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The Effect of Different Hydroponic Types and Nutrient Concentrations on the Chemical Composition and Antioxidant Capacity of Purwoceng (*Pimpinella pruatjan*) Extract

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ABSTRACT

Purwoceng (*Pimpinella pruatjan*) is an Indonesian native plant but difficult to cultivate. Hydroponics can be used as a solution for purwoceng cultivation. Hydroponic types and nutrient concentrations can affect purwoceng's productivity. This study aimed to determine total phenolic content (TPC) and total flavonoid content (TFC), changes in steroid, and antioxidant capacity due to two hydroponic types (nonrecirculating and recirculating drip) and three nutrient concentrations (1,000, 1,500, and 2,000 ppm). Purwoceng was determined for its moisture content, ash content, and dry weight, and extracted with ethyl acetate for gas chromatography-mass spectrometry analysis and with ethanol for TPC, TFC, and antioxidant capacity. The data were analyzed statistically and grouped using principal component analysis (PCA) and heat map analysis (HMA). Nonrecirculating drip with high nutrient concentration at the aerial part was the best treatment to produce high TPC, TFC, and antioxidant capacity. There were no significant changes in steroid content. PCA showed that purwoceng extracts could be grouped in to two groups, i.e. aerial and root groups. HMA showed that purwoceng extracts could be distinguished from the abundance of palmitic acid and stigmasterol. The antioxidant capacity of purwoceng was directly proportional to the TPC value and also related to the presence of phytol, stigmasterol, and palmitic acid.

1. Introduction

Purwoceng (*Pimpinella pruatjan*) is an Indonesian native plant that grows in the highlands. The physical appearance of the purwoceng is a small herb growing horizontally on the ground, like Centella asiatica. Purwoceng has been reported to contain steroids, flavonoids, phenolics, glycosides, saponins, and tannins (Ma'mun *et al.* 2006), oxygenated monoterpenes, oxygenated sesquiterpenes, and sesquiterpenes (Nurcahyanti *et al.* 2018). Due to some of those compounds, purwoceng has the potential as an antioxidant (Wahyuningrum *et al.* 2016). Unfortunately, this plant is difficult to find because purwoceng is difficult to cultivate outside its natural habitat because it has specific growing requirements and it is categorized as endangered medicinal plant (Nuryadin and Nabiila 2018) which grows endemically at mountainous area. In addition, this plant is very sensitive to environmental changes (Wahyu *et al.* 2013). Therefore, appropriate cultivation technology is needed to increase purwoceng production and quality. One solution to overcome the extinction of this plant is cultivating purwoceng in a greenhouse using hydroponics due to controlled environmental factors, such as the type of hydroponics and nutrient concentrations.

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Hydroponic growers widely use drip irrigation. This method provides the advantage of saving water because it minimizes evaporation (Wang *et al.* 2020). Water and nutrient from the reservoir are provided to individual plant roots in appropriate proportion with the help of the pump (Sharma *et al.* 2018). The drip irrigation system can be divided into recirculating and nonrecirculating drip irrigation. Recirculating drip irrigation is a system that utilizes nutrient concentration run off to be reused, while nonrecirculating drip irrigation is a drip irrigation system that does not reuse nutrient concentration runoff. Factors such as differences in the hydroponic types and the nutrient concentrations can affect the chemical content of the purwoceng extract. Purwoceng cultivation with hydroponics was carried out by Sumarni *et al.* (2023) with with the result that purwoceng can be cultivated in a greenhouse. Unfortunately there have been no reports on the secondary metabolites produced by hydroponically cultivated purwoceng, along with their phenolic, flavonoid, and antioxidant content. Therefore, this study aimed to determine total phenolic content, total flavonoid content, steroid changes, and antioxidant capacity due to two different hydroponic types (recirculating and nonrecirculating drip) and three nutrient concentrations given (1,000, 1,500, and 2,000 ppm).

2. Materials and Methods

2.1. Cultivation and Harvesting

The study was conducted from December 2020 to March 2021. The research location was in a greenhouse on the Lembang medium plain 800 m above sea level. The experiment used a randomized block design with three replications. The factors were hydroponic types and nutrient concentrations, as seen in Table 1.

The Purwoceng seedlings were obtained from farmers on Dieng Plateau in Central Jawa, Indonesia which was 1.5 months old. Seedlings were given acclimatization treatment for four days before being transplanted into the installation to be used for research. The controlled conditions during planting in the greenhouse were the growing media, nutrient concentration, temperature, and light intensity. The nutrient solution used was AB Mix for General Tubers from Agrifam (Bogor, Indonesia). The solution contained macronutrients, such as NO_3^{2-} (188 ppm), NH_4^+ (21 ppm), P (62 ppm), K (236 ppm), Ca (176 ppm), Mg (49 ppm), and S (76 ppm), and micronutrients, such as Fe (3.7 ppm), Mn (0.5 ppm), Zn (0.1 ppm), Cu (0.05 ppm), B (0.5 ppm), and Mo (0.05 ppm). After 240 days after planting, the aerial and root parts were harvested. The growth and yield of Purwoceng plants which included plant height, number of leaves, and harvest data (dry weight of aerial part, dry weight of roots, and dry weight of plants), were analyzed by F test and Duncan's Multiple Distance Test (DMDT) levels 5%.

