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# Bioactivities of *helichrysum cymosum* cultivated in aquaponic, hydroponic and field systems

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**Background:** Aquaponics and hydroponics are potential alternative techniques for sustainable cultivating of medicinal plants.

**Setting:** The experiments were carried out on the Bellville campus of the Cape Peninsula University of Technology, Cape Town, South Africa.

**Aim:** The aim of this study was to evaluate crop yield, secondary metabolite contents, and the antifungal activities of extracts from *Helichrysum cymosum* (*H. cymosum*) grown in aquaponic and hydroponic systems compared with field-collected plants.

**Methods:** *Helichrysum cymosum* seedlings were cultivated in hydroponic and aquaponic systems for 6 weeks under greenhouse conditions. The data on plant growth parameters, phytochemical analyses of the leaves, anti-*Fusarium oxysporum* (*F. oxysporum)* activity of ethanolic extracts, and antioxidant capacities were recorded.

**Results:** The results showed that the heights of plants grown in aquaponics and hydroponics did not differ substantially ($p > 0.05$). The total polyphenol contents varied significantly ($p < 0.01$) among the three cultivation techniques, with the field-collected plants yielding the highest contents (452.10 mg GAE/g ± 53.37 mg GAE/g). The flavonol contents differed significantly among the three cultivation techniques ($p < 0.05$), with the highest flavonol contents in the field-collected plants (250.62 mg QE/g ± 58.12 mg QE/g). The plants grown in aquaponics had the highest number of compounds (104). The microdilution bioassay showed that the ethanolic extracts of field-harvested *H. cymosum* had higher fungistatic activity against *F. oxysporum*. The highest antioxidant capacity was recorded in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay for plants cultivated in hydroponics ($p < 0.05$), while field-collected plants showed a significantly higher value of 2719.42 µmol ± 278.72 µmol AAE/g ($p < 0.05$) in the ferric ion reducing antioxidant parameter (FRAP) assay.

**Conclusion:** The field-collected plants performed better in phytochemical contents. However, cultivation of *H. cymosum* using a hydroponic system may be feasible based on the antioxidant results.

**Contributions:** The study contributes to developing an alternative strategy for cultivating plants and promoting sustainable farming.

**Keywords:** *Helichrysum cymosum*; Asteraceae; secondary metabolites; aquaponics; hydroponics.

## Introduction

A growing number of citizens in developing countries, including South Africa, rely on medicinal plants to treat countless diseases (Taylor et al. 2001). Numerous wild medicinal plants are under severe pressure caused by over-harvesting and habitat degradation. The risk of extinction of many species is at an all-time high, warranting urgent interventions to achieve sustainable utilisation of medicinal plant resources (Chen et al. 2016). Soilless cultivation is an innovative approach that could limit the exploitation of some endangered medicinal plants from the wild. Numerous opportunities exist for commercialising medicinal plants in aquaponic and hydroponic systems (Nchu, Matanzima & Laubscher 2018). As a production method that combines hydroponics and aquaculture, aquaponics is one of the most resource-effective plant cultivation methods (Stadler et al. 2015). Hydroponics is increasingly used to substitute typical agricultural soil cultivation (Pardossi et al. 2005). In hydroponics, plants are grown in a sterile nutrient solution or substrate culture. Both aquaponics and hydroponics provide opportunities to manipulate nutrient availability, crop yield, and quality.

Previous research studies have shown that cultivating medicinal plants under controlled conditions in aeroponic or hydroponic systems improves quality, bioactivity, and biomass output on a commercial scale (Jousse et al. 2010; Polycarpou et al. 2005). These approaches are beneficial in areas with significant environmental stresses, such as cold, heat, and desert (Polycarpou et al. 2005). They are also compatible with various medicinal plant species; the viability and advantages of these cultivation systems for synthesising secondary metabolites have been proven (Hayden 2006; Léonhart et al. 2003; Maggini, Kiferle & Pardossi 2014; Dorais et al. 2001). However, few studies have compared the cultivation of indigenous medicinal plants in aquaponic, hydroponic, and field systems.

The genus *Helichrysum* Mill. belongs to the *Asteraceae* family, which consists of roughly 600 different species, of which 250 are found in South Africa (Lourens, Viljoen & Van Heerden 2008). *Helichrysum cymosum* (L) D. Don is native to South Africa and is a member of the *Asteraceae* family. It is among the country's most sought-after medicinal species. The leaves are small, elliptic-oblong or linear-oblong in shape, with an acute, sometimes acuminate, apex, mucronate, somewhat constricted, and clasping base (Heyman 2013). *Helichrysum cymosum* is used to treat pain, coughs, colds, fever, headache, menstrual pains, wound dressing, and infection prevention (Heyman 2013; Maroyi 2019b). Other ethnomedicinal uses of *H. cymosum* include treatment of a blocked nose, cardiovascular problems, diarrhoea, dizziness, eye problems, flatulence, kidney problems, menstrual pain, pertussis, pulmonary problems, skin infections, urinary problems, varicose veins, vomiting, weak bones, and boosting the immune system (Heyman 2013). The leaves, stems, and twigs of *H. cymosum* are sold as herbal medicines in the informal herbal medicine markets in the Gauteng and the Western Cape provinces in South Africa (Maroyi 2019a). The plant's volatiles have long been used to treat respiratory and wound infections (Kutluk et al. 2018).

Essential oils from *H. cymosum* have antibacterial characteristics and could be used to treat tropical diseases such as malaria (Van Vuuren et al. 2006). Crude extracts of *H. cymosum* and compounds isolated from the species have been found to have antibacterial (Maroyi 2019a; Sindambiwe et al. 1999; Stafford, Jäger & Van Staden 2005; Van Vuuren 2007; Van Vuuren et al. 2006), antioxidant (Tchoumbougnang et al. 2010), antifungal (Runyoro et al. 2010; Van Vuuren 2007; Van Vuuren et al. 2006; Tchoumbougnang et al. 2010), antiviral (Sindambiwe et al. 1999), anti-HIV and cytotoxic (Heyman 2009; Heyman et al. 2015), anti-inflammatory (Stafford et al. 2005), and antimalarial effects (Van Vuuren et al. 2006). The antimicrobial properties of *H. cymosum* have made the plant one of South Africa's most sought-after medicinal plant species. Numerous compounds have been isolated from the alcoholic extract of the leaves and roots of *H. cymosum* including sesquiterpenes and chalcones (Jakupovic et al. 1989; Popoola et al. 2015; Van Vuuren et al. 2006). Several other compounds have been discovered in Helichrysum species, including phenolics, flavonoids, phthalides, pyrone derivatives, terpenoids, and fatty acids (Czinner et al. 2001).

To the best of our knowledge, this is the first study to compare the effects of three different cultivation approaches (aquaponics, hydroponics, and field methods) on the growth, secondary metabolite contents and antifungal activity of *H. cymosum*.

# Research methods and design

## Research design

Four-week-old, rooted cuttings of *H. cymosum* were grown using two cultivation systems (hydroponic and aquaponic), demonstrating two treatments. The data on plant growth (crop yield: plant height, fresh and dry weight), secondary metabolite contents, and antifungal activities were obtained at the end of the experiment. The secondary metabolite contents and antifungal activities of plants cultivated aquaponically and hydroponically were compared with those of field-cultivated plants. *Helichrysum* seedlings were acquired from Shadowlands Wholesale Nursery Pty. Ltd. in Zevenwacht Link Road, Kuilsriver 7580 Western Cape. The plant specimens were mounted and placed in the Herbarium (Voucher no. 7069) of the Cape Peninsula University of Technology (CPUT), Bellville campus, Cape Town, South Africa. The roots were carefully cleaned with deionised water and separated by hand to eliminate potting soil debris before the commencement of the experiment. The plants were arranged in a completely randomised design inside a research greenhouse, where they were exposed to natural sunlight entering through the polycarbonate ceiling of the greenhouse.

## Greenhouse experiment

The experimental protocol described by Zantanta et al. (2022) was used in this study. The experiment was conducted in a greenhouse on the Bellville campus of the CPUT. For the hydroponic system, 15 seedlings of *H. cymosum* were transplanted individually into 23 cm diameter pots containing a substrate mix of pine bark, perlite, and vermiculite in a ratio of 2:1:1. The plants were watered daily using 400 mL of deionised water and supplied with Nutrifeed® fertiliser (Starke Ayres Pty. Ltd., Cape Town, South Africa). The fertiliser was mixed with deionised water at 10 g/5 L. Each plant received a volume of 100 mL of the nutritional solution fortnightly, with a pH of 6.5 and an EC value of 2 mS $cm^{-1}$, measured with a Milwaukee EC 50 and pH 55 kits supplied by Spraytech Pty. Ltd., Cape Town, South Africa. A recirculating aquaponic system was used in the aquaponic system. The system consisted of a fish tank containing a submersible pump, an air pump (Regent 7500), and plant grow beds (four black 50 L plastic containers with perforated lids to fit the net pots). The submersible pump pumped the wastewater (nutrient-rich water) from the tank to a grow bed, deep culture design, through a PVC pipe. Fifteen *Helichrysum* seedlings were transplanted into net pots

containing a mixture of perlite and coco coir (50:50 ratio) as substrate. The plants were continuously watered from the bottom through the drain holes in the net pots immersed in the nutrient-rich water pumped from the fish tank. Ten-to-fifteen-cm Goldfish fingerlings (*Carassius auratus*) and fish food (Koi and Goldfish powder, small pellets) supplied by Stodels Nursery Pty. Ltd., Doncaster Road, Kenilworth 7708, Western Cape, South Africa, were used in this study. Twenty Goldfish (*Carassius auratus*) were placed in each tank (1000 L capacity). The EC level of the nutrient solution in the fish tank was 0.8 mS $cm^{-1}$, and the pH was 6.3. The fish were fed twice daily at 08:30 am and 16:00 pm. The aquaponic setup was replicated four times. The experiment lasted 6 weeks. At the end of the experiment, plant height (cm) and fresh and dry weights (g) of aquaponically and hydroponically produced plants were recorded and compared. However, the growth parameters of field plants were not assessed because the plants were already cultivated and established on the premises of the Bellville campus of CPUT before the commencement of the study. The harvested plant materials were used for tissue nutrient and secondary metabolite (polyphenol and flavonol) content analyses and were screened for antifungal activities. The greenhouse conditions were 15 °C – 26 °C and 74% relative humidity.

