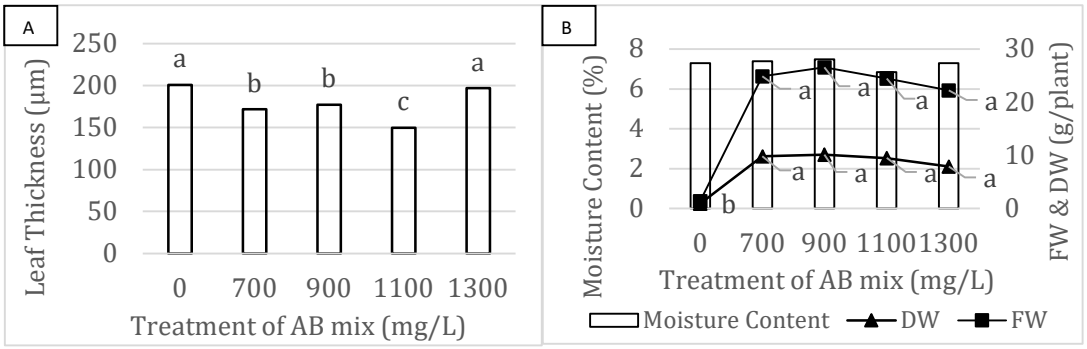

Figure 2. Leaf thickness (A), moisture content, fresh weight (FW), and dry weight (DW) of *A. platyphyllum* leaf (B). Different bar charts followed by different letters are significantly different based on DMRT 5%.

Based on the analysis presented in Fig. 1A, the leaf thickness of *A. platyphyllum* leaves treated with the AB-mix solution concentration 700, 900, and 1100 mg/L was significantly different compared to untreated leaves. The increasing concentration of the AB-mix solution led to an increase in plant growth, resulting in a higher leaves number. Plants with the AB-mix nutrient treatment tend to have broader leaves, causing a denser growth which in turn results in reduced sunlight exposure (Wu *et al*., 2019). According to Dörken & Lepetit, (2018), different light exposure conditions lead to distinct morphological and physiological differences in leaves. Leaves exposed to intense sun exposure were thicker than shade leaves, like leaves from untreated plants with the highest leaf thickness. This might be attributed to more sunlight exposure due to nutrient deficiencies, resulting in stunted growth with a lower number of leaves. In contrast to the 700, 900, and 1300 mg/L treatments, the plants treated with 1300 mg/L exhibited inferior growth

performance. This was evident from their significantly lower dry and wet weights than the AB-mix treatments (Fig. 1B), leading to a relatively reduced leaf count. Consequently, these plants were more susceptible to sunlight exposure, thereby inducing an increase in leaf thickness.

Fresh and Dry Weight at Harvest

The fresh and dry weight of *A. platyphyllum* leaves after harvested increased significantly with the concentration of AB-mix nutrient solution (Figure 1B). Plants treated with nutrient solution had much higher wet and dry leaf weights than plants without AB-mix. The fresh weight of a plant is directly linked to the plant's ability to absorb water from the planting medium. An increased number of leaves positively correlated with higher fresh weight. Furthermore, enhanced nutrient availability contributes to a raised fresh weight of the plants (Khasanah et al., 2018). While, dry weight is a crucial metric for assessing plant growth and development, as it accurately quantifies the accumulated synthesized organic compounds within plants. Additionally, plant dry weight offers valuable insights into the nutritional status of plants. It serves as an indicator for establishing the relationship between nutrient availability and the plant's growth (Sitorus *et al.*, 2014).

Moisture Content

The moisture content from the dry basis of *A. platyphyllum* leaves by the gravimetric method was not significantly affected by the concentration of AB-mix nutrient solution. The five treatments produced plants with moisture content below 10%, indicating that the dried leaves had good quality (Komala & Haryoto, 2020). Leaf water content plays a critical role in assessing a plant's ability to resist drought and salinity, as water stress limits transpiration, leading to stomatal closure and reduced water evaporation from leaf surfaces. This stress adversely impacts photosynthesis and crop productivity. Drought and salinity-tolerant plants exhibit consistent water content and higher dry matter accumulation (Ahmed *et al*., 2013). Furthermore, leaf water content is a valuable guide for optimizing crop fertilizer application and irrigation. Consequently, the assessment and management of leaf water content hold significant importance in crop cultivation (Jin *et al*., 2017).

Total Phenolic and Flavonoid Concentration

Figure 2 shows the variations in the TPC and TFC of *A. platyphyllum* species at different concentrations of AB-mix solution. The highest TPC was found in *A. platyphyllum* without AB-mixed treatment and significantly differed from the other four concentrations (Fig. 2A). Furthermore, the results of the analysis of variance stated that the application of different concentrations of AB-mix fertilizer had no significant effect on the TFC (Fig. 2B). However, the highest concentration of flavonoids was obtained in plants without AB-mix fertilizer application.

