horticulturae

MDPI

Article

Response to Various Water Regimes of the Physiological Aspects, Nutritional Water Productivity, and Phytochemical Composition of Bush Tea (*Athrixia phylicoides* DC.) Grown under a Protected Environment

Muneiwa Rumani [1,*], Tafadzwanashe Mabhaudhi [2,3], Maanea Lonia Ramphinwa [4], Anza-Tshilidzi Ramabulana [5], Ntakadzeni Edwin Madala [6], Lembe Samukelo Magwaza [7,8] and Fhatuwani Nixwell Mudau [1,*]

1 School of Agricultural Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Pietermaritzburg 3209, South Africa
2 Centre of Transformative Agricultural and Food Systems, University of KwaZulu-Natal, Private Bag X01, Pietermaritzburg 3209, South Africa
3 Centre on Climate Change and Planetary Health, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK
4 Department of Plant and Soil Science, Faculty of Sciences, Agriculture and Engineering, University of Venda, Private Bag X5050, Thohoyandou 0950, Limpopo, South Africa
5 Department of Nutrition, Faculty of Health Sciences, University of Venda, Private Bag X5050, Thohoyandou 0950, Limpopo, South Africa
6 Department of Biochemistry and Microbiology, Faculty of Sciences, Agriculture and Engineering, University of Venda, Private Bag X5050, Thohoyandou 0950, Limpopo, South Africa
7 Department of Food Systems and Development, Faculty of Natural and Agricultural Sciences, University of Free State, P.O. Box 330, Bloemfontein 9300, South Africa
8 Plant Science Laboratory, Cranfield University, Cranfield MK43 AL, UK
* Correspondence: muneiwaabigail@gmail.com (M.R.); mudauf@ukzn.ac.za (F.N.M.)

Abstract: The influence of water regimes on plants is crucial for integrating bush tea (*Athrixia phylicoides* DC.) into strategies in Sub-Saharan Africa to tackle food and nutritional insecurity by considering physiological aspects, nutritional yield, nutritional water productivity, and metabolite composition. The objective of the study was to determine the physiological aspects, including leaf gas exchange and chlorophyll fluorescence, nutritional yield, nutritional water productivity, and metabolite composition of bush tea under varying water regimes. The tunnel experiment was laid out in a randomized complete block design (RCBD) with treatments consisting of three water regimes: 100% of crop water requirement (ET_a), 30% of ET_a, and a control (no irrigation), all replicated three times. The morphological aspects were recorded on a weekly basis. However, yield, nutrient content, nutritional water productivity (NWP), and phytochemical composition were determined at harvest. The phytochemical analysis by liquid chromatography mass spectrometry (LC-MS), coupled with visualization of the detected chemical spaces through molecular networking, indicated *Athrixia phylicoides* DC. to be rich in various bioactive compound derivatives, including methyl chlorogenate, flavonoids, tartaric acid, caffeoylquinic acid, and glutinane. The results showed that 30% ET_a enhanced plant growth, nutrient content, and nutritional water productivity compared to other water treatments. Nevertheless, 100% ET_a yielded more (95.62 kg ha^{-1}) than 30% ET_a (60.61 kg ha^{-1}) and control (12.12 kg ha^{-1}). The accumulation of chlorogenic acids was higher under 30% ET_a compared to 100% ET_a and control. Therefore, this study is the first to determine the accumulation of various bioactive compounds in bush tea leaf extracts under varying water regimes. This confirms that in areas with low water availability, bush tea is well adapted for production without limiting nutrients.

Keywords: herbal tea; water treatments; water stress; nutrient content; water-use efficiency; growth development; yield; molecular network

check for updates

Citation: Rumani, M.; Mabhaudhi, T.; Ramphinwa, M.L.; Ramabulana, A.-T.; Madala, N.E.; Magwaza, L.S.; Mudau, F.N. Response to Various Water Regimes of the Physiological Aspects, Nutritional Water Productivity, and Phytochemical Composition of Bush Tea (*Athrixia phylicoides* DC.) Grown under a Protected Environment. *Horticulturae* **2024**, *10*, 590. https://doi.org/10.3390/horticulturae10060590

Academic Editors: Mengfei Li, Jianhe Wei, Xiaofang Wang and Ma Yu

Received: 6 May 2024
Revised: 29 May 2024
Accepted: 30 May 2024
Published: 4 June 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Bush tea (*Athrixia phylicoides* DC.) is a nutritious indigenous crop grown in South Africa that is used as medicine by many households [1]. Traditionally, it is well known as an herbal tea that purifies blood, treats boils, headaches, infected wounds, and cuts, and creates a foam bath [2–4]. Its roots are used as an aphrodisiac by Vhavenda people [5] and the Zulu people use a decoction of the root as a cough remedy and purgative [6]. Research by Tshivhandekano et al. [6] and Lerotholi et al. [7] has shown that the plant tissues contain a significant number of bioactive compounds.

Despite the medicinal properties, bush tea grows naturally at various altitudes with varying rainfall regimes and soil characteristics [8]. Variations in seasonal temperature and vapor pressure deficits may affect plant growth, yield, and the quality of harvested bush tea [9]. Gatabazi et al. [10] reported that cultivation practices and climate change (increased risk of drought) affect the life cycles and distribution of medicinal plant species across a range of habitats. Therefore, a protected environment is one strategy for mitigating the changing agricultural landscape.

South Africa is classified as a water-scarce country, which significantly limits crop production and threatens household food security [11]. The country's annual average rainfall ranges around 500 mm, far below the world's average of 860 mm per annum [12]. Furthermore, Bendou [13] discovered that drought is one of the major factors in limiting plant growth and yield in agricultural production for various crop species. In addition, drought affects plants at various stages of development, from germination to maturity, resulting in decreased crop growth and productivity [14]. Therefore, it is critical to determine the effect of cultural practices such as varying water regimes under a protected tunnel environment to produce high-quality bush tea production.

Previous research on bush tea production determined the response of plant growth, yield, and quality to diverse cultural techniques such as nutritional management in a protected cultivation and field environment [6,15], pruning [16], harvesting methods [17], processing [18], and seasonal variation [15,19]. However, the research on the impact of different water regimes on plant growth, yield, and secondary metabolites of bush tea under a protected tunnel environment is still lacking. Irrigation is a significant factor that affects the growth, yield, and quality of medicinal and aromatic plants [20]. *Camellia sinensis* (tea) responds positively to irrigation and thrives well in dry land conditions [21]. The amount of water used by the crop and lost through the soil or plant (as evapotranspiration) during the growth period is an important factor in determining yield output and nutrient losses [22]. Therefore, in contrast to the prevailing understanding, our study hypothesizes that bush tea grown under a protected environment will exhibit diminished physiological aspects, reduce nutritional water productivity, and alter phytochemical composition when subjected to varying water regimes. Specifically, we anticipate that decreased water availability may lead to physiological stress, reduced nutrient uptake, and changes in secondary metabolite production in bush tea plants. Therefore, the current study aims to assess how different water levels of water availability influence the plant growth, nutrient content, water-use efficiency, and the accumulation of bioactive compounds in the bush tea plant.

2. Materials and Methods

2.1. Experimental Site

The protected environment experiment was conducted during the spring and summer seasons of the year 2022, in a tunnel situated at the University of KwaZulu-Natal's Controlled Environment Research Unit (CERU) (29.58° S, 30.42° E), in Pietermaritzburg, South Africa. Weather data were sourced from the University of KwaZulu-Natal (UKZN) agrometeorology weather service during the spring and summer seasons of the year 2022. The temperatures range from ~33/18 °C (day/night) and relative humidity (60–80%) (Figure 1).

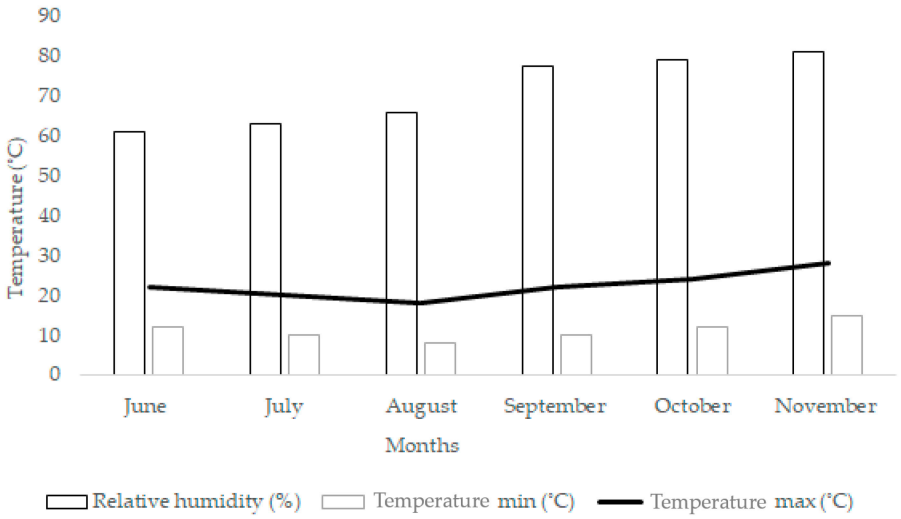


Figure 1. Climatic data: the average monthly temperature during the growing season.

2.2. Plant Material Description

Bush tea cuttings were collected from the Agricultural Research Council (ARC) in Nelspruit, Mpumalanga Province (25.4518° S. 30.9697° E), South Africa. The tunnel experiment was conducted in 11 m long-raised beds with a fixed width of 0.75 m. Transplanting commenced after 35 days of planting to avoid transplanting shock [23]. Cuttings were transplanted into long-raised beds and kept for 40 days. Thereafter, uniform plants were selected for the water treatment trial layout. Planting beds were prepared before establishing a trial. Regular manual weeding was done to keep the trials weed free. Karate (30 mL/15 L water) was applied eight weeks after planting and twice at weekly intervals to control mealy bugs. The soil was submitted to the Institute for Commercial Forestry Research (ICFR) Analytical and Research Laboratory to determine chemical and physical properties and soil texture, which are presented in Table 1. However, the soil fertility test (Table 1) indicated that the soil required nitrogen, which was applied to the soil with a concentration of 4 g of limestone ammonium nitrate (LAN), based on the recommendation of Mudau et al. [3].

