

## Secondary Metabolites and Antioxidant Activity of Purwoceng (*Pimpinella Pruatjan*) Root Extracts from Various Hydroponic Planting Techniques

Irmanida Batubara<sup>1,2✉</sup>, Shadila Fira Asoka<sup>1</sup>, Eni Sumarni<sup>3</sup>, Herry Suhardiyanto<sup>4</sup>, Mohamad Solahudin<sup>4</sup>, Slamet Widodo<sup>4</sup>, Supriyanto Supriyanto<sup>4</sup>, Eti Rohaeti<sup>1,2</sup>, Yudiwanti Wahyu<sup>5</sup>, Folkes Laumal<sup>6</sup>, Erniati Erniati<sup>6</sup>

<sup>1</sup>Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, INDONESIA

<sup>2</sup>Tropical Biopharmaca Research Center, IPB University, Gedung CRC Lantai 2, Kawasan STP IPB, Bogor, INDONESIA

<sup>3</sup>Department of Agricultural Technology, Faculty of Agriculture, General Soedirman University, Purwokerto, INDONESIA

<sup>4</sup>Department of Mechanical and Biosystem Engineering, Faculty of Agricultural Engineering and Technology, IPB University, Bogor, INDONESIA

<sup>5</sup>Department of Agronomy, Faculty of Agriculture, IPB University, Bogor, INDONESIA

<sup>6</sup>Doctoral Program, Department of Mechanical and Biosystem Engineering, Faculty of Agricultural Engineering and Technology, IPB University, Bogor, INDONESIA

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### ABSTRACT

*Purwoceng (Pimpinella pruatjan) is Indonesia's native herbs that grow in the highlands but its existence is hard to find. The reason is that purwoceng is difficult to cultivate. A controlled factor in cultivation, such as hydroponic types and nutrient concentration, can be used as a solution to this problem. Hydroponic types and nutrient concentration treatments can affect the secondary metabolites and antioxidant activity of the purwoceng root extract produced. This study aimed to determine total phenolic and flavonoid content, as well as antioxidant activity in three different hydroponic systems (nonrecirculating drip, recirculating drip, and nutrient film technique (NFT)) and two nutrient concentrations (1.5‰ and 2.0‰). The combination of recirculating drip with low nutrient concentration was the best treatment to produce an extract with high phenolic and flavonoid content. Purwoceng root extracts from nonrecirculating with high nutrient concentrations produced high antioxidant activity. The characteristics of extracts from recirculating with low nutrient concentrations were similar to those from the nonrecirculating drip. In contrast, extracts from recirculating with high nutrient concentrations were closer to extracts from NFT, proven by principal component and heat map analysis. Antioxidant activity related to total phenolic content, also the presence of betaine and bergapten in purwoceng root extracts.*

✉Corresponding Author:  
[ime@apps.ipb.ac.id](mailto:ime@apps.ipb.ac.id)

## 1. INTRODUCTION

Purwoceng (*Pimpinella pruatjan*) is one of Indonesia's native herbs, belonging to Apiaceae family. It grows in the highlands with low temperatures such as in Dieng, Central Java, Indonesia. Purwoceng grows horizontally, in clumps, and is no more than 50 cm high. Purwoceng has rounded green leaves like *Centella asiatica* and a single root like *Panax sp* (Widodo *et al.*, 2021). Oxygenated monoterpenes, oxygenated sesquiterpenes, and sesquiterpenes have been reported to be contained in purwoceng extract (Nurcahyanti *et al.*, 2018). Steroids, flavonoids, phenolics, glycosides, saponins, and tannins also had a fairly high abundance in purwoceng extract (Fathonah & Sugiyarto, 2019). This plant is used traditionally in Indonesia because it has properties as an aphrodisiac and increases stamina (Usmiati & Yuliani, 2010). This plant has the potential as a medicinal resource for stamina resistance because it can increase aggressiveness in male mice (Kanedi *et al.*, 2017). Arfah *et al.* (2013) also stated that purwoceng extract could masculinize *Betta splendens*. In addition, purwoceng also has antioxidant activity (Wahyuningrum *et al.*, 2016). Unfortunately, this plant is difficult to find and categorize as endangered (Darwati & Roostika, 2016). This is because the demand for this plant in the herbal medicine industry is high but not balanced with proper cultivation. Purwoceng cultivation is difficult, and therefore their existence is rare. This plant is environmentally changes sensitive (Wahyu *et al.*, 2013). Appropriate cultivation technology is needed to increase purwoceng production and quality. The solution is to cultivate purwoceng using the hydroponic method due to controlled environmental factors in a greenhouse.