## Plant tissue analysis

Fresh aerial plant materials (leaves) obtained from the aquaponic and hydroponic systems were sent to a certified commercial laboratory (Bemlab [Pty] Ltd. in Somerset West, South Africa) for the analysis of macro- and micro-elements. The aerial parts (leaves) of *H. cymosum* were washed with Teepol solution, rinsed with de-ionised water, and dried in an oven at 70 °C overnight. The dried leaves were then powdered and ashed at 480 °C for extraction using filter paper in a 50:50 HCl solution (Campbell & Plank 1998). The concentrations of potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), and boron (B) were measured in mg/kg (Campbell & Plank 1998; Miller et al. 1993). Total combustion in a Leco N analyser was used to determine the concentrations of nitrogen in the leaves. A conversion factor of 10 000 was used to convert the amounts of N, P, K, Ca, and Mg from percentages to mg/kg (Xego, Kambizi & Nchu 2017). Three replicates from each treatment were analysed.

## In vitro fungal screening using micro-dilution method

The microdilution method was used to assess the extracts' minimum inhibitory concentration (MIC) as described by Eloff (1998) and Nchu et al. (2010). Five grams of milled *H. cymosum* leaf materials from three replicates were extracted with 25 mL ethanol (analytical grade, 99.9%) overnight, then filtered and the solvent evaporated. The dry extract was reformulated with ethanol to 6 mg/mL. The extract (6 mg/m) was transferred to the first row of a 96-well microplate with wells containing 100 µL of sterile distilled water. After that, the extracts were serially diluted twofold. A *Fusarium oxysporum* strain (UPFC no. 21) maintained at CPUT's Department of Horticultural Sciences was used in the microdilution assay. Fungal conidia obtained from stock agar plates were transferred to Nutrient Broth (Merck Pty. Ltd, Cape Town, South Africa) and incubated at 25°C for 4 h. One hundred microliters (100 µL) of conidial suspension ($10^5$ conidia/mL) was added to each of the 96 wells of the microplates containing the plant extract. Dithane (Stodels Nursery Pty. Ltd, Garden Centre, South Africa) (200 mg/25 mL) was used as a positive control and the solvent (ethanol) as a negative control. Each microplate well was filled with 40 µL of 0.2 mg/mL p-iodonitrotetrazolium chloride (INT) (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa) diluted in sterile distilled water, sealed in a plastic bag, and incubated at 37 °C and 100% RH. In the presence of fungus development, the colourless tetrazolium salt was reduced to a red-coloured formazan product.

At 18 h of incubation of the microtiter plates, the MIC values were recorded by visually comparing the pink colour of the wells. Three replicates of each treatment were used in the antifungal bioassay (MIC).

## Determination of antioxidant activities (ferric reducing antioxidant power, 2,2-diphenyl-1-picrylhydrazyl, and trolox equivalent antioxidant capacity)

### Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) analysis is based on the protocols described by Benzie and Strain (1996). In a 96-well microplate, 10 µL of the crude extract was combined with 300 µL FRAP reagent (0.3 µM acetate buffer, pH 3.6) (Saarchem, South Africa), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 0.1 µM HCl (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa), 20 mM Iron (III) chloride hexahydrate (FeCl at 593 nm). As a standard, L-ascorbic acid (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa) was employed at concentrations ranging from 0 to 1000 µM. The absorbance was determined. The results were represented in milligrams of ascorbic acid equivalent per gram of dry weight (mg of AAE/g DW). Three replicates from each treatment were analysed.

### 2,2-diphenylpicrylhydrazyl assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of the tested samples was determined according to Katalinić et al. (2004). A solution of 0.135 mM DPPH produced in a dark container was used to create the DPPH radical (Olatunji & Afolayan 2019). About 300 µL of DPPH solution were combined with 25 µL of the crude extract and graded concentrations (0 and 500 µM) of Trolox standard (6-Hydrox-2,5,7,8- tetramethylchroman-2-20 carboxylic acid). After a 30-min of the incubation period, the absorbance at 517 nM was determined as µM/Trolox equivalent per gram dry weight (µM TE/g DW) to express the results.

### Trolox equivalent antioxidant capacity

The trolox equivalent antioxidant capacity (TEAC) assay was carried out using the method described by Re et al. (1999). The solutions of 7 mM 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid and 140 mM potassium–peroxodisulphate ($K_2S_2O_8$) (Merck, South Africa) were used as stock solutions. The working solution was then made by mixing 88 µL of $K_2S_2O_8$ with 5 µL of ABTS solution. The two solutions were thoroughly mixed and left to react at room temperature in the dark for 24 h. The standard was Trolox (6-Hydrox-2,5,7,8-tetramethylchroman-2-20 carboxylic acid) at concentrations ranging from 0 to 500 µM. The crude extracts (25 µL) were allowed to react with 300 L of ABTS at room temperature for 30 min before being read in a plate reader at 734 nm at 25°C. The results were represented as µM/Trolox equivalent per gram dry weight (µM TE/g DW).

## Secondary metabolite contents

### Determination of total polyphenol and flavonol contents

The total polyphenol contents of dried *H. cymosum* samples (leaves) was determined using the Folin-Ciocalteu procedure (Singleton, Orthofer & Lamuela-Raventós 1999). Twenty-five microliters of aqueous extracts were mixed with 125 µL of Folin–Ciocalteu reagent (Merck Pty. Ltd., Cape Town, South Africa) in a 96-well microplate and diluted 1:10 with distilled water in a 96-well microplate. The well was filled with 100 µL of aqueous $Na_2CO_3$ (7.5%) after 5 min (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa). The plates were incubated for 2 h at room temperature before being examined at 765 nm with a Multiskan plate reader (Thermo Electron Corporation, Waltham, Massachusetts, USA). The results were represented as mg of gallic acid equivalents per gram dry weight (mg GAE/g DW) using 0 mg/L, 20 mg/L, 50 mg/L, 100 mg/L, 250 mg/L, and 500 mg/L gallic acid in 10% ethanol (Espinoza et al. 2019; Singleton et al. 1999).

The total flavonol contents of dried leaves of *H. cymosum* plants was evaluated using a standard of quercetin 0 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 40 mg/L, and 80 mg/L in 95% ethanol (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa). A volume of 12.5 µL of crude aqueous extracts were combined with 12.5 µL of 0.1% HCl (Merck Pty. Ltd., Cape Town, South Africa) in 95% ethanol and 225 µL of 2% HCl in the sample wells, which were incubated at room temperature for 30 min. At a temperature of 25°C, the absorbance was measured at 360 nm. The results were represented in milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW) (Espinoza et al. 2019). Three replicates from each treatment were analysed.

## Gas chromatography–mass spectrometry (GC–MS) analysis (Headspace) and secondary metabolite analysis

### Sample preparation

Fresh plant materials (leaves) were harvested and freeze-dried overnight at an −80°C temperature. After that, 1 g was weighed into a solid phase micro-extraction (SPME) vial, along with 2 mL of 12% ethanol solution at pH 3.5 and 3 mL of 20% NaCl solution. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre was used to analyse the headspace of all the samples (grey). Leaf samples from three plants from each treatment were analysed.

### Chromatographic separation

To determine the relative abundance of secondary metabolites, a method reported by Matrose et al. (2021) was used in the separation of volatile compounds using a gas chromatography (6890N, Agilent Technologies Network) coupled to an Agilent Technologies Inert XL/CI Mass Selective Detector Analytics PAL autosampler, and the separation of volatiles present in the samples was achieved using a polar ZB-WAX (30 m, 0.25 mm ID), at a flow rate of 1 mL/min, helium was used as the carrier gas. With a 5:1 ratio, the injector temperature was kept at 250°C. The temperature of the oven was programmed as follows: 35°C for 6 min, then 3°C/min to 70°C for 5 min, then 4°C/min to 120°C for 1 min, and lastly 20°C/min to 240°C and maintained for 2.89 min. The Mass Selective Detector (MSD) was in full scan mode when the incident occurred.

## Statistical analysis

The experimental data for the plant growth parameters (plant height, fresh and dry weight) tissue nutrient contents and secondary metabolite contents were analysed using the Kruskal–Wallis test, at a significance level of $p < 0.05$. Furthermore, multiple comparisons of the means were carried out using the Mann–Whitney test. PAST was used to carry out these computations (Hammer, Harper & Ryan 2001), and the number of volatiles in the aquaponics, hydroponics, and field plants were compared using Pearson's chi-square test.

## Ethical considerations

Ethical clearance to conduct this study was obtained from the Cape Peninsula University of Technology, Research Ethics Committee (No. 212011286/06/2020).

# Results

## Plant height

Aquaponics and hydroponics had no significant effect on the vegetative growth of *H. cymosum* plants (Degree of Freedom [*df*] = 1; $\chi^2 = 1.63$; $p = 0.21$) (Table 1). The results showed that

**TABLE 1:** Growth parameters of *helichrysum cymosum* grown in aquaponics and hydroponics for 6 weeks under greenhouse condition.

| Treatments | Plant height (cm) | Fresh weight (g) | Dry weight (g) |
|---|---|---|---|
| T1 | 22.83 ± 1.59† | 11.60 ± 0.80† | 6.04 ± 0.47† |
| T2 | 26.46 ± 0.94† | 13.82 ± 0.71‡ | 7.23 ± 0.39† |

Note: Values shown are mean ± SE. Means followed by the same lowercase letters (†, ‡) in the same column are not significantly different ($p > 0.05$) following comparison using the Mann–Whitney test.

T1, aquaponic; T2, hydroponic.