Table 1. Physical and chemical properties of the soil in the long-raised beds.

Clay (%)	Organic C (%)	pH (KCl)	P (cmol/kg)	Ca (cmol/kg)	K (cmol/kg)	Mg (cmol/kg)	Na (cmol/kg)	N (%)
38.5	2.70	5.19	539.62	1059	0.36	1.11	0.23	0.19

2.3. Experimental Description

The experiment was laid out in a randomized complete block design (RCBD) consisting of three water regimes (100%, 30%, and control (no irrigation)) replicated three times. Water was supplied using a drip irrigation system three times a day (7, 11, and 3 am) to minimize losses in evaporation and drainage; the system included filters, a pump, two solenoid valves, two water meters, a control box, Netafim inline drippers with four split drippers, and a mainline [24]. The system's maximum operating pressure was set to 200 kPa, with a discharge rate of 2 L hr^{-1} on average, while the drip rate was 33 mL min^{-1} with an average rate per dripper of 2 L hr^{-1}. The irrigation events were the same, but the irrigation interval was different between water regimes. Under control, water was supplied two times a day at 7 am and 11 am, with a discharge rate set to give a total of 2 L a day using a watering can for over 40 days after transplanting; thereafter, irrigation was intentionally withheld to induce water stress on the bush tea plants. The spacing was 0.5 m between rows by 0.5 m within rows (40,000 plants/ha plant population). The

irrigation schedule was determined by the daily crop water requirement from the product of normal tea (unshaded) crop factors (K_c), as previously reported by Singh [25], and monthly average reference evapotranspiration (ET_o), because the crop factor is not yet determined on bush tea (Table 2). The agrometeorology automatic weather station on site was used to collect reference evapotranspiration data. All treatments were watered to field capacity before the treatment was applied to reduce evaporation and drainage losses, and ensure moisture is accessible in the soil during peak periods of the day. As a result, the crop water requirements, ET_a, were calculated using Equation (1).

$$ET_a = ET_o \times K_c \quad (1)$$

where K_c = crop factor based on Allen et al. [26], ET_a = crop water requirement (mm), ET_o = reference evapotranspiration (mm), and K_c = crop factor.

Table 2. Crop water requirements of bush tea in response to varying water treatments.

	K_c	ET_o	ET_a	Duration	Total Water Applied
		mm	mm	Days	Mm
Initial	0.95	4.8	4.56	40	408
Mid-season	1.00	6.57	6.57	51	525.21
Late season	1.00	7.89	7.89	25	341.60
Water applied (ET_a)					
• **100%**					714.72
• **30%**					241.416
• **0%**					0

2.4. Data Collection

2.4.1. Gas Exchange and Chlorophyll Fluorescence Measurements

Gas exchange and chlorophyll fluorescence were measured using the LI-6400 XT Portable Photosynthesis System (Licor Bioscience, Inc., Lincoln, NE, USA) integrated with an infrared gas analyzer (IRGA) attached to a leaf chamber fluorometer (LCF) (640040B, 2 cm^2 leaf area, Licor Bioscience, Inc., Lincoln, NE, USA). The temperature of the leaf was kept at 25 °C. Water flow rate and relative humidity were maintained at 500 µmol and 43%, respectively. The leaf-to air vapor pressure deficit in the cuvette was maintained at 1.7 kPa to avoid stomatal closure due to low air humidity. For fluorescence measurements, the samples were dark adapted by placing them in complete darkness for 15–30 min prior to measurement. Measurements were taken on the third half-fully formed leaf from the plant's tip between 08.30 and 11.30 am by clamping the leaf inside the sensor head, under 100%, 30%, and control. Three plants per replication were used for taking measurements. The following gas exchange measurements were determined: stomatal conductance (*gs*), net CO_2 assimilation rate (*A*), transpiration rate (*T*), intercellular CO_2 concentration (*Ci*), the ratio of intercellular and ambient CO_2 concentrations (*Ci/Ca*), and the ratio of net CO_2 assimilation rate to intercellular CO_2 concentration (*A/Ci*). The ratio of *A* and *gs* was used to compare intrinsic water-use efficiency (*WUEi*) and the ratio of *A* and T was used to calculate instantaneous water-use efficiency (*WUEins*). Additional chlorophyll fluorescence measurements were estimated according to Evans [27], including *Fv′/Fm′*, the maximum quantum efficiency of photosystem II photochemistry, the effective quantum efficiency of photosystem II photochemistry (Φ_{PSII}), photochemical quenching (*qP*), non-photochemical quenching (*qN*), and electron transport rate (*ETR*). The ratio of *ETR* and *A* was used to calculate a relative measure of electron transport to oxygen molecules. Gas exchange and chlorophyll fluorescence measurements were measured on fully expanded leaves.

2.4.2. Yield and Yield Components

Bush tea plants were harvested 153 days after planting. Determination of bush tea yield components was conducted by sampling 3 plants on each replication per treatment. Measurements recorded on a plot basis included yield (fresh below-ground mass), number of roots per plant, and number of marketable roots; marketable roots were defined as whole (undamaged) and without harvest wounds, pest, and disease damage [28]. The fresh mass of marketable roots was used to determine actual yield (Ya), which was then converted to kg ha^{-1}.

The percentage of marketable storage roots was computed using Equation (2).

$$(\text{Number of marketable roots per plot})/(\text{Total number of roots per plot}) \times 100\% \quad (2)$$

2.4.3. Determination of Nutritional Composition (NC)

Bush tea leaves were oven dried at 40 °C for 24 h, and thereafter were ground into powder with a mortar and pestle. Ground samples (0.5 g) were put in a quartz crucible and charred in a wild Barfield muffle furnace at 650 °C for 120 min. Acid digestion was performed according to Huang et al. [29] with minor modifications. The ash was dissolved in 10 mL dilute aqua regia (HNO_3 and HCl mixed in ratio 1:3) in a 25 mL volumetric flask. Contents of the flask were brought to volume by adding distilled H_2O and heated in a water bath for 1 h at 85 °C. Samples were chilled to 25 °C before analysis using atomic absorption spectrometry (AAS) [30]. The nutrients analyzed per dry matter basis included macro-nutrients (calcium, magnesium, and potassium) and micronutrients (copper, zinc, iron, manganese, and sodium). Therefore, the samples were sent to the University of KwaZulu-Natal, Pietermaritzburg Campus, Chemistry Laboratory Department for Nutrition Analysis.

2.4.4. Determination of Nutritional Water Productivity (NWP)

Nutritional water productivity was calculated based on the formula by Renault and Wallender [31], shown in Equation (3).

$$NWP = Y_a/ET_a \times NC \quad (3)$$

where NWP is the nutritional water productivity (nutrition m^{-3} of water evapotranspiration), Ya is the actual harvested yield (kg ha^{-1}), ET_a is the water applied based on crop water requirement (m^3 ha^{-1}), and NC is the nutritional content per kg of product (nutrition unit kg^{-1}).

2.4.5. Metabolite Extraction

A method suggested by Makita et al. [32] was used to determine metabolite extraction. The bush tea leaves were freeze dried and then crushed to a fine powder. An amount of 2 g of the powder were then extracted using 20 mL (1:10 m/v) of 80% aqueous methanol (Romil SpS, Cambridge, UK). Samples were rotated at 70 rpm in a digital rotisserie tube rotator for the whole night. The homogenate was centrifuged at 5000× g for 20 mins to remove debris. The supernatant was subsequently filtered using 0.22 µm nylon filters into glass vials with 500 µL inserts. At least 3 independent replicates for each sample group were prepared, and these were stored at 4 °C [33]. Equal quantities of each sample were mixed to generate quality control (QC) samples, which were used in conjunction with experimental samples to evaluate the quality of the data collected [34].

2.4.6. Liquid Chromatography-Quadruple Time-of-Flight Tandem Mass Spectrometry (LC-MS/MS)

A liquid chromatography-quadruple time-of-flight tandem mass spectrometry (QTOF)–MS instrument, model LC-MS 9030 (Shimadzu Corporation, Kyoto, Japan) was used. The chromatographic separation was performed on a Shim Pack Velox C18 column (100 mm × 2.1 mm with a particle size of 2.7 µm) to analyze the prepared bush tea

leaf extracts. The column oven was set at 40 °C, as described by Ramabulana et al. [33]. A binary solvent gradient, consisting of 0.1% formic acid in water (Eluent A) and 0.1% formic acid in acetonitrile (Eluent B) was used at a constant flow rate of 0.4 mL min^{-1}. The gradient was set for 53 min, and the flow rate was set at 0.3 mL min^{-1} under the following separation conditions: 10% B for 3 min, 10%–60% B over 3–40 min, 60% B from 40–43 min. Then, the gradient increased to 90% B between 43–45 min and was kept at 90% B for 3 min. The gradient was reset to its initial state 48–50 min later. This was followed by a 3 min column re-calibration period. A mass spectrometer detector was used for monitoring analyte elations under the following conditions: ESI (electrospray ionization) negative modes; interface voltage of 3.5 kV; nitrogen gas was used as nebulizer at a flow rate of 3 L min^{-1}; heating gas flow at 10 L min^{-1}; heat block temperature at 400 °C; CDL temperature at 250 °C; detector voltage of 1.70 kV; and the TOF temperature at 42 °C. In tandem with MS (MS/MS) experiments, typical mass accuracies with a mass error below 1 ppm were obtained using a mass calibration solution of sodium iodide (NaI). High resolution was obtained for MS and (MS/MS) experiments using a mass-to-ratio (m/z) range of 100–1000 Da.