Hydroponic farmers generally use drip systems and nutrient film technique (NFT) hydroponics. A drip system can save water usage because the pump helps deliver water and nutrient from the reservoir to individual plant roots in appropriate proportion (Sharma *et al.*, 2018). Therefore, it minimizes evaporation (Wang *et al.*, 2020). The drip irrigation system can be categorized as recirculating drip and nonrecirculating drip irrigation. Recirculating drip irrigation is a system that reuses nutrients from the previous batch, while nonrecirculating drip irrigation is a drip irrigation system that does not reuse nutrient runoff. NFT was developed in the mid-1960s and aimed to overcome the shortcomings of other hydroponic systems. Unlike the drip irrigation system, NFT doesn't need to use a lot of growing media, it is easy to disinfect roots, and the consistent flow prevents salt buildup in the root area (Kour *et al.*, 2022). Suprayogi & Suprihati (2021) stated that plant growth and yields in hydroponics are affected by the slope of the gutters in the NFT system. This indicates that the chemical content of the purwoceng extract may also change depending on the factors imposed during plant growth such as differences in the hydroponic system and the nutrient concentration given. Therefore, this study aimed to determine total phenolic and flavonoid content, and antioxidant activity in three different hydroponic systems (recirculating drip, nonrecirculating drip, and NFT) and two nutrient concentrations (1.5‰ and 2.0‰).

## 2. MATERIALS AND METHODS

### 2.1. Cultivation and Harvesting

The study was conducted in a greenhouse in the Lembang medium plain ( $\pm 1500$  m above sea level) from December 2020 to March 2021. The randomized block design with three replications was used. The factors were hydroponic type and nutrient concentration (Table 1).

**Table 1.** The experiment designs

Hydroponic types	Nutrient concentration (‰)	
	1.5 (1)	2.0 (2)
Nonrecirculating (A)	A1	A2
Recirculating (B)	B1	B2
NFT (C)	C1	C2

Purwoceng seedlings aged 1.5 months were obtained from farmers from the Dieng Plateau, Central Java. Seedlings acclimatized for four days. The seedlings were transferred to the research installation after acclimatization. The types of hydroponics used were nonrecirculating drip, recirculating drip, and NFT hydroponics. Growing media, nutrient volume, temperature, and light intensity were the factors that were controlled during the study. Purwoceng roots were harvested after 240 days of planting. The *F* test and Duncan's Multiple Range Test (DMRT) at a 5% level were used to perform an equivalence test of the population variants.

## 2.2. Moisture Content Determination

The thermogravimetric method was used to determine moisture content. The sample was left in a hot air oven at 105 °C. This process was carried out until the sample was dry. After that, the sample was put into a desiccator and weighed (Sumarni *et al.*, 2023).

## 2.3. Ash Content Determination

The thermogravimetric method was used to determine ash content. The sample was burned and put in a furnace at 45 °C for about three hours till no more organic components. Then, the ash's weight and content are determined (Sumarni *et al.*, 2023).

## 2.4. Total Phenolic Content Determination

Before determine the phenolic and flavonoid content and identify the compounds contained in purwoceng root, the sample was extracted with ethanol with a ratio of 1:10 (1g root : 10 ml ethanol). Then, the Folin–Ciocalteu colorimetric method was used to determine total phenolic content (TPC). A total of 100 µl prepared purwoceng root extract was added with 0.2 ml of Folin–Ciocalteu reagent and 2 ml of distilled water. The mixture was incubated for 3 min at room temperature. After incubation, the mixture was added with 1 ml of 20% sodium carbonate and then incubated at the same temperature for 1 h. After the second incubation, TPC was determined using a spectrophotometer (Shimadzu UV–1240) at a wavelength of 765 nm. The gallic acid standard solution was prepared with a concentration varying from 15.625; 31,25; 62,5; 125; 250; and 500 ppm. TPC was determined by considering the standard gallic acid curve and converted to units of % gallic acid equivalent (GAE)/plant by dividing it by the plant's dry weight.