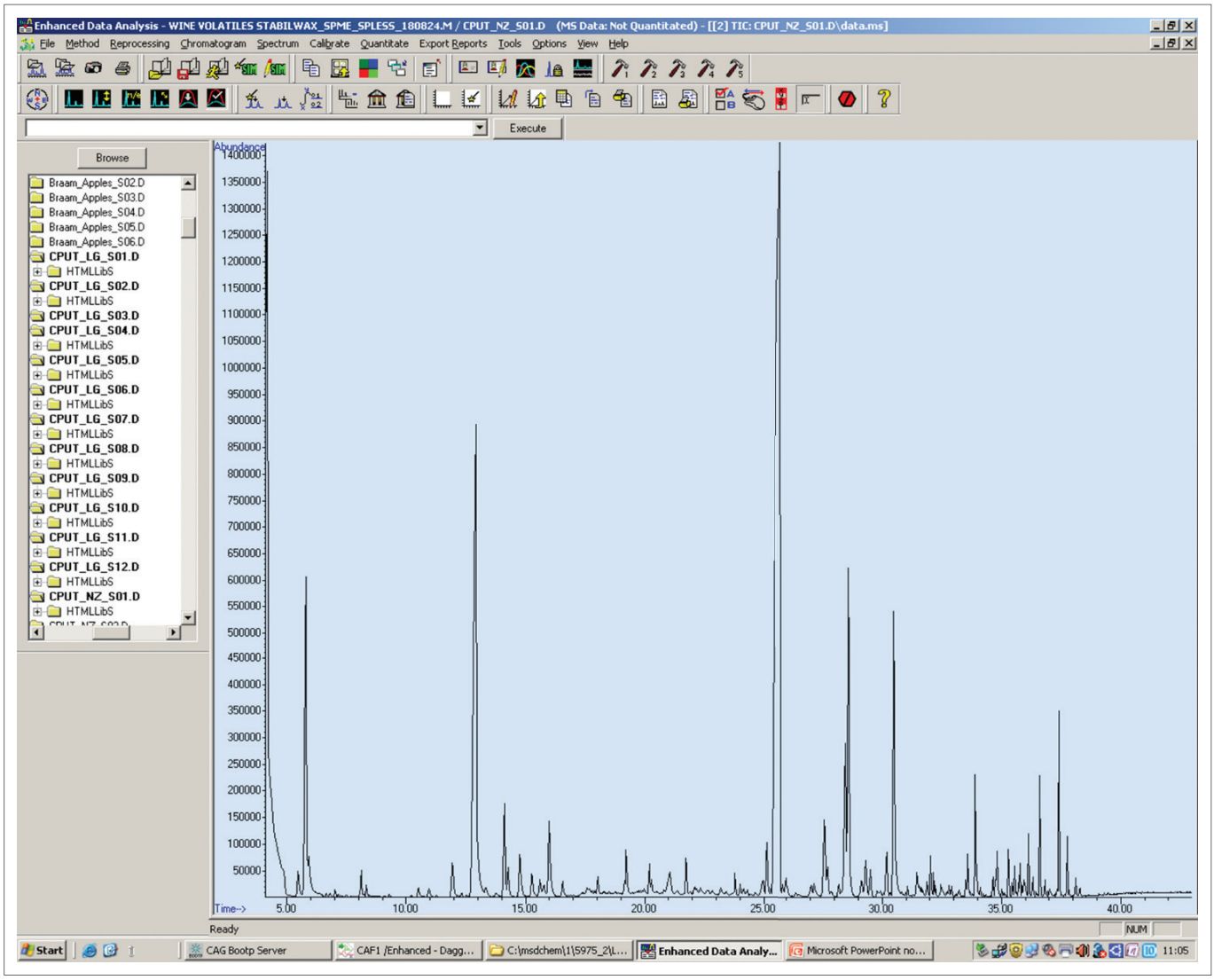


**FIGURE 1:** Chromatogram for *H.cymosum* field plants (T3).

the heights of plants grown in aquaponics and hydroponics did not differ substantially ($df = 1; 2 = 1.128; p > 0.05$) (Table 1, Figure 1). However, the mean shoot lengths of hydroponic plants (26.46 cm) were higher than those of aquaponic plants (22.83 cm).

## Fresh and dry weight

Plant height did not vary significantly between hydroponic and aquaponic plants ($p > 0.05$) (Table 1). When the fresh weights were compared, there was a significant difference between the *H. cymosum* plants grown in aquaponics and hydroponics treatments 6 weeks after treatment ($df = 1$; $\chi^2 = 3.85$; $p = 0.05$). Hydroponic plants had significantly higher fresh weight (13.82 g ± 0.7 g) than aquaponic plants (11.60 g ± 0.80 g) (Table 1, Figure 1).

There were no significant differences ($df = 1$; $\chi^2 = 3.75$; $p > 0.05$) in dry weights between aquaponics and hydroponic-grown *H. cymosum* plants, but the highest mean values were observed in hydroponic-grown plants (7.23 g ± 0.39 g) (Table 1, Figure 1).

## Tissue analysis

### Macronutrients

The plants cultivated in hydroponics had significantly higher macronutrient contents (P, K, and Mg) in the hydroponic plants than the aquaponic plants: P ($df = 1; \chi^2 = 14.29; p = 0.01$), K ($df = 1$; $\chi^2 = 34.34$; $p < 0.05$), and Mg ($df = 1$; $\chi^2 = 34.68; p < 0.05$) (Table 2, Figure 2). However, there were no significant differences in carbon (C), nitrogen (N), and calcium (Ca) contents between aquaponics and hydroponics plants ($df = 1; p > 0.05$). In general, macronutrient intake was found to be higher in hydroponic (T2) plants (Table 2, Figure 2). .

### Micronutrients

Tissue nutrient concentrations of Na ($df = 1$; $\chi^2 = 21.65$; $p = 0.01$), Cu ($df = 1$; $\chi^2 = 12.52$; $p = 0.02$), Zn ($df = 1$; $\chi^2 = 15.50; p = 0.01$) varied significantly (Table 3, Figure 3), with higher levels occurring in aquaponic plants. In contrast, B was significantly lower (20.37 mg/kg ± 2.51 mg/kg) in aquaponic grown plants. There was no significant difference in Mn and Fe uptake between aquaponics and hydroponics produced plants ($p > 0.05$).

**TABLE 2:** Tissue nutrient contents of aerial parts (leaves) of *helichrysum cymosum* grown in aquaponics and hydroponics for 6 weeks under greenhouse condition.

| Treatments | Nutrient quantity (mean ± SE) (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | C | N | P | K | Ca | Mg |
| T1 | 445 200 ± 13452.26† | 14 900 ± 1385.64† | 2200 ± 230.94† | 13 150 ± 1991.86† | 9800 ± 750.56† | 1300 ± 115.47† |
| T2 | 447 400 ± 3002.22† | 14 950 ± 259.80† | 3100 ± 57.73‡ | 24 900 ± 230.94‡ | 8950 ± 548.48† | 2150 ± 86.60‡ |

Note: Means followed by the same lowercase letters †, ‡ in the same column are not significantly different ($p > 0.05$) following comparison using the Mann–Whitney test.
T1, aquaponic; T2, hydroponic; SE, standard error; N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, Magnesium; C, carbon.

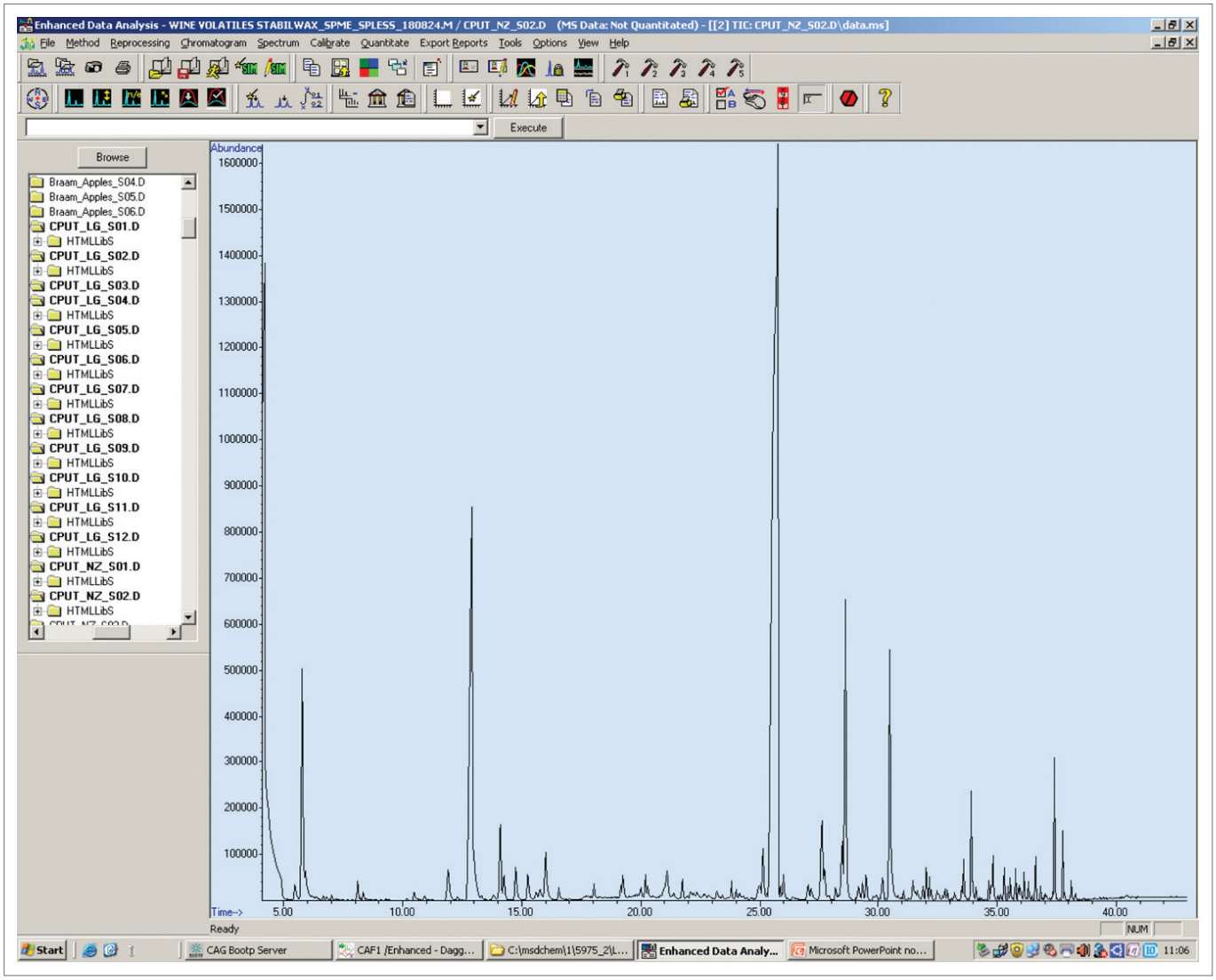


**FIGURE 2:** Chromatogram for *H.cymosum* field plants (T3).

**TABLE 3:** Tissue nutrient contents of aerial parts (leaves) of *helichrysum cymosum* grown in aquaponics and hydroponics for 6-weeks under greenhouse conditions.