Table 1. The experiment design

Hydroponic types	Nutrient concentrations (ppm)			Plant part
	1,000 (1)	1,500 (2)	2,000 (3)	
Nonrecirculating drip (A)	A1L	A2L	A3L	Aerial (L)
	A1R	A2R	A3R	Root (R)
Recirculating drip (B)	B1L	B2L	B3L	Aerial (L)
	B1R	B2R	B3R	Root (R)

2.2. Moisture Content Determination

The method used was the thermogravimetric. The sample was kept in a hot oven at 105°C until dried. After cooling, the sample was placed in a desiccator and weighed to determine the moisture content (Sumarni *et al.* 2023).

2.3. Ash Content Determination

The method used was the thermogravimetric. The sample was burned and put in a furnace at 45°C for about 3 h till no more organic components. Then, the ash content was determined (Sumarni *et al.* 2023).

2.4. Extract Preparation for Total Phenolic Content, Total Flavonoid Content, and Antioxidant Capacity Analysis

Fresh purwoceng aerial or roots were extracted with ethanol (1 g sample:10 ml ethanol). The extracts obtained were collected. The extracts obtained were used for total phenolic content, total flavonoid content, and antioxidant capacity analysis.

2.5. Total Phenolic Content Determination

Total phenolic content (TPC) value was measured using the Folin–Ciocalteu colorimetric method. Sample extracts prepared for total phenolic content (100 µL) were mixed with 0.2 ml of Folin–Ciocalteu reagent and 2 ml of distilled water and then incubated at room temperature for 3 min. After adding 1 ml of 20% sodium carbonate to the mixture, total polyphenols were determined after one h of

incubation at room temperature. The absorbance was measured at 765 nm with a spectrophotometer (Shimadzu UV–1240, Japan). The gallic acid standard solution was prepared with a concentration varying from 15.6 to 500 mg/L. TPC value was determined by considering the standard gallic acid curve and converted to units of g gallic acid equivalent (GAE)/ plant by multiplying it by the plant's dry weight.

2.6. Total Flavonoid Content Determination

The total flavonoid content (TFC) value was determined using the method used by Nursid *et al.* (2022). A total of 10 µL sample extract, 60 µL methanol, 10 µL aluminum chloride (10% w/v), 10 µL potassium acetate (1 M), and 110 µL distilled water were mixed on a 96-well micro-plate. Samples were incubated in the dark at room temperature for 30 minutes. The absorbance was measured at a wavelength of 415 nm using a UV-Vis spectrophotometer. Quercetin standard solution was prepared with various concentrations from 5 to 80 mg L^{-1}. TFC wass determined by considering the standard quercetin curve and converted to g quercetin equivalent (QE)/ plant by multiplying it by the dry weight of the plant.

2.7. Antioxidant Capacity

The antioxidant capacity of each harvested material was determined using a DPPH method used by Batubara *et al.* (2020). In brief, 100 µL ethanol extract of harvested materials was added to 100 µL of 2,2-diphenyl-1-picrylhydrazyl in 1.25 × 10^{-4} mol of methanol at the 96-well microplate (Costar-USA). After 30 min, the absorbance at 517 nm was measured using the microplate reader (BMG Labtech, Germany). The ascorbic acid standard solution was prepared with a concentration varying from 3.125 to 100 mg L^{-1}. The antioxidant capacity was determined by considering the standard ascorbic acid curve and the results expressed in µg ascorbic acid equivalent (µg AAE/plant).

2.8. Compounds Identification Using Gas Chromatography-Mass Spectrometry

Purwoceng was extracted using ethyl acetate. Purwoceng extracts were analyzed using the Shimadzu Gas Chromatography-Mass Spectrometry QP2020NX (Japan). The carrier gas used was Helium, and the column used was sh-Rxi®-5Sil MS (30 m × 0.25 mm × 0.25 µm). The column oven and injector temperatures were 40°C and 280°C, respectively. The injection mode used was split, and the pressure was 49.3 kPa. The column flow rate used was 1.00 ml/min. Metal quadrupole with pre-rod was used as a mass analyzer with a mass range of m/z of 1.5 to 1090. The detector used was an electron multiplier with a low noise overdrive lens of 8 × 10^6.

