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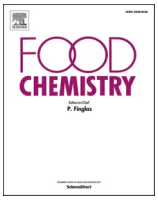

Magnesium and manganese induced changes on chemical, nutritional, antioxidant and antimicrobial properties of the pansy and Viola edible flowers

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ABSTRACT

Pansy and viola edible flowers were grown hydroponically with different levels of Mg and Mn. The nutritional composition was determined using standard methods. Free sugars, fatty acids, organic acids, tocopherols, and phenolic compounds were analyzed using various HPLC and GC devises. The extract's antimicrobial, antioxidant, cytotoxicity, and anti-inflammatory activity were assessed. The results indicated that Mg enrichment negatively affected plant growth and mineral accumulation but improved photosynthetic performance. The edible flowers contained significant amounts of protein, low levels of fat, and varying sugar contents, such as glucose and fructose. Various fatty acids and phenolic compounds were identified, with different concentrations depending on the treatment. The flowers exhibited antioxidant potential, antimicrobial activity, cytotoxic effects, and anti-inflammatory properties. The correlations between the investigated parameters not only expand knowledge on Mg and Mn interaction but also catalyze significant advancements in sustainable agriculture and food health, fostering a healthier and more conscious future.

1. Introduction

Research into new foods that are healthy has expanded due to the growing interest in nutraceuticals and functional foods and their importance, and linked to the prevention and care of chronic diseases (Benvenuti, Bortolotti, & Maggini, 2016). In addition to nutritional value and useful attributes, consumers seek out novel emotions. Edible flowers meet these needs by bringing fresh and appealing colors, textures, and vibrancy to meals (González-Barrio et al., 2018). The use of edible flowers has been documented for thousands of years; however, they only make up a small portion of the market and are seen as a culinary curiosity nowadays. In reality, there are several historical examples of the usage of inflorescences in food preparation and garnishing, dating from the ancient civilizations of the Greeks and Romans (Fernandes, Casal, Pereira, Saraiva, & Ramalhosa, 2017). By enhancing culinary dishes with color, smell, flavor, and visual appeal, edible flowers enhance the sensory characteristics of food, however, they are highly perishable and have a short shelf life. Indeed, neither any official lists of edible and non-edible flowers by any international body nor any legal requirements for edible flowers marketing is documented (Fernandes, Casal, Pereira, Pereira, Saraiva & Ramalhosa, 2019), indicating the necessity to take adequate measures for the safe cultivation, preservation, composition and uses. The properties of edible flowers might be affected by several abiotic and biotic factors, to that sense the nutritive value, antioxidant, antimicrobial, cytotoxical are only few of the important attributes of interest when evaluating ''new foods''.

Viola cornuta L. (horned pansy), *Viola tricolor* L. (Johnny Jumpup), and *Viola* x *wittrockiana* Gams (garden pansy) are the three species of edible violas, with the latter to have the largest flower size (around 11.5 cm) and the formers smaller flower size of 2.5 cm (Vukics, Kery &

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Guttman, 2008). *Viola tricolor* was studied as a good model plant to investigate microevolutionary processes. *Viola tricolor* may prove to be a good and affordable source of amino acids for use in food, cosmetics, pharmaceuticals, or medicine and revealed positive impact on human health, including its ability to treat respiratory issues, eczema, asthma, and rheumatic pain as well as cytotoxic activities against cancer cells and immunosuppressive activity (Dziągwa-Becker, Weber, Zajączkowska, & Oleszek, 2018).

The garden pansy (*Viola* × *wittrockiana*) is one of the most widely grown cool-season garden crops for landscaping and one of the top five bedding plants in both developed and developing nations (Gandolfo, Hakim, Geraci, Feuring, Giardina & Benedetto,2016). Pansies are edible flowers that can be used to decorate desserts or salads, or when crystallized to be consumed as a sweet treat (Fernandes et al., 2019). Pansies flower are high in health-promoting compounds, like anthocyanins, carotenoids, flavonoids, potassium, and phosphorus (Rop, Mlcek, Jurikova, Neugebauerova, & Vabkova, 2012). Rop et al. (2012) reported that, out of the 12 edible flowers examined, *Viola* x *wittrockiana* flowers have the highest concentration of mineral components.

Mineral accumulation in edible parts of a plant tissue is of great interest, on top of other nutritive and biochemical properties. Several strategies are used to fortify crops with minerals, including direct application in soil or soilless culture, foliar application, and balanced mineral antagonisms/availability. However, the application of synthetic fertilizers in excess or the uncontrolled use of manure or organic wastes which are overdosed on minerals, including heavy metal, can negatively affect crop production, food quality and safety with environmental constraints.

Magnesium (Mg) is the fourth most prevalent cation in living organisms (human body: calcium > potassium > sodium > magnesium), the second and the sixth most abundant cation in the hydrosphere and in the lithosphere, respectively (Farzadfar, Zarinkamar, & Hojati, 2017). Mg has a functional role on chlorophyll molecules and involved in several enzymatic reactions including nucleotide metabolisms and nucleic acid folding (Farzadfar et al., 2017). Magnesium is required by numerous enzymes, which is also present in our bones and teeth (Rop et al., 2012).

Manganese (Mn), an essential microelement in plants, involved in many biochemical pathways as an enzyme cofactor such as chlorophyll biosynthesis and photosynthetic capacity, has been linked to normal plant growth and development, stress tolerance, and structural and catalytic functions (Rajpoot, Rani, Srivastava, Pandey, & Dubey, 2018). Mn is the second most prevalent transition metal after iron in the crust of the earth, with soil levels ranging from 0.45 to 4.0 g/kg (Shao, Yamaji, Shen, & Ma, 2017). However, under acidic soil conditions, Mn availability is increased and can be under toxic levels in acidic soils with detrimental impacts on plant growth and productivity (Li et al., 2010). At Mediterranean climate conditions, periods of extremely hot and dry summer weather are typically followed by severe winter rainstorms that cause soil to become waterlogged and change the soil's redox potential, increasing the amount of bioavailable Mn (Faria et al., 2020).

The objectives of this study, which took into account all the aforementioned details, were to enhance specific chemical properties or bioactivities for the edible flowers of pansy and viola under the increased levels of Mg, Mn and/or their combination, in hydroponics. The nutritional composition, identify and quantify the chemical properties, bioactive, cytotoxic and anti-inflammatory activities for the edible flowers were determined.

2. Materials and methods

2.1. Plant material and growing conditions

The experiment took place at the greenhouse hydroponic infrastructures equipped with an automated climatic control system at Cyprus University of Technology, Cyprus. The hydroponic system adapted was the Nutrient Film Technique (NFT), with 16 individual NFT channels (twins white plastic channels) that were aligned with 16 catchments (60 L) to set up 16 independent hydroponic units. Four treatments were used in the present study, with each treatment had four replications (independent hydroponic units) and each replication accompanied 8 plants for viola and 8 plants for pansies, giving a final plant density of 25 plants/m^2. The climatic conditions during the trials were: air temperature fluctuated among 16.9 and 32.7 °C during this period.