## 2.5. Total Flavonoid Content Determination

Determination of the total content of flavonoid (TFC) refers to Nursid *et al.* (2022). A total of 10 µL sample extract was mixed with 60 µL methanol, 10 µL aluminum chloride (10% w/v), 10 µL potassium acetate (1 M), and 110 µL distilled water on a 96-well micro-plate. The samples were homogenized and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at a wavelength of 415 nm.

The quercetin standard solution was prepared with a concentration varying from 5, 10, 20, 40, 60, and 80 ppm. TFC was determined by considering the standard quercetin curve and converted to units of % quercetin equivalent (QE)/plant by dividing it by the plant's dry weight.

## 2.6. Identification of Compounds in Purwoceng Root Extracts

The UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS Thermo Scientific instrument with Accucore C18 Thermo Scientific column (2.1 mm × 100 mm, 1.5 µm) (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to analyze compounds in purwoceng root extracts. Methanol (LC-MS grade) was used as the sample solvent. The prepared solution was filtered using a PTFE membrane (0.2 µm). The analysis involved a column temperature of 30 °C and a time of 30 min. The ionization source and mass analyzer used in this study were electrospray ionization (ESI) with 5500 V of spraying voltage and quadrupole-orbitrap. The mobile phase used was 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The injection volume was 5 µl with a flow rate of 0.2 ml/min. Gradient elution was used with a composition as Table 2. At 8.01–16.00 minutes, the eluent composition changed linearly to 100% B. Then at 16.01–18.00 minutes, the eluent composition was maintained at 100% B.

**Table 2.** Mobile phase composition

Minutes	Phase A (%)	Phase B (%)
0.00–1.00	80	20
1.01–8.00	50	50
8.01–16.00	0	100
16.01–18.00	0	100
18.01	80	20

## 2.7. Antioxidant Activity

The antioxidant capacity of each harvested material was determined using a DPPH method used by [Batubara \*et al.\* \(2020\)](#). Briefly, 100 µL of the ethanol extract of the sample and 100 µL of 2,2-diphenyl-1-picrylhydrazyl in  $1.25 \times 10^{-4}$  mol of methanol were mixed in a 96-well microplate (Costar-USA). The mixture was incubated for 30 minutes and then the absorbance of the mixture was measured using the microplate reader (BMG Labtech, Germany) at a wavelength of 517 nm. The ascorbic acid standard solution was prepared with a concentration varying from 3.125; 6.25; 12.5; 25; 50; and 100 ppm. The antioxidant activity was determined by considering the standard ascorbic acid curve and the results expressed in µg ascorbic acid equivalent (AAE/g DW).

## 2.8. Statistical Analysis

One way ANOVA and *t*-test were performed using Microsoft Excel to determine the variability of the data. The data analyzed is a collection of data obtained from three replicate experiments. Orange data mining software was also used to perform multivariate analysis, such as principal component and heat map analysis. Finally, the abundance of compounds data was preprocessed and grouped based on the similarity of the sample characteristics.

### 3. RESULTS AND DISCUSSION

#### 3.1. Growth and Quality of Purwoceng Planted in Highland Greenhouse

Purwoceng could grow well in three different hydroponic systems, i.e. nonrecirculating, recirculating, and NFT altitudes only 800 m above the sea level. Recirculating drip and NFT gave a lower dry weight than nonrecirculating drip hydroponics. The 1.5‰ gave a higher dry weight than the 2.0‰, except for the nonrecirculating drip (Table 3). The highest dry weight was obtained from the circulating drip with a nutrient concentration of 1.5‰, which was 6.55 g (Table 3). The lowest dry weight value was found in NFT with the nutrient concentration of 2.0‰, which was 2.80 g (Table 3). The differences were due to in the type of hydroponics and the concentration of nutrients used. In nonrecirculating drip, the weight of dry roots was heavier than in other types of hydroponics. This was because the nutrients used in the first batch were not reused in the next batch so the nutrients obtained by the plants were still fresh and plentiful. [Han et al., \(2016\)](#) stated that the nutrient treatments affected carbohydrate distribution so the plant weight changes.

