



## Article

# Enhancement of Antioxidant Potential, Phytochemicals, Nutritional Properties, and Growth of *Siphonochilus aethiopicus* (Schweinf.) B.L.Burttt with Different Dosages of Compost Tea

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**Abstract:** The wild population of *Siphonochilus aethiopicus* (Zingiberaceae) is being eroded due to several pharmacological benefits and the hidden economy credited to its ethnobotanical uses in Southern Africa. This has called for the adoption of sustainable ways of cultivating the species without compromising its bioactive constituents. In this study, compost tea was brewed and applied at different dosages to potted *S. aethiopicus* to enhance its growth quality, phytochemical content, and elemental compositions. Treatments comprised 0.25, 0.50, 0.75 and 1.00 ( $\frac{v}{v}$ ) graded concentrations of compost tea, while water and undiluted compost tea were the control treatments. Results showed that dosages of compost tea had no significant effect on chlorophyll content or fresh and dry weights of rhizomes of *S. aethiopicus*. The longest leaves were recorded in plants irrigated with water only, while the shortest leaves were recorded in plants irrigated by 50% compost tea. A similar trend was observed in leaf width, except that equivalent values were recorded in all compost tea treatments while plants irrigated with undiluted compost tea were tallest. The highest and lowest flavanols were respectively recorded in 0.50 and 0.25 compost tea-treated plant samples, while undiluted compost tea yielded the highest flavonol and phenolic acids. The highest antioxidant contents were produced by the 0.25 compost tea-treated samples in the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), FRAP (Ferric Reducing Antioxidant Power) and ORAC (Oxygen Radical Antioxidant Capacity) assays, while the lowest were observed in plants irrigated with water only, although all compost tea-treated plants had equivalent effects on the ORAC content. The highest N, P, K, and Mn contents were produced in the 0.25-treated samples, while the minerals were least accumulated in samples treated with water only. All treatments had equivalent effects on Ca, Zn, and B yield, whereas the highest and equivalent accumulations of Mg and Na were recorded in the control treatments. Iron (Fe) and Cu were most influenced significantly by water whereas P, Ca and Zn tissue concentration was not significantly influenced by treatments. These results indicate that compost tea can optimize growth, mineral accumulation, phytochemicals, and antioxidants in *S. aethiopicus*. This approach serves as a greener and sustainable way of conserving overexploited indigenous medicinal plants such as *S. aethiopicus* to mitigate overexploitation of its wild relatives and preserve its genome from imminent extinction.

**Keywords:** compost tea; hidden herbal economy; rhizomes; wild ginger; Zingiberaceae

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## 1. Introduction

*Siphonochilus aethiopicus* (Schweinf.) B.L.Burttt, also known as 'wild ginger', is a medicinally important plant with restricted distribution to South Africa, especially in Northern and Mpumalanga Provinces [1,2]. Other local names include Wildegemmer (Afrikaans), Natal ginger and Isiphephetho or Indungulo in Zulu language [2,3]. Its cone-shaped rhizome is the most sought-after part for various medicinal uses, including antiplasmodial, antifungal and antibacterial effects [4,5]. There is renewed interest in the food industries to

use the rhizome as a potential source of new flavour to improve the shelf life and sensory values of human diets [6–8].

The wild population of *S. aethiopicus* is on the fringe of extinction due to overexploitation and destructive methods of harvesting by indiscriminate harvesters who uproot the rhizomes and fleshy roots for sale at local herbal markets [2,5]. Apart from making difficult the vegetative propagation of the plant through nodes on the rhizome, this practice is further exacerbated by traditional healers who believe that only plants harvested from the wild can provide the desired medicinal benefits, thereby increasing the frequency of overexploitation [9–11]. The proliferation of informal markets where indigenous medicinal plants are traded, encouraged by the relaxing of various conservation policies regulating exploitation of medicinal plants [12–14], has plundered the wild vegetation of these valuable species.

The rising demand for the rhizomes of *S. aethiopicus*, due to its chemical diversity and numerous potential pharmacological uses, as well as the hidden herbal economy, attributed to its ethnobotanical uses among rural dwellers, has triggered its selective exploitation in the wild [5,6]. This has necessitated the development of different cultivation methods to preserve its wild population and rapidly eroding genome from intrinsic extinction. The use of compost tea to improve growth and the medicinal quality of plants has been widely reported in botanical literature. This was considered as an alternative and sustainable way of ensuring continuous availability of *S. aethiopicus* in all seasons, alongside other means of conserving medicinal plants such as replacement of plant parts [15,16], biostimulants and fertilizer applications, among others [14,17]. In this study, compost tea was brewed and applied at different dosages to potted *S. aethiopicus* as a way of enhancing its growth quality, phytochemical content, and elemental compositions. It is thought that these findings will complement the existing methods of cultivating *S. aethiopicus* under controlled conditions, thereby boosting its commercial production.

## 2. Materials and Methods

### 2.1. Plant Materials and Experimental Design

Sixty rhizomes of *S. aethiopicus* used for the study were purchased from Afro-Indigenous Nursery, KwaZulu-Natal, South Africa. The experimental trial was carried out under carefully regulated environmental conditions in the tropical greenhouse of the Department of Horticultural Sciences, Cape Peninsula University of Technology (CPUT), Bellville (33°55'56" S, 18°38'25" E). The rhizomes were weighed after delivery and then planted in 15 cm pots containing pine bark chips, vermiculite, and perlite substrate mixed in equal proportion (Table 1, Figure 1). Thereafter, rhizomes were irrigated three times a week with water for two months without fertilizer or compost tea, before being moved into the tropical greenhouse [18]. The experiment was laid in a completely randomized block design using a factorial arrangement where each treatment comprised 10 pots (N = 10). Every week, plants were subsequently irrigated with 100 mL of compost tea; however, plants treated with only tap water and crude compost extract were used as negative and positive controls respectively [18–20].

