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Comparative seasonal analysis of IC50, total antioxidant capacity, phenolics, and flavonoids of some vegetable plants from the aquaponics system

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ABSTRACT

Seasonal factors such as temperature, solar UV-light intensity, and daylight length can induce changes in the water quality properties and, hence, the nutritional compositions of plants. This comparative study was carried out for the consecutive four (4) seasons (winter, spring, summer, and autumn) to determine the influence of seasonal variations on the 50% inhibitory concentration (IC50), total antioxidant capacity (TAC), total phenolics content (TPC), and total flavonoids content (TPC) of the red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) collected from a coupled commercial aquaponics system. The IC50, TAC, TPC, and TFC concentration levels indicated a significant ($P<0.05$) difference in the summer compared with the winter, spring, and autumn. The RCF extract indicated the lowest IC50, thus greater scavenging power in comparison to RTF, GLS, and GLL extracts. Similarly, the RCF showed the highest TAC and TPC, while the GLL showed the highest TFC. In this study, variations in seasons have induced changes in the IC50, TAC, TPC, and TFC concentration levels of the RCF, RTF, GLS, and GLL extracts.

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1. Introduction

An aquaponics system is a bio-integrated ecosystem. It links recirculating fish aquacultures with hydroponics plants production (Pattillo, 2017; Goddek et al., 2019). Hydroponic plants such as vegetables, flowers, or herbs, and aquatic species such as fish can be grown together in this soilless, water-based system (Rakocy et al., 2006). The fish in the aquaponics technology produces wastes that different bacterial species can convert into nutrients to be used up by plants, which in turn improves the water quality for the fish. This closed-loop system provides a renewable and sustainable method of food production (Medina et al., 2016; Rakocy et al., 2006).

Climatic change is one of the factors that can affect food production and supply at local, regional, and global levels. For instance, abiotic environmental factors such as high temperatures, precipitation pattern changes, and reduced water availability can influence food productivity, quality, accessibility, and availability (US EPA, 2017). Secondary metabolites, bioactive compounds or phytochemicals in plants varies because of changes in the environmental factors (Nchabeleng et al., 2012; Jayanthy et al., 2013; Sampaio et al., 2016). The most successful adaptation of plants is the synthesis of different phytochemicals to withstand or sustain both biotic and abiotic stress (Mohiuddin, 2019; Huang et al., 2020). Thus, secondary metabolites production in plants allows them to survive different seasonal conditions (Yadav and Agarwala, 2011).

Different temperature levels due to seasonal shifts have indicated an effect on the secondary metabolite compositions in plants (Usano-Alemany et al., 2014). Djurdjevic et al. (2012), detected an optimal total phenolics content of Conyza Canadensis L. plants during the flowering and fruiting period (rainy season). In another study, Akiode et al. (2021), reported cool period as the best season for tannins and alkaloids synthesis from *Azadirachta indica* and *Eucalyptus globulus* plants.

Red chilies contain many essential nutrients such as carotenoids (Marisa et al., 2001), tocopherols (Ling and Suhaila, 2001), phenolics and flavonoids (Ananthan et al., 2014). The pungent flavor of red chili is related to the compound capsaicinoid (Garces-Claver et al., 2006). The carotenoids are the pigments synthesized during tomato fruit ripening and responsible for the final red color (Peryeen et al., 2015). A tomato is a vital sources of lycopene (Agarwal and Rao, 2000), tocopherols, and β-carotene bioactive compounds (Burns et al., 2003; Hwang et al., 2012). It is also a good source of phenolics compound (Martinez-Valverde et al., 2002). Thus, tomato plays a critical function against various eyesight disorders, tumors, cancer, and cardiovascular disease (Agarwal and Rao, 2000; Martinez-Valverde et al., 2002; Peryeen et al., 2015). This fruit (tomato) can additionally reduce obesity and hyperglycemia (Cummings and Schwartz, 2003).

The dark green color of the leafy spinach indicates a high concentration of health-promoting carotenoids and chlorophyll (Ramaiyan et al., 2020). Carotenoids are anti-inflammatory, anti-cancerous (Ramaiyan et al., 2020), and prevent macular degeneration and cataracts (Wu et al., 2015). Leafy spinach also contains kaempferol; which reduces the risk of cancers and chronic diseases (Naoki et al., 2009; Ramaiyan et al., 2020), nitrates; which enhance heart health and lower blood pressure (Nathan and John, 2015), flavonoids; which are anti-carcinogenic and promote cardiovascular well-being (Ramaiyan et al., 2020). The antioxidant compounds reported in the leafy lettuce include phenolics (Liu et al., 2007), flavonoids (Llorach et al., 2008), β-carotene, and α-tocopherol (USDA, 2019). Evidence from in vitro, preclinical, and clinical studies suggest that lettuce has potential anti-inflammatory (Pepe et al., 2015), blood pressure-lowering (Lee et al., 2009), anti-diabetic (Cheng et al., 2015), and anti-cancer (Brennan et al., 2000) properties. Hence, consumption of different types of edible vegetable plants can be of crucial advantages, especially in developing countries with high-rate poverty, nutritional marginalization, and ever-increasing human populations (Braglia, 2022; Kumar et al., 2020).

There were no reports in the existing literature on the impacts of seasonal differences on the 50% inhibitory concentration (IC50), total antioxidant capacity (TAC), total phenolics content (TPC), and total flavonoids content (TPC) of vegetable crops from the aquaponics system. Notwithstanding, reports were detected on the influence of cultural shifts on pre-harvest and post-harvest factors on vitamin C content of horticultural crops (Lee and Kader, 2000), effect of plant growth temperature on antioxidant capacity in Strawberry (Wang and Zheng, 2001).