2.9. Statistical Analysis

The abundance values for each compound were analyzed using ANOVA: single factor. The significant differences in compound abundance among the sample were determined using a t-test. ANOVA and t-test analysis was performed using Microsoft Excel. Minitab 19 software was used for principal component analysis (PCA), and Orange Data Mining was used for heat map analysis to differentiate the extracts. Imported data were preprocessed using normalized data from the software. The data obtained were grouped using heat map analysis and PCA with a percentage of the variance of both principal components (PC) of at least 70%.

3. Results

3.1. Growth of Purwoceng Plants Cultivated in Highland Greenhouses

Purwoceng could grow well outside their natural habitat in altitudes only 800 m above sea level. Purwoceng could grow in nonrecirculating and recirculating hydroponics with nutrients of 1,000-2,000 ppm. There was an interaction between hydroponic-type treatment and nutrient concentrations on aerial and root part dry weight.

The results showed that the aerial part had a higher dry weight than the root part, except for B2 (Table 2). Recirculating drip hydroponics gave a lower dry weight than nonrecirculating drip

Table 2. Interaction between hydroponic types and nutrient concentrations on the dry weight of aerial and root part of purwoceng

Treatment combination	Root part dry weight (g)	Aerial part dry weight (g)
A1	5.80^{b}	7.04^{b}
A2	5.47^{ab}	10.72^{d}
A3	6.09^{b}	8.97^{c}
B1	5.77^{b}	6.72^{b}
B2	6.55^{c}	5.18^{a}
B3	4.12^{a}	7.56^{b}

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

hydroponics. The nutrient concentration of 1,500 ppm gave a higher dry weight than the other. The highest dry weight from the aerial part was obtained from the nonrecirculating drip and nutrients of 1,500 ppm, which was 10.72 g (Table 2). Furthermore, the highest root part dry weight was obtained from the circulating drip and nutrients of 1,500 ppm, which was 6.55 g (Table 2).

3.2. Harvested Material Quality

The harvested materials determined their quality using specific proximate analysis such as moisture and ash content. In addition, the total phenolic content (TPC) and total flavonoid content (TFC) values were also determined.

Recirculating drip irrigation had a higher moisture and ash content than nonrecirculating drip irrigation; on the other hand, we could find higher TPC and TFC value at nonrecirculating drip irrigation (Table 3). The moisture content and TPC value were directly proportional to the nutrient concentration given. This gave the result that the nutrients of 2,000 ppm produced the highest moisture content and TPC value (Table 3). Meanwhile, the nutrients of 1,500 ppm gave the highest results on ash content and TFC value. The nutrients of 1,000 ppm gave the lowest values for moisture content, ash content, and TPC value (Table 3). This was presumably because the plants lacked nutrients, so the results obtained were of little value. The highest moisture content, ash content, TPC value, and TFC value were found in the aerial part (Table 3). The highest moisture content was found in nonrecirculating drip with nutrients of 1,500 ppm at the root part (A2R;12.10%), while the highest ash content, TPC value, and TFC value were found in nonrecirculating drip with nutrients of 1500 ppm at aerial part (A2L), with values of 14.49%, 5.15%GAE/plant, and 3.27%QE/plant, respectively (Table 3).

3.3. Secondary Metabolites in Purwoceng Extract Using Gas Chromatography-Mass Spectrometry

The resulting chromatogram shows that the compound content in the extract was not entirely different because there was a similarity in the separation pattern of each peak and only differed in intensity (Figure 1).

There were 44 compounds identified in purwoceng aerial and root extracts based on the results of GC-MS analysis. A total of nine compounds belong to the terpenoid group, one of them was α-zingiberene. In addition, six compounds belong to the steroid group, one of which was stigmasterol. Stigmasterol had the highest average abundance (34.63%) in purwoceng aerial and root extracts. Compounds that had a high average abundance in all extracts is fatty acid, i.e., 10-(E)-12-(Z)-conjugated linoleic acid (20.74%) and palmitic acid (17.53%).

3.4. Principal Component and Heat Map Analysis

Principal component analysis (PCA) is a multivariate analysis with the principle of subtracting variables from several observations. The reduced data forms a new component called the principal component (PC). This study used PCA to find patterns and differentiate compounds in different purwoceng treatment conditions. The results obtained were in the form of plot scores which provide information about the grouping patterns of purwoceng extracts.

