Effect of Vermicompost Application on Bioactive Properties and Antioxidant Potential of MD2 Pineapple Fruits

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Abstract: Vermicompost is an organic waste produced from earthworms that can enhance the soil condition and is rich with essential plant nutrients, thus increasing produce quality and shelf life. In this study, a one-year field trial was conducted to elucidate the effects of vermicompost supplementation on the composition of bioactive compounds and antioxidant activities of pineapple (*Ananas comosus* var. MD2) fruits, compared to control and application of chemical fertilizer. Based on the results, pineapple fruits produced from plants supplemented with chemical fertilizer showed the strongest radical scavenging properties against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), followed by vermicompost and control plants. Application of chemical fertilizer and vermicompost also produced fruits with a very high content of chlorophylls and β-carotene compared to control plants. However, the amounts of bioactive compounds present in fruits produced with chemical fertilizer are higher than in fruits produced with vermicompost. Total phenolics content and Ferric Reducing Antioxidant Power (FRAP) reducing power were lowest in fruit extracts produced from pineapple plants supplemented with vermicompost. These results suggested that vermicompost cannot completely replace chemical fertilizer for the production of fruits with a high content of phytoconstituents but could be used as an additional supplement to reduce environmental pollution and ensure agricultural sustainability.

Keywords: vermicompost; organic agriculture; *Ananas comosus*; phytochemical; plant nutrient; bioactivity; sustainability

1. Introduction

Pineapple (*Ananas comosus* (L.) Merr.) belongs to the Bromeliaceae or Bromeliad family and is generally cultivated for its fruit. The fruit can be eaten fresh, canned, frozen or made into juice, syrups or candied [1]. Today, pineapple is found in almost all subtropical and tropical areas of the world and has become one of the leading tropical fruits in international commerce [1]. The introduction of MD2 variety into the fresh pineapple market has increased its demand. The MD2 hybrid variety developed by Del Monte Fresh Produced International Inc. is currently the most preferred tropical fruit for both domestic and international markets [2]. A study by Wardy et al. [3] showed that MD2 variety has a great potential in the horticultural industry compared to Sugarloaf and Smooth Cayenne.

The MD2 variety is in high demand due to its good quality characteristics, such as blemish-free flesh, uniform fruit size with golden yellow pulp, a very pleasant aroma when ripened, and longer shelf-life. Furthermore, it has a high sugar content (15–17 Brix) with low acidity 0.4–0.45% [4]. It is also a good source of vitamin A, C, calcium, phosphorus, iron, potassium, and thiamine.

Vermicompost is an organic waste produced by earthworms from interaction with microorganisms in a mesophilic process, to produce fully stabilized and organic soil amendments that are rich with essential plant nutrients. Vermicomposting aids in diverting organic wastes from landfills and serves as a quick and cost-effective method of composting. Various studies have reported that soil amendment using vermicompost promotes soil quality, resulting in improved soil structure, increased microbial activity, and enhanced plant available nutrients, which in turn improves plant production compared to conventional chemical fertilization [5]. Promotion of microbial activity due to the usage of vermicompost has also been linked to producing healthier plants and increasing plant resistance towards pests and diseases [5]. For example, vermicompost application had a positive effect on yield parameters of tomatoes [6], wheat [7], maize [8], and peppermint [9]. Peppermint plants grown on vermicompost showed higher total fresh yield and produced higher chlorophyll *a*, chlorophyll *b*, and carotenoids compared to the supplementation of inorganic fertilizers and unfertilized plants [9]. However, it was also reported that the application of vermicompost did not significantly affect the total phenols and antioxidant capacity of the plants. The benefits that plants receive from vermicompost application depend on the plant's ability to extract from the fertilizing substrate and the substances needed for growth and development [10]. Nevertheless, the vast benefits of vermicompost have garnered attention as a greener replacement for chemical fertilizers.