Impacts of cultural cycles and nutrient solutions on plant growth, yield, and fruit quality of alpine strawberry (*Fragaria vesca* L.) grown in hydroponics (Caruso et al., 2011), influence of cultural cycles and nutrient solution electrical conductivity on plant growth, yield, and fruit quality of 'Friariello' pepper grown in hydroponics (Amalfitano et al., 2017). Impact of seasonal and temperature-dependent variation in root defense metabolites on herbivore preference in *Taraxacum officinale* (Huang et al., 2020.), effect of seasonal changes on the quantity of secondary metabolites from neem and eucalyptus plants (Akiode et al., 2021), and influence of different seasons on polyphenol content and antioxidant potential of ethanolic, methanolic, ethyl acetate, and aqueous extracts of leaves, stems, and roots of *Premna integrifolia* (Singh et al., 2022). Hence, the present study intends to reports or identify the impacts of seasonal changes on IC50, TAC, TPC, and TFC contents of some vegetable plant extracts from the aquaponics system.

2. Material and methods

2.1. Study site

The site of the study aquaponics system was Makhanda town, Eastern Cape of South Africa. The research was conducted for consecutive four (4) seasons (winter, spring, summer, and autumn). The winter study started on the 31st of August 2020. In the spring period, the experiment commenced on the 30th of November 2020. The summer investigation began on the 1st of March 2021. Lastly, the autumn analysis was initiated on the 27th of May 2021.

2.2. Plant material

The sample plants of this research were Bird's eye red chili (*Capsicum frutescens* L.), red cherry tomato fruit (large) (*Solanum lycopersicum*), green leafy silver-beet spinach (*Spinacia oleracea*), and green leafy Locarno lettuce (*Lactuca sativa* L.) (Figure 1).

Figure 1. Vegetable plants for the comparative seasonal evaluation of 50% inhibitory concentration, total antioxidant capacity, phenolics, and flavonoids, a = Thai or Bird's eye red chili; b = Cherry tomato (large); c = Silver-beet spinach in a wicking bucket to support the growing roots; d = Locarno lettuce.

The Bird's eye red chili was obtained from gravel stone media-bed I denoted as GMB-I. Each collected red cherry tomato fruit and green leafy silver-beet spinach was from gravel stone media-bed II and III, represented as GMB-II and GMB-III, respectively. Besides, the green leafy Locarno lettuce was sourced from a polystyrene sheet on deep-water culture-1 (DWC-1). Each plant sample was then placed in a clean polythene bag, transported to the laboratory, and rinsed separately with Milli-Q water to remove unwanted materials or contaminants. Finally, each plant material was preserved at -20 ^{o}C before analysis.

The study vegetable plants selection is based on their nutritional value composition and or antioxidant properties. Also, these plants are among the most common vegetables regularly consumed and or used for different types of food menus preparation all over the globe.

2.3. Chemical reagents and apparatus

The chemical reagents used for this study include ascorbic acid (>99.5%, Merck, Lot No. 1047302, South Africa), quercetin (Willow Outcrop, South Africa), Gallic acid (CAS-No.149-91-7, Germany), 2,2-diphenyl-1-picrylhydrazyl (Glentham Life Sciences, CAS No. 1889-66-4, England), Folin-Ciocalteu's phenol reagent (Merck, Lot No. HC6043320, Germany), aluminum chloride (Saarchem, Batch No.1021022, South Africa). Other chemical reagents were sodium hydroxide (Merck, Batch No. MB1M610352), ammonium molybdate and sodium molybdate (Saarchem, South Africa), sodium carbonate (Merck, Batch No. QG1Q610988), and HPLC grade methanol (CAS-No. 67-56-1, Germany). The consumables include Milli-Q water (EMD-Millipore, Model 13681), filter paper (Whatman No. 1, Maidstone, England), micropipettes, Eppendorf tubes, and falcon tubes. The equipment consists of an analytical balance (Radwag, 220 g × 0.1 mg, Model, AS/220/C/2, Poland), a 96-well plate reader (Epoch Model, USA), a vortex machine (Model No. S10100A, BioRAD, USA), a BÜchi heating water bath (B-491, Switzerland), and a BÜchi rotavapor (R-210, Switzerland).

2.4. Preparation of standard stock and working solutions

Each standard stock solution for the 50% inhibitory concentration (IC50) and total phenolics content (TPC) was prepared by dissolving 2.0 mg of Gallic acid in 1.0 mL of methanol. However, the stock solution for the total antioxidant capacity (TAC) was generated by dissolving 2.0 mg of ascorbic acid in Milli-Q water (1.0 mL). Nevertheless, the standard stock for total flavonoid content (TFC) was made by dissolving 2.0 mg of quercetin in 1.0 mL of methanol.

The IC50 working solution (500 μg mL^{-1}) was prepared from its standard stock to produce concentrations of 2.5, 10, 50, 100, 150, and 300 μg mL^{-1} by dilution with Milli-Q water. The TAC working solution (1,000 μg mL^{-1}) was obtained from its stock solution, different concentrations of 50, 100, 150, 200, 300, and 600 μg mL^{-1} were made by dilution with Milli-Q water. Lastly, each standard working solution (500 μg mL^{-1}) for TPC and TFC was prepared using each respective standard stock solution to generate concentration levels of 10, 50, 100, 200, 400, and 500 μg mL^{-1} by dilution with Milli-Q water.