Young seedlings of viola (*Viola tricolor* var. Hortensis) and pansy (*Viola* × *wittrockiana*) (series Delta Mix) were grown in peat-based media and were purchased from commercial nursery. Seedlings were transplanted into netted pot and placed in the NFT channels holes. Plants were grown for 1 week under complete NS to allow recovery from transplant stress. The composition of the nutrient solution in water was: NO_3^--N = 14.00, K^+ = 8.31, PO_4^{3-}-P = 2.26, Ca^{2+} = 3.74, Mg^{2+} = 2.50, SO_4^{2-}-S = 2.22 and Na^+ = 1.91 mmol/L, respectively; and B = 18.51, Fe = 71.55, Mn = 18.00, Cu = 4.73, Zn = 1.54, and Mo = 0.51 μmol/L, respectively. Following the 1 week, plants were subjected for 75 days in the modified NS for Mg and Mn concentrations, resulting in the following four treatments: 1) 2.5 mmol/L Mg; 18 μmol/L Mn (named as control or -Mg-Mn), 2) high Mg of 5.0 mmol/L; 18 μmol/L Mn (named as + Mg), 3) high Mn of 100 μmol/L; 2.5 mmol/L Mg (named as + Mn), and 4) high + Mg + Mn of 5.0 mmol/L Mg + 100 μmol/L Mn (named as + Mg + Mn). The NS was checked every day for the pH and electric conductivity (EC) levels, and adjusted accordingly, by using HNO_3 (5 % *v/v*) for pH maintenance, and relevant modified NS for the EC balance. The target pH and EC of the nutrient solution were 5.8 and 2.2 mS/cm respectively.

2.2. Plant growth, physiology, and tissue analysis

Following 75 days of plant growth, leaf stomatal conductance was measured by using a Delta-T AP4 dynamic porometer (Delta-T Devices-Cambridge, UK). To assess leaf photochemistry, relative chlorophyll content (optical chlorophyll meter SPAD-502, Minolta, Osaka, Japan) and leaf chlorophyll fluorescence with the maximum photochemical quantum yields F_v/F_m of PSII were measured with the OptiSci OS-30p Chlorophyll Fluorometer (Opti-Sciences). Moreover, plant tissue (six replications/treatment; each replication was a pool of two plants tissue; 0.1 g) was incubated in heat bath at 65 ∘C for 30 min, in the dark, with 10 mL dimethyl sulfoxide (DMSO) for chlorophyll extraction. Leaf photosynthetic pigments such as chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), total chlorophyll (*t*-Chl) and carotenoids content were calculated (Chrysargyris, Papakyriakou, Petropoulos & Tzortzakis, 2019). Following the flower set (day 33 after transplanting), flowers were harvested every 4–5 days (total nine harvests), and the number of flowers and yield (g) per plant was recorded. At the experiment completion, plant upper part fresh weight (fw) and dry weight (dw) were determined.

Mineral content in leaves and flowers were determined in three replications per treatment (three pooled plants/replication). Samples were dried to constant weight (at 65 °C for 4 d) and sub-samples (~0.5 g) were ashed in a furnace (Carbolite, AAF 1100, GERO, Germany) at 450 °C/5h and acid (2 mol/L HCl) digested. Mineral assessment for phosphorus (P) and nitrogen (N) was performed according to Chrysargyris et al. (2019). Potassium (K), sodium (Na), magnesium (Mg), and calcium (Ca) were determined by Ion Chromatography (ICS-3000, Dionex Aquion, Sunnyvale, CA, USA) and quantified through an electrical conductivity detector (IonPac CG12A (4 x 250 mm, Dionex, Corporation) guard columnand IonPac CS19 (4 x 250 mm, Dionex, Corporation) analytical column. Manganese (Mn) copper (Cu), and zinc (Zn) were determined by an atomic absorption spectrophotometer (PG Instruments AA500FG, Leicestershire, UK). Data were expressed in g/kg and mg/kg of dry weight, for macronutrients and micronutrients, respectively.

2.3. Extracts preparation

The sample was extracted by maceration with an 80 % ethanol solution (EtOH:H2O, 80:20, *v/v*). The solution was added to the sample at a ratio of 1 g/30 mL and placed under continuous stirring for 1 h, after which it was filtered (Whatman n○ 4). The residue was re-extracted according to the conditions described above. The ethanolic fraction was evaporated using a rotary vacuum evaporator (Büchi, R-210, Flawil, Switzerland) and the aqueous fraction was lyophilized (-47 °C, 0.045 bar). The extract was then used to determine the phenolic compounds and bioactive properties; the other analyses were based on the dried natural sample (de Oliveira et al., 2023).

2.4. Centesimal composition

To determine the centesimal composition of the flower samples, standard methods were applied (AOAC, 2016). Crude protein (N × 6.25) was determined using the macro-Kjeldahl method, crude fat was extracted with petroleum ether in a Soxhlet apparatus; ash content was calculated by incineration at 550 °C, and total carbohydrates amount was estimated by difference. Finally, the total energy value was calculated using the equation: Energy (kcal) = 4 × (g proteins + g carbohydrates) + 9 × (g lipids).

2.5. Chemical characterization

2.5.1. Sugar composition

Free sugars were determined by high-performance liquid chromatography coupled to a refractive index detector (HPLC-RI; Knauer, Smartline system 1000, Berlin, Germany), using melezitose as internal standard; as following the procedures described by Mandim et al. (2022). Results are expressed in g per 100 g dry weight.

2.5.2. Fatty acids

After Soxhlet extraction, the samples were subjected to transesterification according to the procedure previously reported by Albuquerque et al. (2023). The analysis was performed using gas chromatography (DANI 1000, Contone, Switzerland), equipped with a slit/ slotless injector and a flame ionization detector (FID at 260 °C). Separation was performed with a Macherey-Nagel column (Duren, Germany) (50 % cyanopropylmethyl- 50 % phenylmethylpolysiloxane, 30 m × 0.32 mm ID × 0.25 μm df). Identification of the fatty acids was done by comparison with the relative retention times of the FAME peaks of the standard samples. The results were processed using CSW 1.7 software (Data Apex 1.7, Prague, Czech Republic), and expressed as relative percentage (%).

2.5.3. Tocopherols

Prior to extraction, hexane solutions of butylated hydroxytoluene and tocol (internal standard) were added to the samples. The samples were homogenized with methanol and hexane, and the clear top layer was transferred to a flask after the addition of a saturated aqueous NaCl solution. The extraction was repeated three times and the combined extracts were dried with nitrogen and dissolved in *n*-hexane. The tocopherols were identified and quantified by HPLC with a fluorescence detector and an analysis program. The results were expressed as mg per 100 g dry weight (Mandim et al., 2022).