2.4.7. Molecular Networking

For all datasets acquired as stipulated in Section 2.4.6, the molecular network was constructed using a methodology suggested by Wang et al. [34] and the online Global Natural Products Social Molecular Networking (GNPS) process (https://gnps.ucsd.edu accessed on 15 June 2023). The data were filtered by removing all MS/MS fragment ions that were within +/− 17 Da of the *m/z* precursor. Only the top 6 fragment ions within the +/− 50 Da window were selected from the window-filtered MS/MS spectra. The MS/MS fragmentation ion tolerance (MS/MS) was set to 0.5 Da, while the mass tolerance for the precursor ion was set to 2.0 Da. Following the recommended procedure by Aron et al. [35], a network with edges with cosines larger than 0.7 and more than 6 associated peaks was produced. Additionally, edges detected between two nodes were preserved in the network when both nodes occurred in their 10 most similar nodes. The maximum molecular family size was eventually set to 100 and the lowest-ranked edges were removed from the molecular families until the molecular family size fell below this threshold. Then, the GNPS spectral libraries were used to search for network spectra. The library spectra were filtered in the same way as the input data. All matches between the library and lattice spectra were required to contain at least 6 matching peaks and a value larger than 0.7 [35].

2.5. Data Analysis

Data were subjected to analysis of variance (ANOVA) using GenStat® version 20 (VSN International, Hemel Hempstead, UK). Least significant difference (LSD) was used to separate means at the 5% significance level. Principal component analysis (PCA) was performed for all metabolites and the molecular network analyses were constructed on the GNPS while the raw data were acquired from the Shimadzu LCMS-9030 qTOF of bush tea extracts under varying water regimes.

3. Results and Discussion

3.1. Gas Exchange and Chlorophyll Fluorescence Measurements

In bush tea production, understanding the morphological mechanisms that contribute to improved adaptation to water-limited environments is critical. Varying water regimes significantly affected gas exchange and chlorophyll fluorescence measurements of bush tea grown under a tunnel environment (Tables 3 and 4). The highest photosynthetic rate was recorded under 30% ET_a, followed by 100% ET_a, and control (no irrigation) was recorded as the lowest. Similarly, stomatal conductance, net CO_2 assimilation rate, transpiration rate, intercellular CO_2 concentration, ratio of intercellular and ambient CO_2 concentrations, intrinsic water-use efficiency, and instantaneous water-use efficiency increased under 30% ET_a, compared to 100% ET_a and control (no irrigation). The measured chlorophyll

fluorescence measurements were *Fv'/Fm'*, maximum quantum efficiency of photosystem II photochemistry, effective quantum efficiency of photosystem II photochemistry, photochemical quenching, non-photochemical quenching, and electron transport rate. The results show the reduction of *A*, *gs*, *T*, and *Ci*, due to no irrigation. It is critical to understand how and when to apply limited amounts of water to maximize the yield of high-quality plant products [36]. Bush tea may thrive well when plants are exposed to 30% ET_a.

The evaluated bush tea exhibited high water-use efficiency under limited water application (30% ET_a). These results suggest that bush tea plants are efficient water users, which can be attributed to their limited water application (Table 4), as the study exhibited maximum *WUEi* and *WUEinst* compared to other water treatments. The study revealed that bush tea plants are capable of reducing loss of water when exposed to no irrigation. Efficient PSII activity under limited water application protects the structure of chloroplasts against oxidative damage by preventing the creation of singlet oxygen [37]. Positive performance under limited water application is probably influenced by maintaining the effectiveness of PSII function and the antioxidant system components. The current study is the first to show that morphological measurements under limited water enhance the morphological development of bush tea under a protected tunnel environment.

3.2. Yield

Varying water regimes had significantly affected yield in bush tea production (Table 5). There was a significant difference in yield aspects of bush tea plants exposed to different water regimes grown under protected tunnel environments. Higher yield was recorded under 100% ET_a (96 kg ha^{-1}), compared to 30% ET_a (60.6 kg ha^{-1}) and control (12.12 kg ha^{-1}). The findings could imply that crop yield increases as irrigation water supply increases [38]. The results concur with those reported earlier by Mabhaudhi et al. [24], who also had similar water regimes on sweet potato production. Higher numbers of roots (46) were recorded on bush tea plants that were exposed to 100% ET_a than plants with 30% ET_a and the control. The percentage of marketable roots differed significantly between the different water treatments, with 100% ET_a recording the most marketable roots, followed by 30% ET_a, and with the lowest marketable roots recorded under control (no irrigation condition). These results are similar to those of Osman [39] and Hassan and Ali [20], who reported that irrigation at higher levels resulted in the highest vegetative growth characters and yield in coriander production compared to the lower levels. Higher yield may be due to full application of water to bush tea, which promotes vegetative growth, yield, and quality [20]. However, 30% ET_a had significantly higher water productivity than 100% ET_a and control (no irrigation). This result could be attributed to the volume of water consumed by bush tea plants when exposed to 30% ET_a, because WP depends on the amount of the output [40]. Thus, the results of the current study concur with the findings by Mabhaudhi et al. [12,24] and Motsa et al. [41].

3.3. Elemental Composition

There was a significant difference among mineral elements of bush tea exposed to varying water treatments (30%, 100% ET_a, and control (no irrigation)). The highest nutrient compositions (calcium, copper, iron, potassium, magnesium, manganese, and zinc) were recorded under 30% ET_a, followed by 100% ET_a, with the lowest elemental composition recorded under control (no irrigation) (Table 6). The study revealed that bush tea may generate more nutrients when exposed to 30% ET_a. These results are similar to those of Mabhaudhi et al. [24], who demonstrated that the increase in nutrients was influenced by water availability, nutrient uptake, and plant physiology in potato cultivars. Additionally, the results highlight the importance of water management strategies to generate the nutritional quality of bush tea plants.

Table 3. Analysis of variance showing mean squares and significant tests for leaf gas exchange and chlorophyll fluorescence measurements of bush tea evaluated under varying water treatments.

Source of Variance	d.f	*gs*	*T*	*A*	*Ci*	*A*/*Ci*	*Ci*/*Ca*	WUE_i	WUE_{inst}
Water treatment	2	0.1401667 **	10.5797 **	3.47367 **	61477.6 **	6.63433 **	376.711 **	97.933 **	175.599 **
Time	1	0.14062678 **	865.2420 *	1509.6744 **	535,691.9 **	1.82133 **	2332.305 **	2055.305 **	18,203.825 **
Water treatment × time	2	0.00019671 *	1.9300 *	0.3664 **	12523.9 **	43.27496 **	339.606 **	2.060 **	3.707 **
Residual	12	0.0003317	0.1835	0.05408	255.2	0.06533	1.119	1.536	2.355
Source of variance	**d.f**	***Fo***	***Fm***	***Fv*/*Fm***	Φ_{PSII}	***qP***	***qN***	***ETR***	
Water treatment	2	197,228 **	390,037 **	0.1698479 **	352.197 **	71.56132 **	78.52056 **	1,853,447 **	
Time	1	232,382 **	570,074 *	0.1557303 *	843.243 **	119.91842 **	81.06889 **	6,754,541 **	
Water treatment × time	2	5079 *	7197 *	0.0007281 *	76.338 **	19.88921 **	10.57056 **	831,555 **	
Residual	12	1514	3333	0.0003534	2.808	0.04054	0.07444	1342	

d.f—degrees of freedom, g_s—stomatal conductance, *T*—transpiration rate, *A*—net CO_2 assimilation rate, A/C_i—CO_2 assimilation rate/intercellular CO_2 concentration, C_i—intercellular CO_2 concentration, C_i/C_a—ratio of intercellular and atmospheric CO_2, WUE_i—intrinsic water-use efficiency, WUE_{ins}—instantaneous water-use efficiency, F_v/F_m—maximum quantum efficiency of photosystem II photochemistry, Φ_{PSII}—the effective quantum efficiency of PSII photochemistry, *qP*—photochemical quenching, *qN*—non-photochemical quenching, *ETR*—electron transport rate to oxygen molecules, * and **—significant at 5 and 1% probability levels, respectively.

Table 4. Means of leaf gas exchange and chlorophyll measurements of bush tea under varying water treatments.

Leaf Gas Exchange Measurements															
Week 1	**ET_a**	***gs***	***T***	***A***	***C_i***	***A/C_i***	***C_i/C_a***	***WUE_i***	***WUE_{inst}***	***Fo***	***Fm***	***Fv/Fm***	***Φ_{PSII}***	***qP***	***qN***	***ETR***
	Control (no irrigation)	0.18a	32.48a	26.27a	318.0a	3.40a	0.85a	20.83a	52.30a	2676.4a	2437a	0.1219a	20.10a	0.137a	1.13a	663.5a
	100%	0.37b	35.01b	28.30b	330.5b	3.56b	0.94b	26.51b	60.23b	2803.0b	2657b	0.250b	24.52b	2.10b	3.30b	943.6b
	30%	0.3487c	36.18c	29.74c	423.9c	6.83c	1.59c	34.10c	64.22c	2983.6c	2951c	0.440c	28.96c	3.41c	6.133c	1036.6c
Leaf Gas Exchange Measurements																
Week 2	**ET_a**	***gs***	***T***	***A***	***C_i***	***A/C_i***	***C_i/C_a***	***WUE_i***	***WUE_{inst}***	***Fo***	***Fm***	***Fv/Fm***	***Φ_{PSII}***	***qP***	***qN***	***ETR***
	Control (no irrigation)	0.35a	47.63a	29.63a	570.9a	5.53a	11.99a	38.94a	117.68a	2837.8a	2848a	0.30a	25.56a	1.20a	2.367a	1205.7a
	100%	0.56b	48.45b	30.45b	677.2b	8.67b	18.34b	40.86b	122.63b	3051.5b	2935b	0.42b	42.76b	8.50b	9.267b	2058.0b
	30%	0.65c	49.19c	31.19c	877.2c	1.5c	41.35c	42.75c	127.25c	3255.4c	3330c	0.65c	46.32c	11.43c	11.667c	3055.5c

g_s—stomatal conductance, *T*—transpiration rate, *A*—net CO_2 assimilation rate, (μmol CO_2 m^{-2} s^{-1}), A/C_i—CO_2 assimilation rate/intercellular CO_2 concentration, C_i—intercellular CO_2 concentration, C_i/C_a—ratio of intercellular and atmospheric CO_2, WUE_i—intrinsic water-use efficiency, WUE_{inst}—instantaneous water-use efficiency, F_v/F_m—maximum quantum efficiency of photosystem II photochemistry, Φ_{PSII}—the effective quantum efficiency of PSII; photochemistry, *qP*—photochemical quenching, *qN*—non-photochemical quenching, *ETR*—electron transport rate. Varying lower-case letters within a column indicates significant differences among water treatments. g_s (mmol m^{-2} s^{-1}), *T* (mmol H_2O m^{-2} s^{-1}), *A* (μmol CO_2 m^{-2} s^{-1}), A/C_i (μmol mol m^{-1}), C_i (μmol mol m^{-1}), WUE_i (μmol (CO_2) m^{-2}), WUE_{inst} (μmol mol^{-1}), *Fv/Fm* (ratio), *ETR* (μmol e^{-1} m^{-2} s^{-1}).