The abundance values from each compound in purwoceng extracts could be used to represent each extract as a single shape in the PCA score plot by the first two principal components. The PC scores for both PC1 and PC2 were 59.9% and 19.9%, respectively, resulting in a total PC of 79.4% (Figure 2). Extracts which closely related in composition had a close position. For

Table 3. Quality of harvested material in each treatment combination

Treatment combination	Moisture content (%)		Ash content (%)		TPC value (g GAE/plant)		TFC value (g QE/plant)	
	Root	Aerial	Root	Aerial	Root	Aerial	Root	Aerial
A1	2.67^{a}	5.22^{a}	5.38^{a}	13.25^{b}	0.78^{a}	3.17^{a}	0.57^{a}	1.85^{b}
A2	12.10^{b}	6.84^{ab}	9.32^{b}	14.49^{b}	0.55^{a}	5.15^{b}	0.56^{a}	3.27^{a}
A3	9.77^{b}	10.51^{b}	8.46^{b}	11.96^{b}	1.55^{b}	4.28^{b}	0.27^{a}	0.92^{c}
B1	9.91^{b}	11.16^{b}	11.97^{b}	9.18^{b}	0.25^{a}	0.84^{c}	0.29^{a}	0.65^{c}
B2	9.92^{b}	11.82^{b}	10.60^{b}	10.65^{b}	0.75^{a}	2.28^{a}	0.38^{a}	0.77^{c}
B3	9.55^{b}	11.43^{b}	10.33^{b}	12.22^{b}	0.55^{a}	3.28^{a}	0.31^{a}	1.18^{b}

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

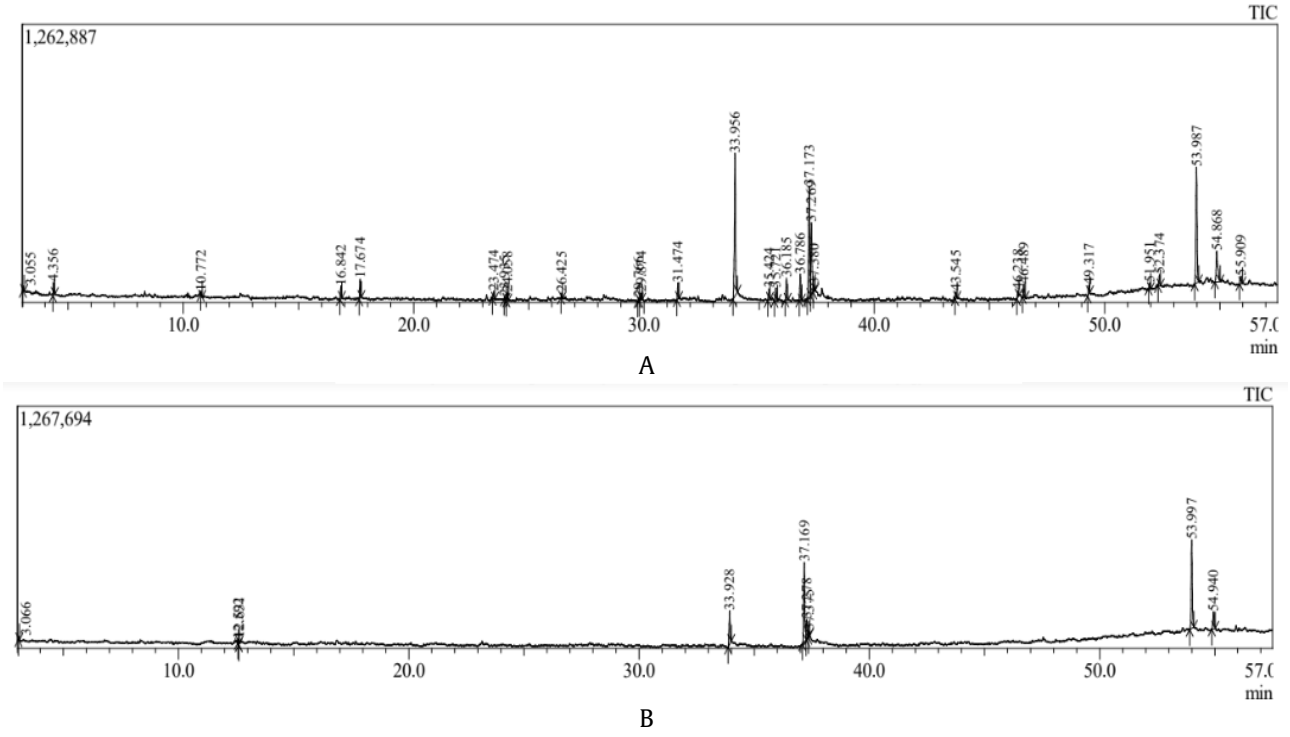

Figure 1. Gas chromatography-mass spectrometry chromatogram of purwoceng aerial part (A) and root extract (B)