2.5.4. Phenolic compounds

The obtained extract (section 2.3.) was dissolved with a solution of EtOH:H_2O (20:80, *v/v*) at a final concentration of 10 mg/mL and filtered using a Clarify-Ny 25 mm Syringe filter (Madureira et al., 2023). Detection was performed using an LC-DAD-ESI/MSn system (Dionex Ultimate 3000 UPLC; Thermo Scientific, San Jose, CA, USA) equipped with a diode array detector (DAD using 280, 330, and 370 nm as wavelengths) and an electrospray ionization mass detector (Linear Ion Trap LTQ XL, ThermoFinnigan, San Jose, CA, USA) working in negative mode. Identification of compounds was done by comparing chromatographic parameters with those available in the literature and with commercial standards if available (apigenin-6-*C*-glucoside, caffeic acid, chlorogenic acid, hesperetin, luteolin-7-*O*-glucoside, naringenin, quercetin-3-*O*-rutinoside, rosmarinic acid). Calibration curves were obtained by DAD using the previously mentioned quantification standards, and the results were expressed in mg/g extract (Table 4).

2.6. Bioactive properties

2.6.1. Cytotoxic and anti-inflammatory activities

2.6.1.1. Cytotoxicity. Five human tumor cell lines (MCF-7, NCI-H460, AGS, CaCo-2) and one non-tumor cell line (VERO) obtained from the Leibniz-Institut DSMZ were used to analyze cytotoxicity. Cells were cultured in specific media and their cytotoxicity was assessed using the sulforhodamine B (SRB) assay. Different concentrations of extracts were tested on cells seeded in a 96-well plate, and the test was carried out after 72 h. The GI_{50} values, which indicate the concentration that causes a 50 % inhibition of cell proliferation, were calculated. Ellipticine served as a positive control in the experiment (Marcelino et al., (2023).

2.6.1.2. Anti-inflammatory activity. Anti-inflammatory activity was performed by measuring the concentration of inflammatory mediator nitric oxide (NO) using a murine macrophage cell line (RAW 264.7) stimulated by lipopolysaccharide (LPS) from *Escherichia coli* membrane (Sigma-Aldrich, Saint Louis, MO, USA). The extracts were re-dissolved in water (8 mg/mL) and were successive diluted to obtain the concentrations to be tested (final concentration between 400 and 6.25 μg/mL), as described by de Oliveira et al. (2023). Dexamethasone (50 mmol/L) was used as a positive control. The results were expressed as EC_{50} values (μg/mL) corresponding to the concentrations of the extracts responsible for 50 % NO production inhibition.

2.6.2. Antioxidant activity

2.6.2.1. Thiobarbituric acid reactive substances (TBARS). For the TBARS assay, pig brain tissues were homogenized with a Tris-HCl buffer solution and centrifuged. An aliquot of the supernatant was incubated with the extracts, $FeSO_4$, and ascorbic acid at 37 °C for 1 h. Trichloroacetic acid and thiobarbituric acid solutions were added, and the mixture was heated. After centrifugation, the absorbance of the supernatant was measured. Trolox was used as a positive control. The results were expressed as EC_{50} values, indicating the concentration of the extract providing 50 % of the antioxidant activity (Mandim et al., 2022).

2.6.2.2. Cellular antioxidant activity (CAA). For cellular antioxidant activity, mouse macrophage cells (RAW 264.7) were used. The cells were cultured in DMEM medium with supplements and incubated at 37 °C with 5 % CO_2. When reaching 70–80 % confluence, they were seeded onto a black plate and incubated for 48 h. The evaluation of extract's ability to inhibit reactive oxygen and nitrogen species was performed following a procedure described previously. After incubation, the cells were treated with different concentrations of extracts and incubated for 1 h. Then, a solution was added, and fluorescence was measured using a microplate reader. The results were expressed as a percentage of inhibition of the oxidation reaction (de la Fuente et al., 2022).

2.6.3. Antimicrobial activity

2.6.3.1. Antibacterial potential. The antibacterial activity of the samples was tested against a variety of bacteria, including both Gram-positive and Gram-negative strains. The minimum inhibitory concentration

(MIC) was determined using a colorimetric assay with p-iodonitrotetrazolium chloride (INT) in a 96-well microplate. Stock solutions of the samples at 20 mg/mL were prepared and serially diluted. These diluted samples, along with the bacterial inoculum, were added to the wells of the microplate and incubated at 37 °C for 24 h. MICs were identified as the concentration that prevented color change, indicating inhibition of bacterial growth. The minimum bactericidal concentration (MBC) was defined as the concentration that could kill the bacterial strains (Pires, Dias, Barros, & Ferreira, 2017).

2.6.3.2. Antifungal potential. In antifungal assays, two microfungi (*Aspergillus fumigatus* and *Aspergillus brasiliensis*) were employed. After being cultured on malt agar for 72 h, fungal spores were collected and added to a 96-well microplate along with diluted extract samples. Serial dilutions were performed, and the plates were incubated at 28 °C for 72 h. The Minimum Fungicidal Concentration (MFC) was determined by subculturing the tested compounds and observing for an additional 72 h at 26 °C. The lowest concentration without visible growth was defined as the MFC, indicating 99.5 % killing of the original inoculum. Ketoconazole was used as the positive control (Fernandes et al., 2022).

2.7. Statistical methods

The chemical composition and bioactivity parameters were determined in three different samples for each treatment and all the analyses were performed in triplicate. Plant growth related parameters were determined in six biological samples for each treatment. Data was analyzed with Statgraphics 5.1. plus (Statpoint Technologies, Inc., Warrenton, VA, USA). The statistical analysis showed significant interactions between the tested factors for all the tested parameters, while the comparison of means was performed with the Tukey's HSD or Duncan test ($p = 0.05$).

3. Results and discussion

3.1. Plant growth, physiology, and mineral accumulation

In viola, Mg enrichment in the NS decreased plant growth and mineral (K, Ca, Mg, Na and Cu) accumulation in flowers but enhanced photosynthetic performance with increases in leaf stomatal conductance, chlorophylls and carotenoids content and accumulation of N, K,

Table 1
Effect of increasing magnesium (5.0 mmol/L Mg), manganese (100 μmol/L Mn) or the combination of + Mg + Mn in the nutrient solution on total upper biomass fresh weight (fw; g/plant), and dry weight (dw; g/plant), leaf stomatal conductance (mmol/m^2/s), chlorophyll fluorescence (Fv/Fm), chlorophylls (Chl a, Chl b, Total Chl) and carotenoids content (mg/g fresh weight) and mineral content (g/kg or mg/kg) (nitrogen-N, potassium-K, phosphorus-P, calcium-Ca, magnesium-Mg, sodium-Na in g/kg, and manganese-Mn, zinc-Zn, and copper-Cu in mg/kg), in viola and pansy plants grown hydroponically in NFT. Mean values (n = 3 or n = 6) in rows followed by different letters are significantly different, $p \leq 0.05$, for viola or pansy. n.d. - not detected.