A limited number of reports were found in the published literature on the effect of vermicompost supplementation on bioactive compounds and antioxidant activities of pineapple fruits. In this study, the effect of vermicompost supplementation on the composition of bioactive compounds and antioxidant activities of the resulting pineapple fruits were investigated and compared to fruits produced with chemical fertilizer. The outcomes of this study provide additional knowledge and understanding about the effects of vermicompost application on the production and availability of bioactive phytoconstituents in crops, especially in pineapple fruits. Thus, this study adds to the knowledge that can provide a better understanding and appreciation of the importance of organic fertilization and sustainable agriculture.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

Ananas comosus var. MD2 plants were cultivated at Glami Lemi Biotechnology Research Centre, University of Malaya, Jelebu, Negeri Sembilan, Malaysia from January 2015 until March 2016 using suckers as the starting materials. A Randomized Complete Block Design (RCBD) with three treatment groups was employed when planting the pineapple in the field. The treatment groups consisted of control plants (T1), plants supplied with NPK fertilizer (T2), and plants supplied with vermicompost (T3). Each treatment plot (block) was 3 m by 2 m and was replicated four times. There was an average of 15 plants in each block, planted in double rows with a plant-to-plant spacing of 60 cm $\times$ 30 cm. All the beds were covered with plastic mulch with 0.03 mm of thickness. The chemical properties and soil texture of the study site prior to cropping were as previously reported [11].

For T2, the plants were treated with commercial chemical fertilizer and foliar spray as recommended by Malaysia Pineapple Industrial Board [11,12] with minor modifications. 20 g of NPK (15:15:15) fertilizer granules per plant were applied at one month, three months, and seven months after planting (MAP). Fifty to 100 milliliters (50−100 mL) of foliar fertilizer mix were sprayed twice at 1.5 months (640 g of hydrated lime, 42 g of copper sulfate, 42 g of zinc sulfate, 21 g of ferrous sulfate in 18 L of water) and 4.5 months (added 640 g urea in 18 L of water, as before) after planting onto the leaves of each plant. For T3, commercial vermicompost was applied twice (10 t ha^{-1}),

at transplanting and seven months after planting (one month before flowering was induced) by incorporating into the top 10 cm of the soil. The nutrient availability of vermicompost was total nitrogen (N) 1.54%, total phosphorus (P) 0.64%, total potassium (K) 6.31%, total magnesium (Mg) 0.58%, total calcium (Ca) 1.39%, total sulfur (S) 0.34%, total zinc (Zn) 0.01%, total boron (B) 0%, total iron (Fe) 0.76%, and total aluminum (Al) 1.04%. The control plants (T1) were not supplied with any fertilizer or vermicompost products.

Flowering was induced after nine months of planting (MAP) by spraying the center of the pineapple plants with 50 mL of Ethrel (2-chloroethyl phosphonic acid) solution (15 mL of Ethrel and 90 g of urea in 9 L of water). The fruits were harvested when they were one-third ripened or after 152 days of flower induction. The plants were watered when necessary using a sprinkler water system, and weeds were manually controlled (through pulling and cutting).

2.2. Sample Extraction

Five grams of freeze-dried samples were subjected to solvent extraction using 150 mL of 99.8% methanol for 48 hours at room temperature, under dark condition using an orbital shaker (722-2T, Protech, Selangor, Malaysia) at 100 rpm. The extracts were filtered using Whatman No. 2 filter paper, and the collected filtrate was stored at −20 °C. The residue was re-extracted and filtered. The extracts were pooled, centrifuged at 9000 rpm, 4 °C for 5 min, and the supernatant was collected before being concentrated to dryness using a Rotavapor® R-3 (Büchi Labortechnik AG, Flawil, Switzerland) at 45 °C [13]. A 99.8% methanol was then used to adjust the concentration of the solvent-free extract to 20 mg/mL, prior to storage in an airtight container at −20 °C until further analysis. As far as possible, all extraction procedures were performed in the dark, to avoid exposure to daylight.

For the analysis of carotenoid content, 1.0 g of freeze-dried fruit samples were rehydrated with 1.0 mL distilled water and soaked overnight at room temperature in 5 mL of acetone:methanol (7:3). Then, the mixture was vortexed and centrifuged at $13,500 \times g$ for 2 min, where the supernatant was then transferred into a 50 mL graduated polypropylene centrifuge tubes covered with foil. The supernatant was centrifuged again at $13,500 \times g$ for 5 min to remove fine particulates. The extract was then stored at 4 °C in the dark, prior to analysis. For the extraction of carotenoids, a 1:1 ratio of hexane and distilled water was added to the sample mixture, vortexed, and centrifuged at $13,500 \times g$ for 1 min. The carotenoid layer (upper layer) was collected and dried under a gentle stream of O_2-free nitrogen gas. Then, the vials were immediately capped and sealed using parafilm before being stored at −80 °C until subsequent analysis.