### 2.2. Extraction of the Compost Tea

The mushroom compost was purchased from Stanler Farms, Cape Town, South Africa. To prepare the treatments, 500 g of mushroom compost was aerobically rested in 40 L of tap water (1:8 g/L) for 24 h [19,20]. The protocol was conducted according to Stanler Farms' prescription. Tap water and crude compost extract were used as negative and positive controls, respectively. Other treatments were derived by adding 1 L of municipal water to 250 mL, 500 mL, 750 mL and 1 L of brewed compost tea respectively, resulting in different concentrations (0.25, 0.5, 0.75 and 1  $\frac{v}{v}$ ) of the compost tea solution. These specific preparations were stored in separate lidded flasks and applied subsequently to the potted plants [18–20]. The plants were assessed weekly over 20 weeks for their growth responses to the above-mentioned formulations.

**Table 1.** Physicochemical analysis of the growth medium.

Treatments	Soil	pH	Resist.	H <sup>+</sup>	P Bray II	K	Exchangeable Cations (cmol(+)/kg)				Cu	Zn	Mn	B	Fe	C%
		(KCl)	(Ohm)	(cmol/kg)	mg/kg	Na	K	Ca	Mg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg		
0.25 ( $\frac{25}{100}$ )	Sand	4.85 ± 0.63	1095.00 ± 559.00	1.19 ± 0.78	123.50 ± 81.30	358 ± 23.50	0.91 ± 0.67	0.92 ± 0.60	12.56 ± 1.82	6.26 ± 2.60	4.12 ± 2.50	99.20 ± 5.97	527.00 ± 46.60	1.74 ± 0.06 <sup>bc</sup>	331.50 ± 70.70	2.82 ± 0.92
0.50 ( $\frac{50}{100}$ )	Sand	4.80 ± 0.57	1525.00 ± 290.00	1.34 ± 0.84	104.50 ± 16.30	282.5 ± 14.80	0.61 ± 0.08	0.73 ± 0.04	12.61 ± 1.69	5.52 ± 0.01	3.16 ± 1.99	56.1 ± 2.59	320.00 ± 22.40	1.57 ± 0.01 <sup>c</sup>	240.00 ± 68.30	4.10 ± 1.82
0.75 ( $\frac{75}{100}$ )	Sand	4.85 ± 0.59	1435.00 ± 163.00	1.17 ± 0.74	114.00 ± 49.50	326.0 ± 33.90	0.56 ± 0.02	0.84 ± 0.09	15.10 ± 0.43	5.40 ± 0.17	4.29 ± 1.87	91.60 ± 5.23	564.00 ± 42.50	2.15 ± 0.13 <sup>a</sup>	355.00 ± 79.90	3.86 ± 2.39
1.00 ( $\frac{100}{100}$ )	Sand	4.85 ± 0.50	975.00 ± 469.00	1.14 ± 0.42	117.50 ± 2.12	421.0 ± 77.80	0.86 ± 0.40	1.08 ± 0.19	11.56 ± 0.16	5.20 ± 0.22	3.22 ± 1.15	85.80 ± 4.86	483.00 ± 33.40	1.61 ± 0.23 <sup>c</sup>	256.22 ± 12.81	2.25 ± 0.14
Compost tea only	Sand	4.95 ± 0.64	435.00 ± 247.00	1.05 ± 0.70	158.50 ± 51.60	558 ± 165.00	1.20 ± 0.42	1.43 ± 0.42	14.26 ± 1.70	6.34 ± 1.29	3.54 ± 1.44	83.50 ± 5.98	487.00 ± 35.20	2.16 ± 0.06 <sup>a</sup>	314.70 ± 55.00	2.10 ± 0.01
Soil + water	Sand	4.80 ± 0.28	1920.00 ± 157.00	0.72 ± 0.11	105.5 ± 44.50	275.5 ± 23.30	0.57 ± 0.04	0.71 ± 0.06	12.06 ± 1.77	5.32 ± 0.04	4.35 ± 0.92	107.60 ± 9.73	547.00 ± 59.70	2.00 ± 0.15 <sup>ab</sup>	294.10 ± 47.00	2.37 ± 0.17
<b>Analysis of Variance (ANOVA) at 95% confidence limit</b>																
<i>p</i> -values		1.000 ns	0.579 ns	0.947 ns	0.871 ns	0.317 ns	0.51 ns	0.316 ns	0.248 ns	0.865 ns	0.954 ns	0.966 ns	0.991 ns	0.010 *	0.450 ns	0.540 ns
<i>F</i> -values		0.02	0.82	0.21	0.34	1.50	0.95	1.50	1.80	0.35	0.19	0.17	0.09	8.71	1.09	0.90
Pooled SD		0.53	314.17	0.65	48.27	56.38	0.36	0.31	1.44	1.19	1.73	61.07	41.62	0.13	59.80	1.29

Note: Means were ranked using Fisher's LSD at 95% confidence limit along the column. Means that do not share a letter are significantly different. \*  $\leq 0.05$  = significant difference at  $p \leq 0.05$ ; ns = not significant.



**Figure 1.** Experimental layout in the greenhouse at CPUT (picture by T. Jasson).

### 2.3. Sample Measurement and Data Collection

Growth parameters such as rhizome diameter, leaf length, leaf width and leaf number were measured with a metre rule, while rhizome weight and chlorophyll content were measured with an electronic precision balance (Model No: FR-H, Fuzhou Furi Electronics Co., Ltd., Fuzhou, China) and SPAD, respectively. The weight of fresh rhizomes of *S. aethiopicus* were measured, and thereafter, oven-dried at 40 °C for one week and re-weighed on the balance meter [21,22].

### 2.4. Determination of Mineral Content

The mineral content of dry plant samples was analysed as described by Tshayingwe et al. [23], using the atomic absorption spectrophotometer facility at BEMLAB ([www.bemlab.ac.za](http://www.bemlab.ac.za)), a commercial laboratory located at Somerset West, Cape Town, South Africa [21].