The harvest materials determined their quality using specific proximate analysis such as moisture and ash content. Nonrecirculating drip irrigation gave the highest average moisture content. A1 had the highest moisture content, while C1 has the lowest (Table 3). This is because drip irrigation prevents the moisture stress of plants ([Dursun & Ozden, 2011](#)). On the other hand, NFT had the highest average ash content with C1 was significantly different from others (Table 3). The moisture and ash content value was inversely proportional to the concentration of nutrients given, except for moisture content from NFT. This is the first report on the relationship between nutrient concentrations in hydroponics and moisture and ash content, particularly in purwoceng.

**Table 3.** Characteristics of harvest material in each treatment combination

Treatment combination	Root part dry weight (g)	Moisture content (%)	Ash content (%)	Total phenolic (% GAE/plant)	Total flavonoid (% QE/plant)
A1	5.47 <sup>ab</sup>	12.10 <sup>a</sup>	9.32 <sup>a</sup>	0.55 <sup>c</sup>	0.55 <sup>a</sup>
A2	6.09 <sup>b</sup>	9.77 <sup>a</sup>	8.46 <sup>a</sup>	2.92 <sup>a</sup>	0.61 <sup>a</sup>
B1	6.55 <sup>c</sup>	9.92 <sup>a</sup>	10.60 <sup>a</sup>	2.88 <sup>a</sup>	0.98 <sup>b</sup>
B2	4.12 <sup>a</sup>	9.55 <sup>a</sup>	10.33 <sup>a</sup>	1.77 <sup>b</sup>	0.66 <sup>a</sup>
C1	4.25 <sup>a</sup>	8.58 <sup>a</sup>	11.58 <sup>b</sup>	2.85 <sup>a</sup>	0.55 <sup>a</sup>
C2	2.80 <sup>d</sup>	9.54 <sup>a</sup>	10.87 <sup>a</sup>	1.18 <sup>b</sup>	0.34 <sup>a</sup>

Note: Numbers followed by the same letter in one column are not different in Duncan's Multiple Distance Test levels 5%

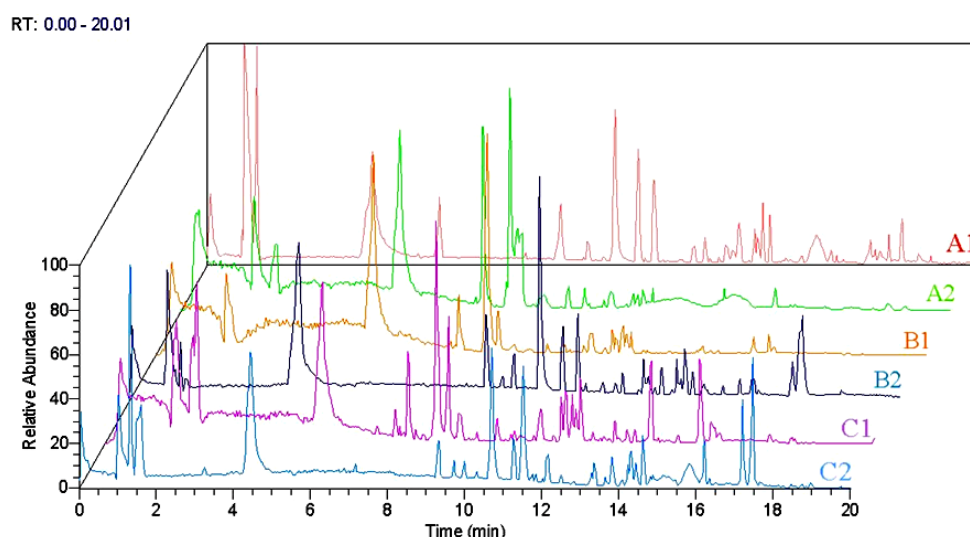
The total phenolic content (TPC) and total flavonoid content (TFC) were also determined. The productivity of the total phenolic content per plant is affected by the treatment of the plants. This was proven by the results of Duncan's Multiple Distance Test, which showed differences in the purwoceng root extracts, which were distinguished by the type of hydroponics and the concentration of nutrients given. Nonrecirculating drip irrigation had the lowest average, while recirculating drip irrigation had the highest average of TPC (Table 3). Phenols act as a stress indicator since their production is maximal in plants exposed to various stresses ([Verma & Shukla, 2015](#)). Recirculating drip irrigation might be an environmental

stress for purwoceng plants because in the recirculating drip irrigation, the drainage water and nutrient doesn't flow away but is reused over time. The nutrient concentration of 1.5‰ gave the highest results on TPC, except for nonrecirculating (Table 3). This was presumably because purwoceng is sufficient and reaches optimum development conditions when given nutrients at a level of 1.5‰ so that TPC are produced optimally. The highest TPC was found in nonrecirculating drip irrigation with 2.0‰ of nutrient concentration (A2), with values of 2.92%GAE/plant (Table 3).