| Treatments | Nutrient concentration (Mean ± SE) (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | Na | Mn | Fe | Cu | Zn | B |
| T1 | 7425 ± 1082.53† | 58.23 ± 4.19† | 214 ± 30.02† | 7.07 ± 0.66† | 60.8 ± 1.27† | 20.37 ± 2.51† |
| T2 | 2375 ± 77.94‡ | 45.75 ± 3.20† | 251.5 ± 23.96† | 4.37 ± 0.38‡ | 46.17 ± 3.49‡ | 36.5 ± 2.60‡ |

Note: Means followed by the same lowercase letters †, ‡ in the same column are not significantly different ($p > 0.05$) following comparison using the Mann–Whitney test.
SE, standard error; T1, aquaponic; T2, hydroponic; Na; sodium; Mn, manganese; Fe, iron; Cu, copper; B, boron; Zn, zinc.

## Secondary metabolites (polyphenols and flavonols)

Plants harvested from the field and hydroponics had considerably higher total polyphenol contents in aerial part (leaves) of *H. cymosum* ($df = 2$; $\chi^2 = 19.76$; $p = 0.00$) (Table 4, Figure 4). Total flavonol contents varied significantly ($df = 2$; $\chi^2 = 6.31$; $p = 0.03$) between aquaponic and hydroponic treatments with higher contents occurring in field plants.

## Volatile compound contents

As shown in Table 5 and Figure 5, compounds that matched the GC-MS mass spectral library (version 2.0d) of more

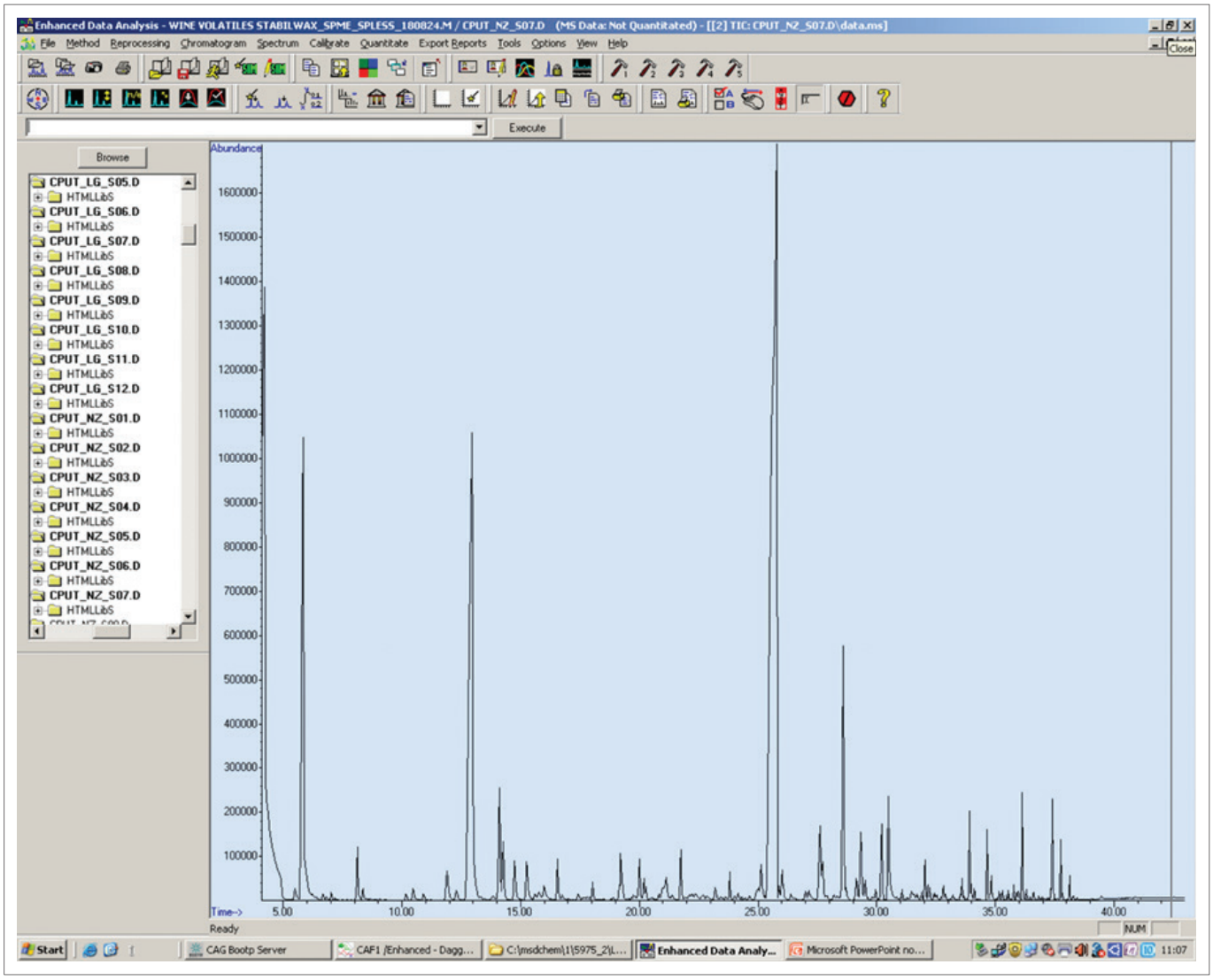


**FIGURE 3:** Chromatogram for *H.cymosum* aquaponics plants (T1).

**TABLE 4:** Mean total polyphenol ± SE and total flavonol ± SE contents of *helichrysum cymosum* leaves cultivated in aquaponics, hydroponics and the field at 6 weeks post-treatment.

| Treatments | Total polyphenol concentration (Mean ± SE) (mg GAE/g) | Total flavonol concentration (Mean ± SE) (mg QE/g) |
|---|---|---|
| T1 | 136.46 ± 42.09† | 71.60 ± 14.45† |
| T2 | 433.49 ± 11.95†,‡ | 164.05 ± 14.89†,‡ |
| T3 | 452.10 ± 53.37‡ | 250.62 ± 58.12‡ |

Note: Means followed by the same lowercase letters (†, ‡) in the same column are not significantly different ($p > 0.05$) following comparison using the Mann–Whitney test.

SE, standard error; GAE, gallic acid equivalents; T1, aquaponic; T2, hydroponic; T3, field.

than 90% were chosen, and a variety of volatile chemicals occurred in the species. Although more volatile chemicals (104) were found in aquaponics-grown plants than in field-grown (101) and hydroponics-grown (102) plants, the difference was not statistically significant using the Pearson chi-square test ($df = 2$; $\chi^2 = 3.53$; $p = 0.17$). The compounds detected included some compounds that are known for antifungal and antioxidant activities, such as nonadecane, 1-octen-3-ol, beta-fencyl acetate, benzaldehyde, alpha-humulene, linalyl propanoate, acoradiene, beta-himachalene, alpha-cedrene, alpha-ced 2,7-dimethyl-1,6-octadiene, trans- (+)-carveol, (-)-phyllocladene, and cyclooctanone were abundant in aquaponic-produced plants when compared with hydroponic- and field-collected plants.

## Antioxidant capacity: Ferric reducing antioxidant power, trolox equivalent antioxidant and 2,2-diphenyl-1-picrylhydrazyl

Plants that were collected from the field (T3) showed significantly higher antioxidant capacity, with a mean FRAP value of 2719.42 µmol ± 278.72 µmol AAE/g in *H. cymosum* plants ($df = 2$; $\chi^2 = F = 21.90$; $p < 0.05$), than hydroponics and aquaponics cultivated plants (Table 6, Figure 6). In the DPPH assay, the results indicated that hydroponic plant extracts yielded a significantly higher mean value ($df = 2$; $\chi^2 = 28.68$; $p < 0.05$) compared with aquaponics and field-collected plant extracts while aquaponics had the least antioxidant capacity (Table 6). The TEAC assay results showed that field-collected and hydroponic plants produced significantly higher values compared with aquaponics and hydroponics plants ($df = 2$; $\chi^2 = 16.25$; $p < 0.05$).