### 2.5. Preparation of Crude Extracts

Crude extracts were prepared by stirring about 0.5 g of finely ground dry plant samples in 50 mL of 80% ethanol and centrifuged for 5 min at 4000 rpm. Unmacerated tissues were separated by running the supernatant through a Whatman No. 1 filter paper inserted in a Buchner funnel connected to an electric vacuum pump. The plant extract was used for subsequent phytochemical and antioxidant assays.

### 2.6. Determination of Total Flavanol Content

The total flavanol concentration was measured with the vanillin assay using the method of Salacha et al. [24] with slight modification. The results were expressed as mg catechin equivalents per gram (mg CE/g).

### 2.7. Determination of Total Flavonol Content

The total flavonol content of the extracts was calculated from the quercetin standard curve generated from concentration ranges of 0, 5, 10, 20, 40, and 80 mg/L of quercetin dissolved in 95% ethanol. A volume of 12.5 µL crude extracts was mixed with 12.5 µL of 0.1% HCl (Merck, Modderfontein, South Africa) in 95% ethanol and 225 µL of 2% HCl

for each sample. The mixture was then incubated at room temperature for 30 min [25] before taking absorbance at 360 nm. The results were expressed in milligrams of quercetin equivalent per gram of dry weight (mg QE/g).

### 2.8. Determination of Total Polyphenol Content

The total polyphenolic composition in the extracts was determined using the Folin-Ciocalteu method, as described by Idris et al. [26]. Crude extract and diluted Folin-Ciocalteu reagent mixed in ratio 1:5 *v/v* was pipetted in a 96-well microplate. This was reacted with 7.5% sodium carbonate solution and incubated at room temperature for 30 min. A Multiskan spectrophotometer (Thermo Electron Corporation, Waltham, MA, USA) was used to read the mixture's absorbance at 765 nm. The standard equation generated from gallic acid prepared in graded concentrations of 0, 20, 50, 100, 250, and 500 mg/L dissolved in 10% ethanol was plotted and values were calculated in mg gallic acid equivalent per gram dry weight (mg GAE/g).

### 2.9. Determination of ABTS Antioxidant Capacity

The antioxidant capacity of ABTS was determined following Jimoh et al. [27] with minor amendments. The stock solution included 7 mM ABTS and 140 mM potassium peroxydisulphate ( $K_2S_2O_8$ ) solutions. A 5 mL ABTS solution was mixed with 88  $\mu$ L of potassium peroxydisulphate to prepare the experimental stock solution. The mixture was left in a dark cupboard at room temperature for 24 h. A Trolox (6-Hydrox-2,5,7,8-tetramethylchroman-2-20 carboxylic acid) standard was prepared in concentration ranges of 0–500  $\mu$ M. A volume of 25  $\mu$ L each of the tested samples and standard was reacted with 300  $\mu$ L ABTS solution in the dark for 5 min at room temperature and the absorbance was measured at 734 nm at 25 °C. The obtained data were expressed as  $\mu$ M of Trolox equivalent per g of dry weight ( $\mu$ M TE/g) of tested samples.

### 2.10. Determination of Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was carried out using the method described by Bulawa et al. [28] and Idris et al. [26] with minor modifications. FRAP reagent was made by mixing 30 mL Acetate buffer (0.3 M, pH 3.6) with 3 mL 2,4,6-tripyridyl-s-triazine (10 mM in 0.1 M Hydrochloric acid), 3 mL Iron (III) chloride hexahydrate ( $FeCl_3 \cdot 6H_2O$ ), and 6 mL of distilled water, and incubated for 30 min. at 37 °C. Thereafter, 300  $\mu$ L of the FRAP solution was mixed with 10  $\mu$ L of the crude extract in a 96-well plate and the absorbance of the mixture was measured at 593 nm. The Ferric Reducing Antioxidant Power of the tested samples was calculated using an L-Ascorbic acid concentration curve ranging from 0 to 100  $\mu$ M (Sigma-Aldrich, Johannesburg, South Africa). Results were expressed as  $\mu$ M ascorbic acid equivalents (AAE) per g dry weight ( $\mu$ M AAE/g).

### 2.11. Determination of Oxygen Radical Absorbance Capacity (ORAC)

The ORAC experiment was assayed following Ou et al. [29] and Prior [30]. Calibration standards ranging from 5 to 25  $\mu$ M were prepared by diluting a stock standard solution of Trolox (500 M) in phosphate buffer (75 mM, pH 7.4). The Fluoroskan ascending plate reader which was supplied by Thermo Fisher Scientific (Waltham, MA, USA) was set at 37 °C. The excitation and emission wavelengths of the fluorescence filters utilized were 485 nm and 538 nm, respectively. A phosphate buffer was used to generate a fluorescein stock solution, which was further diluted to a final concentration of 14  $\mu$ M per well. About 25 mg/mL of 2,2'-azobis (2-amidino-propane) dihydrochloride dissolved in phosphate buffer was used to generate the peroxy radical from 4.8 mM 2,2'-azobis (2-amidino-propane) dihydrochloride constituted in each well. After 5 min, the fluorescence of the wells, each of which was filled with 12  $\mu$ L diluted hydrophilic extract, was measured. The ORAC fluorescence values were extrapolated from the regression equation ' $y = ax^2 + bx + c$ ' estimated as  $\mu$ M Trolox equivalents per g dry weight ( $\mu$ M TE/g DW) calculated with the area under the curve.

### 2.12. Data Analysis

Data were analyzed using MINITAB 17 statistical package. A one-way analysis of variance was computed on MINITAB to compare means, which were then ranked using Fisher's Least Significant Difference (LSD) pairwise comparison. Means were considered significantly different at  $p \leq 0.05$ .