	Viola				Pansy			
Parameters	**Control (-Mg-Mn)**	**+Mg**	**+Mn**	**+Mg + Mn**	**Control (-Mg-Mn)**	**+Mg**	**+Mn**	**+Mg + Mn**
Upper biomass Fw	27.53 ± 5.24^{a}	18.09 ± 2.58^{b}	15.17 ± 3.18^{b}	18.45 ± 1.44^{b}	11.79 ± 2.72^{ab}	13.50 ± 2.94^{a}	11.65 ± 2.62^{ab}	8.61 ± 1.66^{b}
Upper biomass Dw	3.82 ± 0.73^{a}	2.61 ± 0.32^{b}	2.28 ± 0.29^{b}	2.41 ± 0.22^{b}	1.88 ± 0.40	1.95 ± 0.33	1.75 ± 0.22	1.46 ± 0.26
Stomatal conductance	41.92 ± 3.06^{b}	47.80 ± 0.65^{a}	42.12 ± 2.28^{b}	43.12 ± 2.23^{b}	43.58 ± 3.21	46.90 ± 8.85	40.68 ± 7.07	44.37 ± 3.95
Chlorophyll fluorescence	0.80 ± 0.01^{ab}	0.81 ± 0.00a^{b}	0.79 ± 0.01^{b}	0.81 ± 0.01^{a}	0.82 ± 0.01^{ab}	0.81 ± 0.01^{ab}	0.80 ± 0.02^{b}	0.83 ± 0.02^{a}
SPAD	44.60 ± 7.32	43.01 ± 5.56	38.56 ± 4.83	43.29 ± 2.97	52.30 ± 2.84^{a}	51.93 ± 3.69^{a}	44.81 ± 8.57^{ab}	40.78 ± 8.48^{b}
Chlorophyll *a*	0.68 ± 0.05^{b}	0.92 ± 0.11^{a}	0.98 ± 0.11^{a}	0.97 ± 0.15^{a}	0.98 ± 0.07	0.86 ± 0.07	0.92 ± 0.11	1.05 ± 0.19
Chlorophyll *b*	0.24 ± 0.02	0.41 ± 0.19	0.34 ± 0.04	0.43 ± 0.14	0.44 ± 0.08	0.31 ± 0.05	0.34 ± 0.05	0.44 ± 0.14
Total Chlorophylls	0.92 ± 0.08^{b}	1.33 ± 0.29^{a}	1.32 ± 0.15^{a}	1.41 ± 0.28^{a}	1.42 ± 0.16	1.17 ± 0.12	1.27 ± 0.17	1.49 ± 0.33
Carotenoids	0.17 ± 0.01^{b}	0.22 ± 0.02^{a}	0.23 ± 0.02^{a}	0.23 ± 0.03^{a}	0.21 ± 0.02	0.19 ± 0.02	0.20 ± 0.02	0.22 ± 0.05
Leaf N	27.46 ± 0.81^{b}	30.82 ± 0.22^{a}	29.78 ± 0.87^{a}	30.71 ± 0.12^{a}	36.72 ± 0.87^{a}	37.04 ± 0.51^{a}	32.83 ± 1.76^{b}	37.42 ± 1.47^{a}
Leaf K	29.43 ± 0.82^{c}	32.70 ± 1.47^{a}	31.73 ± 0.72^{ab}	29.12 ± 0.85^{bc}	37.52 ± 0.75^{b}	37.45 ± 1.43^{b}	35.71 ± 0.25^{c}	42.25 ± 0.58^{a}
Leaf P	18.83 ± 0.71^{ab}	18.63 ± 0.96^{ab}	17.30 ± 0.96^{b}	20.19 ± 0.15^{a}	28.33 ± 1.62^{a}	22.11 ± 0.74^{b}	19.01 ± 0.76^{c}	23.32 ± 1.32^{b}
Leaf Ca	6.27 ± 0.17^{bc}	7.08 ± 0.27^{a}	5.82 ± 0.36^{c}	6.48 ± 0.22^{b}	7.36 ± 0.12^{a}	6.38 ± 0.20^{b}	7.24 ± 0.06^{a}	6.63 ± 0.23^{b}
Leaf Mg	3.27 ± 0.11^{b}	4.44 ± 0.08^{a}	2.69 ± 0.08^{c}	3.12 ± 0.04^{b}	4.68 ± 0.12^{ab}	4.61 ± 0.05^{bc}	4.51 ± 0.02^{c}	4.80 ± 0.06^{a}
Leaf Na	4.05 ± 0.18^{b}	4.03 ± 0.27^{b}	4.36 ± 0.24^{b}	5.02 ± 0.38^{a}	2.93 ± 0.15^{b}	3.47 ± 0.21^{a}	3.58 ± 0.11^{a}	3.30 ± 0.31^{ab}
Leaf Mn	94.97 ± 2.85^{c}	83.15 ± 3.73^{c}	163.61 ± 10.52^{a}	149.57 ± 6.94^{b}	100.12 ± 6.62^{b}	97.34 ± 4.69^{b}	135.73 ± 4.11^{a}	55.44 ± 11.10^{c}
Leaf Zn	78.89 ± 5.89^{c}	72.11 ± 5.41^{c}	96.84 ± 9.08b	115.96 ± 13.01^{a}	71.92 ± 6.61	81.83 ± 3.54	83.02 ± 9.28	81.44 ± 1.80
Leaf Cu	7.75 ± 0.24	9.77 ± 1.72	9.29 ± 1.95	6.72 ± 0.46	18.49 ± 1.83^{a}	13.84 ± 0.43^{b}	11.44 ± 1.58^{c}	14.64 ± 0.51^{b}
Flower N	27.32 ± 0.29^{a}	25.86 ± 1.04^{ab}	24.73 ± 1.11^{b}	25.21 ± 0.80^{b}	21.24 ± 0.36^{ab}	22.74 ± 1.39^{a}	22.44 ± 0.95^{ab}	20.17 ± 0.12^{b}
Flower K	20.93 ± 0.10^{a}	20.46 ± 0.10^{b}	20.51 ± 0.33^{b}	20.98 ± 0.21^{a}	19.44 ± 0.19^{a}	19.06 ± 0.23^{b}	17.93 ± 0.06^{c}	18.03 ± 0.06^{c}
Flower P	10.02 ± 0.78	10.40 ± 1.55	10.20 ± 1.08	10.51 ± 1.18	9.28 ± 0.03	9.36 ± 0.63	9.07 ± 0.36	9.85 ± 0.74
Flower Ca	3.49 ± 0.06^{a}	2.96 ± 0.05^{c}	3.27 ± 0.05^{b}	3.01 ± 0.04^{c}	2.61 ± 0.07^{b}	2.41 ± 0.08^{c}	2.83 ± 0.09^{a}	2.56 ± 0.01^{bc}
Flower Mg	1.81 ± 0.02^{a}	1.72 ± 0.01^{b}	1.74 ± 0.04^{b}	1.81 ± 0.03^{a}	1.63 ± 0.04^{b}	1.59 ± 0.01^{b}	1.73 ± 0.04^{a}	1.76 ± 0.01^{a}
Flower Na	2.99 ± 0.04^{c}	3.16 ± 0.05^{b}	3.36 ± 0.03^{a}	3.14 ± 0.05^{b}	4.30 ± 0.03^{a}	3.19 ± 0.04^{b}	4.33 ± 0.08^{a}	4.26 ± 0.00^{a}
Flower Mn	82.64 ± 2.62^{b}	75.28 ± 11.41^{b}	116.06 ± 8.77^{a}	123.37 ± 13.19^{a}	97.58 ± 4.64^{b}	81.80 ± 2.54^{c}	113.73 ± 6.05^{a}	106.00 ± 6.59^{ab}
Flower Zn	77.45 ± 3.22	79.26 ± 3.31	73.90 ± 2.57	74.30 ± 3.00	92.17 ± 1.29^{a}	85.06 ± 1.21^{b}	81.84 ± 1.96^{b}	91.49 ± 3.51^{a}
Flower Cu	21.63 ± 1.78^{a}	13.17 ± 0.38^{b}	13.35 ± 1.11^{b}	11.56 ± 0.54^{b}	3.06 ± 0.35^{b}	9.23 ± 3.61^{a}	3.32 ± 0.43^{b}	n.d.