The difference in the purwoceng plants' treatments did make a difference in the TFC. The highest results on TFC were given by the nutrient concentration of 1.5‰, except for nonrecirculating (Table 3). However, recirculating drip irrigation gave the highest average of TFC (Table 3). The highest TFC was found in recirculating drip irrigation with 1.5‰ (B1), with values of 0.98%QE/plant (Table 3). From the explanation above, it can be concluded that the hydroponic type and nutrient concentration are factors that can cause changes in the total phenolic and flavonoid content

### 3.2. Secondary Metabolites of Purwoceng Root Extracts

Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) was used to identify secondary metabolites in purwoceng root extracts. This was a non-targeted analysis because the goal was to discover the metabolites in the purwoceng root extracts. The chromatogram obtained provides information regarding retention time, separation pattern, and abundance. These pieces of information are used as initial information for compound identification. The same retention time and separation pattern as available databases indicates the same compound. The peak intensity is interpreted as the abundance of a compound in a sample. Therefore, peak intensity was directly proportional to the abundance of a compound in the sample. The resulting chromatogram shows that the compound content in the extract was not completely different because there is a similarity in the separation pattern of each peak and only differs in intensity (Figure 1).

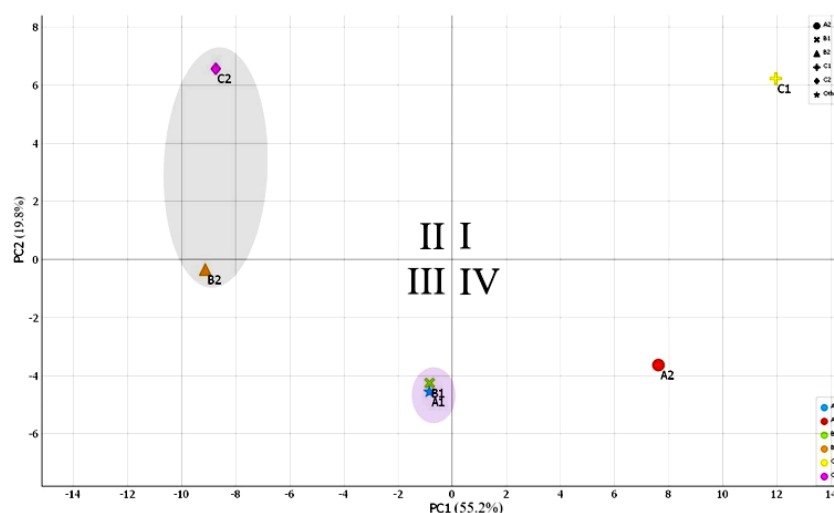


**Figure 1.** LC-MS/MS chromatogram for purwoceng root extrcats

There were 106 compounds identified in purwoceng root extracts based on the results of LC-MS/MS analysis (Table S1). Compounds in purwoceng root extract identified in this study included phenolic, flavonoid, amino acid, and fatty acid compounds. The most abundant compounds in the phenolic, flavonoid, amino acid, and fatty acid, namely vanillin (13.26%), hymecromone (5.85%), DL-stachydrine (4.25%), and oleamide (1.53%), respectively (Table S1). Vanillin was the highest average abundance (13.26%), followed by choline (9.29%), and betaine (8.76%) in purwoceng root extracts (Table S1). The difference in compound content from the results of the LC-MS/MS analysis in this study was thought to be due to different treatments such as the hydroponic systems and the different nutrient concentrations given.

### 3.3. Principal Component Analysis (PCA)

Principal component analysis (PCA) is a multivariate analysis with dimensionality-reduction features by transforming a set of variables into smaller variables that still contain important information. PCA aims to emerge as the most influential component for expressing large data sets. These new components, called principal components (PC), will hopefully reveal advanced information and reduce the noise in the data set (Kurita, 2020). PCA was used in this study to differentiate compounds in purwoceng root extract after being given different treatments.