## 3. Results

### 3.1. Impact of Compost Tea on Vegetative Growth of *S. aethiopicus*

Leaf Length, Leaf Width, Leaf Number, Plant Height, Chlorophyll and Rhizome Diameter

Except for the leaf number, treatments had significant effects on the measured growth parameters such as leaf length, leaf width, plant height, and rhizome diameter (Table 2). The highest values of rhizome diameter were recorded in the two control treatments which comprised compost tea only, and growth media and water (Table 2). However, other treatments with graded dosages of compost tea had equivalent effects on rhizome diameter. Both leaf length and width were highly impacted by the control treatment in which growth media were treated with water only, whereas the concentrated compost tea had the highest impact on the plant height (Table 2). Other treatments had equivalent effects on the leaf width whereas significant variability was observed in the impacts of treatments on leaf length, leaf width, plant height and rhizome diameter. Similarly, treatments had no significant influence on fresh and dry weights of rhizomes of *S. aethiopicus*. However, there was variability in the fresh rhizome weight values, whereas dry rhizomes had equivalent values (Table 2). In addition, treatments had no significant effects on chlorophyll concentrations in sampled leaves of *S. aethiopicus*, as the leaves had equivalent chlorophyll content (Table 2; Figure 2).

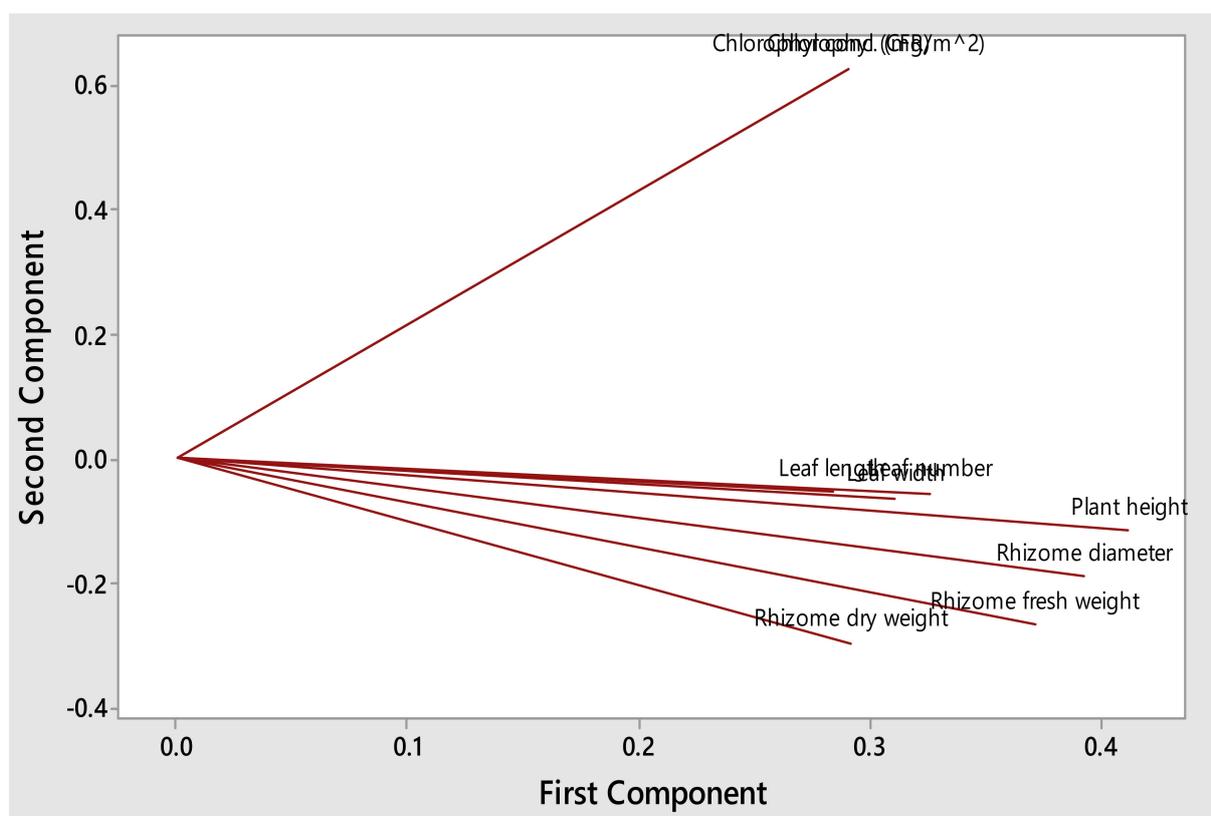


Figure 2. Principal component analysis for measured growth parameters in *S. aethiopicus*.