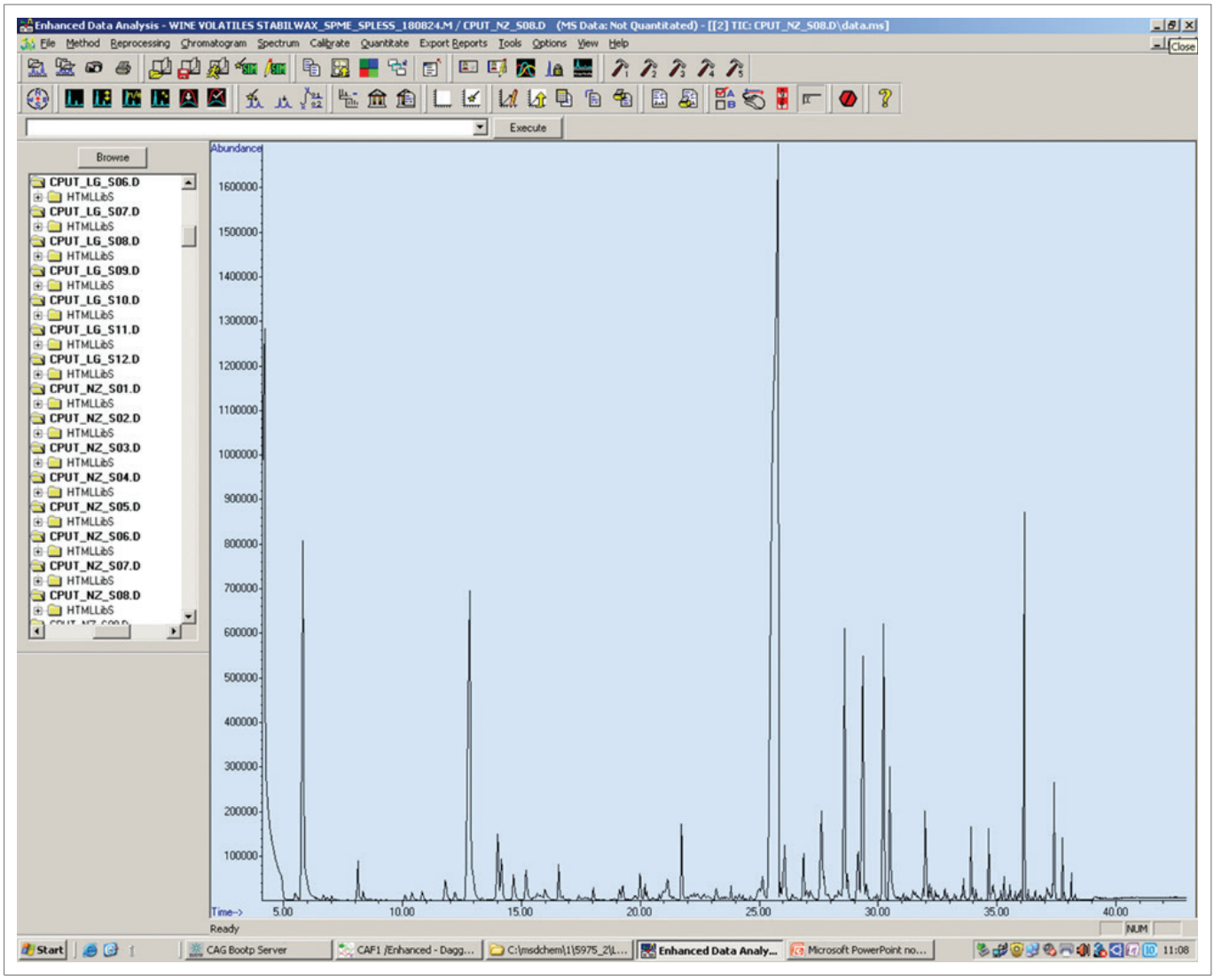


**FIGURE 4:** Chromatogram for *H.cymosum* aquaponics plants (T1).

**TABLE 5:** Volatile compounds in *helichrysum cymosum* plants grown under field, aquaponics and hydroponic.

| Compounds | Aquaponics peak area in the chromatogram | Hydroponics peak area in the chromatogram | Field plants peak area in the chromatogram | Retention times |
|---|---|---|---|---|
| Decane | 1.26 ± 0.025† | 0.83 ± 0.02‡ | 1.17 ± 0.05† | 5.48 |
| Alpha-pinene | 12.53 ± 2.18† | 24.33 ± 6.09† | 20.06 ± 0.78† | 5.79 |
| Nonadecane | 0.51 ± 0.01‡ | 0.19 ± 0.01† | 0.20 ± 0.03† | 6.68 |
| Camphene | 0.35 ± 0.04† | 0.11 ± 0.01‡ | 0.25 ± 0.00† | 7.01 |
| 4-methyl-octane | 4.34 ± 0.08‡ | 1.19 ± 0.43† | 1.64 ± 0.08† | 7.38 |
| Beta-pinene | 4.34 ± 0.08‡ | 1.22 ± 0.42† | 1.64 ± 0.08† | 8.13 |
| Undecane | 0.55 ± 0.02† | 0.57 ± 0.01† | 0.41 ± 0.00‡ | 8.34 |
| Alpha-phellandrene | 0.51 ± 0.03† | 0.36 ± 0.05† | 0.00 ± 0.00‡ | 8.47 |
| Myrcene | 0.90 ± 0.02§ | 0.21 ± 0.05† | 0.50 ± 0.03‡ | 9.59 |
| Alpha-terpinene | 0.77 ± 0.15† | 0.52 ± 0.05† | 0.41 ± 0.03† | 10.9 |
| Limonene | 2.77 ± 0.05† | 0.90 ± 0.16‡ | 2.49 ± 0.19† | 11.9 |
| Beta-phellandrene | 0.89 ± 0.03§ | 0.51 ± 0.07‡ | 0.13 ± 0.00† | 12.28 |
| 1,8-cineole | 57.177 ± 3.68‡ | 31.28 ± 8.85† | 31.28 ± 8.85†,‡ | 12.9 |
| o-ethyltoluene | 0.14 ± 0.01†,‡ | 0.00 ± 0.00† | 0.24 ± 0.07‡ | 12.19 |
| Cis-ocimene | 6.49 ± 1.07‡ | 2.01 ± 0.41† | 4.86 ± 0.20†,‡ | 14.17 |
| Gamma-terpinene | 2.45 ± 1.22† | 0.02 ± 0.01† | 1.41 ± 0.05† | 11.87 |
| Styrene | 2.65 ± 0.04§ | 0.89 ± 0.10† | 2.09 ± 0.06‡ | 14.72 |
| Trans-beta-ocimene | 2.70 ± 0.06§ | 0.88 ± 0.10† | 2.22 ± 0.12‡ | 14.72 |
| Para-cymene | 3.32 ± 0.16† | 14.76 ± 4.69† | 9.16 ± 0.87† | 15.24 |
| 1,2,3-trimethylbenzene | - | - | 0.62 ± 0.06‡ | 9.13 |

Table 5 continues on the next page →

**TABLE 5 (Continues...):** Volatile compounds in *helichrysum cymosum* plants grown under field, aquaponics and hydroponic.