2.5. Preparation of samples

Each sample (10.0 g) of multiple fresh-weight red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) was homogenized using a mortar and pestle. Each homogenate was incubated in 50 mL of the HPLC-grade methanol, allowed to stand for 1 h for complete extraction, and centrifuged at 4oC at 4,000 rpm for 20 min. Each extract was filtered using a Whatman No. 1 filter paper (11 μm pore size). Each filtrate was then evaporated to dryness with rotary evaporator under reduced pressure at 35 ^{o}C. The obtained dried powder of each sample was suspended (1.0 mg mL^{-1}) in HPLC-grade methanol for 50% inhibitory concentration (IC50), total antioxidant capacity (TAC), total phenolics content (TPC), and total flavonoids content (TPC) analysis. Finally, the remaining dried fractions of each sample was stored at -4 ^{o}C. All preparations and reactions were carried out under penumbra of light.

2.5.1. DPPH scavenging activity assay

This assay was performed as reported by Kalita et al. (2014), with modification in reagent stock and working concentrations. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) stock solution was made by dissolving 6.8 mg of the DPPH in 100 mL of HPLC-grade methanol. The absorbance of the DPPH stock reagent solution was obtained as 0.9982 ± 0.05 at 517 nm.

To a 200 µL of each sample extract with different concentrations (2.5, 10, 50, 100, 150, and 300 µg mL^{-1}) in the eppendorf tube, DPPH stock reagent (1.2 mL) was added. Each mixture was vortexed vigorously for 30 s and incubated in the dark at room temperature (25±5 °C) for 30 min. The absorbance of each mixture was read photometrically in triplicate at 517 nm against the reagent blank using a 96-well plate reader.

2.5.2. Phospho-molybdenum assay

The phospho-molybdenum assay procedure for the total antioxidant capacity (TAC) test was performed as described by Umamaheswari and Chatterjee (2008) with modifications in the incubation temperature and time. To 100 µL of each sample extract, 1.0 mL of the reagent solution (0.1 M phosphate buffer, 0.6 M H_2SO_4, 28 mM Na_2MoO_4, and 4.0 mM $Al_2(MoO_4)_3$) were added and mixed. The reaction mixtures were incubated at 95 °C for 120 min in a water bath and cooled to room temperature. Each mixture absorbance was read in triplicate at 765 nm wavelength against the reagent blank, using a 96-well microplate reader

2.5.3. Total phenolics content analysis

The total phenolics content (TPC) of each extracts was assayed using the Folin-Ciocalteu (FC) reagent as reported by Kim et al. (2003) and Blainski et al. (2013) with modification in extracts concentration and incubation time. Each sample extract (200 µL) was mixed with 400 µL Folin-Ciocalteu reagent solution. Each mixtures was kept at 25 °C for 15 min, 0.2 mL of 7% Na_2CO_3 reagent was then added and mixed. Finally, each mixture was diluted with 10 mL of Milli-Q water and re-incubated at 25 °C for 2 h, 15 min. Each mixture absorbance was read in triplicate at 725 nm wavelength against a reagent blank.

2.5.4. Total flavonoids content analysis

The total flavonoid content (TFC) was determined using a procedure described by Chang et al. (2002), with modification in the extract concentration and volume of reagents. To 100 µL of each extract, 400 µL of Milli-Q water, 35 µL of 5% $NaNO_2$, and 35 µL of 10% $AlCl_3.6H_2O$ were added, mixed, and incubated for 6 min. In addition, 215 µL of NaOH (1.0 M) was added to each reaction mixture, diluted with 250 µL of Milli-Q water, and mixed. Finally, the reaction mixtures were allowed to stand for 15 min at room temperature. The absorbance of each sample mixture was read at 510 nm in triplicate against a reagent blank using a 96-well microplate reader.

2.6. Statistical analysis

Data evaluations were conducted using repeated-measures analysis of variance (RM ANOVA). The level of significance used was 5%. When the RM ANOVA indicated a significant difference among the comparative four (4) seasons, a post-hoc test using an unpaired student's t-test was performed to determine the significantly different season(s).

3. Results and discussion

3.1. DPPH scavenging activity

The DPPH scavenging activity of each extract was calculated with the following relation.

$\%I=(AC-AS)/AC\times100$

Where: I = inhibition, AC = absorbance of the control, AS = absorbance of the sample

Finally, the 50% inhibitory concentration (IC50) of each study plant extracts was calculated using GraphPad Prism (Software version 9.2.0332, San Diego, CA 92108, USA). The IC50 concentration level of each sample extract of red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) was expressed as µg Gallic acid equivalent (GAE) mg^{-1} fw. Charts for the IC50 were depicted in Figures 2 to 5. There was a significant ($P<0.05$) difference in the IC50 level of the RCF, RTF, GLS, and GLL extracts among the comparative four (4) seasons (Table 1). The detected significant ($P<0.05$) difference was between winter and spring (Table 1). Similarly, the winter was significantly ($P<0.05$) different from summer as well as the winter in comparison to autumn (Table 1).

In addition, there was a significant (P<0.05) difference in the spring comparison summer likewise, between the spring and autumn (Table 1). Finally, a significant (P<0.05) difference existed in the summer compared with autumn (Table 1). The lowest IC50 level was revealed in the summer period. The red chili fruit (RCF) extract demonstrated the lowest IC50 value in this period (summer), hence the highest inhibitory action compared with the standard (Gallic acid). However, the highest (lowest inhibitory action) IC50 value was in the winter season, revealed by the green leafy lettuce (GLL). Seasonal variations on the IC50 level of the RCF, RTF, GLS, and GLL extracts from aquaponics system was lacking in the existing literature. Although, Higher or warmer temperature enhances photosynthesis (Jamloki et al., 2021) and reduced water availability (Goddek et al., 2019) in plants. Both cases inevitably encourages the synthesis and increased level of the secondary metabolites in plants (Jamloki et al., 2021).