**Table 2.** Impact of compost tea on vegetative growth of *S. aethiopicus*.

Treatments ( $\frac{v}{v}$ )	Leaf Length (cm)	Leaf Width (cm)	Leaf Number	Plant Height (cm)	Rhizome Diameter (cm)	Fresh Rhizomes (g)	Dry Rhizomes (g)	Chlorophyll Concentration (mg/m <sup>2</sup> )
Compost tea only	13.00 ± 4.47 <sup>bc</sup>	2.95 ± 0.67 <sup>b</sup>	4.90 ± 1.73	44.25 ± 17.30 <sup>a</sup>	3.22 ± 0.70 <sup>a</sup>	20.38 ± 14.13	2.47 ± 1.78	277.70 ± 58.70
Growth media + water	21.05 ± 3.15 <sup>a</sup>	3.69 ± 0.62 <sup>a</sup>	5.20 ± 1.32	40.85 ± 10.28 <sup>ab</sup>	3.22 ± 0.77 <sup>a</sup>	16.57 ± 8.33	2.19 ± 1.22	268.00 ± 46.00
0.25	11.43 ± 3.53 <sup>bc</sup>	2.73 ± 0.63 <sup>b</sup>	4.20 ± 1.14	25.95 ± 8.28 <sup>c</sup>	2.26 ± 0.73 <sup>b</sup>	9.93 ± 4.21	2.00 ± 1.87	268.20 ± 58.70
0.50	10.81 ± 3.87 <sup>c</sup>	2.54 ± 0.50 <sup>b</sup>	4.50 ± 1.90	27.85 ± 15.33 <sup>c</sup>	2.28 ± 0.88 <sup>b</sup>	12.31 ± 17.67	1.44 ± 2.23	238.90 ± 33.50
0.75	12.21 ± 3.77 <sup>bc</sup>	2.93 ± 0.58 <sup>b</sup>	4.60 ± 1.65	29.10 ± 12.89 <sup>c</sup>	2.26 ± 0.71 <sup>b</sup>	12.86 ± 11.86	2.46 ± 3.20	236.60 ± 70.10
1.00	14.35 ± 2.87 <sup>b</sup>	2.76 ± 0.45 <sup>b</sup>	5.90 ± 2.81	30.55 ± 11.57 <sup>bc</sup>	2.28 ± 0.67 <sup>b</sup>	11.03 ± 8.03	1.36 ± 1.09	243.30 ± 60.90
<b>Analysis of Variance (ANOVA) at 95% confidence limit</b>								
<i>p</i> -values	0.000 <sup>*</sup>	0.001 <sup>*</sup>	0.380 <sup>ns</sup>	0.010 <sup>*</sup>	0.002 <sup>*</sup>	0.348 <sup>ns</sup>	0.714 <sup>ns</sup>	0.39 <sup>ns</sup>
<i>F</i> -values	10.62	4.74	1.09	3.40	4.32	1.14	0.58	1.07
Pooled StDev	3.65	0.58	1.84	12.96	0.75	11.58	2.03	54.67

Note: Means were ranked using Fisher's LSD at 95% confidence limit along the column. Means that do not share a letter are significantly different. \* ≤ 0.05 = significant difference at  $p \leq 0.05$ ; ns = not significant.

### 3.2. Impact of Compost Tea on Phytochemicals and Antioxidant Content of *S. aethiopicus*

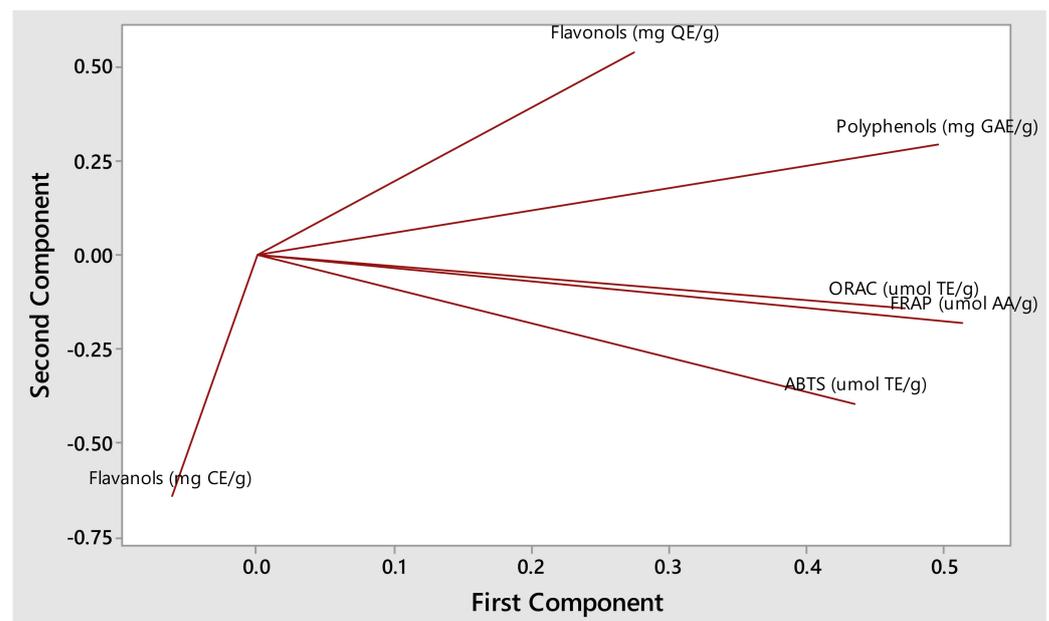
#### 3.2.1. Flavanols

The total flavanols in *S. aethiopicus* varied significantly in the tested samples treated with different dosages of compost tea. The highest and lowest flavanol values were recorded in 0.50 and 0.25 compost tea-treated samples, whereas flavanol content varied significantly in other treatments, including the control treatments (Table 3, Figure 3).

**Table 3.** Effects of compost tea on phytochemicals and antioxidant content.

Treatments ( $\frac{g}{g}$ )	Flavanols (mg CE/g)	Flavonols (mg QE/g)	Polyphenols (mg GAE/g)	ABTS (umol TE/g)	FRAP (umol AA/g)	ORAC (umol TE/g)
Compost tea only	1.39 ± 0.63 <sup>bc</sup>	4.83 ± 1.36 <sup>a</sup>	7.34 ± 0.86 <sup>a</sup>	31.66 ± 5.20	29.07 ± 1.85 <sup>ab</sup>	208.16 ± 24.22 <sup>a</sup>
Growth media + water	1.14 ± 0.05 <sup>bc</sup>	3.08 ± 0.56 <sup>bc</sup>	5.929 ± 0.39 <sup>c</sup>	28.69 ± 1.81	24.56 ± 0.94 <sup>c</sup>	145.93 ± 19.09 <sup>b</sup>
0.25	1.05 ± 0.07 <sup>c</sup>	2.68 ± 0.20 <sup>c</sup>	7.27 ± 0.63 <sup>ab</sup>	36.27 ± 10.43	29.48 ± 4.66 <sup>a</sup>	212.50 ± 48.00 <sup>a</sup>
0.50	1.87 ± 0.14 <sup>a</sup>	3.17 ± 0.36 <sup>bc</sup>	5.89 ± 1.04 <sup>c</sup>	29.76 ± 4.16	27.39 ± 3.41 <sup>abc</sup>	189.2 ± 34.10 <sup>a</sup>
0.75	1.37 ± 0.38 <sup>bc</sup>	3.58 ± 0.13 <sup>b</sup>	6.45 ± 0.80 <sup>bc</sup>	29.07 ± 4.59	28.89 ± 3.19 <sup>ab</sup>	191.72 ± 15.34 <sup>a</sup>
1.00	1.43 ± 0.11 <sup>b</sup>	2.98 ± 0.56 <sup>bc</sup>	5.70 ± 0.22 <sup>c</sup>	32.37 ± 2.32	25.77 ± 2.03 <sup>bc</sup>	193.20 ± 33.40 <sup>a</sup>
<b>Analysis of Variance (ANOVA) at 95% confidence limit</b>						
<i>p</i> -values	0.002 *	0.000 *	0.001 *	0.196 ns	0.036 *	0.013 *
<i>F</i> -values	5.12	8.02	6.13	1.58	2.77	3.48
Pooled StDev	0.31	0.66	0.714329	5.52	2.94	31.0129