Table 5. Yield and yield components of varying water treatments (100% ET_a, 30% ET_a, and control (no irrigation).

Water Treatments (ET_a)	Yield (kg ha^{-1})	Number of Roots	Marketable Roots (%)	WP (kg m^{-3})
Control	12.12a	15.33a	13.33a	0a
30%	60.61b	32.67b	29b	95.62b
100%	95.62c	45.67c	41.67c	202.03c
LSD ($p < 5\%$)	0.02	0.04	0.02	0.01

Water productivity (WP) represents the total biomass. The yield, number of roots, marketable roots and WP values are statistically significant with *p*-values < 0.001, according to the LSD (5% level). Different lower-case letters within a column indicates significant differences among water treatments.

3.4. Nutritional Water Productivity

Nutritional water productivity for the macronutrients (calcium, magnesium, and potassium) and micronutrients (copper, zinc, iron, manganese, and sodium) measured in this study varied significantly between different water treatments (Table 7). Interestingly, high NWP_{ca}, NWP_{cu}, NWP_{fe}, NWP_{Mn}, and NWP_{Zn} were observed under 30% ET_a relative to 100% ET_a (Table 7). However, at 100% ET_a, high NWP_k and NWP_{Mg} were observed (Table 7). This only demonstrates that potassium and magnesium, which have a common function in the functioning and contraction of muscles, are stimulated extremely effectively with the full application of water [42,43]. The results of the current study used similar varying water treatments in maize and sweet potato production [24,44].

Table 6. Elemental composition of bush tea with varied water treatments (30% ET_a, 100% ET_a, and control (no irrigation) of crop water requirements).

Water Treatments (ET_a)	Ca (mg L^{-1})	Cu (mg L^{-1})	Fe (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Mn (mg L^{-1})	Zn (mg L^{-1})
Control (no irrigation)	155a	0.52a	8.96a	134a	42a	1.19a	1.14a
100%	359a	0.59a	10.41a	669a	215a	2.4b	1.88b
30%	731a	0.72a	16.97b	1512b	608b	3.61c	2.6c
LSD ($p = 0.05$)	0.20	0.31	0.01	0.01	0.003	0.005	0.05

Elemental compositions represented: calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), and zinc (Zn). Different lower-case letters within a column indicates significant differences among water treatments.

Table 7. Macro- and micronutrients determined on a dry mass basis of bush tea under varying water treatments (30% ET_a, 100% ET_a, and control (no irrigation) of crop water requirements).

Water Treatments	NWP_Ca (mg L^{-1})	NWP_Cu (mg L^{-1})	NWP_Fe (mg L^{-1})	NWP_K (mg L^{-1})	NWP_Mg (mg L^{-1})	NWP_Mn (mg L^{-1})	NWP_Zn (mg L^{-1})
Control (no irrigation)	155a	0.52a	8.96a	134a	42a	1.19a	1.14a
100%	359a	0.59a	10.41a	669a	215a	2.4b	1.88b
30%	731a	0.72a	16.97b	1512b	608b	3.61c	2.6c
LSD ($p = 0.05$)	0.20	0.31	0.01	0.01	0.003	0.005	0.05

NWP_ micro- and macronutrients (mg L^{-1}): This denotes the analysis of variance concentration of micro- or macronutrients in parts per million/milligrams per liter of water. Different lower-case letters within a column indicates significant differences among water treatments.

3.5. Multivariate Data Analysis

The computed model (score plot) of the PCA for bush tea plant extracts indicated that 38.7% and 20.8% of the variation were explained by PC1 and PC2, respectively (Figure 2).

The PCA model revealed an obvious separation among the three varying water regimes, as shown in Figure 2. This indicates that the metabolic constituents and their distribution in bush tea plant tissues under varying water regimes varied significantly. This observation is also reflected in the variation of intensities of separated metabolites [45]. Similarly, PCA score plots were computed and validated for bush tea plant extracts under varying water regimes, as indicated in Figure 2. The results suggest that different groups of molecules can be found under different water regimes. The similarities of molecule groups were represented through score plots, as shown in Figure 2, which indicated the general clustering within the datasets of bush tea plant extracts under varying water regimes.

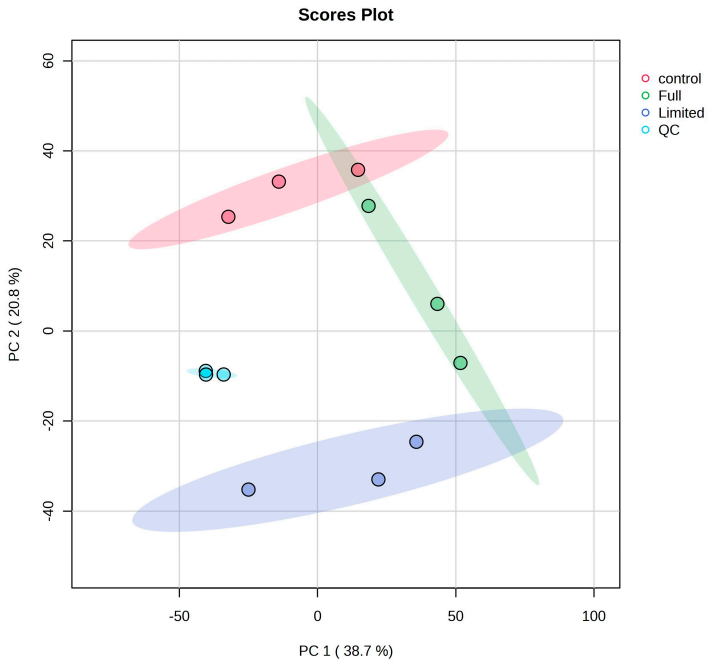


Figure 2. Multivariate data analysis of bush tea plant extracts under varying water regimes. A principal component analysis (PCA) scores scatterplot dataset obtained from LC−MS experiments with PCA 1 and PCA 2 explaining 38.7% and 20.8% of the variation, indicating the general clustering within the datasets of bush tea tissues of varying water regimes.

3.6. Major Chemical Class of Bush Tea Metabolomics

A significant number of metabolites were found in negative MS mode. However, mass spectra were obtained in both ESI modes (+/−). Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry analysis of bush tea leaves under varying water regimes revealed 60 compounds, all of which were confirmed by their retention times, masses, and molecular formulae. Different water treatments resulted in differential regulation of secondary metabolite production in bush tea, which was observed by the presence and absence of different metabolites. The identified chlorogenic acids were found under varying water regimes with significant differences in retention time. The significant presence of chlorogenic acids might be due to varying water regimes on bush tea plants. Our results concur with earlier observations by Zhang et al. [46] that all herbal teas contain multiple bioactive compounds, including chlorogenic acids. Similarly, Meinhart et al. [47], reported that chlorogenic acids have various health benefits and may cure diabetes, cardiovascular disease, cancer, and obesity. This study is the first to demonstrate varying water regimes as an essential factor in chlorogenic acid production in bush tea leaf extracts.

3.6.1. Molecular Networking

Molecular networking was used to arrange MS/MS spectra into a network-shaped map based on spectral similarity, which shows structural similarity between molecules, as part of the data analysis workflow for untargeted MS/MS-based metabolomics studies [35]. The MS-Cluster algorithm evaluated similarities between and within sample spectra [48]. Ions were grouped into consensus spectra, represented as nodes, within a predefined mass tolerance. Based on their similarity scores (cosine scores ≥ 0.7), structurally related compounds that have comparable gas-phase chemistries were classified into molecular families. In the computed molecular networking (Figure 3), 1148 consensus spectra (nodes) were generated, of which 808 were clustered and grouped into 130 independent molecular families (with a minimum of two nodes connected by an edge) using GNPS spectral matching. Self-loop nodes at the bottom of the network were used to represent spectra that were not classified into molecular families. Figure 3 shows the computed MN; 81 of the nodes were allegedly annotated through automated library spectral matching, providing some insight into the chemical identities of the bush tea leaf extracts, but also alluding to the metabolome complexity of these plants and the lack of comprehensive spectral libraries. The study of the chemical interactions between each MS/MS spectrum and the visualization of the complete metabolome found in a sample revealed structurally linked molecular families in bush tea leaf extracts under varying water regimes.

3.6.2. Impact of Water on Communic Acids

Communic acid compounds are tiny amino acids called cysteine bioactive compounds, which are found in several organic foods such as fruits, vegetables, grains, and legumes [49]. However, in herbal teas, they have not yet been identified. Table 8 displays the classification of communic acids based on their parent ion at a m/z of 301 and the fragments observed had a base peak at a m/z of 164 and 225. These compounds were only detected under full water application. The results could suggest that communic acid compounds are present when the bush tea plant is irrigated fully compared to when the plant is stressed or when it is half irrigated.

3.6.3. Impact of Water on Caftaric Acids

Hydroxycinnamoyl-tartaric acid esters are biologically active compounds, which are shown to have various health benefits and antioxidant effects [50]. Caftaric acids were identified by their parent ions, $[M\text{-}H]^-$, at a m/z of 311, based on their fragmentation patterns, Rt, shown in Table 8, and the chemical structure as illustrated in Figure 3C. Four tartaric acids were identified in all water treatments of bush tea leaf extracts. This could suggest that under different watering regimes, tartaric acid esters will still be biosynthesized in bush tea plants. These tartaric acids have not yet been identified in the leaves of bush tea.