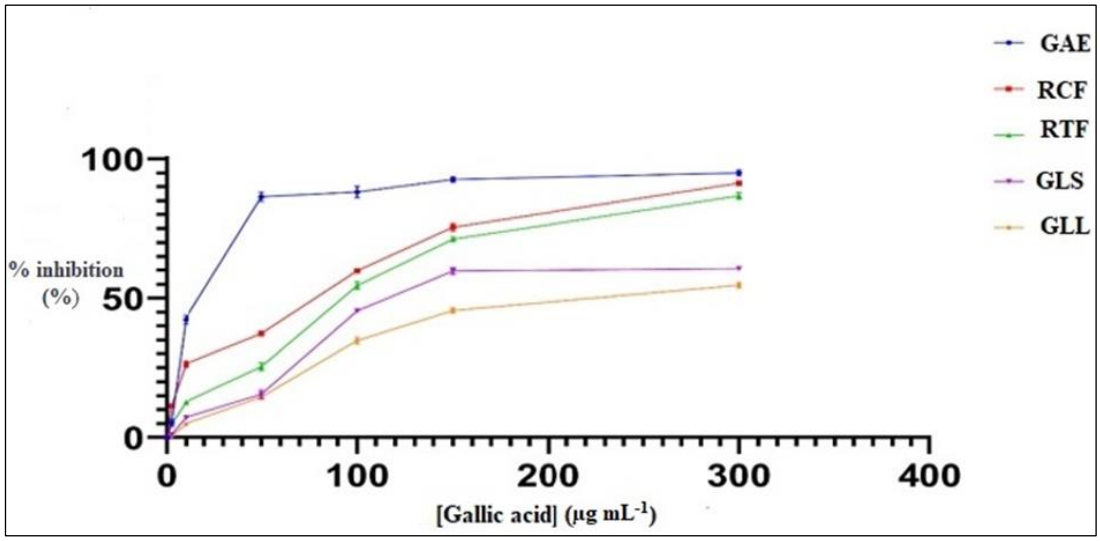

Figure 2. Winter season IC50 charts. The IC50 of each sample extract was determined with a GraphPad Prism. GAE denotes Gallic acid external standard, RCF, RTF, GLS, and GLL are methanolic extracts of red chili fruit, red tomato fruit, green leafy spinach, and green leafy lettuce, respectively.

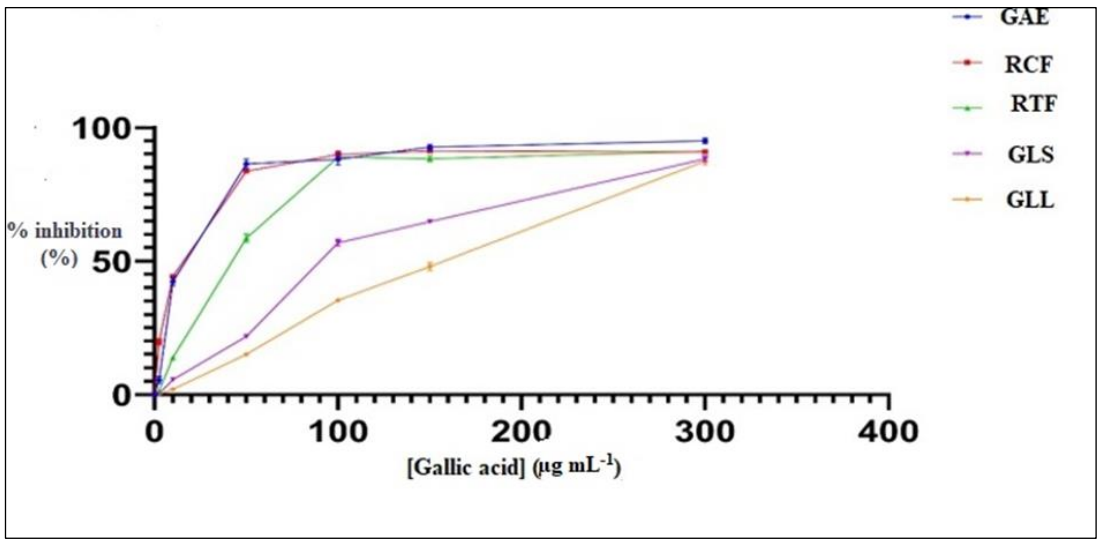

Figure 3. Spring season IC50 charts. The IC50 of each sample extract was determined with a GraphPad Prism. GAE represents Gallic acid external standard, RCF, RTF, GLS, and GLL are methanolic extracts of red chili fruit, red tomato fruit, green leafy spinach, and green leafy lettuce, respectively.

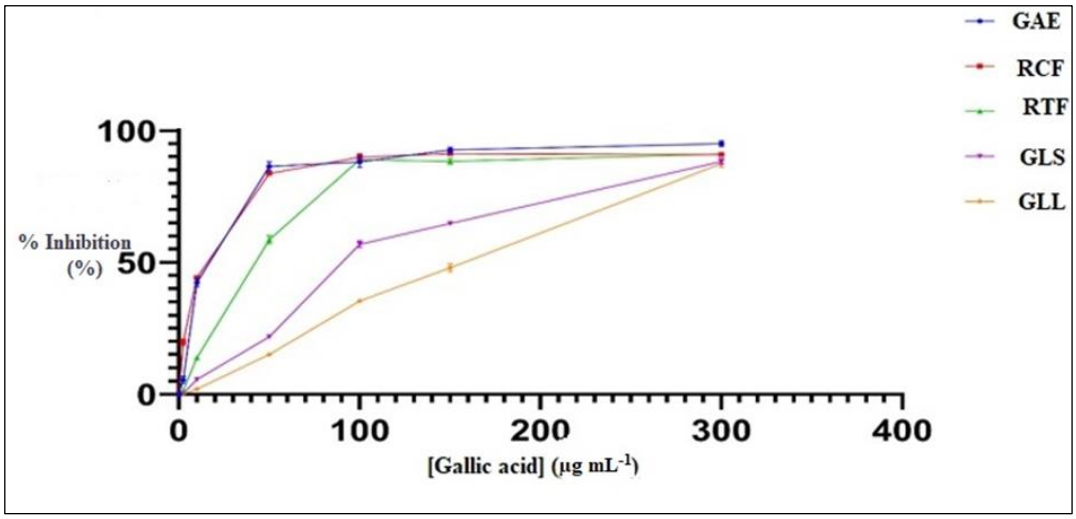

Figure 4. Summer season IC50 charts. The IC50 of each sample extract was determined with a GraphPad Prism. GAE denotes Gallic acid external standard, RCF, RTF, GLS, and GLL are methanolic extracts of red chili fruit, red tomato fruit, green leafy spinach, and green leafy lettuce, respectively.