Phenols are a class or large group of secondary metabolites commonly found in plants. A distinguishing feature of the phenolic compounds is that they possess an aromatic ring containing at least one hydroxyl group. This group of compounds consists of several classes, such as phenolic acids, flavonoids, and tannins (Bodoira & Maestri, 2020). Phenols often involve various roles, such as pigmentation, plant self-defense against environmental stress, antioxidant, anticancer, antiviral, and anti-inflammatory effects (Aryal *et al*., 2019). Various concentrations of AB-mix solution significantly decreased the TPC but not significantly affect TFP. *A. platyphyllum* plants without treatment had the highest phenolic and flavonoid concentration. This might be caused by plants growing in nutrient-poor environments tend to experience environmental stress and produce more secondary metabolites (Montgomery & Biklé, 2021).

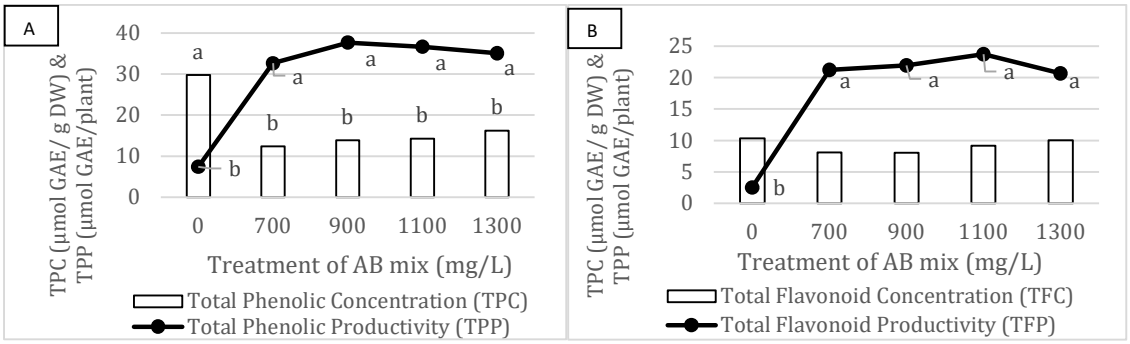

Figure 3. Total phenolic concentration (TPC) and total phenolic productivity (TPP) of *A. platyphyllum* (A), total flavonoid concentration (TFC), and total flavonoid productivity (TFP) of *A. platyphyllum* (B). Different bar/line charts followed by letters differ significantly based on DMRT 5%.

The thickness of *A. platyphyllum* leaves correlates with the levels of TPC and TFC. According to Agati *et al*., (2020), phenolic compounds and flavonoids are typically stored in separate compartments within the leaf, such as vacuoles and cell walls. This leads to an increase in the accumulation of TPC and TPC in the sample. Plants without AB-mix fertilizer and 1300 mg/L treatment had the highest leaf thickness due to receiving the most sunlight intensity compared to other treatments. An increase in leaf thickness enhances the photosynthesis rate, leading to a greater energy demand for the production of secondary metabolites.

Phenolic and Flavonoid Productivity

Secondary metabolite productivity can be determined by multiplying the dry weight of the plant at harvest by the levels of these secondary metabolites. The variance analysis for total phenolic productivity (TPP) and total flavonoid productivity (TFP) of *A. platyphyllum* are shown in Figure 2. The lowest phenolic and flavonoid productivity was obtained in untreated plants, five and ten times lower compared to plants treated with AB-mix solution for TPP and TFP, respectively. The inverse relationship observed between the levels of secondary metabolites and their productivity in *A. platyphyllum* can be attributed to the leaf's thickness and low dry weight produced by plants without a nutrient solution during harvest. Plants with low biomass from poor nutrition exhibit a diminished capacity to produce significant quantities of phenolic compounds and flavonoids. This inverse relationship between low biomass and high levels of phenolics and flavonoids demonstrates the limited ability of such plants to generate secondary metabolites, leading to low production values (Bahmani *et al*., 2020).

On the other hand, the highest value of phenolic and flavonoid productivity was found in the AB-mixed nutrient solution with a concentration of 900 and 1100 mg/L, respectively. This was closely related to the dry leaf biomass of the treated plants of 900 and 1100 mg/L during harvest, which had a relatively high weight. Bahmani *et al*., (2020) reported that plants with high biomass produce lower phenolic compounds but have increased phenolic productivity. Although the plants treated with 1100 mg/L made the highest levels of flavonoids, the difference was statistically not significant compared to the 900 mg/L concentration. However, both concentrations were lower than the treatment without the AB-mix nutrient solution.