Note: Means were ranked using Fisher's LSD at 95% confidence limit along the column. Means that do not share a letter are significantly different. \* ≤ 0.05 = significant difference at  $p \leq 0.05$ ; ns = not significant.



**Figure 3.** Principal component analysis for phytochemicals and antioxidants in *S. aethiopicus*.

#### 3.2.2. Flavonols

Samples treated with undiluted compost tea yielded the highest flavanol while the lowest value was recorded in the 0.25 compost tea-treated plants (Table 3, Figure 3). There was significant variability in the effects of different dosages of compost tea on the total flavanol in *S. aethiopicus* at  $p \leq 0.05$ .

#### 3.2.3. Polyphenols

Both 0.25 and 0.50 treatments yielded the lowest equivalent values in phenolic acid, while the highest was observed in plants treated with undiluted compost tea (Table 3). However, there was significant variation in the effects of different concentrations of compost tea on total phenolic content of the plant.

### 3.2.4. The ABTS, FRAP, and ORAC Antioxidant Contents

The highest antioxidant contents were produced by the 0.25 compost tea-treated samples in the FRAP and ORAC assays, while the lowest were observed in plants irrigated with water only, although all compost tea-treated plants had equivalent effects on the ORAC content (Table 3, Figure 3). Treatments influenced both FRAP and ORAC contents significantly ( $p < 0.05$ ); however, the ABTS content was not affected significantly ( $p > 0.05$ ).

### 3.3. Effects of Varying Dosages of Compost Tea on Mineral Compositions of *S. aethiopicus*

Different concentrations of compost tea affected the accumulation of minerals in *S. aethiopicus*. The highest N, P, K, and Mn contents were produced in the 0.25-treated samples, while the minerals were least accumulated in samples treated with water only. All treatments had equivalent effects on Ca, Zn, and B yield, whereas the highest and equivalent accumulations of Mg and Na were recorded in the control treatments. Iron (Fe) and Cu were most significantly influenced by water. At  $\alpha < 0.05$ , the differences in mean values of P, Ca and Zn accumulated in various treatments were not significant (Table 4, Figure 4).