2.3. Phytochemical Analysis

Chemical tests were performed on the methanolic extracts of pineapple pulp to identify the presence of bioactive secondary metabolites based on standard assay procedures described by Solihah et al. [14].

2.4. Determination of Chlorophyll a and b

Methanolic solutions of fruit extracts were analyzed using a UV-Vis spectrophotometer (Perkin Elmer™, Waltham, MA, USA) at 470, 652.4, and 665.2 nm. The concentrations of chlorophylls *a* and *b* were calculated based on the formula by Lichtenthaler and Buschmann [15]:

$$\text{Chlorophyll } a \text{ (μg/g)} = 16.72 \, A_{665.2} - 9.16 \, A_{652.4} \tag{1}$$

$$\text{Chlorophyll } b \text{ (μg/g)} = 34.09 \, A_{652.4} - 15.28 \, A_{665.2} \tag{2}$$

2.5. Chromatographic Determination of Carotenes

HPLC-MS analysis was conducted using an Agilent 1200 series HPLC system (Agilent Technologies, Santa Clara, CA, USA), based on the method described by Othman et al. [16].

Sample (10 μL) was injected into a 5 μm, 4.6 × 250 mm ZORBAX SB-C$_{18}$ end capped reverse phase column (Agilent Technologies, Santa Clara, CA, USA) at 20 °C. The mobile phase consisted of 9:1 *v/v* acetonitrile:water (eluent A) and 100% ethyl acetate (eluent B). The analysis was conducted using a gradient program: from 0–40% solvent B (0–20 min), from 40–60% solvent B (20–25 min), from 60–100% solvent B (25–25.1 min), 100% solvent B (25.1–35 min), 100% solvent B (35–35 1 min). Simultaneous monitoring was performed at 350 to 550 nm at a flow rate of 1.0 mL/min. The column was allowed to re-equilibrate in 100% solvent A for 10 min before the injection of the next sample. The contents of carotenoid compounds were calculated based on standard curves obtained from HPLC analysis of carotenoid standards and are expressed as μg per g dry weight samples. The peak height of less than 10 mAU was not detected. The fruit extracts were screened for eight types of carotenoid; neoxanthin, violaxanthin, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, lycopene, and lutein.

2.6. Total Phenolic Content (TPC)

Total phenolic content (TPC) of the fruit methanolic extracts was determined using the Folin–Ciocalteu (FC) method as described by Singleton et al. [17]. Briefly, 20 μL of sample (20 mg/mL) was mixed with 1.58 mL of distilled water and 100 μL of 2 N Folic-Ciocalteu reagent. Then, 300 μL of 20% (*w/v*) Na$_2$CO$_3$ was added (30 s to eight min) into the solution. The solution was incubated for two hours at room temperature, in the dark condition. The absorbance of the samples was read using a UV/Vis spectrophotometer (Lambda 25, Perkin Elmer, Waltham, MA, USA) at 765 nm. The blank and standard were prepared with a similar method. A standard solution of gallic acid (r^2 = 0.99) was used to prepare the calibration curve. The TPC content of the samples was expressed in mg gallic acid equivalent (GAE)/g of dried extract and was calculated based on the following formula:

$$\text{Total phenolic content (mg GAE/g dry extract)} = (cV)/m \tag{3}$$

where c is the concentration of gallic acid obtained from the calibration curve (mg/mL), V is the volume of extract (mL), and m is the weight of extract (g).

2.7. DPPH Radical Scavenging Assay

The DPPH assay was performed following Brand-Williams et al. [18] with some modification. The positive control used was ascorbic acid. A 150 μL of 3 mM solution of DPPH radical solution in methanol was added into 50 μL of methanolic extract of samples (2 mg/mL to 12 mg/mL), standard solution (0.01 mg/mL to 1.00 mg/mL), and control (99.8% methanol) in different wells for triplicates. Then, the solution was left to stand for 30 min in the dark at 27 °C. The changes in the absorbance of the samples were measured at 515 nm using the microplate spectrophotometer (Multiskan^{TM} GO, Thermo Scientific, Waltham, MA, USA). Scavenging of free radicals by DPPH as a percentage of the radical scavenging activities was calculated using the formula below:

$$\text{DPPH radical scavenging activity (\%)} = ((A_o - A_1)/A_o) \times 100 \tag{4}$$

where A_o is the absorbance of the control, A_1 is the absorbance of samples.