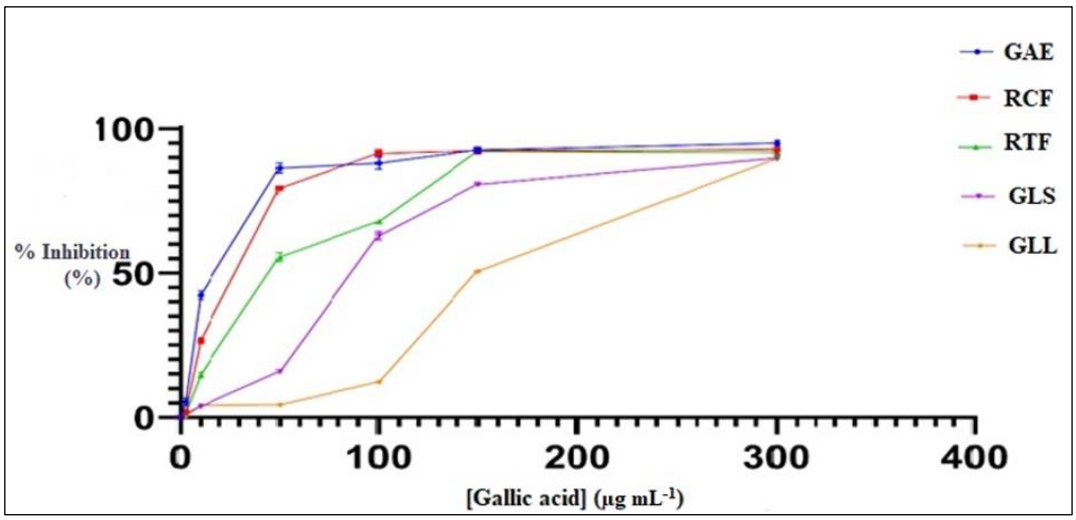

Figure 5. Autumn season IC50 charts. The IC50 of each sample extract was determined with a GraphPad Prism. GAE presents Gallic acid external standard, RCF, RTF, GLS, and GLL are methanolic extracts of red chili fruit, red tomato fruit, green leafy spinach, and green leafy lettuce, respectively.

Table 1. Seasonal variations in the 50% inhibitory concentration of the plant extracts

	IC_{50} (µg mg^{-1} fw)			
	GA = 19.52±9.42			
	Sample extracts (n = 3)			
Seasons	RCF	RTF	GLS	GLL
Winter	98.44±0.42[a]	170.59±4.41[a]	259.29±6.62[a]	286.34±4.44[a]
Spring	69.80±1.20[b]	92.72±2.75[b]	115.00±0.81[b]	175.16±0.52[b]
Summer	18.40±0.05[c]	37.62±0.84[c]	90.74±1.77[c]	152.56±3.75[c]
Autumn	24.19±0.61[d]	47.36±1.29[d]	98.34±1.27[d]	165.28±1.36[d]

IC50 = 50% inhibitory concentration, fw = fresh weight, GA = Gallic acid, RCF = red chili fruit, RTF = red tomato fruit, GLS = green leafy spinach, GLL = green leafy lettuce, and n = number of repeats for each sample extract. Results were presented as a mean±SD. Values with different superscript letters between seasons in a column are significantly ($P<0.05$) different.

The secondary metabolites sometimes called the defensive compounds possessed the capability to defend biotic and abiotic stresses in plants as well as helping plants to survive oxidative stress-mediated damages (Jamloki et al., 2021). Thus, the lower IC50 level detected in the summer could be related to increased synthesis of these defensive compounds. In this study, variations in the IC50 levels of the RCF, RTF, GLS, and GLL extracts can be linked to the influence of seasonal changes.

3.2. Total antioxidant activity

Figure 6 depicts the linear calibration curve for total antioxidant activity. The total antioxidant capacity (TAC) level for each of red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) extracts was expressed as µg ascorbic acid equivalent (AAE) mg^{-1} fw. A significant ($P<0.05$) difference in the TAC level of the RCF, RTF, GLS, and GLL extracts was detected among the comparative four (4) seasons (Table 2). The TAC level for each RCF, RTF, GLS, and GLL extracts revealed a significant ($P<0.05$) difference in the winter compared with the spring and between the winter and summer (Table 2). Comparably, a significant ($P<0.05$) difference was detected in the winter in comparison to autumn (Table 2). Furthermore, the spring is significantly ($P<0.05$) different from summer (Table 2). Similarly, a significant ($P<0.05$) difference was showed between the spring and autumn (Table 2). Lastly, there was a significant ($P<0.05$) difference in the summer compared with autumn (Table 2).