| Compounds | Aquaponics peak area in the chromatogram | Hydroponics peak area in the chromatogram | Field plants peak area in the chromatogram | Retention times |
|---|---|---|---|---|
| alpha-fenchene | 0.20 ± 0.05† | 0.11 ± 0.01† | 0.52 ± 0.06‡ | 15.66 |
| Alpha-terpinolene | 0.23 ± 0.12† | 0.10 ± 0.03† | 0.40 ± 0.02† | 15.76 |
| Cyclohexanone | 0.82 ± 0.02‡ | 0.35 ± 0.03† | 3.89 ± 0.12§ | 15.76 |
| 2,6,6-trimethylcyclohexanone | 0.17 ± 0.09† | - | 0.01 ± 0.00† | 15.99 |
| cis-3-hexenyl_acetate | 0.44 ± 0.02‡ | - | - | - |
| trans-2-heptenal | 0.43 ± 0.01§ | 0.16 ± 0.01‡ | 0.01 ± 0.01† | 11.6 |
| 6-methyl-5-hepten-2-one | 1.02 ± 0.01† | 0.88 ± 0.15† | 0.69 ± 0.02† | 17.41 |
| Allo-ocimene | 0.94 ± 0.08† | 2.71 ± 0.15‡ | 0.55 ± 0.06† | 19.19 |
| Cis-3-hexenol | 0.16 ± 0.00§ | 0.08 ± 0.00‡ | 0.04 ± 0.00† | 19.74 |
| 4-methyl-1,5-heptadiene | 2.09 ± 0.00§ | 0.83 ± 0.08‡ | 0.19 ± 0.07† | 20 |
| 3-ethyl-o-xylene | 0.26 ± 0.03‡ | 0.11 ± 0.00† | 0.08 ± 0.01† | 20.83 |
| Para-cymenyl | 0.40 ± 0.08‡ | 1.85 ± 0.17† | 2.02 ± 0.23† | 20.96 |
| Tetradecane | 2.48 ± 0.26† | 2.11 ± 0.22† | 2.34 ± 0.30† | 21.05 |
| 1-octen-3-ol | 4.07 ± 0.79‡ | 1.59 ± 0.16† | 1.12 ± 0.07† | 21.74 |
| Beta-fencyl acetate | 3.79 ± 0.79‡ | 0.84 ± 0.44† | 0.03 ± 0.01† | 21.9 |
| 6-methyl-5-hepten-2-ol | 0.30 ± 0.00† | 0.27 ± 0.08† | 0.31 ± 0.08† | 22.12 |
| 2,5-dimethyl-p-xylene | 0.51 ± 0.03† | 0.26 ± 0.01‡ | 0.41 ± 0.02† | 22.28 |
| Alpha-ylangene | 0.45 ± 0.03‡ | 0.09 ± 0.04† | 0.06 ± 0.00† | 22.63 |
| Italicene | 0.01 ± 0.00‡ | 0.00 ± 0.00† | 0.00 ± 0.00† | 22.99 |
| Benzaldehyde | 1.19 ± 0.08‡ | 0.70 ± 0.07† | 0.48 ± 0.05† | 23.16 |
| Allyl_isopentanoate | 0.16 ± 0.02† | 0.03 ± 0.01† | 0.16 ± 0.09† | 23.66 |
| Gamma-curcumene | 0.43 ± 0.10‡ | - | 0.22 ± 0.03†,‡ | 20.05 |
| l-linalool | 0.14 ± 0.01† | 0.05 ± 0.01‡ | 0.14 ± 0.01† | 24.32 |
| Alpha-copaene | 0.37 ± 0.01‡ | 0.07 ± 0.01† | 0.05 ± 0.01† | 24.37 |
| cis-sabinene_hydrate | 0.28 ± 0.01§ | 0.08 ± 0.02‡ | 0.00 ± 0.00† | 24.63 |
| alpha-farnesene | 0.59 ± 0.17†,‡ | 0.56 ± 0.05† | 1.13 ± 0.11‡ | 24.96 |
| Fenchol | 1.96 ± 0.04† | 1.65 ± 0.07† | 2.78 ± 0.30‡ | 25.13 |
| Beta-caryophyllene | 115.48 ± 24.27† | 76.78 ± 7.70† | 90.04 ± 13.21† | 25.71 |
| (+)-aromadendrene | 1.49 ± 0.82† | 32.11 ± 18.52† | 0.23 ± 0.05† | 25.8 |
| Delta-elemene | 0.51 ± 0.08†,‡ | 0.78 ± 0.11‡ | 0.34 ± 0.01† | 25.84 |
| (-)-Isoledene | 0.05 ± 0.03† | 0.05 ± 0.00† | 0.20 ± 0.00† | 26.59 |
| Ethyl-caprate | 2.31 ± 0.94† | 0.336 ± 0.07† | 0.01 ± 0.00† | 26.87 |
| Pinocarveol | 0.62 ± 0.12† | 0.36 ± 0.06† | 0.78 ± 0.14† | 27.04 |
| Gamma-elemene | 0.64 ± 0.02† | 0.22 ± 0.01† | 5.44 ± 0.51‡ | 27.11 |
| Alpha-humulene | 8.50 ± 1.21‡ | 2.44 ± 0.30† | 5.23 ± 0.42†,‡ | 27.74 |
| Linalyl-propanoate | 1.85 ± 0.15‡ | 0.75 ± 0.23† | 1.42 ± 0.13†,‡ | 27.81 |
| Alpha-humulene | 6.11 ± 3.05† | 0.23 ± 0.11† | - | 20.23 |
| Acoradiene | 0.32 ± 0.04‡ | 0.05 ± 0.02† | 0.03 ± 0.01† | 27.91 |
| 1,8-menthadien-4-ol | 0.09 ± 0.02† | 0.11 ± 0.05† | 0.41 ± 0.11† | 28.09 |
| Beta-himachalene | 19.27 ± 2.15‡ | 10.87 ± 0.70† | 5.79 ± 1.18† | 28.14 |
| Beta-himachalene | 18.98 ± 2.06‡ | 10.59 ± 0.74† | 5.64 ± 1.27† | 23.75 |
| Alpha-terpineol | 18.98 ± 2.06‡ | 10.59 ± 0.74† | 16.19 ± 1.01†,‡ | 19.05 |
| Ledene | 8.45 ± 4.00†,‡ | 0.20 ± 0.02† | 16.26 ± 1.18‡ | 28.65 |
| (+)-2-carene | 0.37 ± 0.21† | - | 0.00 ± 0.00† | 28.73 |
| Valencene | 3.61 ± 1.11† | 1.09 ± 0.18† | 1.00 ± 0.01† | 24.09 |
| Alpha-gurjunene | 3.57 ± 1.14† | 1.08 ± 0.18† | 0.93 ± 0.01† | 29.08 |
| Eremophilene | 3.57 ± 1.14† | 1.09 ± 0.19† | 0.98 ± 0.03† | 29.11 |
| Beta-Selinene | 3.28 ± 1.07† | 0.98 ± 0.15† | 1.27 ± 0.27† | 29.11 |
| Neryl-acetate | 15.71 ± 6.02† | 4.56 ± 0.63† | 1.62 ± 0.08† | 29.93 |
| Cis-alphabisabonele | 14.56 ± 5.68† | 4.42 ± 0.58† | 1.47 ± 0.17† | 29.31 |
| Beta-bisabonele | 1.13 ± 0.01† | 0.36 ± 0.07‡ | 1.22 ± 0.05† | 29.37 |
| Alpha-cedrene | 0.53 ± 0.01§ | 0.13 ± 0.02† | 0.31 ± 0.03‡ | 29.54 |
| 7-epi-alpha-selinene | 15.11 ± 5.98† | 4.69 ± 0.67† | 1.82 ± 0.12† | 29.93 |
| delta-cadinene | 15.11 ± 5.98† | 4.68 ± 0.67† | 1.88 ± 0.14† | 30.24 |
| Alpha-curcumene | 9.86 ± 1.46† | 4.68 ± 0.42‡ | 14.04 ± 0.72† | 30.24 |
| Ar-curcumene | 9.71 ± 1.18‡ | 4.74 ± 0.37† | 14.01 ± 0.61§ | 30.57 |
| Gamma-selinene | 10.01 ± 1.62† | 4.73 ± 0.38‡ | 14.43 ± 0.62† | 30.62 |
| Alpha-cadinene | 0.22 ± 0.08† | 0.04 ± 0.00† | 0.15 ± 0.06† | 30.95 |
| Nerol | 0.21 ± 0.09 | 0.19 ± 0.02 | 0.31 ± 0.02 | 31.06 |
| 2-phenylethyl-acetate | 0.10 ± 0.05† | 0.10 ± 0.04† | 0.00 ± 0.00† | 31.18 |
| Isogeraniol | 0.11 ± 0.01‡ | 0.18 ± 0.00§ | 0.03 ± 0.01† | 31.29 |
| Trans-beta-damascenone | 0.11 ± 0.00‡ | 0.20 ± 0.00§ | 0.04 ± 0.00† | 31.29 |

Table 5 continues on the next page →

**TABLE 5 (Continues...):** Volatile compounds in *helichrysum cymosum* plants grown under field, aquaponics and hydroponic.

| Compounds | Aquaponics peak area in the chromatogram | Hydroponics peak area in the chromatogram | Field plants peak area in the chromatogram | Retention times |
|---|---|---|---|---|
| 1s-(cis)-calamenene | 0.39 ± 0.13† | 0.38 ± 0.01† | 1.17 ± 0.02‡ | 31.5 |
| Trans- (+)-carveol | 0.04 ± 0.01‡ | 0.00 ± 0.00† | 0.15 ± 0.01§ | 31.61 |
| p-cymen-8-ol | 0.48 ± 0.05† | 0.20 ± 0.00‡ | 0.55 ± 0.04† | 31.85 |
| 4-phenyl-2-btanone | 0.38 ± 0.00‡ | 0.19 ± 0.01† | 0.55 ± 0.02§ | 31.87 |
| Ethyl-laurate | 3.700 ± 1.14‡ | 2.02 ± 0.18†,‡ | 0.53 ± 0.03† | 31.98 |
| (e)-geranyl-acetone | 4.92 ± 1.83‡ | 2.30 ± 0.28†,‡ | 1.35 ± 1.35† | 32.02 |
| Ascaridole | 4.44 ± 1.57‡ | 2.12 ± 0.24†,‡ | 1.34 ± 0.03† | 32.02 |
| Benzyl-alcohol | 0.61 ± 0.07‡ | 0.38 ± 0.01† | 0.28 ± 0.00† | 32.24 |
| 4-ethyl-o-xylene | 0.36 ± 0.06‡ | 0.14 ± 0.01† | 0.04 ± 0.01† | 32.24 |
| Ethyl-3-phenylpropionate | 0.38 ± 0.08‡ | 0.16 ± 0.02† | 0.02 ± 0.00† | 32.4 |
| Phenylethyl-alcohol | 0.55 ± 0.02‡ | 0.24 ± 0.01† | 0.26 ± 0.02† | 32.4 |
| Alpha-calacorene | 0.33 ± 0.04† | 0.13 ± 0.00‡ | 0.34 ± 0.03† | 32.8 |
| Palustrol | 0.11 ± 0.00† | 0.06 ± 0.00† | 0.27± 0.03† | 32.92 |
| Alpha-cubene | 0.16 ± 0.00† | 0.05 ± 0.00† | 0.31 ± 0.05‡ | 33.23 |
| Caryophyllene-oxide | 3.55 ± 0.18‡ | 1.29 ± 0.04† | 3.56 ± 0.20‡ | 33.91 |
| (+)-ledol | 0.01 ± 0.00† | 0.01 ± 0.00† | 0.02 ± 0.00† | 34.53 |
| Alpha-caryophyllene-alcohol | 0.78 ± 0.05† | 0.52 ± 0.02† | 1.37 ± 0.09‡ | 34.82 |
| Fonenol | 2.20 ± 0.85‡ | 0.65 ± 0.10† | 1.13 ± 0.09† | 34.83 |
| Longifolenaldehyde | 0.04 ± 0.02† | 0.05 ± 0.01† | 1.15 ± 0.09‡ | 34.87 |
| n-benzylidenecyclohexylamine | 0.02 ± 0.00† | 0.01 ± 0.00† | 0.16 ± 0.01‡ | 35.01 |
| Cyclooctanone | 0.43 ± 0.07‡ | 0.09 ± 0.01† | 0.07 ± 0.02† | 35.16 |
| Caryophyll-5-en-2-beta-ol | 0.44 ± 0.05† | 0.32 ± 0.01† | 0.77 ± 0.05‡ | 35.78 |
| t-cadinol | 0.48 ± 0.03† | 0.32 ± 0.01† | 0.77 ± 0.05‡ | 35.78 |
| Eugenol | 0.06 ± 0.01† | 0.1 ± 0.03† | 0.44 ± 0.00‡ | 35.94 |
| (+)-calarene | 10.97 ± 4.27† | 5.92 ± 0.56† | 1.13 ± 0.15† | 36.13 |
| Eudesm-7(11)-en-4-ol | 10.97 ± 4.27† | 5.93 ± 0.56† | 1.13 ± 0.16† | 36.13 |
| Beta-cadinene | 10.99 ± 4.28§ | 5.94 ± 0.56‡ | 0.71 ± 0.40† | 36.19 |
| Epi-bicyclosesquiphellandrene | 0.43 ± 0.05†,‡ | 0.22 ± 0.00† | 0.53 ± 0.07‡ | 36.3 |
| Carvacrolok | 0.16 ± 0.04† | 0.07 ± 0.01† | 0.11 ± 0.01† | 36.46 |
| Alpha-eudesmol | 0.42 ± 0.04† | 0.41 ± 0.05† | 2.23 ± 0.46‡ | 36.62 |
| Beta-eudesmol | 0.11 ± 0.05† | 0.00 ± 0.00† | 2.37 ± 0.47‡ | 36.73 |
| Decanoic-acid | 0.43 ± 0.23† | 0.03 ± 0.00† | 0.14 ± 0.06† | 37.08 |
| (-)-phyllocladene | 0.05 ± 0.00‡ | 0.02 ± 0.01†,‡ | 0.00 ± 0.00† | 38.44 |
| 2,7-dimethyl-1,6-octadiene | 0.01 ± 0.00† | 0.00 ± 0.00† | 0.01 ± 0.00† | 38.57 |
| xanthorrhizol | 0.03 ± 0.00† | 0.02 ± 0.00† | 0.01 ± 0.00† | 40.9 |
| **Total number of compounds** | **104** | **102** | **101** | **-** |

Note: Means followed by the same lowercase letters (†, ‡) in the same column are not significantly different ($p > 0.05$) following comparison using Pearson's chi-square test.