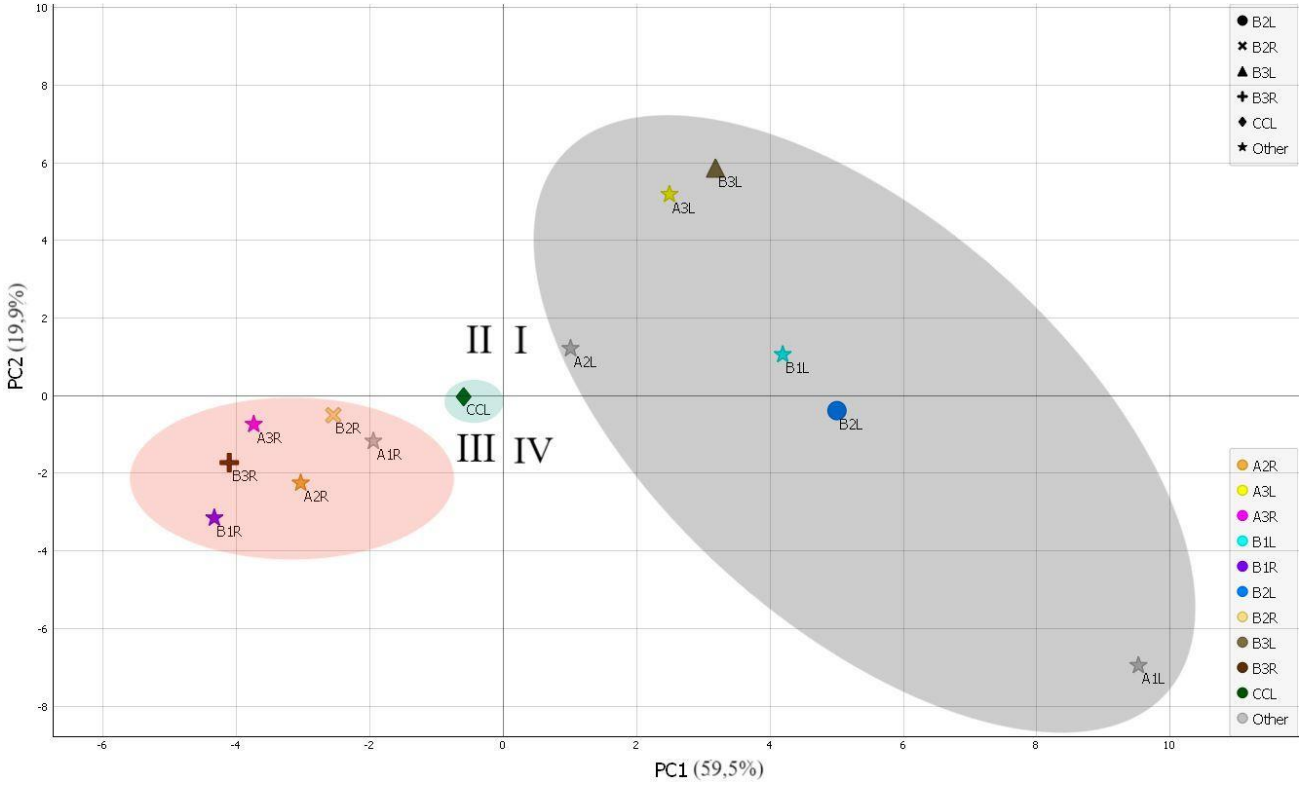

Figure 2. PCA score plot

example, the aerial extract was in quadrants I and IV, while the root extract was grouped in quadrants II (Figure 2). Based on the results obtained, grouping tends to be based on the part of the plant not because of the hydroponic type or concentration used. CCL was an aerial extract whose mix of leaves and roots came from the market. CCL position was between the aerial extract, whose leaves came from different hydroponic types, and root extracts (Figure 2). The highest concentrations have adjacent positions in both the leaves (A3L and B3L) and roots (A3R and B3R).

Heat map analysis was used to find out more about the compound differences that form the basis for grouping extract on the PCA plot score. Figure 3 shows the result of the heat map analysis. Based on the hierarchical cluster analysis in the heat map, the samples were divided into two large clusters: the root and the aerial cluster (Figure 3). The A3R was closely related to the A2R and then the A1R. B3R was closely related to nonrecirculating hydroponics extracts, then B1R and B2R. B3L was closely related to A3L, then A2L. However, B2L was closely related to B1L, then A1L. Aerial extracts had a low abundance of 10-(E)-12-(Z)-conjugated linoleic acid and stigmasterol characterized by a greenish color in the cells and a high abundance of linoleic acid and palmitic acid compounds characterized by a yellowish color in the cells (Figure 3). CCL had the highest abundance of linoleic acid among other aerial extracts, characterized by a yellower cell color (Figure 3).