The graph of DPPH radical scavenging activity percentage against concentration was plotted using non-linear regression (third-degree polynomial) as previously described by Samad et al. [19]. The 50% inhibition concentration (IC$_{50}$) values in mg/mL were determined and reported.

2.8. ABTS Radical Scavenging Assay

For ABTS assay, the procedure described by Miller et al. [20] was followed, but with few modifications. The stock solutions (7.4 mM ABTS solution and 2.6 mM potassium persulfate) were separately prepared prior to analysis and were used in preparing the working solution. For analysis, 1:1 ratio of the stock solutions were mixed and incubated for 12–16 hours at room temperature in

the dark before use. The solution was then diluted with deionized water (18.2 MΩcm^{-1}) until an absorbance of 0.70 $\pm$ 0.02 units at 734 nm was obtained using a spectrophotometer (Multiskan^{TM} GO, Thermo Scientific, Waltham, MA, USA). Fresh ABTS solution was prepared for each assay. Fruit extracts (20 μL) at six different concentrations (2 mg/mL to 12 mg/mL) were allowed to react with 200 μL of ABTS solution in the dark for 10 min. Then, the absorbance was read at 734 nm using a spectrophotometer. A standard curve was plotted using non-linear regression (third-degree polynomial) between the percentage of inhibition and concentration (r^2 = 0.99). The results were reported in terms of 50% inhibition concentration (IC$_{50}$) values in mg/mL. The positive control used was ascorbic acid.

2.9. FRAP Assay

The FRAP assay was performed based on the method described by Benzie and Strain [21] with some modification. Stock solutions consisting of 300 mM acetate buffer (3.1 g C$_2$H$_3$NaOO and 16 mL C$_2$H$_4$O), pH 3.6, 10 mM 2, 4, 6tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM FeCl$_3$.6H$_2$O solution were separately prepared before the analysis. Prior to each analysis, a fresh working solution consisting of acetate buffer, TPTZ solution, FeCl$_3$.6HO solution, and distilled water (10:1:1:1.2) was prepared and warmed at 37 °C before use. Fruit extracts (10 μL) were allowed to react with 300 μL of FRAP solution in the dark for 30 min. Absorbance readings of the colored product (ferrous tripyridyltriazine complex) were measured at 593 nm using microplate spectrophotometer (Multiskan^{TM} GO, Thermo Scientific, Waltham, MA, USA). The standard curve was plotted using a linear regression between 0.01 and 0.10 mg/mL of ferrous sulphate FeSO$_4$.7H$_2$O (r^2 = 0.99). FRAP values were expressed in milligram of ferrous equivalent Fe (II) per gram of dried extract.

2.10. Statistical Analysis

All data in this paper are reported as the mean $\pm$ standard error of the mean. Duncan Multiple Range Test (DMRT) and one-way analysis of variance (ANOVA) were used to compare the results at a 95% confidence level. Correlation analysis was done among all data using Pearson correlation coefficient in bivariate linear correlation (SPSS Statistics version 24 for Windows, IBM Inc., Armonk, NY, USA).

3. Results

3.1. Detection and Quantification of Bioactive Compounds in Fruit Samples

The phytochemical constituents in the methanolic extracts of A. *comosus* var. MD2 fruits are presented in Table 1. The results of qualitative analysis on each fruit pulp extract showed the presence of phenols, flavonoids, and tannins. Alkaloids were absent in all treatments. Tannins were observed to be strongly present in fruit extracts produced from plants treated with chemical fertilizer while flavonoids were strongly detected in fruit extracts grown with vermicompost.

Chlorophyll *a* and *b* showed similar trends (Table 2) whereby higher amounts were detected in fruit extracts of plants treated with chemical fertilizer, followed by vermicompost and control plants. Results indicated that the TPC of fruits ranged between 4.859 mg GAE/g of dried extract to 8.895 mg GAE/g of dried extract (Table 2). The fruit extracts produced from plants supplied with chemical fertilizer showed significantly higher TPC compared with other treatments.

Table 1. Phytochemical analysis of the methanolic extract of MD2 pineapple fruits produced from plants supplied with different types of fertilizers in the field.