Ca and Mg in leaves, in comparison to the control treatment (Table 1). Indeed, Mn adding in the NS increased chlorophylls content and N, K, Mn and Zn content in leaves and Mn content in flowers but minerals such as N, K, Ca, Mg and Cu were decreased in flowers. An intermediate situation was observed when both Mg and Mn were added in the NS.

In pansy, the effects of Mg and/or Mn were less pronounced than in viola. In pansy, Mg enrichment in the NS decreased P, Ca and Cu in leaves, decreased K, Ca, Na, Mn and Zn in flowers but increased leaf Na content and flower Cu in comparison to the control treatment. Pansy grown under Mn enriched NS had lower N, K, P, Mg and Cu content in leaves and K and Zn content in flowers, but higher Na and Mn in leaves and Ca, Mg and Mn in flowers, in comparison with the control treatment (Table 1). Combining increased + Mg + Mn levels in the NS, the content of K in leaves and Mg in flowers were increased while Ca, P and Mn content in leaves and K content in flowers were decreased.

Mineral composition in the present study was in similar levels as reported in previous studies (González-Barrio et al., 2018), with Mg, K, P and Ca ranging from 2.69 to 4.80 g/kg, 29.12–42.25 g/kg, 17.30–28.33 g/kg, and 5.82–7.36 g/kg, respectively for viola and pansy. For maximum plant growth and development, a Mn content of more than 30 mg/kg dry weight is needed (Marschner, 1995). The Mn concentration in the shoots was inside the range reported by Mills and Jones (1996) (41.00——203.00 mg/kg). Therefore, in the present study, Mn content in leaves and flowers were ranged from 83.15 to 163.61 mg/kg and 75.28–123.37 mg/kg, respectively in viola and 55.44–135.73 mg/kg and 81.80–113.73 mg/kg, respectively in pansy. Similarly, Mn content in *Viola* x *wittrockiana* shoots and flowers was ranged in 60–110 mg/kg dw and 50–90 mg/kg dw, respectively (Plaza, Carmassi, Diara, Pardossi, Lao & Jiménez-Becker, 2021). Observing these results, both viola and pansy can be considered as a good source of minerals. Minerals are components of the human skeleton and of enzyme systems, are necessary for human biological processes like osmotic pressure management and have also been linked to the prevention of some diseases like cancer or cardiovascular disorders (Rop et al., 2012).

Increased levels of Mg and Mn in nutrient solutions can have both positive and negative effects on plant growth and mineral accumulation in flowers. Higher levels of Mg have been associated with improved tomato development by promoting root architecture adjustments and biomass partitioning (Carvalho et al., 2019). However, increased levels of Mg and + Mg + Mn in the nutrient solution negatively affected the accumulation of other minerals, particularly in flowers. Conversely, increased Mn levels in the nutrient solution resulted in higher Mn accumulation in flowers, indicating a fortified edible flower. The high concentration of Mn^{2+} can compete with similar ions like Mg^{2+} or Ca^{2+} for active sites, affecting their intended functions (Faria et al., 2020). Phytotoxic levels of Mn in beans led to reduced calcium uptake and translocation, affecting cell wall formations and auxin levels (Faria et al., 2020). In this study, viola plants exhibited decreased leaf Ca accumulation, while pansy plants did not show this effect, indicating species-specific responses to mineral levels. Higher mineral levels in the nutrient solution can also increase the EC, which may affect the nutritive and pharmaceutical properties of edible flowers (Kentelky, Szekely-Varga, Morar & Cornea-Cipcigan, 2022). In viola, increased Mg levels resulted in a higher number of flowers and flower yield, as observed in pansy as well.

3.2. Centesimal composition and soluble sugars

In this study, the nutritional profile of the flowers in different mineral treatments were evaluated, which showed significant differences between the different treatments for both species, except for the protein profile for pansy which showed no difference between the four applied treatments (Table 2). As the flowers are less consumed plant organ, it is observed that they have a considerable protein content, low fat values and mineral contents. However, they are an excellent source of carbohydrates and energy. In addition, the sugar contents varied between the different species and treatments, two of which were tentatively identified, glucose and fructose, where glucose in pansy in the Mn and Mg treatments was not detected. According to the literature, fructose is one of the most common sugars in flowers (González-Barrio et al., 2018), which was also detected in this study; however, in many treatments, glucose presented higher values. Also, da Silva, Fischer, and Zambiazi (2020), stated that the glucose contents were higher compared to the contents of the other sugars, this being the most abundant sugar, followed by fructose which comes in agreement with the present study. The differences in the parameters of the nutrient profile, can be explained by the fertigation treatments and crops to which they were submitted, having these direct interferences in the development and accumulation of different nutrient contents in the flowers in the course of the development of their different tissues (the differences between the centesimal composition and soluble sugars in the different treatments and flower species become more visible as elucidated in (Supplementary material - **Figure S1**). The majority of flowers are predominantly composed of water, ranging from 70 to 95 %, combined with proteins, lipids, carbohydrates, starch, vitamins A, B, C and E, as well as other important minerals for the maintenance of a balanced diet (Koike, 2015; Rop et al., 2012). This nutritional content can vary according to growing conditions and the composition of micronutrients available in the soil (Rivas-García et al., 2021). The carbohydrate fraction of flowers is composed mainly of simple sugars (fructose, glucose, and sucrose), with fructose being the most abundant monosaccharide detected in flowers (Pires et al., 2017).

3.3. Chemical characterization

3.3.1. Fatty acids

Twenty different fatty acids were identified (Table 3), of which the majority for both flowers and treatments were palmitic acid followed by oleic and linoleic acid. In addition, linolenic and 5,8,11,14-Eicosatetraenoic showed significant concentrations in some treatments within the analyzed profiles. Saturated fatty acids represent the majority for both different treatments, representing 50 % of the total composition in viola and 40 % in pansy. Monounsaturated and polyunsaturated fatty acids (Supplementary material - **Figures S2 and S3**), for viola and pansy,

Table 2
Centesimal composition and soluble sugars by Viola and Pansy.