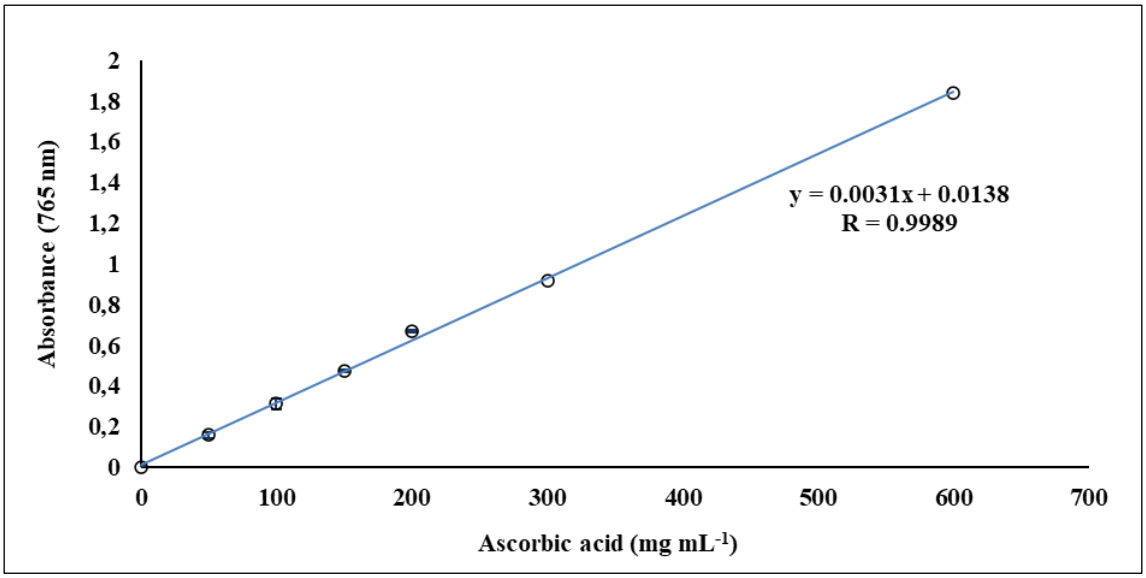

Figure 6. A standard curve for total antioxidant capacity (phospho-molybdenum assay). The standard curve was generated over a concentration range of 50 to 600 mg mL-1 using ascorbic acid as a standard, prepared in the Milli-Q water. Various standard concentrations were evaluated photometrically in triplicate.

Table 2. Seasonal differences in the total antioxidant capacity of the vegetable extracts

	TAC (µg mg^{-1} fw)			
	Sample extracts (n = 3)			
Seasons	RCF	RTF	GLS	GLL
Winter	527.10±0.01^{a}	158.06±0.03^{a}	94.95±0.01^{a}	69.68±0.03^{a}
Spring	566.13±0.03^{b}	179.78±0.01^{b}	122.80±0.03^{b}	107.20±0.01^{b}
Summer	591.18±0.07^{c}	232.37±0.02^{c}	249.57±0.05^{c}	137.42±0.01^{c}
Autumn	503.98±0.10^{d}	225.27±0.03^{d}	180.11±0.05^{d}	98.14±0.01^{d}

TAC = total antioxidants capacity, fw = fresh weight, RCF = red chili fruit, RTF = red tomato fruit, GLS = green leafy spinach, GLL = green leafy lettuce, and n = number of repeats for each sample extract. Results were presented as a mean±SD. Values with different superscript letters between seasons in a column are significantly ($P<0.05$) different.

The summer season revealed the highest TAC level, the RCF extract possessed the highest TAC amount (Table 2). The lowest TAC activity was in the winter period, the GLL extract indicated the lowest activity. There were no reports on the effect of seasonal differences on the TAC level of the RCF, RTF, GLS, and GLL extracts from the aquaponics source. However, Kamath et al. (2015) reported the TAC activity of fresh weight red chilli (40.28±2.18 mg g^{-1}), tomato (8.88±0.73 mg g^{-1}) and spinach (30.49±2.26 mg g^{-1}) extracts sourced from local foodstuff. From the above report, the farming method and cultivar type of the plant material were not indicated. Additionally, Khanam et al. (2012), cited the TAC activity of salad spinach (3.66 µg g^{-1}) and lettuce (11.56 µg g^{-1}) of a dry weight basis from non specified farming system and cultivar, purchased from supermarket. Furthermore, the TAC activity of the fresh tomato fruit from organic (2.59±0.06 mol g^{-1}) and aquaponics (2.87±0.09 mol g^{-1}) farming was detected by Braglia et al. (2022), with unidentified cultivar. Crops culture solution/farming method and cultivar differences influences allelochemicals levels in plants (Nida et al., 1999; Kawaoka and Funabashi, 2020). Variations in TAC levels of the investigated sample extracts are positively associated with seasonal changes.

3.3. Total phenolics content

The total phenolics content standard curve was showed in Figure 7. The total phenolics content (TPC) level of the red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) extracts was presented as µg Gallic acid equivalent (GAE) mg^{-1} fw. The investigated RCF, RTF, GLS, and GLL extracts indicated a significant ($P<0.05$) difference in the TPC among the comparative four (4) seasons (Table 3).