**TABLE 6:** Mean ferric reducing antioxidant power ± standard error (SE) (µmol AAE/g), ABTS ± SE (µmol TE/g) and 2,2-diphenyl-1-picrylhydrazyl ± SE (µmol TE/g) of aerial parts (leaves) of *helichrysum cymosum* leaves grown using different cultivation methods.

| Treatments | FRAP (µmol AAE/g) | TEAC (µmol TE/g) | DPPH (µmol TE/g) |
|---|---|---|---|
| T1 | 1043.71 ± 189.81‡ | 1402.77 ± 244.92‡ | 539.13 ± 169.37‡ |
| T2 | 2657.30 ± 99.59† | 3190.66 ± 41.80† | 1764.97 ± 25.12† |
| T3 | 2719.42 ± 278.72† | 3446.77 ± 408.85† | 1651.2 ± 136.65† |

Note: Means followed by the same lowercase letters († and ‡) in the same column are not significantly different ($p > 0.05$) following comparison using the Mann–Whitney test.

FRAP, ferric reducing antioxidant power; TEAC, trolox equivalent antioxidant capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; T1, aquaponic; T2, hydroponic; T3, field; SE, standard error.

## In vitro fungal activity using the micro-dilution assay

### The minimum inhibitory concentration of *helichrysum cymosum*

There was a significant difference in the minimum inhibitory concentrations among the three cultivation methods when the ethanol extracts of *H. cymosum* plants were assessed against *F. oxysporum* ($df = 2.5$; $\chi^2 = 7.5$; $p = 0.03$). Field plants (T3) were more bioactive at 18 h incubation period with a MIC value of 0.37 mg/mL. In contrast, aquaponics plants (T1) were least active, with a MIC value of 0.75 mg/mL, which was equivalent to the commercial fungicide (Dithane) used as a positive control (Table 7). Generally, field-collected plants had the best fungistatic results.

# Discussion

This study reports various effects of aquaponics and hydroponics on growth metrics, including plant height and dry and fresh weights. Plants cultivated in hydroponic systems yielded higher fresh and dry weights and mean heights than aquaponic plants. However, except for the fresh weight that was significantly higher in hydroponics plants, there was no statistical difference ($p > 0.05$) between these two cultivation methods in the plant heights and dry weights. Interestingly, despite higher levels of macronutrients such as N, P, and Mg occurring in the hydroponic plants, these did not translate to increased growth of plants. These elements influence plant growth and development (Nget et al. 2022; Uchida 2000; Yousaf et al. 2021). Previous research has shown that the availability of N in aquaponics medium, light intensity, root zone temperature, air temperature, nutrient availability, growth stage, and growth pace are factors that affect how plants absorb nutrients (Buzby & Lin 2014). Although aquaponics outperformed hydroponics in plant

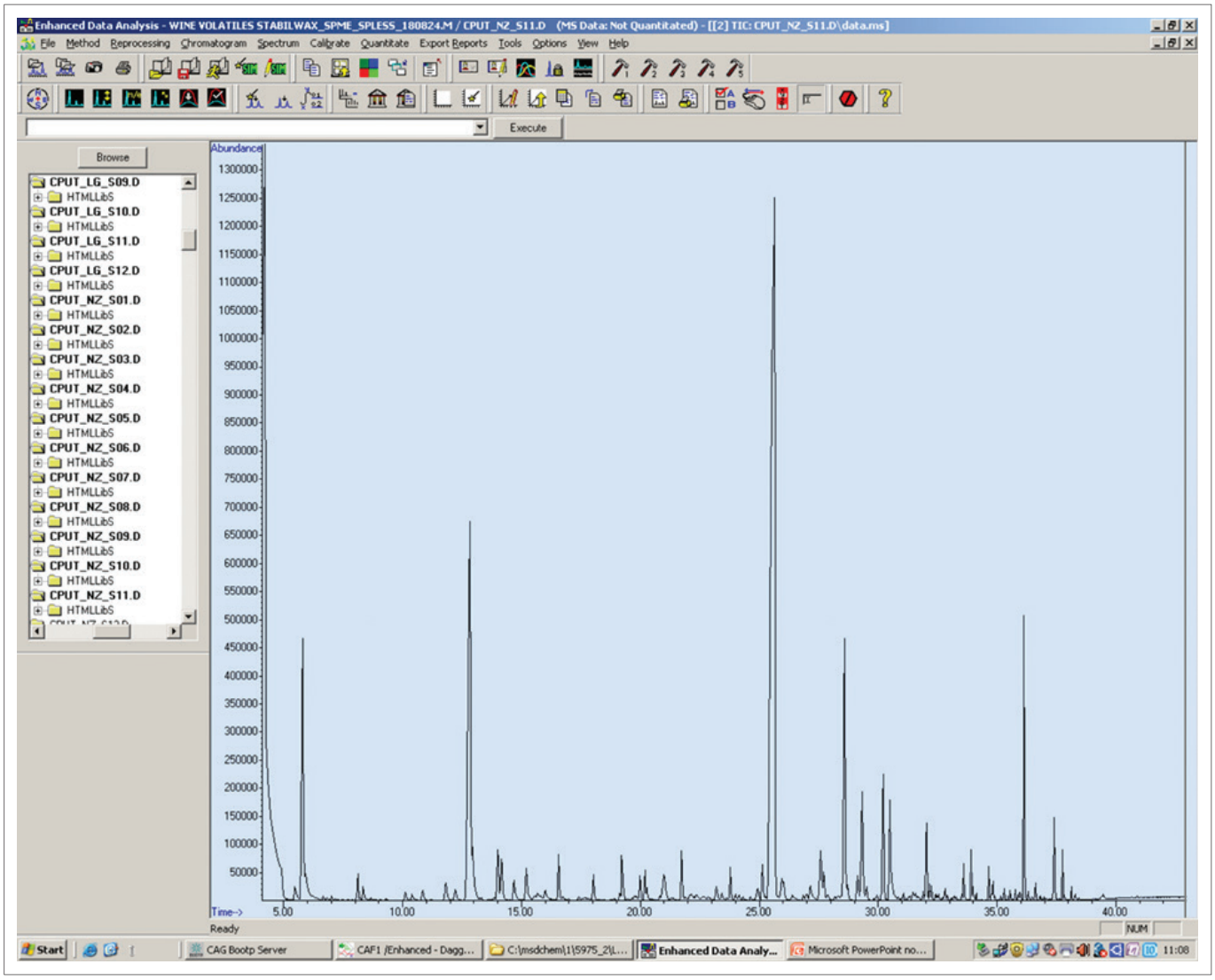


**FIGURE 5:** Chromatogram for *H.cymosum* hydroponics plants (T2).

**TABLE 7:** Anti-*Fusarium oxysporum* activity (mean MIC ± SE) of ethanol extracts of *helichrysum cymosum* plants that were cultivated under aquaponic, hydroponic and field systems.

| Treatments | MIC (Mean ± SE) (mg/mL) 18 h |
|---|---|
| T1 | 0.75 ± 0‡ |
| T2 | 0.56 ± 0.125†,‡ |
| T3 | 0.37 ± 0.00† |
| Positive control (Dithane) | 0.75 ± 0.00‡ |
| Negative control (Ethanol) | 0 |

Note: Means followed by the same lowercase letters (†, ‡ in the same column are not significantly different ($p > 0.05$) following comparison using the Mann–Whitney test.

MIC, minimum inhibitory concentration; SE, standard error; T1, aquaponic; T2, hydroponic; T3, field.

micronutrient uptake, previous research has demonstrated that plant species may behave differently in the system (Ibáñez Otazua et al. 2022). According to Delaide et al. (2016), adding mineral nutrients to an aquaponic solution to achieve the same nutrient concentrations as hydroponics can occasionally result in higher yields. The primary source of N and P in the aquaponic system is fish feed. When the fish feed is introduced to the system, a sizable portion of it is consumed by the fish for development and metabolism and the waste is eliminated as soluble and solid faeces.

Higher secondary metabolite concentrations (total phenolic and flavonol contents) in field-collected *H. cymosum*, obtained in this study could, for example, be related to abiotic stresses such as exposure to drought and nutrient deficiency, insect and fungal exposure (Akula & Ravishankar 2011; Bennett & Wallsgrove 1994; Chalker-Scott & Fuchigami 2018). The production of secondary metabolites is a plant's mechanism for adjusting to unfavourable environmental changes (Edreva et al. 2008; Kosová et al. 2018). Through signalling mechanisms and pathways, this process involves the production of complex chemicals. Interestingly, higher production of bioactive secondary metabolites may translate to higher antifungal and antioxidant activities.