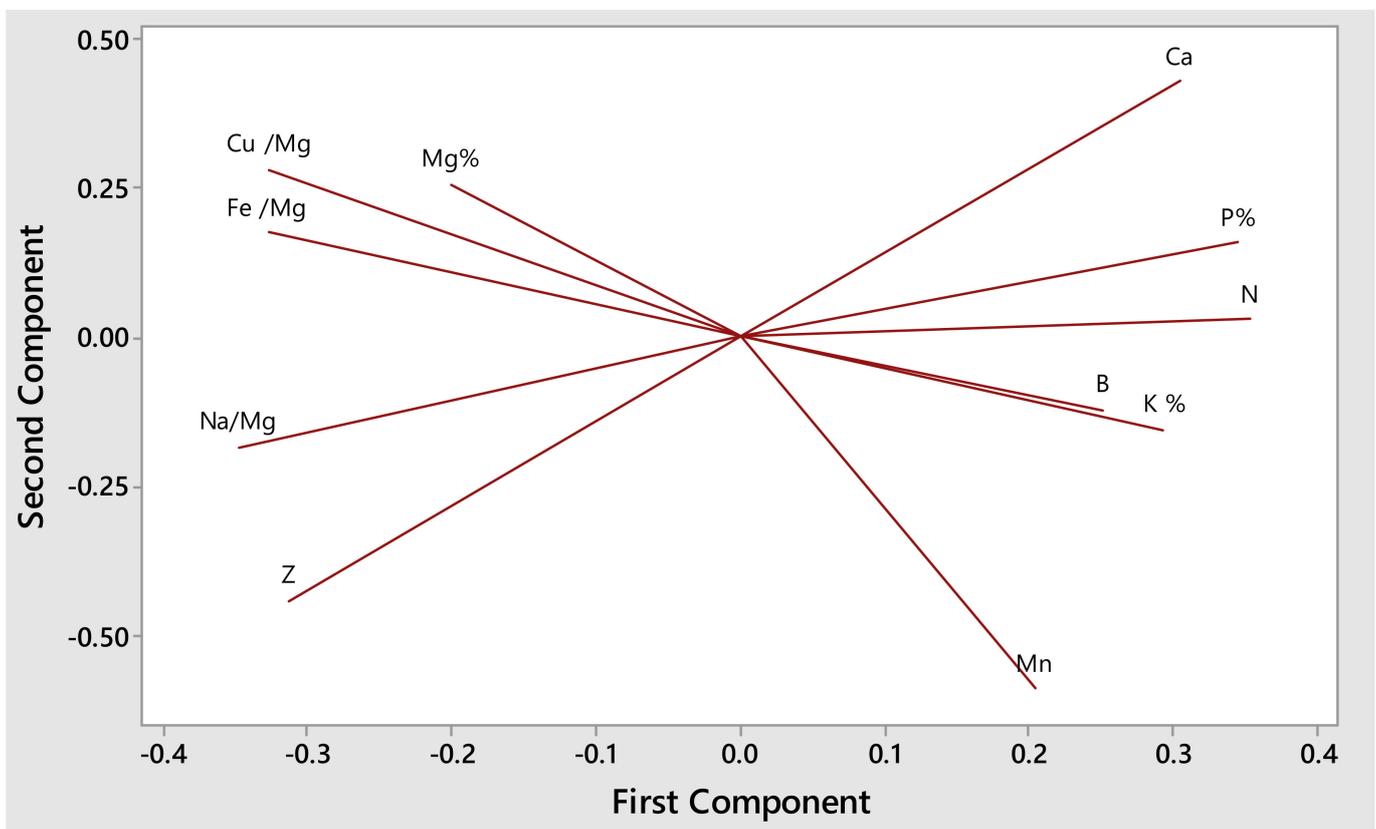


Figure 4. Principal component analysis of minerals in *S. aethiopicus*.

**Table 4.** Effects of different dosages of compost tea on mineral compositions of *S. aethiopicus*.

Treatments ( $\frac{g}{g}$ )	N%	P%	K %	Ca%	Mg%	Na (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	B (mg/kg)
Compost tea only	1.030 ± 0.34 <sup>bc</sup>	0.29 ± 0.07 <sup>ab</sup>	3.13 ± 0.55 <sup>bc</sup>	0.49 ± 0.07 <sup>a</sup>	0.34 ± 0.013 <sup>a</sup>	1346.00 ± 98.50 <sup>a</sup>	963.00 ± 28.10 <sup>cd</sup>	311.00 ± 39.50 <sup>b</sup>	3.17 ± 1.17 <sup>b</sup>	41.33 ± 5.57 <sup>a</sup>	9.50 ± 1.23 <sup>a</sup>
Growth media + water	0.87 ± 0.45 <sup>c</sup>	0.22 ± 0.09 <sup>b</sup>	2.22 ± 0.99 <sup>c</sup>	0.47 ± 0.16 <sup>a</sup>	0.34 ± 0.07 <sup>a</sup>	1331.00 ± 74.70 <sup>a</sup>	617.00 ± 27.20 <sup>d</sup>	1469.00 ± 13.93 <sup>a</sup>	5.33 ± 3.01 <sup>a</sup>	77.2 ± 7.58 <sup>a</sup>	8.17 ± 2.14 <sup>a</sup>
0.25	1.45 ± 0.20 <sup>a</sup>	0.31 ± 0.01 <sup>a</sup>	4.35 ± 0.74 <sup>a</sup>	0.52 ± 0.17 <sup>a</sup>	0.29 ± 0.01 <sup>b</sup>	395.80 ± 82.60 <sup>b</sup>	1932.00 ± 27.30 <sup>a</sup>	83.33 ± 4.97 <sup>b</sup>	2.00 ± 0.00 <sup>b</sup>	41.33 ± 5.57 <sup>a</sup>	13.17 ± 4.54 <sup>a</sup>
0.50	1.11 ± 0.34 <sup>abc</sup>	0.27 ± 0.06 <sup>ab</sup>	3.72 ± 1.13 <sup>a</sup>	0.50 ± 0.05 <sup>a</sup>	0.32 ± 0.05 <sup>ab</sup>	783.00 ± 76.20 <sup>ab</sup>	1857.00 ± 48.90 <sup>a</sup>	258.00 ± 41.70 <sup>b</sup>	2.50 ± 0.84 <sup>b</sup>	77.20 ± 7.58 <sup>a</sup>	9.67 ± 1.21 <sup>a</sup>
0.75	1.22 ± 0.27 <sup>abc</sup>	0.29 ± 0.05 <sup>a</sup>	3.65 ± 0.64 <sup>ab</sup>	0.47 ± 0.21 <sup>a</sup>	0.30 ± 0.04 <sup>ab</sup>	898.00 ± 75.90 <sup>ab</sup>	1534.00 ± 54.50 <sup>ab</sup>	194.00 ± 26.80 <sup>b</sup>	2.17 ± 0.41 <sup>b</sup>	72.20 ± 7.73 <sup>a</sup>	10.33 ± 1.97 <sup>a</sup>
1.00	1.29 ± 0.073 <sup>ab</sup>	0.31 ± 0.03 <sup>a</sup>	4.59 ± 0.74 <sup>ab</sup>	0.42 ± 0.16 <sup>a</sup>	0.31 ± 0.03 <sup>ab</sup>	589.20 ± 61.70 <sup>ab</sup>	1358.00 ± 50.20 <sup>bc</sup>	105.50 ± 12.83 <sup>b</sup>	2.67 ± 0.52 <sup>b</sup>	50.83 ± 3.92 <sup>a</sup>	10.83 ± 0.75 <sup>a</sup>
<b>Analysis of Variance (ANOVA) at 95% confidence limit</b>											
<i>p</i> -values	0.038 <sup>*</sup>	0.122 <sup>ns</sup>	0.000 <sup>*</sup>	0.884 <sup>ns</sup>	0.210 <sup>ns</sup>	0.107 <sup>ns</sup>	0.000 <sup>*</sup>	0.005 <sup>*</sup>	0.003 <sup>*</sup>	0.737 <sup>ns</sup>	0.022 <sup>*</sup>
<i>F</i> -values	2.74	1.91	6.55	0.34	1.53	2.00	9.30	4.31	4.68	0.55	3.12
Pooled StDev	0.30	0.06	0.82	0.15	0.04	78.27	39.37	23.29	1.39		2.33

Note: Means were ranked using Fisher's LSD at 95% confidence limit along the column. Means that do not share a letter are significantly different. \* ≤ 0.05 = significant difference at  $p \leq 0.05$ ; ns = not significant.