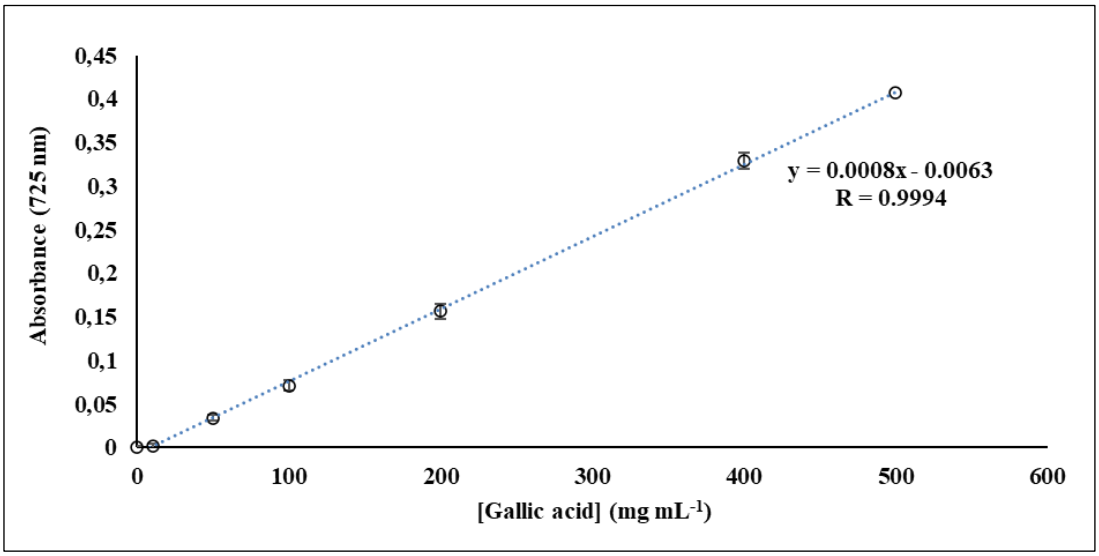

Figure 7. A total phenolics content standard curve. The standard curve was generated over a concentration range of 10 to 500 mg mL^{-1} with gallic acid as an external standard, prepared in the Milli-Q water. Different standard concentrations were determined using Folin-Ciocalteau calorimetric method in triplicate.

There was a significant ($P<0.05$) difference in the RCF, RTF, GLS, and GLL extracts in the winter compared with spring, between the winter and summer, and the winter in comparison to autumn (Table 3). In addition, the spring is significantly ($P<0.05$) different from summer (Table 3). Similarly, a significant ($P<0.05$) difference was detected between spring and autumn (Table 3). The RCF extract had the highest TPC level in the summer season (Table 3). However, the GLL extract indicated the lowest TPC in the winter period. There was no report of the influence of seasonal variations on the TPC of the investigated sample extracts from the aquaponics system. Notwithstanding, Kamath et al. (2015) reported the presence of phenolics in fresh red chili (2.87±0.18 mg g^{-1}), tomato (4.71±0.32 mg g^{-1}), and spinach (5.84±0.42 mg g^{-1}) extracts sourced from the local foodstuff. The cultivar and farming method of samples were not revealed. Moreover, Braglia et al. (2022) detected phenolics from the fresh weight tomato extract sourced from organic (0.61±0.09 mg g^{-1}) and aquaponics (0.41±0.07 mg g^{-1}) farming with different concentration level measurement unit.

Furthermore, Al-Mamary (2002) revealed the presence of phenolics in the fresh weight chili (116.03±2.47 mg 100 g^{-1}), tomato (28.85±0.93 mg 100 g^{-1}), spinach (10.31±1.46 mg 100 g^{-1}), and lettuce (56.45±0.76 mg 100 g^{-1}) extracts sourced from local market. The cultivar name and farming system of the vegetable plants were not provided. Furthermore, Khanam et al. (2012) revealed the presence of phenolics in fresh weight spinach (95.78±2.95 µg g^{-1}) and lettuce (80.84±4.75 µg g^{-1}) plants obtained from supermarket. The farming method and cultivar type were also not revealed. Cultivar type and farming practice determine the secondary metabolite amounts in plants (Nida et al., 1999; Kawaoka and Funabashi, 2020). This study has indicated that seasonal changes have induced differences in the TPC level of the examined sample (RCF, RTF, GLS and GLL) extracts.

Table 3. Seasonal dynamics in the total phenolics content of the plant extracts

	TPC (µg mg^{-1} fw)			
	Sample extracts (n = 3)			
Seasons	RCF	RTF	GLS	GLL
Winter	251.25±0.02^{a}	59.58±0.01^{a}	73.75±0.03^{a}	16.25±0.01^{a}
Spring	404.17±0.01^{b}	100.75±0.04^{b}	137.08±0.01^{b}	65.67±0.03^{b}
Summer	422.50±0.02^{c}	117.17±0.06^{c}	185.42±0.10^{c}	66.00±0.06^{cb}
Autumn	338.33±0.00^{d}	144.00±0.00^{d}	220.17±0.011^{d}	97.08±0.00^{d}

TPC = total phenolic content, fw = fresh weight, RCF = red chili fruit, RTF = red tomato fruit, GLS = green leafy spinach, GLL = green leafy lettuce, and n = number of repeats for each sample extract. Results were presented as a mean±SD. Values with different superscript letters between seasons in a column are significantly ($P<0.05$) different.

3.4. Total flavonoids content

The linear regression curve of the total flavonoid content (TFC) was depicted in Figure 8. Each of red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) extracts TFC value was calculated as µg quercetin equivalent (QE) mg^{-1} fw. A significant ($P<0.05$) difference was detected in the TFC level of the RCF, RTF, GLS, and GLL extracts among the comparative seasons (Table 4). There was a significant ($P<0.05$) difference in the TFC level of the RCF and RTF between winter and spring, the winter compared with summer, and winter in comparison to autumn (Table 4).