3.6.4. Impact of Water on Mono-acyl Chlorogenic Acids

p-Coumaric acids have various biological activities such as antioxidant, anti-inflammatory, and anti-cancer activities [51]. Table 8 shows the identification of metabolites (7–10) with a molecular ion $[M\text{-}H]^-$ at a m/z of 337, identified as 5-p-Coumaroylquinic acids, based on their fragmentation patterns, Rt, shown in Table 8. All these compounds were identified under varying water regimes. The results could reveal that the water levels that have been used in this study integrate bioactive compounds. These results concur with previous findings by Ramphinwa et al. [52], who reported that bush tea plants contain these compounds on selected shades in relation to no irrigation.

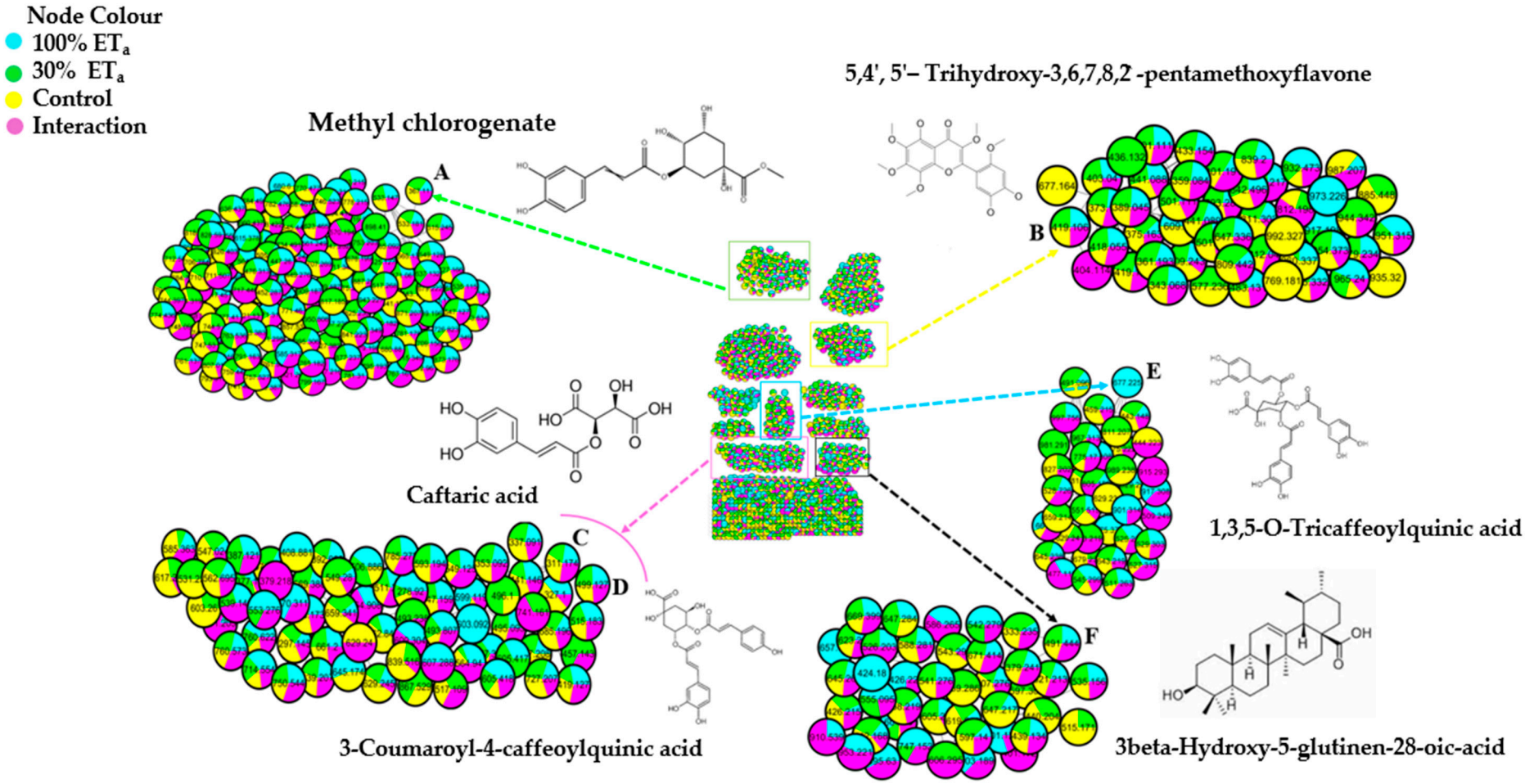


Figure 3. Molecular network of bush tea leaf extracts under varying water regimes analyzed by liquid chromatography–tandem mass spectrometry using electrospray ionization in negative mode, with a molecular family (Center). Identified highlighted nodes are bioactive compounds consisting of (**A**) methyl chlorogenate, (**B**) flavonoids, (**C**) tartaric acid, (**D**,**E**) caffeoylquinic acid, (**F**) glutinane. Node colors represent the varying water regimes bush tea plants were subjected to before leaf extracts were made and the respective MS2 spectral counts indicating the presence and absence of metabolites.

Table 8. Classification of various bioactive compounds consisting of derivatives of quinic acid (QA), flavonoids, hydroxycinnamic acids (HCAs), and tartaric acids from bush tea leaf extracts under varying water regimes of bush tea leaf extracts.

No.	Mass to Charge (*m*/*z*)	Retention Time (mn)	Fragmentation Ion	Molecular Formular	Compound Name	Water Treatments (ET_a)		
						30%	100%	Control (No Irrigation)
1	301.2226	21.494	164, 225	$C_{20}H_{30}O_2$	Communic acid		•	
2	311.039	16.745	179, 135	$C_{13}H_{12}O_9$	Caftaric acid	•		
3	311.039	10.54	187, 231, 267	$C_{13}H_{12}O_9$	Cis-caftaric acid	•		
4	311.035	10.318	132, 135	$C_{13}H_{12}O_9$	Caftaric acid			•
5	311.039	10.491	129, 191, 209	$C_{13}H_{12}O_9$	(-)-3,5-dicaffeoyl quinic acid	•	•	•
6	311.038	16.348	163	$C_{13}H_{12}O_9$	Caftaric acid	•	•	•
7	337.0988	13.311	143,191, 209	$C_{16}H_{18}O_8$	4-p-coumaroylquinic acid		•	
8	337.0991	5.42	191	$C_{16}H_{18}O_8$	5-coumaroylquinic acid	•		
9	337.0992	2.188	153, 191	$C_{16}H_{18}O_8$	5-p-trans-coumaroylquinic acid	•		
10	337.0992	8.103	138, 153, 191	$C_{16}H_{18}O_8$	5-p-coumaroylquinic acid			•
11	353.0942	7.02	191	$C_{16}H_{18}O_9$	Caffeoylquinic acid		•	
12	353.0942	5.515	191, 175, 173, 135	$C_{16}H_{18}O_9$	3-O-caffeoylquinic acid	•		
13	353.0945	7.049	353, 191, 179	$C_{16}H_{18}O_9$	4-caffeoylquinic acid	•		
14	353.0946	5.144	191,129, 209	$C_{16}H_{18}O_9$	3-O-caffeoyl-muco-quinic acid			•
15	353.0945	4.945	191	$C_{16}H_{18}O_9$	trans-4-caffeoylquinic acid	•	•	•
16	353.0946	4.747	191	$C_{16}H_{18}O_9$	(+)-5-caffeoyl quinic acid	•	•	•
17	367.08	3.694	119, 191, 135	$C_{17}H_{20}O_9$	Chlorogenic acid methyl ester	•	•	•
18	419.1063	13.419	149, 317	$C_{20}H_{20}O_{10}$	5,4′,5′-trihydroxy-3,6,7,8,2′-pentamethoxyflavone	•		
19	433.1222	14.441	133, 161, 241	$C_{25}H_{22}O_7$	5′-hydroxycudraflavone A		•	
20	491.3500	24.423	116, 299, 433	$C_{33}H_{48}O_3$	3beta-hydroxy-5-glutinen-28-oic acid		•	

Table 8. *Cont.*

No.	Mass to Charge (*m*/*z*)	Retention Time (mn)	Fragmentation Ion	Molecular Formular	Compound Name	Water Treatments (ET_a)		
						30%	100%	Control (No Irrigation)
21	491.3508	24.336	152, 279	$C_{33}H_{48}O_3$	3beta-hydroxy-5-glutinen-28-oic acid	●	●	●
22	499.1346	10.2	353, 337, 191, 357	$C_{25}H_{24}O_{11}$	3-coumaroyl-4-caffeoylquinic acid			●
23	515.1295	20.345	191, 355, 533	$C_{25}H_{24}O_{12}$	Dicaffeoylquinic acid		●	
24	515.1290	9.781	353, 191	$C_{25}H_{24}O_{12}$	Dicaffeoylquinic acid 1	●		
25	515.1302	9.732	353, 191	$C_{25}H_{24}O_{12}$	Dicaffeoylquinic acid 1			●
26	515.1296	9.723	133, 161, 191, 353	$C_{25}H_{24}O_{12}$	Dicaffeoylquinic acid 1			●
27	515.1299	9.269	191, 353, 417	$C_{25}H_{24}O_{12}$	Dicaffeoylquinic acid	●	●	●
28	515.1295	9.381	129, 191, 357	$C_{25}H_{24}O_{12}$	Dicaffeoylquinic acid 1	●	●	●
29	529.13	17.39	179,173	$C_{26}H_{26}O_{12}$	Dicaffeoylquinic acid 1	●		
30	533.1042	5.033	108, 167, 191	$C_{24}H_{22}O_{14}$	Dicaffeoylquinic acid 1		●	
31	533.1042	8.329	191	$C_{24}H_{22}O_{14}$	Dicaffeoylquinic acid 1	●		
32	533.1045	7.051	371, 209, 173, 135	$C_{24}H_{22}O_{14}$	Dicaffeoylquinic acid 1			●
33	533.1045	9.091	191, 375	$C_{24}H_{22}O_{14}$	Di-caffeoyl glucarate (VII)	●	●	●
34	677.16	10.44	191, 353	$C_{34}H_{30}O_{15}$	Tricaffeoylquinic acid 1			●
35	677.12	23.199	255, 329	$C_{34}H_{30}O_{15}$	Tricaffeoylquinic acid 1			●

● Shaded squares indicate the presence of metabolites under varying water regimes of bush tea leaf extracts.