Chemical Constituents	Control	Chemical Fertilizer	Vermicompost
Phenol	+	+	+
Flavonoids I	+	++	+++
Flavonoids II	+	+	+
Tannins	++	+++	++
Alkaloids I	-	-	-
Alkaloids II	-	-	-
Alkaloids III	-	-	-

Note: - absent; + present.

Table 2. Pigments content, total phenolics content (TPC), and β-carotene content of MD2 pineapple fruit extract grown with different types of fertilizers.

| Bioactive Compounds	Control	Chemical Fertilizer	Vermicompost
Chlorophyll *a* (µg/g)	1.055 ± 0.078 [c]	2.438 ± 0.038 [a]	1.866 ± 0.000 [b]
Chlorophyll *b* (µg/g)	3.194 ± 0.096 [c]	7.203 ± 0.073 [a]	5.496 ± 0.000 [b]
Total Chlorophyll (µg/g)	4.249 ± 0.041 [c]	9.640 ± 0.040 [a]	7.362 ± 0.000 [b]
Chlorophyll *a*/*b* ratio	0.332 ± 0.035 [a]	0.339 ± 0.009 [a]	0.340 ± 0.000 [a]
Total phenolics (mg GAE/g DE)	5.983 ± 0.001 [b]	8.895 ± 0.002 [a]	4.859 ± 0.001 [c]
β-carotene (µg/g DW)	2.32 ± 0.17 [c]	44.21 ± 0.69 [a]	28.43 ± 2.41 [b]

Note: Mean ± standard error of mean (n = 3 replicates) within each row followed by a different letter indicate significant differences by Duncan multiple range test at $p \leq 0.05$. GAE, gallic acid equivalents; DE, dry extract, DW, dry weight.

Moreover, the extracts were also subjected to HPLC analysis to detect and quantify individual carotenoids that are present in the samples, that is, neoxanthin, violaxanthin, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, lycopene, and lutein. However, of the eight types of carotene screened, only β-carotene was detected in the fruit extracts from all treatments (Figure 1). The pulp extract of fruits grown with chemical fertilizer showed a higher amount of β-carotene (44.21 ± 0.69 µg/g DW) compared to vermicompost (28.43 ± 2.41 µg/g DW) and control (2.32 ± 0.17 µg/g DW).