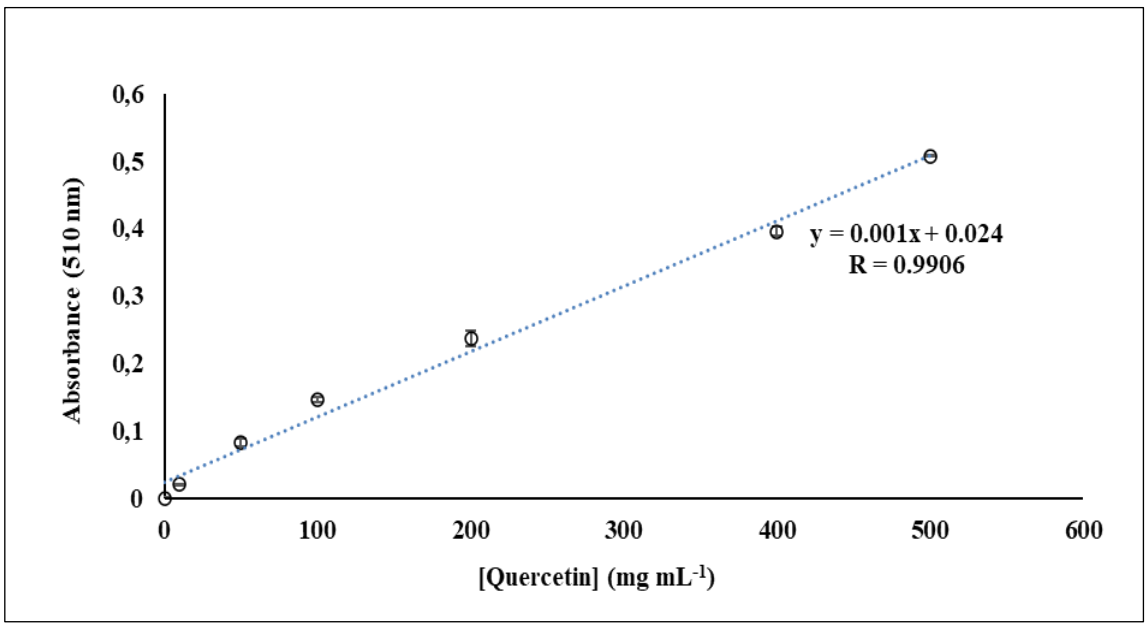

Figure 8. A total flavonoids content standard curve. The standard curve was generated over a concentration range of 10 to 500 mg mL^{-1} with quercetin as a reference standard, prepared in the Milli-Q water. Various standard concentrations were analyzed in triplicate photometrically. A method of $NaNO_2$-$AlCl_3$-NaOH was used.

Table 4. Seasonal variations in the total flavoniods content of the vegetable extracts

	TFC (μg mg^{-1} fw)			
	Sample extract (n = 3)			
Seasons	RCF	RTF	GLS	GLL
Winter	80.33±0.02[a]	11.00±0.01[a]	51.33±0.01[a]	149.67±0.03[a]
Spring	256.33±0.18[b]	18.69-±0.02[b]	134.00±0.02[b]	427.33±0.01[bd]
Summer	272.33±0.01[c]	44.67±0.02[c]	129.00±0.02[cb]	475.33±0.07[c]
Autumn	263.67±0.01[bd]	023.33±0.00[bd]	187.33±0.02[d]	419.00±0.07[d]

TFC = total flavonoids content, fw = fresh weight, RCF = red chili fruit, RTF = red tomato fruit, GLS = green leafy spinach, GLL = green leafy lettuce, and n = number of repeats for each sample extract. Results were presented as a mean±SD. Values with different superscript letters between seasons in a column are significantly (P<0.05) different.

Additionally, a significant (P<0.05) difference was indicated between the spring and summer (Table 4). Furthermore, the summer was significantly (P<0.05) different from the autumn period (Table 4). The GLS extract TFC level indicated a significant (P<0.05) difference in the winter in comparison to spring and winter compared with summer (Table 4). Similarly, the winter was significantly (P<0.05) different from the autumn (Table 4). In addition, a significant (P<0.05) difference was observed in the spring in comparison to autumn and between the summer and autumn (Table 4). The GLL extract TFC amount indicated a significant (P<0.05) difference between the winter and spring, the winter compared with the summer, and the winter in comparison to autumn (Table 4). Lastly, there was a significant (P<0.05) difference in the TFC value of GLL between spring and summer as well as summer compared with autumn (Table 4). The highest TFC amount was detected in the summer (Table 4). The GLL extract revealed the highest TFC level in this period (Table 4). The RTF showed the lowest TFC amount in the winter season. Reports on the effect of seasonal changes on the TFC levels was lacking. However, Khanam et al. (2012), reported the presence of flavonoids in the fresh spinach (102.77±3.95 μg g^{-1}) and lettuce (44.85±1.56 μg g^{-1}) extracts purchased from supermarket. The cultivar name and farming practice of the vegetable plants were not disclosed. Farming system and cultivar variation affects the phytochemical levels in agricultural production (Nida et al., 1999; Kawaoka and Funabashi, 2020). Variation in the TFC concentration level of the researched sample extracts was observed among seasons.

5. Conclusion

This research investigated the effect of seasonal differences on the IC50, total antioxidant capacity (TAC), total phenolics content (TPC), and total flavonoid content (TFC) of the red chili fruit (RCF), red tomato fruit (RTF), green leafy spinach (GLS), and green leafy lettuce (GLL) extracts from the aquaponics system. The summer indicated the highest IC50, TAC, TPC, and TFC amounts compared with winter, spring, and autumn. The RCF extract revealed the highest IC50, TAC, and TPC. While the GLL extract showed the highest TFC value in this season (summer). Thus, seasonal variations have induced changes in the IC50, TAC, TPC, and TFC concentration levels of the RCF, RTF, GLS, and GLL extracts. Therefore, this research findings demonstrated that aquaponics system food production and or harvesting in warmer periods (with increased light intensity and duration) can enhance antioxidants value composition, required for proper growth, development, and protection against diseases when consumed appropriately and sufficiently.

Compliance with Ethical Standards

Conflict of Interest

The author declares no conflict of interest.

Authors' Contributions

The methodology, investigation, conceptualization, data analysis, and data curation were done by the author. In addition, the author write, review, edit, and validate the original draft of this research article.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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