Samples	Protein g/100 g dw	Fat g/100 g dw	Ash g/100 g dw	Carbohydrates g/100 g dw	Energy (Kcal)	Fructose g/100 g dw	Glucose g/100 g dw
Viola -Mg-Mn (control)	15.6 ± 01^{a}	4.4 ± 0.1^{b}	7.5 ± 0.3^{a}	72.51 ± 0.01^{b}	391.95 ± 1.49^{b}	6.03 ± 0.02^{b}	7.60 ± 0.08^{c}
Viola + Mg	14.8 ± 0.4^{ab}	4.48 ± 0.02^{b}	7.3 ± 0.1^{a}	73.42 ± 0.69^{ab}	393.12 ± 0.50^{b}	7.34 ± 0.12^{a}	8.73 ± 0.27^{b}
Viola + Mn	14.19 ± 0.04^{b}	6.14 ± 0.18^{a}	6.9 ± 0.2^{b}	72.80 ± 0.37^{b}	403.23 ± 0.04^{a}	nd	9.1 ± 0.1^{a}
Viola + Mg + Mn	15.1 ± 0.5^{ab}	3.46 ± 0.02^{c}	7.3 ± 0.2^{a}	74.11 ± 0.53^{a}	$387.97 \pm 1.00c$	nd	9.2 ± 0.1^{a}
Pansy -Mg-Mn (control)	12.5 ± 0.1^{a}	4.86 ± 0.32^{b}	7.8 ± 0.3^{a}	74.80 ± 0.07^{b}	392.93 ± 2.95^{b}	nd	15.3 ± 0.8^{b}
Pansy + Mg	13.0 ± 0.9^{a}	3.44 ± 0.13^{c}	6.3 ± 0.5^{c}	77.31 ± 1.97^{a}	392.00 ± 1.44^{b}	10.23 ± 0.09^{c}	nd
Pansy + Mn	12.5 ± 0.1^{a}	6.47 ± 0.01^{a}	6.8 ± 0.3^{bc}	74.3 ± 0.5^{b}	405.18 ± 1.13^{a}	10.75 ± 0.03^{b}	nd
Pansy + Mg + Mn	11.8 ± 0.2^{a}	2.80 ± 0.12^{d}	7.3 ± 0.2^{ab}	78.04 ± 0.02^{a}	384.70 ± 0.12^{c}	11.10 ± 0.01^{a}	21.73 ± 0.21^{a}

*Nd: not detected.

Table 3
Profile in individual fatty acids and tocopherol compounds by Viola and Pansy.

Fatty acids (%)								
Samples	**Viola -Mg-Mn (control)**	**Viola + Mg**	**Viola + Mn**	**Viola + Mg + Mn**	**Pansy -Mg-Mn (control)**	**Pansy + Mg**	**Pansy + Mn**	**Pansy + Mg + Mn**
C12:0	2.57 ± 0.02^{b}	2.62 ± 0.21^{b}	3.00 ± 0.31^{a}	2.80 ± 0.01^{ab}	3.90 ± 0.40^{b}	nd	nd	4.29 ± 0.42^{a}
C13:0	1.40 ± 0.04^{b}	1.427 ± 0.002^{b}	1.93 ± 0.05^{a}	1.10 ± 0.10^{c}	0.75 ± 0.04^{c}	0.90 ± 0.01^{b}	1.42 ± 0.09^{a}	0.84 ± 0.05^{bc}
C14:0	4.14 ± 0.24^{d}	4.6 ± 0.1^{c}	5.10 ± 0.10^{b}	5.69 ± 0.21^{a}	4.82 ± 0.48^{a}	3.06 ± 0.21^{b}	2.89 ± 0.14^{b}	5.36 ± 0.54^{a}
C15:0	0.74 ± 0.05^{b}	nd	nd	1.20 ± 0.09^{a}	nd	nd	nd	nd
C15:1	nd	nd	nd	nd	nd	nd	nd	nd
C16:0	36.05 ± 2.20^{c}	38.46 ± 0.34^{bc}	42.33 ± 2.13^{ab}	45.23 ± 4.28^{a}	25.91 ± 1.42^{c}	31.10 ± 0.61^{a}	26.83 ± 0.85^{bc}	28.78 ± 1.6^{ab}
C16:1	1.47 ± 0.05^{a}	1.37 ± 0.19^{a}	0.90 ± 0.02^{c}	1.25 ± 0.01^{b}	1.26 ± 0.03^{b}	1.41 ± 0.01^{a}	1.03 ± 0.06^{c}	1.40 ± 0.03^{a}
C17:0	2.04 ± 0.05^{c}	4.6 ± 0.4^{a}	3.03 ± 0.27^{b}	1.20 ± 0.03^{d}	1.09 ± 0.10^{a}	nd	0.70 ± 0.06^{b}	1.21 ± 0.11^{a}
C17:1	nd	nd	nd	nd	nd	nd	2.83 ± 0.04^{a}	
C18:0	7.66 ± 0.41^{b}	8.33 ± 0.10^{b}	9.48 ± 0.54a	9.28 ± 0.24^{a}	6.38 ± 0.11^{c}	7.70 ± 0.11^{a}	6.10 ± 0.02^{d}	7.08 ± 0.12^{b}
C18:1n9c	16.22 ± 1.06^{a}	14.76 ± 0.17^{b}	13.17 ± 0.25^{c}	11.12 ± 0.59^{d}	14.92 ± 0.22^{b}	15.00 ± 0.10^{b}	9.6 ± 0.1^{c}	16.56 ± 0.24^{a}
C18:2n6c	15.43 ± 0.76^{a}	7.331 ± 0.001^{b}	3.80 ± 0.20c	7.18 ± 0.24^{b}	22.20 ± 0.20^{c}	21.33 ± 0.08^{d}	26.90 ± 0.08^{a}	24.59 ± 0.20^{b}
C18:3n6	0.91 ± 0.04^{c}	0.78 ± 0.01^{d}	1.16 ± 0.01^{a}	1.05 ± 0.10^{b}	nd	1.10 ± 0.10^{a}	1.01 ± 0.02^{b}	24.59 ± 0.20^{b}
C18:3n3	5.24 ± 0.43^{a}	1.87 ± 0.02^{b}	nd	1.85 ± 0.08^{b}	9.94 ± 0.04^{b}	7.58 ± 0.11^{c}	11.05 ± 0.35^{a}	nd
C20:0	1.26 ± 0.14^{bb}	1.30 ± 0.10^{b}	1.42 ± 0.12^{b}	1.79 ± 0.12^{a}	1.03 ± 0.06^{b}	1.21 ± 0.06^{a}	0.94 ± 0.01^{b}	1.05 ± 0.10^{b}
C20:5n3	nd	nd	nd	nd	nd	nd	nd	1.70 ± 0.13^{a}
C20:4n6	5.15 ± 0.34^{b}	5.34 ± 0.35^{b}	7.28 ± 0.44^{a}	7.61 ± 0.14^{a}	3.84 ± 0.20^{b}	4.52 ± 0.33^{a}	4.79 ± 0.15^{a}	4.38 ± 0.06^{a}
C22:0	1.90 ± 0.12^{b}	2.13 ± 0.05^{ab}	2.41 ± 0.21^{a}	2.15 ± 0.20^{ab}	1.42 ± 0.04^{c}	1.79 ± 0.13^{a}	1.66 ± 0.12^{ab}	1.57 ± 0.05^{bc}
C22:2	nd	2.55 ± 0.08^{a}	2.60 ± 0.04^{a}	nd	1.53 ± 0.10^{b}	1.81 ± 0.16^{a}	2.02 ± 0.06^{a}	nd
C24:0	1.90 ± 0.07^{b}	2.22 ± 0.15^{a}	2.17 ± 0.14^{a}	2.03 ± 0.13^{ab}	1.26 ± 0.09^{b}	1.60 ± 0.13^{a}	1.46 ± 0.11^{ab}	1.39 ± 0.12^{ab}
SFA	58.88 ± 2.23^{c}	65.6 ± 0.7^{b}	71.12 ± 0.92^{a}	71.96 ± 3.58^{a}	46.2 ± 1.6^{b}	47.27 ± 0.53^{b}	41.64 ± 0.37^{c}	51.3 ± 1.8^{a}
MUFA	11.82 ± 2.36^{c}	16.13 ± 0.02^{a}	14.01 ± 0.3^{ab}	11.87 ± 1.31^{bc}	16.18 ± 0.24^{b}	16.42 ± 0.10^{b}	13.38 ± 0.24^{c}	17.97 ± 0.26^{a}
PUFA	29.31 ± 4.58^{a}	18.32 ± 0.68^{b}	14.81 ± 0.65^{b}	16.17 ± 2.27^{b}	37.64 ± 1.31^{b}	36.32 ± 0.43^{c}	45 ± 0.61^{a}	30.8 ± 1.5^{d}
Tocopherol compounds expressed mg/100 g fw								
Alpha	11.01 ± 004^{a}	8.99 ± 0.03^{b}	7.988 ± 0.004^{c}	7.72 ± 0.13^{d}	nd	11.8 ± 0.1^{a}	10.74 ± 0.08^{b}	nd
Total tocopherol compounds								
	11.01 ± 004^{a}	8.99 ± 0.03^{b}	7.988 ± 0.004^{c}	7.72 ± 0.13^{d}	nd	11.8 ± 0.1^{a}	10.74 ± 0.08^{b}	nd