3.2. Antioxidant Activity of Fruit Extracts

The fruit pulp extracts were also examined for radical scavenging and antioxidant activities using three different assay methods, namely DPPH, ABTS, and FRAP. Table 3 shows the IC_{50} values for DPPH assay and ABTS assay, as well as FRAP values. For the DPPH and ABTS assays, the antioxidant capacity of the test extracts is expressed as IC_{50}, which are the concentration of the samples required to scavenge 50% of the DPPH and ABTS free radicals. A lower IC_{50} denotes a more potent antioxidant. Fruits from plants supplemented with fertilizer or vermicompost showed similar DPPH IC_{50} values, with significantly stronger DPPH radicals scavenging activities than control plants. The fruits from plants treated with chemical fertilizer showed the least IC_{50} values against ABTS radicals, followed by vermicompost and control. The FRAP assay estimates the antioxidant power, which is the reducing ability of the substances involved in the transfer of electron in the reaction [22]. Based on Table 3, fruit extracts from all treatments showed no significant differences in their reducing power ($p \leq 0.05$). However, the lowest reducing power among all treatments was exhibited by fruit extracts from vermicompost treatment, with 0.276 ± 0.020 mg of $FeSO_4$ equivalent/g of dried extract.
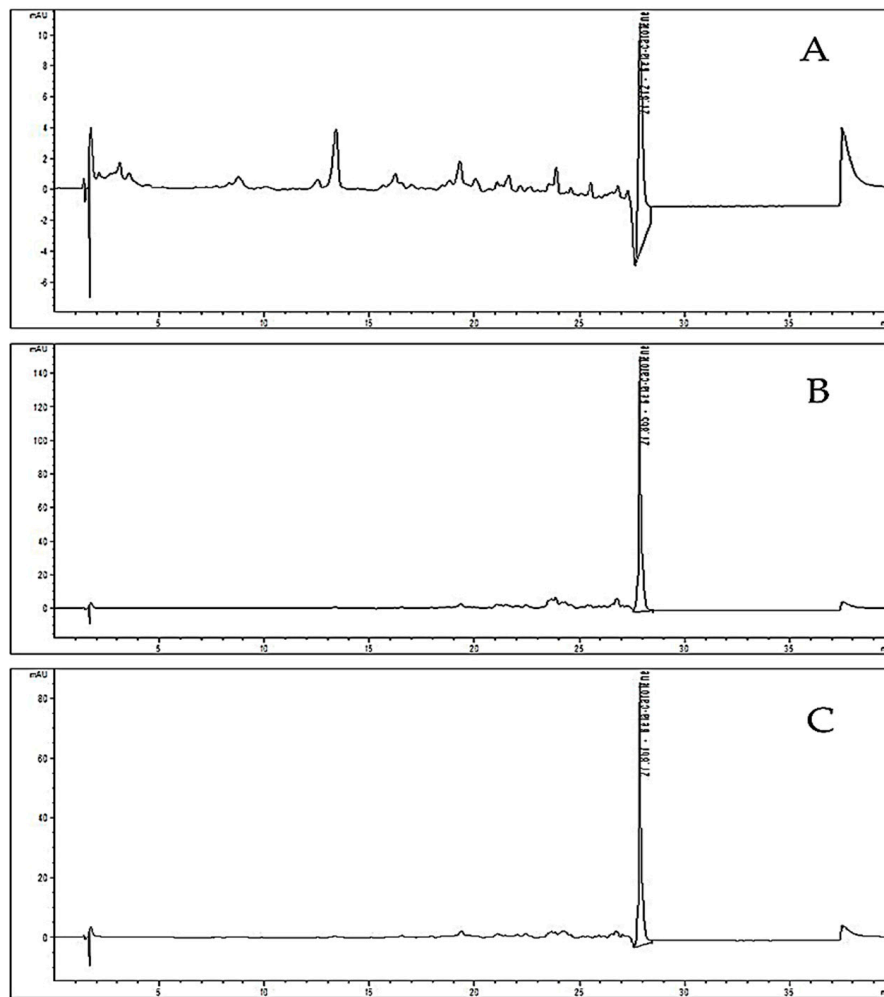
Figure 1. HPLC chromatograms of carotenoids in pineapple pulp extracts retrieved from plants treated with different types of fertilizers in the field. (**A**) control (**B**) chemical fertilizer (**C**) vermicompost. The peak height of less than 10 mAU was not detected.

Table 3. Antioxidant capacities determined by DPPH, ABTS, and FRAP assays in methanolic extracts of MD2 pineapple fruits produced from plants grown with different types of fertilizers.

Antioxidant Assay	Ascorbic Acid (Standard)	Control	Chemical Fertilizer	Vermicompost
DPPH, IC_{50} (mg/mL)	0.034 ± 0.002 [c]	5.133 ± 0.101 [a]	2.909 ± 0.050 [b]	3.239 ± 0.213 [b]
ABTS, IC_{50} (mg/mL)	0.057 ± 0.004 [d]	8.393 ± 0.100 [a]	5.777 ± 0.130 [c]	7.290 ± 0.188 [b]
FRAP (mg FE/g dE)	36.198 ± 4.398 [a]	0.368 ± 0.005 [b]	0.402 ± 0.017 [b]	0.276 ± 0.020 [b]

Note: Mean $\pm$ standard error of mean within each row followed by a different letter indicate significant differences at $p \leq 0.05$, ($n = 3$). FE, ferric equivalent; dE, dry extract.

3.3. Correlation Analysis with NPK Contents in the D-Leaves of MD2 Pineapple Plant

A correlation analysis was conducted between all parameters measured and also the NPK contents in the D-leaves of the pineapple plants during the emergence of inflorescence [11]. Based on the correlation analysis conducted (Table 4), it was determined that total nitrogen content in the D-leaves of pineapple plant during flowering significantly and strongly correlates with the amounts of β-carotene ($r^2 = 0.670$, $p \leq 0.05$), chlorophyll *b* ($r^2 = 0.699$, $p \leq 0.05$), and total chlorophyll ($r^2 = 0.690$, $p \leq 0.05$) contents of the fruit extracts, indicating that an increase in N content will strongly influence and increase β-carotene, chlorophyll *b*, and total chlorophyll contents of the fruits. Furthermore, significantly strong correlations were also observed between potassium (K) content in the D-leaves of the pineapple

plant and β-carotene ($r^2 = 0.720$, $p \leq 0.05$) and chlorophylls content of the fruit extracts. However, although there were strong correlations observed between phosphate (P) content in the D-leaves and the amount of bioactive compounds present in the fruit extracts, these correlations were not significant.