In this study, the antioxidant capacity of the tested samples showed a strong correlation with the polyphenol and flavonol contents. Hydroponics and field-collected plants were not significantly different in antioxidant capacities; however, they performed better than aquaponic-cultivated plants. Previous studies have reported that the secondary metabolite components of *Cantella asiatica*, such as anthocyanins, flavonoids, and phenolic substances, are

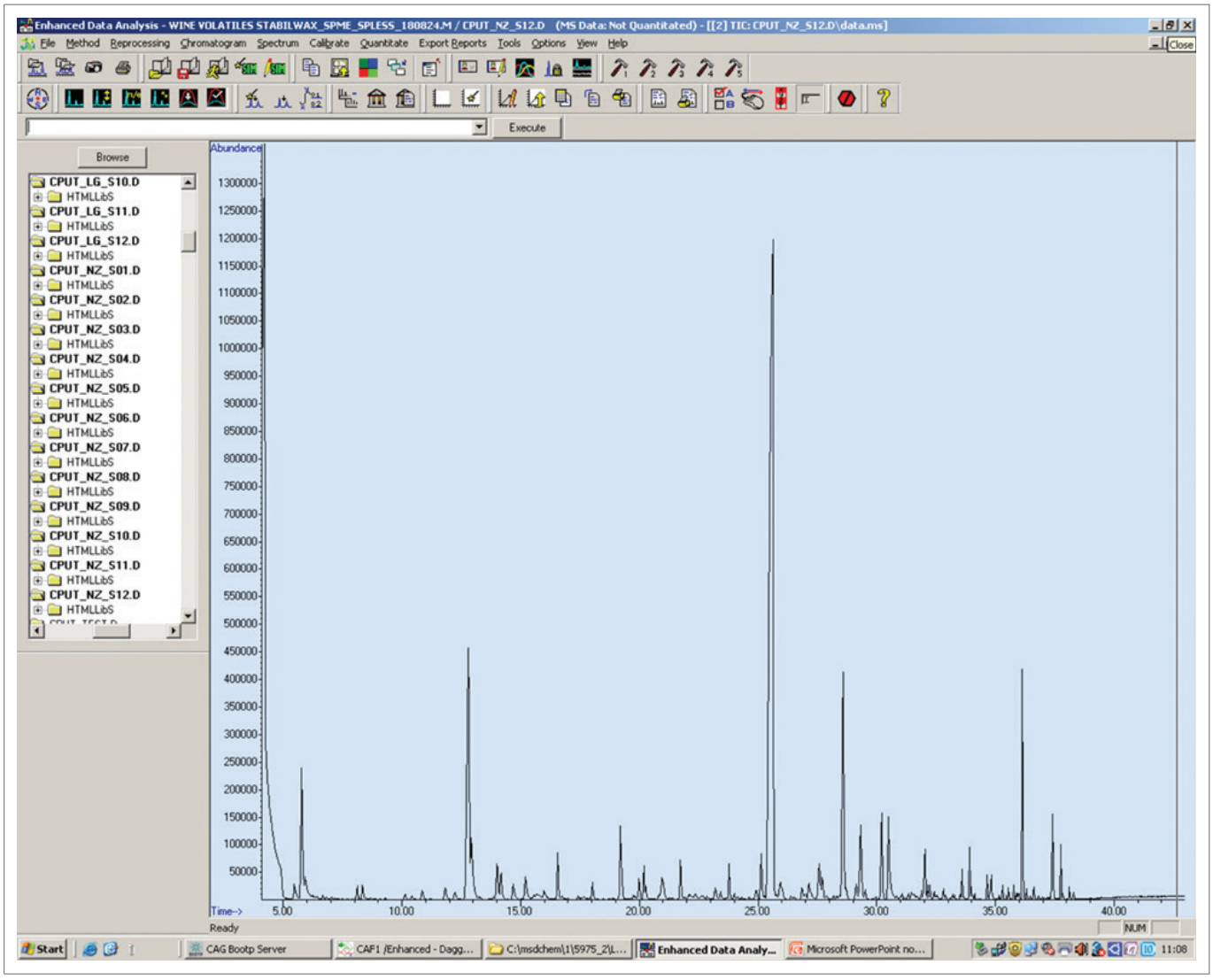


**FIGURE 6:** Chromatogram for *H.cymosum* hydroponics plants (T2).

thought to be responsible for its antioxidant action (Shin et al. 2021). Additionally, it is believed that phenolic compounds play a significant role in the sensory qualities and antioxidant activity of wines (Pandeya et al. 2018). Recently, Ibarra-Cantún et al. (2020) found that apple bagasse's antioxidant activity increased progressively with levels of polyphenols, which is consistent with the results obtained in this study. The FRAP and ABTS bioassays showed that plants harvested from the field had higher antioxidant capacity than plants produced in aquaponics and hydroponic systems. These results suggest that plant extracts of *H. cymosum* have quite high antioxidant activity and high phenol and flavonoid contents, which are important natural antioxidants that are often exploited in treating ailments associated with oxidative stresses and disorders (Kripasana & Xavier 2020).

The MIC findings demonstrated that ethanol extract from field-grown *H. cymosum* species performed better, and these results correlated with the total polyphenol contents. It is worth observing that when plants are subjected to environmental stresses, they acquire large amounts of bioactive compounds (Ncube et al. 2011). Several studies have investigated the antifungal activities of *Helichrysum* spp., including *H. cymosum*; for example, in a study reported by Van Vuuren et al. (2006), field-collected *H. cymosum* was active against 11 pathogens, with MIC values ranging from 0.156 mg/mL to 0.313 mg/mL. Notably, these results corroborate this study's anti-*F. oxysporum* bioassay results reported MIC values ranging from 0.375 mg/mL to 0.75 mg/mL. Matanzima (2014), who previously tested hydroponically grown plants against *F. oxysporum* reported MIC values ranging from 0.078 mg/mL to 03.31 mg/mL. This suggests that the variation in phytochemical profiles in plant extracts in the different treatments may explain the differences in bioactivities.

Based on the GC-MS analysis, *H. cymosum* is a rich source of volatile compounds; the plants obtained from the three cultivation methods (aquaponics, hydroponics, and field cultivated plants) contained up to 104 compounds with 90% match with the mass spectra library. These compounds included nonadecane, 4-methyl-octane, beta-pinene, cis-ocimene, 1-octen-3-ol, beta-fencyl acetate, benzaldehyde,

alpha-humulene, linalyl propanoate, acoradiene, beta-himachalene, alpha-cedrene, alpha-ced 2,7-dimethyl-1,6-octadiene, trans- (+)-carveol, (-)-phyllocladene, and cyclooctanone were among the compounds that were dominating in aquaponics produced plants when compared with hydroponics and field-collected plants. Alpha-humulene and beta-pinene have been reported as compounds with potent antifungal properties (Ruiz-Vásquez et al. 2022; Zuzarte et al. 2021). Aquaponic plants produced the highest number of volatile compounds (106).

The three cultivation methods, however, did not differ statistically ($p > 0.05$). In general, plants obtained from the field had the lowest overall number of volatiles (101). Trans-caryophyllene is a sesquiterpene found in the essential oils of many therapeutic genera, including the *Helichrysum* genus. Many studies have documented its pharmacological effects, including its antibacterial (Moo et al. 2020), anti-helicobacter pylori (Woo et al. 2020), antioxidant and anti-inflammatory (Ames-Sibin et al. 2018), analgesic and anticancer potential, neuroprotective (Machado et al. 2018). Also, some of these chemicals, such as nanodecane, were previously found as aliphatic hydrocarbons in the essential oil of *Helichrysum* plants by hydrodistillation (Radušienė & Judžentienė 2008). However, nanodecane has also been discovered to be a plant secondary metabolite with antioxidant and antifungal properties in plants other than the *Helichrysum* genus (Ganesan & Raja 2021). *Helichrysum cymosum* chemical constituents were previously determined using GC-MS (Van Vuuren et al. 2006). Remarkably, alpha-humulene, trans- (+)-carveol, 1-octen-3-ol, and beta-pinene are among the compounds discovered, which match some of the substances reported in this study. By comparing the percentages of the compounds and their retention indices, Bougatsos et al. (2004) identified 65 phytochemicals as essential oil components, some of which were also detected in this study: Beta-pinene, cis-ocimene, 1-octen-3-ol, and trans- (+)-carveol. Also, although aquaponics had a higher quantity of volatile compounds than hydroponics and field-collected plants, the chemical compositions of *H. cymosum* oils were very similar between the three growing systems. It is, however, worth observing that a few compounds occurred in aquaponics but not in hydroponics or field-collected plants, such as (+)-2-carene; 1,2,3-trimethylbenzene; 2,6,6-trimethylcyclohexanone; cis-3-hexenyl-acetate; trans-2-heptenal; octenyl acetate cis-3-hexenol; 3-octanol-istd; gamma-curcumene.

# Conclusion

The key findings of this study revealed that aquaponic-cultivated *H. cymosum* yielded extracts with significantly lower antioxidant capacity and polyphenol contents than hydroponics and field-cultivated plants. However, there were no significant differences between field and hydroponic systems regarding antioxidant capacities and polyphenol contents. The fungistatic activity of the ethanol extracts against *F. oxysporum* varied with the cultivation methods. This study should be replicated for other *Helicrysum* spp. to identify suitable species for hydroponic and aquaponic cultivation. The results of the present study suggest that cultivation of *H. cymosum* using hydroponic system may be feasible.

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## Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

## Authors' contributions

Z.N., L.K. and F.N. conceptualised the study and designed the experiment. Z.N. performed the experiment, interpreted the data, and prepared the draft manuscript. F.N., N.G.E.R.E. and L.K. corrected the manuscript and supervised the research. N.G.E.R., L.K. and F.N. revised the final version of the manuscript.

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## Data availability

The data that support the findings of this study are openly available from the Cape Peninsula University of Technology eSango at https://doi.org/10.25381/cput.23766105.v1.

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