**Figure 2.** PCA score plot

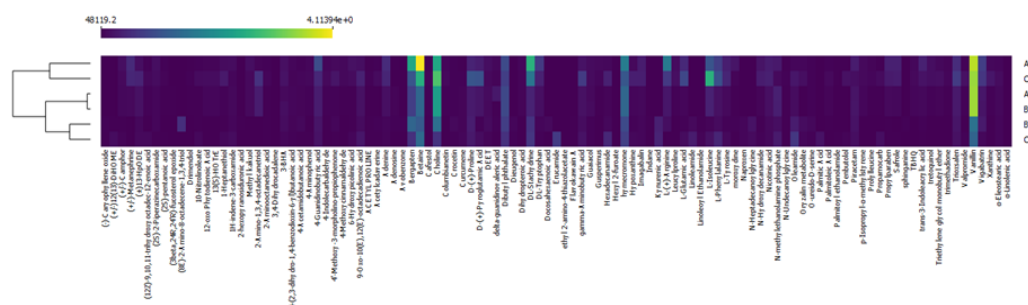
Purwoceng root extracts could be differentiated using principal component analysis (PCA). The total PC score in the score plot was 75.0% with PC1 and PC2 in the amount of 55.2% and 19.8%, respectively (Figure 2). Different types of hydroponic treatment and nutrient concentrations resulted in different grouping patterns of purwoceng root extracts. For example, purwoceng root extracts which came from NFT were in quadrants I and II (above), while nonrecirculating and recirculating drip grouped in quadrants III and IV (below) (Figure 2). Purwoceng root extracts from drip irrigation (A1 and B1) with low nutrient concentrations had very close positions (Figure 2). Interestingly, the grouping pattern changed when the nutrient concentration applied was increased. For example, purwoceng root extract from recirculating drip (B2) was closer to extract from NFT (C2). The closer position of each extract reflects almost



similar extract characters. This similarity can be analyzed using a heat map with the help of hierarchical component analysis.

### 3.4. Heat Map Analysis

The column section of the heat map contained information about the abundance of metabolites in different extracts, while the row section contained information about the abundance of metabolites in each extract. Figure 3 shows the result of the heat map analysis. The color in the heat map shows the abundance of compounds in each cell. The color ranges from dark which indicates low abundance to bright which indicates high abundance. [Balkhyour et al., \(2021\)](#) conducted a heat map analysis to classify the essential oils of six accessions of *Pimpinella anisum* L. at three stages of seed development and CO<sub>2</sub> growth conditions. However, no one has reported heat map analysis to classify purwoceng root extract in hydroponic treatment and different nutrient concentrations.



**Figure 3.** Heat map of compounds in purwoceng root extracts

The results of the heat maps analysis showed that purwoceng extract tended to cluster based on the concentration of nutrients given. Extracts derived from recirculating drip had similar characteristics to extracts derived from nonrecirculating or NFT, depending on the amount of nutrient concentration provided. A1 and B1 had adjacent positions because their chemical composition and abundance were very similar. B2 and C2 were in the same cluster due to a lack of vanillin among the other extracts. However, A2 and C1 were close because of the high abundance of betaine and L-isoleucine. Nonrecirculating extracts had a higher hymechromone abundance compared to other extracts. The abundance of betaine was seen in the NFT extract, which had much betaine but lacked in the recirculating extract.

A1 could be distinguished from other extracts because it is the only extract containing delta-guanidino valeric acid. A2 had betaine as its identifying compound because its abundance in this extract was very high. The identifying compound for B1 was 3-BHA because this extract contains 3-BHA which was higher than other purwoceng root extracts. The only extract contained (8E)-2-amino-8-octadecene-1,3,4-triol was B2. The very abundant presence of L-isoleucine could differentiate C1, while C2 could be distinguished because this extract contains a small amount of vanillin.