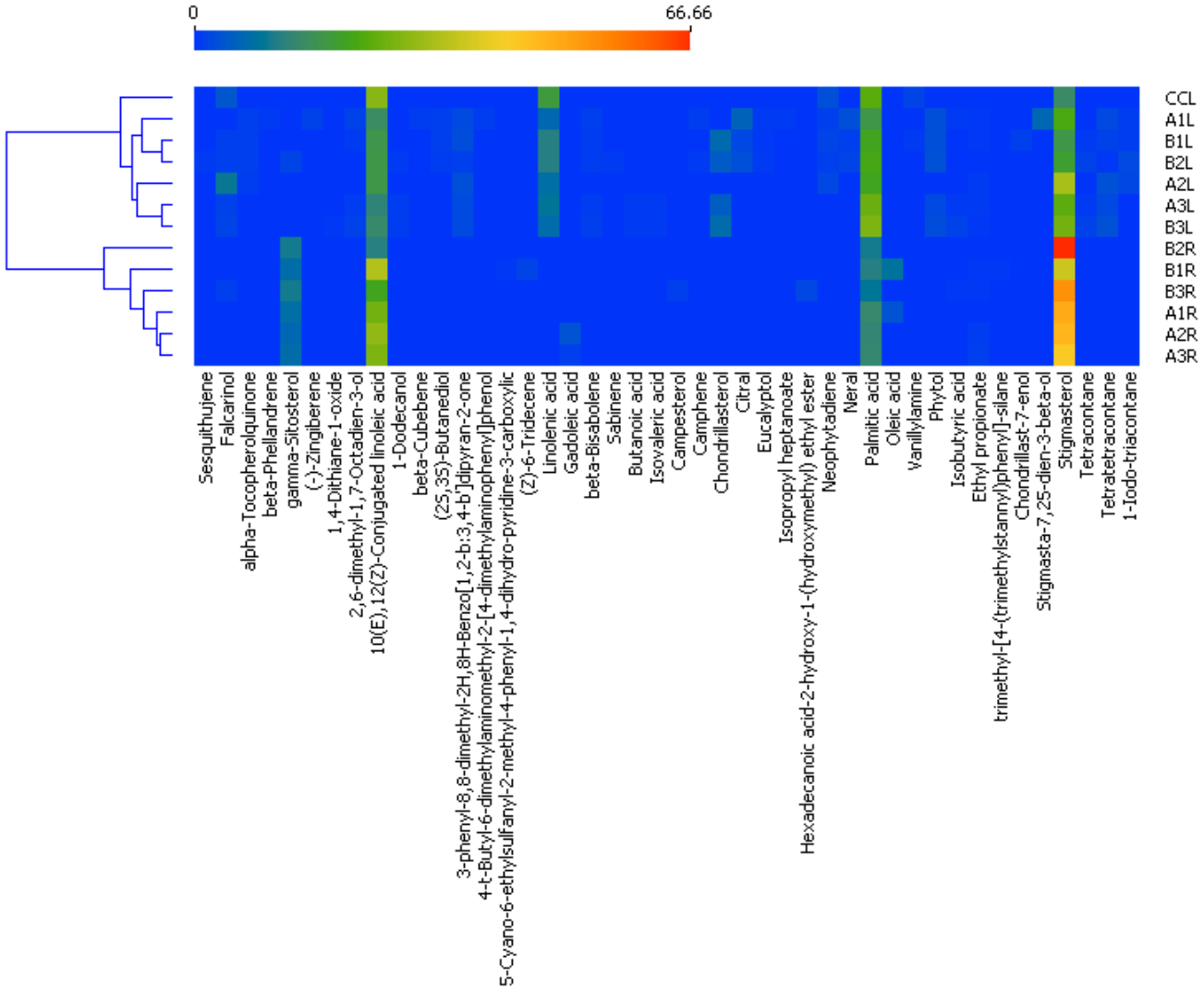

Figure 3. Heat map of compounds in purwoceng aerial and root extract

3.5. Antioxidant Capacity of Purwoceng Extract

Purwoceng extracts were thought to have antioxidant capacity. The greater the capacity for ascorbic acid, the greater the antioxidant capacity in a sample. Overall, purwoceng aerial extracts had higher antioxidant capacity than purwoceng root extracts (Table 4). Purwoceng extracts from nonrecirculating drip hydroponics had a higher average antioxidant capacity than recirculating drip hydroponics (Table 4). In addition, antioxidant capacity increased with increasing nutrient concentration, except for purwoceng aerial extract from nonrecirculating drip hydroponics (B2L) (Table 4).

In general, the antioxidant capacities produced by purwoceng extract from various types of hydroponics and nutrient concentrations were significantly different from each other based on the results of the Duncan's Multiple Distance test at the 5% level, except for A1 and B3. This indicated that there was an effect of the type of hydroponic treatment and nutrient concentration on the antioxidant capacity of the purwoceng extract.