#### 4. Discussion

The use of compost tea to improve crop production without compromising yield and fruit quality has been widely reported in literature as conventional agronomic practices [31,32]. This may not be unconnected with its organic applications, which include use as a biostimulant, bioprotectant, biofertilizer, or as inhibitor of disease-causing organisms [33–35]. However, the dosage of compost tea that could enhance the growth and accumulation of antioxidant phytochemicals and nutritious minerals in *S. aethiopicus* remained uncertain, despite several conservation approaches proposed to increase yield and medicinal properties of the species [6,18,36]. This study has provided a panacea to addressing the impending extinction and overexploitation of its wild relatives, as undiluted compost tea significantly improved the rhizome size and height of the species. Therefore, this corroborates existing reports that compost tea is cost-effective and can substitute the use of chemical fertilizers for cultivation [33,33,37].

Over the years, the rhizome of *S. aethiopicus* has been the main target of harvesters for its excellent medicinal applications [7]. Flavonoids, siphonochilone and diarylheptanoids are the main bioactive chemicals found in the rhizome of *S. aethiopicus* [38]. These compounds make the species a good candidate for the development of multimolecular plant-based drugs capable of healing inflammation, malaria and other disorders associated with the central nervous system [8,38,39]. However, findings from this study suggest that compost tea significantly improves the bioaccumulation of flavonoids (flavanols and flavonols) in the rhizome of *S. aethiopicus* treated with undiluted compost tea (flavonols) and 0.50 ( $\frac{5}{10}$ ) (flavanols) compared with other treatments. This observation agrees with conclusions drawn by [40,41] respectively that compost tea improves the level of flavonoids in lettuce and spinach crops.

In the past three decades, polyphenols have increasingly attracted the attention of functional food researchers and manufacturers owing to their antioxidant properties, their critical roles in the prevention and systemic healing of chronic diseases, and their relative abundance in plant-based diets as scavengers of reactive oxygen species [42–44]. Bioaccumulation of polyphenols in *S. aethiopicus* is significantly influenced by compost tea as the highest polyphenolic content was recorded in rhizomes treated with undiluted compost tea. This is in accordance with [45] who asserted that dosages of different compost tea optimized the accumulation of polyphenols in plants. Likewise, Nofal et al. [46] reported a similar trend in *Phaseolus vulgaris* L., where the application of compost tea was reported to have elevated the level of phenolics while dampening the damping-off disease caused by *Rhizoctonia solani* (J.G. Kühn). However, compost tea had no significant effect on phenolics in corms of *Hypoxis hemerocallidea* (Fisch., C.A. Mey. & Avé-Lall.) equally treated with different concentrations of compost tea [18].

The antioxidant content of *S. aethiopicus* rhizome was consistently low in plants irrigated with water only, which is a manifestation of a lack of compost tea in the treatments. This corroborates the positive correlation between phytochemicals and the antioxidant properties of plants [47,48], and upholds the notion that phytochemicals are precursors to antioxidant effects in plants [49,50]. The existence of positive correlation between ABTS and FRAP results, each of which respectively reflects the levels of endogenous glutathione, and  $\alpha$ -tocopherol in the plasma indicates that the plant could serve as a natural anti-oxidative stress recipe due to its high flavonoid content [51,52].

Uptake of minerals is influenced by physicochemical composition of soil or growth substrate [53,54]. Bioavailability of these minerals is an important component of human diets as they play diverse functions in food metabolism, food fortification, nutrient supplementation, and maintenance of internal environment [55,56]. It was therefore necessary to investigate variation and diversity of mineral elements in the rhizome *S. aethiopicus* to promote its use in food fortification. This will provide households and food industries with an alternative way of achieving nutrient diversity and composite minerals that are affordable.

The amount of N and P peaked in rhizomes of *S. aethiopicus* irrigated with 0.25 ( $\frac{v}{v}$ ) treatment, while equivalent K and Mn values were obtained in rhizomes treated with 0.25 ( $\frac{v}{v}$ ) and 0.50 ( $\frac{v}{v}$ ) compost tea dosages. However, the application of compost tea did not affect Fe and Cu levels in the rhizome as both minerals peaked under water treatment only, while the highest amount of Na was obtained in the control treatments. In contrast, Ca, Zn and B levels were not significantly affected as equivalent values were obtained in all treatments including the control. These minerals play critical roles in healthy human living as they supplement key ingredients necessary for life. They aid transmission of nerve impulses (Ca, K, Na, P, Mg), skeletal functions (Ca and Mg), immune system activity (Cu, N, Zn, Ca, Mg), formation of blood cells (Fe), and they act as signaling molecules (Na, K), provide extracellular fluid cation and ionic balance (Na, K), and contribute to the alleviation of dietary deficiencies [55,57–60].

## 5. Conclusions

Findings from this study indicate that application of compost tea can optimize mineral accumulation, phytochemicals, and antioxidants in *S. aethiopicus*, although measured growth parameters were not significantly enhanced. The highest polyphenols, which are precursors for antioxidant activity in plants, were recorded in rhizomes treated with undiluted compost tea, suggesting that the impact of compost tea on phytochemical accumulation cannot be underestimated. Minerals were least accumulated in plant samples irrigated with water only, whereas the highest N, P, K, and Mn contents were produced in the 0.25 ( $\frac{v}{v}$ ) treated samples. This suggests that *S. aethiopicus* requires minimal dosage of compost tea for the accumulation of essential minerals such as N, P, K, and Mn. Irrigating a population of *S. aethiopicus* could serve as a greener and sustainable way of cultivating and conserving overexploited indigenous medicinal plants such as *S. aethiopicus*. This will help to mitigate overexploitation of its wild relatives, preserve its genome, and rescue the plant from imminent extinction. Further studies are recommended on chemical composition of the compost tea to unravel key metabolites that enhance the yield and accumulation of phytochemicals and antioxidants when used to irrigate *S. aethiopicus*.

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