3.6.5. Impact of Water on Caffeoylquinic Acids

Caffeoylquinic acids occur in our daily life and are considered beneficial for human health [53]. According to Chan et al. [54] and Kinki et al. [55], caffeoylquinic acids are strong antioxidants with various beneficial effects. In this study, metabolites (11–16) were identified as caffeoylquinic acids by their parent ions, at a m/z of 353, based on their fragmentation patterns, Rt, shown in Table 8. The caffeoylquinic acids were found to be present under all varying water regimes of bush tea leaf extracts. The findings may be used to demonstrate how water use, whether limited or extensive, as well as stressful environments, affect the build-up of caffeine-quininic acids. Interestingly, our findings are consistent with earlier observations of Ramphinwa et al. [52], who reported that these compounds are present in bush tea plants under different shades in relation to no irrigation.

3.6.6. Impact of Water on Methyl Chlorogenate Acids

Methyl chlorogenate acids are highly fascinating bioactive compounds found in plants, with biological functions and antioxidant properties [56]. According to Salem et al. [57] and Zhang et al. [58], methyl chlorogenate acids have anti-inflammatory properties; however, in bush tea, they have not been identified. Table 8 and Figure 3A show that methyl chlorogenate acids were identified by their parent ions, $[M\text{-}H]^-$, at a m/z of 367, and fragmented to give product ions at a m/z of 119, 191, and 135. The results of the current study revealed that they are only present when the bush tea plant is irrigated with limited water (30% ET_a), because under full application of water and no irrigation, these compounds were not present. This could suggest limited water as a recommended water application level in bush tea plants to synthesize methyl chlorogenate acid compounds. The current study is the first to reveal that geometric isomers of methyl chlorogenate acids exist on bush tea leaf extracts under varying water regimes, where 30% ET_a had the most significant effect compared to other water regimes.

3.6.7. Impact of Water on Flavonoids

Flavonoids contain several kinds of health properties, including anti-cancer properties, anti-inflammatory properties, and reduced risk of chronic diseases [59]. Table 8 and Figure 3B display the impact of water on flavonoids such as 5,4′,5′-trihydroxy-3,6,7,8,2′-pentamethoxyflavone, based on their parent ions at a m/z of 419, with the product ions at a m/z of 149 and 317. These compounds were detected only in 30% ET_a bush tea leaf extracts. The results could suggest that when the bush tea plant is irrigated under limited water, it generates these compounds. The presence of 5,4′,5′-trihydroxy-3,6,7,8,2′-pentamethoxyflavone compound was reported for the first time in bush tea leaf extracts.

Metabolites (19) were identified as 5′-hydroxycudraflavone A, based on the parent ions at a m/z of 433 and on their fragmentation patterns, Rt, shown in Table 8. These 5′-hydroxycudraflavone A derivatives were only present under full water application of bush tea leaf extracts. The findings may simply show that these compounds are not present in bush tea leaf extracts when there are limited water application or no irrigation conditions.

3.6.8. Impact of Water on Triterpenoid and 3-coumaroyl-4-caffeoylquinic Acids

A pentacyclic triterpenoid was identified as 3-beta-hydroxy-5-glutinen-28-oic acid. This compound is derived from a natural anticancer botulin, which has pharmacological properties, including cholesterol reduction [60]. Metabolites (20–21) were detected by their parent ion at a m/z of 491 based on their fragmentation patterns, Rt, shown in Table 8. The chemical structures are illustrated in Figure 3F. Two of these molecules were observed in all varying water regimes of bush tea leaf extracts. The results of the current study could suggest different water treatments lead to differential regulation of secondary metabolite production in bush tea leaf extracts, as observed by the presence and absence of certain metabolites. The current study is the first to reveal that geometric isomers of 3beta-hydroxy-5-glutinen-28-oic acid exist on bush tea leaf extracts under varying water regimes.

Metabolites (22) were annotated as 3-coumaroyl-4-caffeoylquinic acid (Table 8) and identified by a parent ion, $[M\text{-}H]^-$, at a m/z of 499 with the chemical structure as illustrated in Figure 3D. According to Nunes et al. [61], 3-coumaroyl-4-caffeoylquinic acid exhibits antioxidants and it is considered a beneficial compound for human health. This compound was only observed under no irrigation conditions. The findings could simply show that these compounds can only be detected under zero application of water because the absence of water will not limit the accumulation of this compound on bush tea plants.

3.6.9. Impact of Water on Feruloyl-caffeoylquinic Acids

Feruloyl-caffeoylquinic acid is a soluble phenolic compound present in many angiosperms [62]. Metabolites (29) were identified by their parent ion at a m/z of 529 with fragmentation ions at a m/z of 179 and 173, and the chemical structures as illustrated in Figure 3. Feruloyl-caffeoylquinic acid was detected only under limited water application. These findings may be because of the limited water that the plant has received compared to no irrigation and full application. The current study is the first to detect feruloyl-caffeoylquinic acid on bush tea leave extracts under varying water regimes. However, it has been identified on other medicinal plants [45].

3.6.10. Impact of Water on Tri-caffeoylquinic Acids

Tri-caffeoylquinic acids are specialized bioactive metabolites. In plants, tri-caffeoylquinic acids play a defensive role against biotic and abiotic stress [63]. Table 8 shows the identified tri-caffeoylquinic acids with parent ions, $[M\text{-}H]^-$, at a m/z of 677, based on their fragmentation patterns, Rt, shown in Table 8, and the chemical structure, as illustrated in Figure 3E. These tri-caffeoylquinic acid derivatives were observed to be only present under water and no irrigation conditions on bush tea leaf extracts. These results might suggest that tri-caffeoylquinic acids enable bush tea plants to adapt under no irrigation conditions with zero application of water. This study is the first to indicate the presence of tri-caffeoylquinic acids on bush tea leaf extracts under varying water regimes.

3.6.11. Impact of Water on Di-caffeoylglucosides and Di-caffeoyl Glucarate Acids

Caffeoylglucosides are a powerful antioxidant and are used to treat cancer [64]. Six (6) compounds with a precursor ion, $[M\text{-}H]^-$, at a m/z of 515 were identified as di-caffeoylquinic acids (22-8) under all varying water regimes used in the study, based on their fragmentation patterns, Rt, shown in Table 8 with different intensities. The findings could indicate that bush tea accumulates these naturally occurring compounds even in the absence of water, under restricted water, and under full water application. These findings concur with the findings of Ramphinwa et al. [19,52], who reported the same mass to charge 515 on bush tea plants in relation to no irrigation.

A similar approach was followed in the identification of dicaffeoylquinic acids (30-33), which were identified by their precursor ions, $[M\text{-}H]^-$, at a m/z of 533, and based on their fragmentation patterns, Rt, shown in Table 8. They were then identified under all varying water regimes of bush tea leaf extracts. Similar detection of these compounds concurs with the findings of Ramphinwa et al. [52], who identified a m/z of 533 on bush tea plants in relation to no irrigation.

4. Conclusions

The study concluded that varying water regimes significantly influence the physiological aspects, nutritional yield, nutritional water productivity, and phytochemical composition of bush tea (*Athrixia phylicoides* DC.). The research demonstrated that bush tea plants grown under a limited water regime (30% of crop water requirement (ET_a)) showed enhanced plant growth, nutrient content, and nutritional water productivity compared to other treatments. Although the 100% ET_a treatment produced the highest yield, the 30% ET_a treatment led to a greater accumulation of bioactive compounds, particularly chlorogenic acids. This study is also the first to determine the accumulation of various

bioactive compounds in bush tea leaves under different water regimes. These findings suggest that in water-scarce regions, bush tea can be effectively cultivated with limited water application without compromising its nutritional benefits. The study highlights the potential of bush tea as a resilient crop in arid environments and recommends further investigation into the effects of various water regimes on different maturity stages, as well as the nutritional and phytochemical properties of bush tea stems and roots.

Author Contributions: Conceptualization, M.R., T.M., and F.N.M.; methodology, M.R., M.L.R., A.-T.R., N.E.M., L.S.M., T.M., and F.N.M.; software, M.R. and A.-T.R.; validation, M.R., M.L.R., A.-T.R., N.E.M., L.S.M., T.M., and F.N.M.; formal analysis, M.R., M.L.R., A.-T.R., N.E.M., L.S.M., T.M., and F.N.M.; investigation, M.R.; data curation, M.R. and A.-T.R.; writing—original draft preparation, M.R.; writing—review and editing, M.R., M.L.R., A.-T.R., N.E.M., L.S.M., T.M., and F.N.M.; supervision, N.E.M., L.S.M., T.M., and F.N.M.; funding acquisition, T.M. and F.N Mudau. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Water Research Commission (WRC) project (C2020/2021-00420) on "Water Utilisation and Nutritional Utilisation of Bush Tea", National Research Fund (NRF) through grant support (number:149817).

Data Availability Statement: Data are contained within the article.