Table 4. Antioxidant capacity of purwoceng extracts

Treatment combination	Antioxidant capacity (µg AAE/plant)	
	Root	Aerial
A1	122.1^{c}	346.2^{c}
A2	157.4^{d}	493.7^{e}
A3	206.2^{e}	413.1^{d}
B1	77.7^{a}	260.7^{b}
B2	107.4^{b}	221.0^{a}
B3	129.5^{c}	359.9^{c}

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

The antioxidant capacity was strongly related to the total phenolic content (TPC). The loading plot of PCA proved this. The position of antioxidant capacity with TPC was very close in the loading plot (Figure 4). In addition, it was also proven by the antioxidant capacity and TPC values, which were directly proportional. For example, on A2L and A3L, it had high values of TPC for 5.15 and 4.13 g GAE/plant, respectively (Table 3) and gave the highest values of antioxidant capacity for 493.7 and 413.1 µg AAE/plant(Table 4).

4. Discussion

Purwoceng can be cultivated ex-situ such as in a greenhouse. This research succeeded in cultivating purwoceng in a greenhouse using hydroponics. Certainly, there were differences in cultivation results due to differences in nutritional treatment and types of hydroponics. The aerial part had a higher dry weight than the root part. Plant dry weight consists mostly of carbon, hydrogen and oxygen. This is in

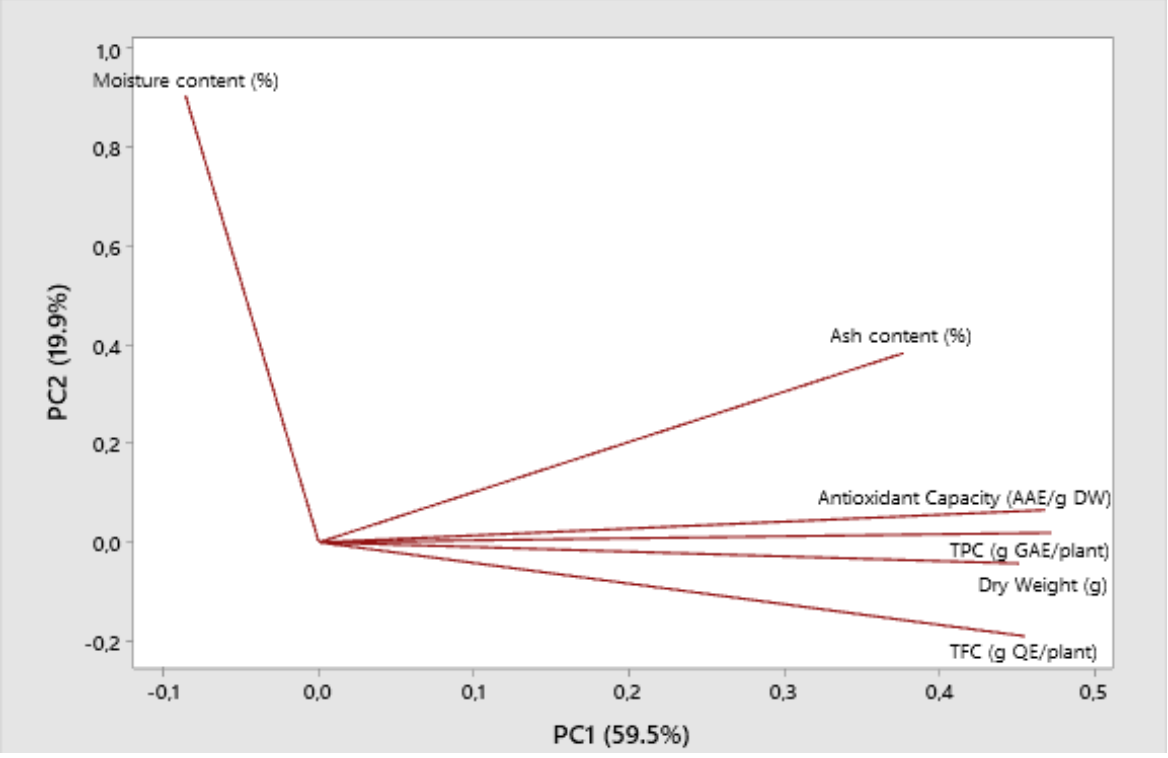

Figure 4. Loading plot of PCA

line with the statement of Ma *et al.* (2018) which stated that the carbon content in leaves is higher than in roots. Moreover, the moisture content and TPC value were directly proportional to the nutrient concentration given. The nutrients of 1,000 ppm gave the lowest values for moisture content, ash content, and TPC value. This was presumably because the plants lacked nutrients, so the results obtained were of little value. This result is in line with Xu *et al.* (2019) who stated that plant productivity was positively correlated with soil nutrient concentrations.

Purwoceng aerial and root extracts were analyzed using gas chromatography-mass spectrometry (GC-MS). This was a non-targeted analysis because the aim was to find the volatile compounds contained in the extracts with no target compound. Based on this study, stigmasterol had the highest average abundance in purwoceng aerial and root extracts. Mariani (2017) also detected a high abundance of stigmasterol compounds in purwoceng, especially in the aerial parts. The difference in chemical composition was thought to be due to different treatments, such as the hydroponic types and nutrient concentrations. However, there were no significant changes in steroids due to differences in the treatment of hydroponic types and nutrient concentrations. The abundance of all identified compounds was grouped using principal component analysis and heat map analysis.