### 3.5. Antioxidant Activity of Purwoceng Root Extracts

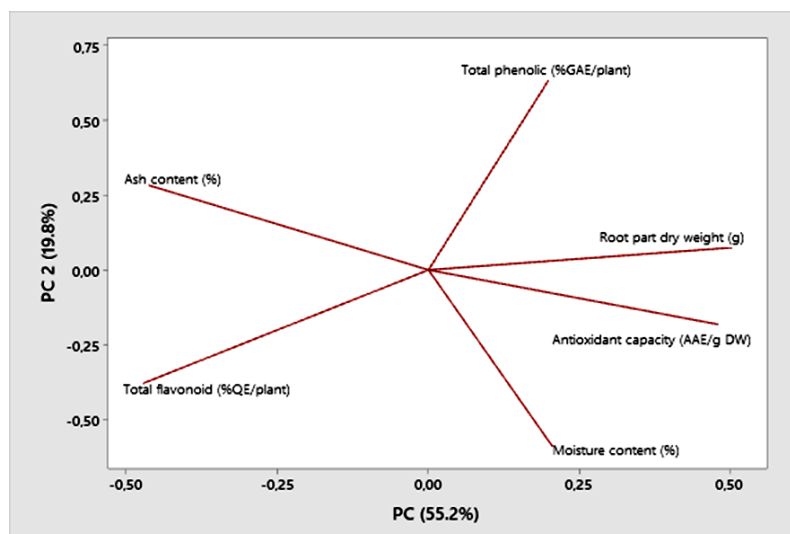
Antioxidant properties of purwoceng root extracts were determined against ascorbic acid and compared. The capacity of ascorbic acid specified antioxidant activity. The ascorbic acid capacity value is directly proportional to the antioxidant activity in a sample. Different treatments, such as hydroponic types and different nutrient



concentrations, lead to differences in the antioxidant activity of purwoceng root extract. Purwoceng root extracts from nonrecirculating drip hydroponics had a higher average antioxidant activity than recirculating drip and NFT hydroponics (Table 4). In addition, antioxidant activity increased with increasing nutrient concentration given, except for NFT. This result is inversely proportional to research [Nguyen & Niemeyer \(2008\)](#) who stated that low concentrations of nutrients, especially nitrogen, produce high antioxidant activity. In this study, the nutrients used were an AB mixture containing nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, boron, manganese, zinc, copper, and molybdenum. it is necessary to conduct further research on the components in the mixture that have the most influence on the value of antioxidant activity.

**Table 4.** Antioxidant capacity of purwoceng extracts against ascorbic acid

Treatment combination	Antioxidant Capacity (AAE/g DW)
A1	28.77
A2	33.86
B1	16.40
B2	31.44
C1	11.09
C2	3.17



**Figure 4.** Loading plot PCA

In loading plots of PCA, the antioxidant activity position was closer to TPC than TFC (Figure 4). This showed that the antioxidant activity of purwoceng root extracts tended to be related to TPC. In addition, it was also proven that the antioxidant capacity and total phenolic content values which were directly proportional ([Vuolo et al., 2019](#)). The greater the total phenolic content value, the higher the antioxidant activity of a sample. That was because phenolic compounds have redox properties that enable them to act as antioxidants ([Vallverdú-Queralt et al., 2014](#)). For example on A2, it had high values of total phenolic content for 0.26% (w/w) (Table 3) and gave the highest values of antioxidant capacity for 33.86 AAE/g DW (Table 4). There was a pattern

indicating compounds suspected of being involved in antioxidant activity, namely betaine and bergapten. According to the LC-MS/MS analysis carried out previously, betaine and bergapten had a high abundance in A2 and B2, respectively, which had high antioxidant activity. Antioxidant activity of bergapten is because it could suppress the growth rate of free radicals that can be found in the body, one of which is reactive oxygen species (ROS) (Zhou *et al.*, 2017). Betaine has been shown to reduce oxidative stress in rat blood (Hassanpour *et al.*, 2020) and rat brains (Alirezaei *et al.*, 2015). Craig (2004) stated that betaine could be an osmolyte to protect living organisms from environmental stress. In addition, betaine can be a methyl donor by participating in the methionine cycle-primarily in the human liver and kidneys (Craig, 2004). Therefore, betaine and bergapten, which could be abundantly found in purwoceng root extracts, were thought to have antioxidant activity.

#### 4. CONCLUSIONS

The hydroponic system and the concentration of nutrients can affect the results of plant production, such as secondary metabolites, total phenolic content, and total flavonoid content. The recirculating drip was the best treatment to produce high phenolic and flavonoid content, while the nonrecirculating drip provided high antioxidant activity. Treatment of low concentrations of nutrients tended to increase the value of total phenolic content and treatments of high concentrations of nutrients tended to increase antioxidant activity. Antioxidant activity of purwoceng root extracts related to the total phenolic content and the presence of betaine and bergapten.

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