***C12:0** Dodecanoic; **C13:0** Tridecanoic; **C14:0** Misterric acid **C15:0** Pendecanoic acid; **C15:1** *cis*-10- Pentadecenoic acid; **C16:0** Palmitic acid; **C16:1** Palmitoleic acid; **C17:0** Heptadecanoic Ácid; **C17:1** Margaroleic acid; **C18:0** Stearic Acid; **C18:1n9c** Oleic Ácid; **C18:2n6t** *trans*-9,12- Octadecadienoic acid; **C18:2n6c** Linoleic acid; **C18:3n6** 6,9,12-Octadecadienoic; **C18:3n3** Linolenic; **C20:0** Arachidium; **C20:5n3** EPA; **C20:4n6** 5,8,11,14-Eicosatetraenoic; **C22:0** Behenic; **C22:2** *cis*-13–16-Docosadienóico; **C24:0** Lignoceric**; SFA** Saturated fatty acids; **MUFA** Monounsaturated fatty acids; **PUFA** Polyunsaturated fatty acids. nd: not detected.

respectively) were determined and the individual compounds as well as their distribution according to different treatments can be observed.

Saturated fatty acids represented 59.43 % of the total fatty acids. Also, as presented in Table 3, high contents of palmitic acid (C16:0) were found, which was the main fatty acid, as well as linoleic acid and its isomer (32.30 %). This value is important because linoleic acid is an essential fatty acid, called omega 6, which cannot be synthesized by the human body and must be supplied only through the diet (Barros, Carvalho & Ferreira, 2010).

Fernandes et al. (2019) conducted a study on the popularization of pansyberry (*Viola* × *wittrockiana*), and in the fatty acid profiles, linoleic acid was predominant (ranging from 18.7 to 51.0 g/100 g fatty acids), followed by palmitic and linolenic acids. These results agree with the present study on the predominance of palmitic acid for viola (ranging from 36.05 to 45.23 %, for the different treatments) and for pansy (ranging from 25.91 to 31.10 %, for the different treatments), followed by oleic and linolenic acid).

3.3.2. Tocopherol compounds

The only tocopherol isoform identified was α-tocopherol for both plants. For viola, the highest results were for the control (-Mg-Mn) treatment, followed by + Mg, +Mn, and finally the combination of + Mg + Mn. For pansy, only the treatments with individual minerals revealed its presence, of which + Mg was the most significant in relation to + Mn, while in the other treatments no compounds were detected (Supplementary material - **Figure S7**) it is possible to identify different tocopherol profiles in the mineral treatments performed).

Tocopherols are natural antioxidants belonging to the vitamin E group and are known for their protective role against oxidative stress and various health benefits. They can protect cells from the harmful effects of free radicals, preventing conditions like cardiovascular diseases, DNA damage, and skin disorders (Lockowandt et al., 2019). Studies on different plants, such as dandelion and *Malva sylvestris*, have shown varying contents of tocopherols in their flowers, with α-tocopherol being the predominant form (Barros et al., 2010).

Da Silva et al. (2020) investigated the bioactive potential of *Viola x wittrockiana* flowers of different colors and found that the proportion of individual tocopherols varied depending on the flower color. α-tocopherol was the predominant tocopherol, with violet and yellow flowers showing the highest concentration. However, "orange" colored flowers had γ-tocopherol as the predominant form. Although there is a lack of studies on the vitamin E composition in edible flowers, it would be interesting to explore its profiles in future research, considering the various benefits that this vitamin provides to the body and its numerous functions.

3.3.3. Phenolic compounds

The bioactivity of edible flowers is highly related to their phenolic compound composition (Koike, 2015). According to the data obtained in the characterization and identification of phenolic compounds present

Table 4
Total phenolic compounds Viola (*Viola tricolor* war. Hortensis) and Pansy (*Viola* x *wittrockiana*).