Table 4. Pearson's correlation coefficient between bioactive compounds composition and antioxidant capacities of fruit extract and NPK content in the D-leaves of MD2 pineapple plant during the emergence of the inflorescence.

Parameters	Chl *a*	Chl *b*	Total Chl	TPC	β-Carotene	DPPH	ABTS	FRAP	Total N	P
Chl *a*	1									
Chl *b*	0.984 **	1								
Total Chl	0.991 **	0.999 **	1							
TPC	0.620	0.634	0.632	1						
β-carotene	0.984 **	0.990 **	0.992 **	0.587	1					
DPPH	−0.927 **	−0.937 **	−0.937 **	−0.371	−0.961 **	1				
ABTS	−0.964 **	−0.963 **	−0.966 **	−0.747 *	−0.949 **	0.862 **	1			
FRAP	0.146	0.158	0.155	0.805 **	0.100	0.129	−0.291	1		
Total N	0.656	0.699 *	0.690 *	0.158	0.670 *	−0.679 *	−0.580	−0.110	1	
P	0.548	0.589	0.581	0.567	0.576	−0.524	−0.613	0.064	0.138	1
K	0.761 *	0.778 *	0.776 *	0.561	0.720 *	−0.636	−0.692 *	0.370	0.504	0.226

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). Note: Chl, chlorophyll; TPC, total phenolics content; N, nitrogen; P, phosphorus; K, potassium.

Correlation analysis was also conducted to determine the relationship between the antioxidant capacities exhibited by the fruits and the bioactive compound constituents. Based on the results presented in Table 4, significant strong correlations were observed between DPPH and ABTS radical scavenging activities and the contents of β-carotene and chlorophylls. However, TPC did not influence DPPH radical scavenging activity. In contrast, it was significantly correlated with ABTS scavenging activity in A. *comosus* var. MD2 fruits. Interestingly, in contrast to the results obtained for DPPH and ABTS scavenging activities, a significantly strong correlation was observed between FRAP and TPC, but very weak correlation was observed between FRAP potential and contents of β-carotene and chlorophylls.

4. Discussion

Phytochemicals are bioactive non-nutrient plant compounds found in fruits and other plant foods that are naturally occurring substances. They could act as antioxidants and anti-inflammatory agents to help reduce the risk of major chronic diseases [23]. They also provide protection against abiotic stresses, such as UV-B irradiation, heat stress, low water potential or mineral deficiency [24].

In this study, the methanolic extract of freeze-dried MD2 pineapple was screened for the presence of primary (e.g., chlorophylls) and secondary metabolites, such as phenols, flavonoids, alkaloids, and tannins. Flavonoids are acclaimed for their antioxidant and antimicrobial activity. Flavonoids test was positive for all treatments in this study. Similar results were obtained in previous studies [25]. Tannins are acclaimed for their free-radical scavenging activities, antiviral, antimicrobial, anti-inflammatory properties, and also used in medicine as astringent [26]. The detection of tannins in fruits indicates their potential health benefits. Tannins were found to be strongly present in the fruit extracts of pineapple plants treated with chemical fertilizer, parallel with their abilities to inhibit the DPPH and ABTS radicals. Alkaloids are a diverse group of secondary metabolites that protect plants against herbivores and pathogens, which are mostly found in herbal or medicinal plants, but with limited occurrence in fruits and vegetables [24]. Alkaloids were not detected in all samples tested in the current study, in contrast to that reported by Gunwantrao et al. [25] and Agnes and Anusuya [26]. This could be due to the different extraction process or the use of different solvents during the extraction procedure.