PCA aims to classify and identify a reduced set of data to represent the original data with fewer variables without losing essential information (Kherif and Latypova 2019). Based on PCA result, there was no clear compound grouping pattern based on hydroponic types and different nutrient concentrations. The compounds were grouped only based on the plant parts, i.e., aerial and root parts. This shows that the secondary metabolites contained in the aerial and root parts are different. To find out the differences in secondary metabolites between one extract and another, heat map analysis can be used. 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. The color ranges from blue, which indicates low abundance, to red which indicates high abundance. Aerial extracts had a low abundance of 10-(E)-12-(Z)-conjugated linoleic acid and stigmasterol characterized by a greenish color in the cells and a high abundance of linoleic acid and palmitic acid compounds characterized by a yellowish color in the heat map cells. CCL had the highest abundance of linoleic acid among other aerial extracts, characterized by a yellower heat map cell color. This was suspected to be the cause of the CCL position, which was not close to other aerial extracts in the PCA score plot previously described. γ-sitosterol and stigmasterol were abundant in root extracts, but linoleic acid was not found in all root extracts. There was no significant color pattern for the different hydroponic types and nutrient concentrations. In aerial extracts, the higher the nutrient concentration provided, the abundance of palmitic acid also increased. It was different from root extracts because when the concentrations of nutrients given increased, the abundance of palmitic acid decreased.

Distinct compounds were obtained from the color pattern displayed on the heat map. A1L was the only extract that contained stigmasta-7,25-dien-3-beta-ol. The same thing happened to B3R because it was the only extract that contained hexadecanoic acid-2-hydroxy-1-(hydroxymethyl) ethyl ester. Falcarinol was most abundant in A2L and isovaleric acid in A3L. The distinct compounds in B1L and B3L were α-tocopherolquinone and chrondrillasterol, respectively, because these two compounds were the most abundant in those extracts. B2L could be distinguished from other extracts because its content contained no tetratetracontane. Gadoleic acid was most abundant in A2R compared to other extracts. The difference between A3R and the others was that it had the highest abundance of ethyl propionate. 10-(E)-12-(Z)-conjugated linoleic acid was most abundant in B1R, while in B2R was stigmasterol.

Purwoceng is thought to have antioxidant capacity. In this study, we analyzed the antioxidant capacity of purwoceng due to differences in nutritional treatment and hydroponic type. In general, different hydroponic types and nutrient concentrations caused differences in the antioxidant capacity of purwoceng extract. Antioxidant capacity increased with increasing nutrient concentration, except for purwoceng aerial extract from nonrecirculating drip hydroponics (Table 4). As already mentioned by Xu *et al.* (2019), nutrient concentration will increase plant productivity. Plant productivity will affect antioxidant capacity. This result is slightly different from Song *et al.* (2020)

who stated that in general antioxidant capacity will decrease when nutrient concentrations increase. Further research is needed regarding the chemical components in hydroponic nutrient mixtures that most influence the value of the antioxidant capacity. A combination of nonrecirculating drip with high nutrient concentration at the aerial part provided high antioxidant capacity.

A loading plot in PCA is very useful for interpreting the correlation between each factor (Gowda *et al.* 2014). The antioxidant capacity was strongly related to the total phenolic content (TPC). Phenolic compounds are well known for their antioxidant capacity. This is due to the presence of hydroxyl groups which are associated with the ability to reduce as hydrogen donors and acceptors (Vuolo *et al.* 2019). Purwoceng extracts with higher antioxidant capacity generally contain several antioxidant compounds based on GC-MS analysis. It had a high abundance of phytol, stigmasterol, and palmitic acid. Phytol is an acyclic diterpene alcohol and a chlorophyll constituent. It exhibited strong antioxidant effects in vitro in its capacity to scavenge hydroxyl radicals and nitric oxide and to prevent the formation of reactive thiobarbituric acid (Santos *et al.* 2013). Stigmasterol protects the brain from damage by reducing oxidative stress and inflammation (Liang *et al.* 2020). According to Hashem *et al.* (2016), palmitic acid could be an antioxidant because it could reduce oxidative stress in experimental rats who were also given a dose of palmitoleic acid. Therefore, phytol, stigmasterol, and palmitic acid were thought to have antioxidant capacity. Optimization of the purwoceng planting environment still needs to be done in order to produce better quality purwoceng. Factors that can be examined such as different growing media, amount of shade, and also harvesting time. The extraction procedure also needs to be optimized in order to get a large amount of extract with good quality.

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