Antioxidant Capacity

The variance analysis of the antioxidant capacity was significantly different (Fig. 3). Based on the linear equation of the standard series of ascorbic acid, the highest antioxidant capacity was found in plants without AB-mix fertilizer, so the results are identical to TPC and TFC. On the other hand, *A. platyphyllum* treated with an AB-mix solution exhibited a comparatively lower antioxidant capacity. However, there was no significant difference observed among the four treatments of AB-mix solution. The increase in antioxidant capacity of *A. platyphyllum* might be related to the increased total phenolic content as measured by DPPH assay. Moreover, the productivity of *A. platyphyllum* in terms of its antioxidant capacity might be evaluated by considering the value of its antioxidant capacity in µmol AAE/plant. This value was obtained by multiplying the antioxidant capacity µmol AAE/g of dry weight (DW) by the harvest dry weight of the plant (DW). The most significant productivity in terms of antioxidant capacity was observed in plants treated with AB-mix solution at a concentration of 1100 mg/L. Meanwhile, the antioxidant productivity of plants without AB-mix solution was significantly lower compared to the other four treatments.

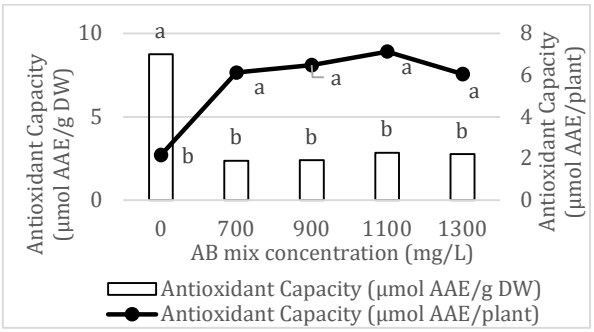

Figure 4. Antioxidant capacity of *A. platyphyllum.* Different bar/line charts followed by letters differed significantly based on DMRT 5%.

The results of the variance analysis indicated that the variation in AB-mix nutrient solution concentration significantly impact the antioxidant capacity and antioxidant productivity of *A. platyphyllum* leaves. Plants without AB-mix solution treatment tended to have lower values of antioxidant productivity compared to the other four treatments, and the results were not different with the productivity of phenolic compounds and flavonoids. These findings related to the low plant biomass at harvest due to nutritional deficiencies, suppressing the production of antioxidants in large quantities. The inverse relationship between antioxidant capacity and antioxidant productivity indicated poor plant performance in producing antioxidants resulting in low productivity values.

Thin-layer Chromatogram Profile

A. platyphyllum leaf samples were also analyzed by thin-layer chromatography using chloroform as the single eluent. The results of the compound separation were observed on a chromatography plate under visible light (Fig. 4A) and were then examined with a UV lamp at a wavelength of 366 nm (Fig. 4B); 254 nm (Fig. 4C). Subsequently, the plate was dipped in a 10% concentrated H_2SO_4 solution and observed under visible light (Fig. 4D), followed by a reading at a wavelength of 366 nm (Fig. 4E). Analysis of the chromatogram using Image J software revealed 17 bands with varying Rf (retention factor) ranges and AUC (area under the curve) values. Bands numbered 7 (Rf 0.31 – 0.32), 14 (Rf 0.72 – 0.74), 16 (Rf 0.86 – 0.87), and 17 (Rf 0.91 – 0.93)

indicated the presence of phenolic/flavonoid group compounds in the sample. As depicted in Figure 4D, the bands corresponding to the phenolic and flavonoid groups in the *A. platyphyllum* sample without AB-mix solution treatment exhibited a denser coloration, especially in bands 7 and 17. The intensified coloration suggests that the concentration of phenolic/flavonoid compounds in these samples was higher compared to the other treatment.

Post-chromatographic derivatization with sulfuric acid has been used to determine the chemical properties of bioactive compounds. Kustrin *et al.,* (2019) reported that the spots from the separated compounds showed various colors ranging from purple, blue, red, and grey to green spots. The color developed after derivatization depends on the chemical nature of the compound. This spot is suitable for detecting various nucleophilic compounds such as sugars, steroids, terpenes, and amines. Multiple colors appear after derivatization, such as purple, indicating phenolic molecules. Blue/red are characteristic of amines, aldehydes, ketones, carbohydrates, and esters (such as alkyl phthalates), while green suggests allyl alcohols. Monoterpenes, triterpenes, and steroids usually appear as blue, purple, and grey specks, while diterpenes produce brown marks and reddish or blue colors under UV at 366 nm (Tshabalala *et al*., 2016).