In a study conducted on *Withania somnifera* (Ashwagandha), utilization of vermicompost has been found to be beneficial in improving germination, plant growth, yield, and content of bioactive Withanolides of the Ashwagandha leaves [27]. This is in line with the findings of the current study

where the addition of vermicompost was found to significantly elevate the amounts of bioactive compounds in the pineapple fruits, compared to control. Results showed that pineapple fruits grown with chemical fertilizer and vermicompost had twice the amounts of chlorophylls, 20 and 10 times the amount of β-carotene than control fruits, respectively. However, the application of chemical fertilizer yielded fruits with slightly higher amounts of chlorophylls, phenolics, and β-carotene compared to fruits grown with vermicompost. These observations were largely due to the difference in nutrient composition of the fertilizers, which affects soil nutrient availability and nutrient uptake in the pineapple crops, to be used for growth and during fruiting [28,29]. We had previously reported that the application of chemical fertilizer significantly increased the total N content in the D leaves of *A. comosus* var. MD2 plants during red bud stage [11]. Nitrogen plays a very important role in growth, reproduction, and maintenance of the photosynthetic capacity of plants [30,31]. However, N content in plants has also been linked in reducing the concentration of anthocyanin in berries and soluble solids in grape juice, due to the increase in vegetative growth [32].

Potassium (K) is essential for fruit production as it affects sugar concentration, regulates pH and fruit acidity, and is involved in the synthesis of phenolic compounds [32]. This is in agreement with the findings in the current study, where a strong correlation was observed between K levels in the pineapple leaves and the total phenolic contents of the fruits, however, the correlation observed was insignificant. Besides that, it was also observed that an increase in K content would significantly increase the chlorophylls and β-carotene contents in the pineapple fruits. In previous studies conducted on tomato plants, it was found that potassium (K) fertilization can affect carotenoid biosynthesis, specifically lycopene [33–36]. It has been reported that the relationship between chlorophyll and carotenoid contents in plants are highly influenced by potassium (K) status of the plant [37]. However, the ratio of chlorophyll to carotenoid content changes during ripening, where chlorophyll content decreases and carotenoids increase with the ripening of the fruit [37]. This is in line with the findings obtained in the study, where the pineapple fruits produced with chemical fertilizer had a higher total chlorophyll content than fruits produced with vermicompost, thus yielding lower carotenoid content in the latter.

Fruits contain many compounds that show antioxidant potentials. The main role of antioxidants is their interaction with oxidative free radicals. Several methods have been developed to estimate the total antioxidant activity of different plant materials. Usually, these methods measure the ability of antioxidants to scavenge specific radicals, to inhibit lipid peroxidation or to chelate metal ions [38]. In order to obtain a more complete picture of the antioxidant capacity of an extract, more than one method should be used [38,39]. Thus, in the present study, the free radical scavenging activity of the pineapple fruit extracts was tested through DPPH, ABTS, and FRAP assays, which are the most widely used assays.

Based on the DPPH assay, fruits from plants supplemented with chemical fertilizer and vermicompost showed similar IC_{50} values, with significantly stronger DPPH radicals scavenging activities than control plants (Table 3). It has been found that chlorophyll *a*, chlorophyll *b*, total chlorophyll, and β-carotene showed a negative significant correlation with DPPH radicals (Table 4). This study supports evidence from previous observations where TPC present in the pineapple extracts is not the main contributor to the radical scavenging activity of the extracts [40]. Nevertheless, many studies had shown correlations between DPPH scavenging activity and TPC in pineapple fruit extracts [39,41–44]. In the current study, a strong significant correlation was observed between FRAP and TPC with $r^2 = 0.805$ at $p \leq 0.01$. This is in contrast with a previous study which found a weak correlation between corrected TPC of fruit extracts of Josephine, Morris, and Sarawak pineapples and the FRAP values ($r^2 = 0.158$) [40].

We had previously reported that organic fertilization, such as the application of vermicompost, had contributed to improving the stability of bioactive compounds of *Clinacanthus nutans* (Sabah snake grass) during the storage of an extract [45]. Thus, it is suggested that future research can include an investigation into the effect of vermicompost on the stability of the bioactive compounds in the

pineapple fruits, especially during transportation of the produce, fruit storage, and fruit processing. This information may be beneficial for plant growers and consumers alike, as it can yield a better understanding and appreciation of the importance of organic fertilization and sustainable agriculture.

5. Conclusions

Based on the results, it can be concluded that the application of vermicompost to pineapple plants produced fruits of good quality with a high content of bioactive compounds and excellent antioxidant properties, compared to control. However, these amounts were slightly lower than in fruits produced with chemical fertilizer. This implies that vermicompost cannot completely replace chemical fertilizer for the production of fruits with a high content of phytoconstituents but could be used as an additional supplement to current fertilization practice to reduce environmental pollution and ensure agricultural sustainability.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; and in the decision to publish the results.

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