Acknowledgments: University of KwaZulu-Natal (UKZN), Pietermaritzburg Campus and University of Venda, Limpopo, South Africa for their technical contributions towards the research, the Water Research Commission (WRC) and the National Research Fund (NRF) for providing funding.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Joubert, E.; Gelderblom, W.C.A.; Louw, A.; de Beer, D. South African Herbal Teas: *Aspalathus linearis*, Cyclopia Spp. and *Athrixia phylicoides*—A Review. *J. Ethnopharmacol.* **2008**, *119*, 376–412. [CrossRef]
2. Steyn, B.; Antonites, A. Plant Use in Southern Africa's Middle Iron Age: The Archaeobotany of Mutamba. *Azania Archaeol. Res. Afr.* **2019**, *54*, 350–368. [CrossRef]
3. Mudau, F.N.; Soundy, P.; Du Toit, E.S.; Olivier, J. Variation in Polyphenolic Content of *Athrixia phylicoides* (L.) (Bush Tea) Leaves with Season and Nitrogen Application. *S. Afr. J. Bot.* **2006**, *72*, 398–402. [CrossRef]
4. Tshikhudo, P.P.; Ntushelo, K.; Kanu, S.A.; Mudau, F.N. Influence of Ecological Parameters on Growth of Bush Tea (*Athrixia phylicoides* DC.) in Limpopo Province, South Africa. *Appl. Ecol. Environ. Res.* **2020**, *18*, 4167–4177. [CrossRef]
5. Rakuambo, Z.J. *Indigenous Knowledge of Bush Tea (Athrixia phylicoides) and Effect of Fertigation Frequency and Growing Medium on Plant Growth*; University of Pretoria: Pretoria, South Africa, 2007; ISBN 9798380883122.
6. Tshivhandekano, I.; Mudau, F.N.; Soundy, P.; Ngezimana, W. Effect of Cultural Practices and Environmental Conditions on Yield and Quality of Herbal Plants: Prospects Leading to Research on Influence of Nitrogen Fertilization, Planting Density and Eco-Physiological Parameters on Yield and Quality of Field-Grown Bush Tea (*Athrixia phylicoides* DC.). *J. Med. Plants Res.* **2013**, *7*, 2489–2493.
7. Lerotholi, L.; Chaudhary, S.K.; Combrinck, S.; Viljoen, A. Bush Tea (*Athrixia phylicoides*): A Review of the Traditional Uses, Bioactivity and Phytochemistry. *S. Afr. J. Bot.* **2017**, *110*, 4–17. [CrossRef]
8. Tshikhudo, P.P.; Ntushelo, K.; Kanu, S.A.; Mudau, F.N. Growth Response of Bush Tea (*Athrixia phylicoides* DC.) to Climatic Conditions in Limpopo Province, South Africa. *S. Afr. J. Bot.* **2019**, *121*, 500–504. [CrossRef]
9. Ramphinwa, M.L.; Mchau, G.R.A.; Mashau, M.E.; Madala, N.E.; Chimonyo, V.G.P.; Modi, T.A.; Mabhaudhi, T.; Thibane, V.S.; Mudau, F.N. Eco-Physiological Response of Secondary Metabolites of Teas: Review of Quality Attributes of Herbal Tea. *Front. Sustain. Food Syst.* **2023**, *7*, 990334. [CrossRef]
10. Gatabazi, A.; Marais, D.; Steyn, M.; Araya, H.; Du Plooy, C.; Ncube, B.; Mokgehle, S. Effect of Water Regimes and Harvest Times on Yield and Phytochemical Accumulation of Two Ginger Species. *Sci. Hortic.* **2022**, *304*, 111353. [CrossRef]
11. Chivenge, P.; Mabhaudhi, T.; Modi, A.T.; Mafongoya, P. The Potential Role of Neglected and Underutilized Crop Species as Future Crops under Water Scarce Conditions in Sub-Saharan Africa. *Int. J. Environ. Res. Public Health* **2015**, *12*, 5685–5711. [CrossRef]
12. Mabhaudhi, T. Drought Tolerance and Water-Use of Selected South African Landraces of Taro (*Colocasia esculenta* L. Schott) and *Bambara groundnut* (*Vigna subterranea* L. Verdc). Ph.D. Dissertation, University of KwaZulu-Natal, Pietermaritzburg, South Africa, 2012.
13. Bendou, O.; Gutiérrez-Fernández, I.; Marcos-Barbero, E.L.; Bueno-Ramos, N.; Miranda-Apodaca, J.; González-Hernández, A.I.; Morcuende, R.; Arellano, J.B. Physiological and Antioxidant Response to Different Water Deficit Regimes of Flag Leaves and Ears of Wheat Grown under Combined Elevated CO_2 and High Temperature. *Plants* **2022**, *11*, 2384. [CrossRef]

14. Chrysargyris, A.; Stamatakis, A.; Moustakas, K.; Prasad, M.; Tzortzakis, N. Evaluation of Municipal Solid Waste Compost and/or Fertigation as Peat Substituent for Pepper Seedlings Production. *Waste Biomass Valorization* **2018**, *9*, 2285–2294. [CrossRef]
15. Mudau, F.N.; Soundy, P.; Du Toit, E.S. Nitrogen, Phosphorus, and Potassium Nutrition Increases Growth and Total Polyphenol Concentrations of Bush Tea in a Shaded Nursery Environment. *HortTechnol* **2007**, *17*, 107–110. [CrossRef]
16. Mohale, K.C.; Hintsa, A.T.; Emanuel, M.A.; Mudau, F.N. Metabolic Profiling of Cultivated Bush Tea (*Athrixia phylicoides* DC.) in Response to Different Pruning Types. *HortScience* **2018**, *53*, 993–998. [CrossRef]
17. Mphangwe, N.I.K. Lung Pruning: A Review of the Practice. *Trfca News* **2007**, *147*, 18–23.
18. Hlahla, L.N. Effect of Fermentation Temperature and Duration on Chemical Composition of Bush Tea *(Athrixia phylicoides DC.*). Ph.D. Dissertation, University of Limpopo, Polokwane, South Africa, 2010.
19. Ramphinwa, M.L.; Madala, N.E.; Mchau, G.R.A.; Ramabulana, A.T.; Mudau, F.N. Effect of UV-Induced Geometrical Isomerization of Hydroxyl-Cinnamic Acid-Containing Molecules of Bush Tea (*Athrixia phylicoides* DC.) Using UHPLC-QTOF-MS. *Sci. Hortic.* **2022**, *301*, 111124. [CrossRef]
20. Hassan, F.A.S.; Ali, E.F. Impact of Different Water Regimes Based on Class-A Pan on Growth, Yield and Oil Content of *Coriandrum Sativum* L. Plant. *J. Saudi Soc. Agric. Sci.* **2014**, *13*, 155–161. [CrossRef]
21. Carr, M.K.V. The Role of Water in the Growth of the Tea (*Camellia sinensis*) Crop: A Synthesis of Research in Eastern Africa. 1. Water Relations. *Exp. Agric.* **2010**, *46*, 327–349. [CrossRef]
22. Dasila, B.; Singh, V.; Kushwaha, H.S.; Srivastava, A.; Ram, S. Water Use Efficiency and Yield of Cowpea and Nutrient Loss in Lysimeter Experiment under Varying Water Table Depth, Irrigation Schedules and Irrigation Method. *SAARC J. Agric.* **2016**, *14*, 46–55. [CrossRef]
23. Maedza, K.V. Effects of Micronutrients on Growth and Quality of Bush Tea (*Athrixia phylicoides* DC.). Master's Thesis, University of South Africa, Pretoria, South Africa, 2015.
24. Mabhaudhi, T.; Chibarabada, T.P.; Modi, A.T. Nutritional Water Productivity of Selected Sweet Potato Cultivars (*Ipomoea batatas* L.). *Acta Hortic.* **2018**, *1253*, 295–302. [CrossRef]
25. Singh, K.K. Water Management in Tea. In *Science of Tea Technology*; Scientific Publishers: Jodhpur, India, 2013.
26. Allen, R.G.; Pereira, L.S.; Raes, D.; Smith, M. Crop Evapotranspiration-Guidelines for Computing Crop Water Requirements-FAO Irrigation and Drainage Paper 56. *Fao Rome* **1998**, *300*, D05109.
27. Evans, J.R. Potential Errors in Electron Transport Rates Calculated from Chlorophyll Fluorescence as Revealed by a Multilayer Leaf Model. *Plant Cell Physiol.* **2009**, *50*, 698–706. [CrossRef]
28. Ossom, E.M.; Pace, P.F.; Rhykerd, R.L.; Rhykerd, C.L. Influence of Mulch on Soil Temperature, Nutrient Content, Growth and Yield of Sweetpotato [*Ipomoea batatas* (L.) Lam.]. *UNISWA Res. J. Agric. Sci. Technol.* **2004**, *6*, 137–146. [CrossRef]
29. Huang, C.-C.; Chen, W.-C.; Wang, C.-C. Comparison of Taiwan Paddy-and Upland-Cultivated Taro (*Colocasia esculenta* L.) Cultivars for Nutritive Values. *Food Chem.* **2007**, *102*, 250–256. [CrossRef]
30. Mandizvo, T.; Odindo, A.O. Seed Mineral Reserves and Vigour of Bambara Groundnut (*Vigna subterranea* L.) Landraces Differing in Seed Coat Colour. *Heliyon* **2019**, *5*, e01635. [CrossRef]
31. Renault, D.; Wallender, W.W. Nutritional Water Productivity and Diets. *Agric. Water Manag.* **2000**, *45*, 275–296. [CrossRef]
32. Makita, C.; Chimuka, L.; Steenkamp, P.; Cukrowska, E.; Madala, E. Comparative Analyses of Flavonoid Content in *Moringa Oleifera* and *Moringa Ovalifolia* with the Aid of UHPLC-QTOF-MS Fingerprinting. *S. Afr. J. Bot.* **2016**, *105*, 116–122. [CrossRef]
33. Ramabulana, A.-T.; Petras, D.; Madala, N.E.; Tugizimana, F. Metabolomics and Molecular Networking to Characterize the Chemical Space of Four Momordica Plant Species. *Metabolites* **2021**, *11*, 763. [CrossRef]
34. Wang, M.; Carver, J.J.; Phelan, V.V.; Sanchez, L.M.; Garg, N.; Peng, Y.; Nguyen, D.D.; Watrous, J.; Kapono, C.A.; Luzzatto-Knaan, T. Sharing and Community Curation of Mass Spectrometry Data with Global Natural Products Social Molecular Networking. *Nat. Biotechnol.* **2016**, *34*, 828–837. [CrossRef]
35. Aron, A.T.; Gentry, E.C.; McPhail, K.L.; Nothias, L.-F.; Nothias-Esposito, M.; Bouslimani, A.; Petras, D.; Gauglitz, J.M.; Sikora, N.; Vargas, F. Reproducible Molecular Networking of Untargeted Mass Spectrometry Data Using GNPS. *Nat. Protoc.* **2020**, *15*, 1954–1991. [CrossRef]
36. Alderfasi, A.A.; Alghamdi, S.S. Integrated Water Supply with Nutrient Requirements on Growth, Photosynthesis Productivity, Chemical Status and Seed Yield of Faba Bean. *Am.-Eurasian J. Agron.* **2010**, *3*, 8–17.
37. Levesque-Tremblay, G.; Havaux, M.; Ouellet, F. The Chloroplastic Lipocalin AtCHL Prevents Lipid Peroxidation and Protects Arabidopsis against Oxidative Stress. *Plant J.* **2009**, *60*, 691–702. [CrossRef]
38. Fereres, E.; Goldhamer, D.A.; Parsons, L.R. Irrigation Water Management of Horticultural Crops. *HortScience* **2003**, *38*, 1036–1042. [CrossRef]
39. Osman, H.E. Gated Pipes Techniques for Improved Surface Irrigation. *Ann. Agric. Sci.* **2000**, *1*, 145–155.
40. Ali, M.H.; Talukder, M.S.U. Increasing Water Productivity in Crop Production—A Synthesis. *Agric. Water Manag.* **2008**, *95*, 1201–1213. [CrossRef]
41. Motsa, N.M.; Modi, A.T.; Mabhaudhi, T. Sweet Potato (*Ipomoea batatas* L.) as a Drought Tolerant and Food Security Crop. *S. Afr. J. Sci.* **2015**, *111*, 8. [CrossRef]
42. Mills, I.W.; Greenland, J.E.; Mcmurray, G.; Mccoy, R.; Ho, K.M.T.; Noble, J.G.; Brading, A.F. Studies of the Pathophysiology of Idiopathic Detrusor Instability: The Physiological Properties of the Detrusor Smooth Muscle and Its Pattern of Innervation. *J. Urol.* **2000**, *163*, 646–651. [CrossRef]