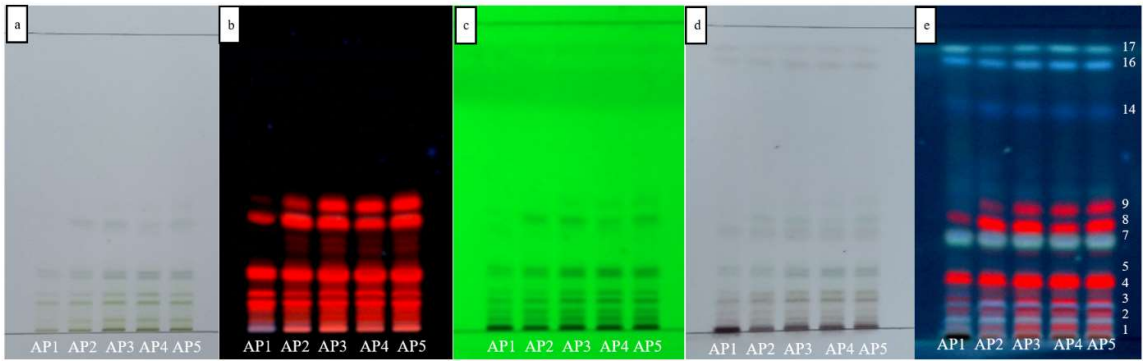

Figure 5. Chromatogram extract of *A. platyphyllum* under visible light (a); UV lamp 366 nm (b); 254 nm (c); dipped with sulfuric acid on visible light (d); and dipped with sulfuric acid on UV 366 nm (e). AP1 is 0 mg/L, AP2 is 700 mg/L, AP3 is 900 mg/L, AP4 is 1100 mg/L, and AP5 is 1300 mg/L.

The *A. platyphyllum* sample produced 17 bands after the separation and derivatization process with sulfuric acid. Phenolic/flavonoid compounds appeared as band numbers 7, 14, 16, and 17. The characteristics were the same as described by Kustrin *et al*., (2019), indicated by the appearance of a purplish spot in the visible light after dipping in H_2SO_4 solution and appeared as a greenish-bright blue band on the plate under a wavelength of 366 nm. While under a 254 nm UV lamp, no spots were detected. Considering the color intensity of the TLC plate, it represented that the sample without AB-mix solution produced relatively thick phenolic/flavonoid bands compared to the other four treatments, especially in bands numbered 7 (Rf 0.31 – 0.32) and 17 (Rf 0.91 – 0.93), the dissimilarity is not significant. The color intensity resulting from the TLC pattern was then quantified using ImageJ software and will deliver a peak of each band in the form of a densitogram. It was then calculated for its area to acquire each band's AUC value. All AUC values were then examined using the MetaboAnalyst software to obtain a heatmap pattern, as shown in Fig. 5.

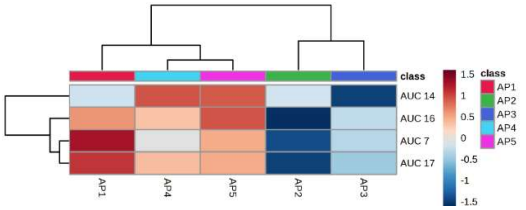

Figure 6. Heatmap analysis of the area under the curve (AUC) from phenolic/flavonoid bands in the TLC plate of the *A. platyphyllum* sample. AP1 is 0 mg/L, AP2 is 700 mg/L, AP3 is 900 mg/L, AP4 is 1100 mg/L, and AP5 is 1300 mg/L.

The results of the heatmap analysis showed that *A. platyphyllum* plants without AB-mix fertilizer had a close relationship with *A. platyphyllum* with a treatment of 1100 and 1300 mg/L. This is indicated by a similar color pattern for both marker bands for phenolic or flavonoid compounds, namely bands 7, 16, and 17. Furthermore, a strong correlation exists among the three bands represented by phenolic and flavonoid compounds, visually depicted by a brief line connecting them. Bands 7, 16, and 17 exhibit identical AUC values and similar characteristics, which are thought to originate from the phenolic/flavonoid group. These findings align with the quantitative assessments of phenolics and flavonoids, as the treatments involving 0, 1100, and 1300 mg/L AB-mix fertilizer yield nearly identical values.

CONCLUSION

Plants without AB-mix solution achieved the highest level of total phenolic, flavonoid, and antioxidant capacity. Conversely, the most significant secondary metabolite productivity was observed at 1100 mg/L. The phenolic/flavonoid compounds were effectively detected through thin-layer chromatography, revealing distinct variations in band intensity among the treatments. The untreated plants displayed thicker bands of phenolic/flavonoid compounds compared to the other treatments, confirmed by the highly intense color pattern exhibited in the heatmap analysis of the untreated samples. Therefore, applying AB-mix solution at 1100 mg/L is considered the most effective treatment for hydroponically grown *A. platyphyllum* to achieve optimal productivity of phenolic compounds, flavonoids, and antioxidant capacity.

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