43. Maughan, R.J.; Shirreffs, S.M. Muscle Cramping during Exercise: Causes, Solutions, and Questions Remaining. *Sports Med.* **2019**, *49*, 115–124. [CrossRef]
44. Sibiya, S.G. Planting Density Effect on Growth and Yield of Taro (*Colocasia esculenta)* Landraces. Ph.D. Dissertation, University of KwaZulu-Natal, Pietermaritzburg, South Africa, 2015.
45. Ramabulana, A.T.; Steenkamp, P.; Madala, N.; Dubery, I.A. Profiling of Chlorogenic Acids from Bidens Pilosa and Differentiation of Closely Related Positional Isomers with the Aid of UHPLC-QTOF-MS/MS-Based in-Source Collision-Induced Dissociation. *Metabolites* **2020**, *10*, 178. [CrossRef]
46. Zhang, L.; Fan, Y.; Su, H.; Wu, L.; Huang, Y.; Zhao, L.; Han, B.; Shu, G.; Xiang, M.; Yang, J.-M. Chlorogenic Acid Methyl Ester Exerts Strong Anti-Inflammatory Effects via Inhibiting the COX-2/NLRP3/NF-KB Pathway. *Food Funct.* **2018**, *9*, 6155–6164. [CrossRef]
47. Meinhart, A.D.; Caldeirão, L.; Damin, F.M.; Filho, J.T.; Godoy, H.T. Analysis of Chlorogenic Acids Isomers and Caffeic Acid in 89 Herbal Infusions (Tea). *J. Food Compos. Anal.* **2018**, *73*, 76–82. [CrossRef]
48. Frank, A.M.; Monroe, M.E.; Shah, A.R.; Carver, J.J.; Bandeira, N.; Moore, R.J.; Anderson, G.A.; Smith, R.D.; Pevzner, P.A. Spectral Archives: Extending Spectral Libraries to Analyze Both Identified and Unidentified Spectra. *Nat. Methods* **2011**, *8*, 587–594. [CrossRef]
49. McIntyre, R.L.; Liu, Y.J.; Hu, M.; Morris, B.J.; Willcox, B.J.; Donlon, T.A.; Houtkooper, R.H.; Janssens, G.E. Pharmaceutical and Nutraceutical Activation of FOXO3 for Healthy Longevity. *Ageing Res. Rev.* **2022**, *78*, 101621. [CrossRef]
50. Mathatha, K.; Khwathisi, A.; Ramabulana, A.T.; Mwaba, I.; Mathomu, L.M.; Madala, N.E. Identification of Putative Acyltransferase Genes Responsible for the Biosynthesis of Homogenous and Heterogenous Hydroxycinnamoyl-Tartaric Acid Esters from Bidens Pilosa. *S. Afr. J. Bot.* **2022**, *149*, 389–396. [CrossRef]
51. Pei, K.; Ou, J.; Huang, J.; Ou, S. P-Coumaric Acid and Its Conjugates: Dietary Sources, Pharmacokinetic Properties and Biological Activities. *J. Sci. Food Agric.* **2016**, *96*, 2952–2962. [CrossRef]
52. Ramphinwa, M.L.; McHau, G.R.A.; Madala, N.E.; Nengovhela, N.; Ogola, J.B.O.; Mudau, F.N. Response of Plant Growth and Development, and Accumulation of Hydroxyl-Cinnamoyl Acid Derivatives to Selected Shade Nets and Seasonality of Field-Grown Bush Tea (*Athrixia phylicoides* DC). *HortScience* **2022**, *57*, 87–96. [CrossRef]
53. Alcázar Magaña, A.; Kamimura, N.; Soumyanath, A.; Stevens, J.F.; Maier, C.S. Caffeoylquinic Acids: Chemistry, Biosynthesis, Occurrence, Analytical Challenges, and Bioactivity. *Plant J.* **2021**, *107*, 1299–1319. [CrossRef]
54. Chan, E.W.C.; Lim, Y.Y.; Chong, K.L.; Tan, J.B.L.; Wong, S.K. Antioxidant Properties of Tropical and Temperate Herbal Teas. *J. Food Compos. Anal.* **2010**, *23*, 185–189. [CrossRef]
55. Kinki, A.; Mezgebe, A.; Lema, T. Antioxidant and Sensory Properties of Herbal Teas Formulated from Dried Moringa (*Moringa stenopetala*) and Stevia (*Stevia rebaudiana* Bertoni) Leaves. *Food Sci. Qual. Manag.* **2020**, *23*, 185–189. [CrossRef]
56. Szymborska, K.; Frański, R.; Beszterda-Buszczak, M. Extraction with Acidified Methanol—An Easy and Effective Method of Methyl Chlorogenate Formation, as Studied by ESI-MS. *Molecules* **2022**, *27*, 7543. [CrossRef]
57. Salem, M.A.; Michel, H.E.; Ezzat, M.I.; Okba, M.M.; EL-Desoky, A.M.; Mohamed, S.O.; Ezzat, S.M. Optimization of an Extraction Solvent for Angiotensin-Converting Enzyme Inhibitors from *Hibiscus Sabdariffa* L. Based on Its UPLC-MS/MS Metabolic Profiling. *Molecules* **2020**, *25*, 2307. [CrossRef]
58. Zhang, S.; Yang, C.; Idehen, E.; Shi, L.; Lv, L.; Sang, S. Novel Theaflavin-Type Chlorogenic Acid Derivatives Identified in Black Tea. *J. Agric. Food Chem.* **2018**, *66*, 3402–3407. [CrossRef]
59. Gutiérrez-Grijalva, E.P.; Picos-Salas, M.A.; Leyva-López, N.; Criollo-Mendoza, M.S.; Vazquez-Olivo, G.; Heredia, J.B. Flavonoids and Phenolic Acids from Oregano: Occurrence, Biological Activity and Health Benefits. *Plants* **2018**, *7*, 2. [CrossRef]
60. Téné, D.G.; Tih, A.E.; Kamdem, M.H.K.; Talla, R.M.; Diboue, P.H.B.; Melongo, Y.K.D.; Dzukoug, C.R.; Mmutlane, E.M.; Ndinteh, D.T.; Bodo, B.; et al. Antibacterial and Antioxidant Activities of Compounds Isolated from the Leaves of Symphonia Globulifera (*Clusiaceae*) and Their Chemophenetic Significance. *Biochem. Syst. Ecol.* **2021**, *99*, 104345. [CrossRef]
61. Nunes, A.R.; Gonçalves, A.C.; Falcão, A.; Alves, G.; Silva, L.R. Prunus Avium l. (Sweet Cherry) by-Products: A Source of Phenolic Compounds with Antioxidant and Anti-Hyperglycemic Properties—A Review. *Appl. Sci.* **2021**, *11*, 8516. [CrossRef]
62. Jnior, H.; de Melo, N.I.; Miller Crotti, A.E. Electrospray Ionization Tandem Mass Spectrometry as a Tool for the Structural Elucidation and Dereplication of Natural Products: An Overview. In *Tandem Mass Spectrometry—Applications and Principles*; InTech: Houston, TX, USA, 2012.
63. Uleberg, E.; Rohloff, J.; Jaakola, L.; Trôst, K.; Junttila, O.; Häggman, H.; Martinussen, I. Effects of Temperature and Photoperiod on Yield and Chemical Composition of Northern and Southern Clones of Bilberry (*Vaccinium myrtillus* L.). *J. Agric. Food Chem.* **2012**, *60*, 10406–10414. [CrossRef]
64. Ştefanescu, B.E.; Szabo, K.; Mocan, A.; Crisan, G. Phenolic Compounds from Five Ericaceae Species Leaves and Their Related Bioavailability and Health Benefits. *Molecules* **2019**, *24*, 2046. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.