Total phenolic compounds viola (*Viola tricolor* war. *Hortensis*) (mg/g dry weigth).										
	Peak	**Rt (min)**	**λmax (nm)**	**[M−H]⁻(*m/z*)**	**MS² (*m/z*)**	**Tentative identification**	**-Mg-Mn (control)**	**+ Mg**	**+ Mn**	**+Mg + Mn**
Non-anthocyanic flavonoids	**1**	6.27	351	771	315(100)	Isorhamnetin-hesoxyl-pentosyl-hexoside[1]	0.82 ± 0.03^{c}	1.08 ± 0.02^{a}	0.97 ± 0.02^{b}	0.86 ± 0.02^{c}
	2	10.47	355	735	301(100)	Quercetin-3-*O*-(2-rhamnosyl) rutinoside[1]	0.77 ± 0.02^{c}	0.97 ± 0.03^{a}	0.86 ± 0.02^{b}	0.82 ± 0.02^{b}
	3	13.38	352	755	609(12),591 (5),489(8),343 (15),301(100)	Quercetin-3-*O*-di-rhamnosyl-glucoside[1]	1.64 ± 0.03^{c}	2.36 ± 0.02^{a}	1.86 ± 0.02^{b}	1.83 ± 0.03^{b}
	4	13.6	349	755	609(12),591 (5),489(8),343 (15),301(100)	Quercetin-3-*O*-di-rhamnosyl-glucoside[1]	1.34 ± 0.03^{a}	1.35 ± 0.03^{a}	0.94 ± 0.02^{b}	1.34 ± 0.03^{a}
	5	14.42	349	797	755(21),489 (5),301(100)	Acetyl-quercetin-3-*O*-(6-*O*-rhamnosylglucoside)-7-*O*-rhamnoside[1]	3.16 ± 0.16^{b}	4.20 ± 0.28^{a}	4.03 ± 0.16^{a}	3.12 ± 0.28^{b}
	6	15.96	322	577	517(5),487 (8),473(30),457 (17),383 (21),353(35)	Violanthin (apigenin-6-*C*-glucosyl-8-*C*-rhamnoside)[2]	1.14 ± 0.03^{ab}	1.11 ± 0.03^{b}	1.17 ± 0.02^{a}	1.17 ± 0.03^{a}
	7	16.51	354	609	301(100)	Quercetin-3-*O*-rutinoside[1]	18.38 ± 0.74^{c}	32.78 ± 0.65^{a}	23.03 ± 0.69^{b}	22.63 ± 0.53^{b}
	8	19.67	345	593	285(100)	Kaempferol-3-*O*-rutinoside[1]	0.533 ± 0.002^{d}	0.669 ± 0.002^{a}	0.594 ± 0.002^{c}	0.631 ± 0.001^{b}
	9	20.66	302sh354	623	315(100)	Isorhamnetin-3-*O*-rutinoside[1]	0.13 ± 0.01^{b}	0.046 ± 0.003^{c}	0.0026 ± 0.0001^{d}	0.249 ± 0.002^{a}
Total anthocyanic flavonoids	**10**	21.2	530	611	465(15),303 (100)	Delphinidin-3-*O*-rutinoside[3]	1.36 ± 0.05^{c}	2.85 ± 0.03^{a}	2.19 ± 0.03^{b}	0.61 ± 0.02^{d}
	11	22.5	529	919	757(26),465 (12),303(100)	Delphinidin-3-(4″-p-coumaroyl)-rutinoside-5-glucoside[3]	2.31 ± 0.02^{c}	6.31 ± 0.46^{a}	4.37 ± 0.23^{b}	1.49 ± 0.04^{d}
	12	25.6	523	903	741(36),449 (12),287(100)	Cyanidin-3-(coumaroyl)-methylpentosyl-hexosyl-5-hexoside[4]	0.580 ± 0.004^{c}	1.11 ± 0.05^{a}	0.773 ± 0.001^{b}	0.480 ± 0.002^{d}
						Σ Total Phenolics				
						Total non-anthocyanic flavonoids	27.91 ± 1.04^{c}	44.54 ± 1.06^{a}	33.45 ± 0.95^{b}	32.64 ± 0.94^{b}
						Total anthocyanic flavonoids	4.3 ± 0.1^{c}	10.28 ± 0.54^{a}	7.34 ± 0.26^{b}	2.6 ± 0.1^{d}
						Total phenolic compounds	32.21 ± 1.14^{c}	54.82 ± 1.6^{a}	40.79 ± 1.21^{b}	35.24 ± 1.01^{b}
Total phenolic compounds pansy (*Viola x wittrockiana*) (mg/g dry weigth).										
	Peak	**Rt (min)**	**λmax (nm)**	**[M−H]⁻(*m/z*)**		**Tentative identification**	**Pansy -Mg-Mn (control)**	**Pansy +Mg**	**Pansy + Mn**	**Pansy +Mg + Mn**
Non-anthocyanic flavonoids	**1**	6,19	358	771	315(100)	Isorhamnetin-hesoxyl-pentosyl-hexoside1	0.80 ± 0.02^{b}	0.726 ± 0.002^{c}	0.719 ± 0.003^{c}	0.86 ± 0.03^{a}
	2	10,54	342	771	625(32),317 (100)	Myricetin-3-*O*-(6-*O*-rhamnosylglucoside)-7-*O*-rhamnoside1	1.84 ± 0.09^{a}	0.92 ± 0.04^{d}	1.22 ± 0.07^{c}	1.45 ± 0.08^{b}
	3	13,31	354	755	609(12),591 (5),489(8),343 (15),301(100)	Quercetin-3-*O*-di-rhamnosyl-glucoside1	5.82 ± 0.24^{b}	4.99 ± 0.33^{c}	4.09 ± 0.26^{c}	6.92 ± 0.24^{a}
	4	14,32	353	797	755(21),489 (5),301(100)	Acetyl-quercetin-3-*O*-(6-*O*-rhamnosylglucoside)-7-*O*-rhamnoside1	3.58 ± 0.10^{b}	4.99 ± 0.33^{a}	2.94 ± 0.02^{c}	5.47 ± 0.46^{a}
	5	15,97	322	577	517(5),487 (8),473(30),457 (17),383 (21),353(35)	Violanthin (apigenin-6-C-glucosyl-8-C-rhamnoside)2	1.00 ± 0.03^{b}	0.80 ± 0.03^{c}	0.81 ± 0.02^{c}	1.08 ± 0.03^{a}
	6	16,53	354	609	301(100)	Quercetin-3-*O*-rutinoside1	18.15 ± 0.64^{b}	26.40 ± 0.95^{a}	15.61 ± 0.79c	19.04 ± 0.58^{b}
	7	19,67	345	593	285(100)	Kaempferol-3-*O*-rutinoside1	0.062 ± 0.004^{b}	0.091 ± 0.005^{a}	0.0051 ± 0.0004^{d}	0.023 ± 0.002^{c}
	8	20,66	302sh354	623	315(100)	Isorhamnetin-3-*O*-rutinoside1	0.08 ± 0.01^{a}	0.069 ± 0.004^{a}	0.011 ± 0.001^{b}	n.d.
Total anthocyanic flavonoids	**9**	13	515	595	449(11), 287 (100)	Cyanidin-3-*O*-rutinoside4	0.488 ± 0.001^{b}	1.18 ± 0.04^{a}	0.477 ± 0.001^{b}	0.479 ± 0.004^{b}

(*continued on next page*)

Table 4 (*continued*)

Total phenolic compounds viola (*Viola tricolor* war. *Hortensis*) (mg/g dry weigth).									
	Peak	**Rt (min)**	**λmax (nm)**	**[M−H]⁻(*m/z*)**	**MS² (*m/z*)**	**Tentative identification**	**-Mg-Mn (control)**	**+ Mg**	**+ Mn**	**+Mg + Mn**
	10	21	530	611	465(15), 303 (100)	Delphinidin-3-*O*-rutinoside3	1.09 ± 0.03[a]	0.79 ± 0.02b	0.64 ± 0.02[d]	0.68 ± 0.01[c]
	11	22	529	919	757(26), 465 (12), 303(100)	Delphinidin-3-(4″-p-coumaroyl)-rutinoside-5-glucoside3	2.13 ± 0.05[a]	2.19 ± 0.03[a]	0.98 ± 0.05[c]	1.33 ± 0.02[b]
	12	25	523	903	741(36), 449 (12), 287(100)	Cyanidin-3-(coumaroyl)-methylpentosyl-hexosyl-5-hexoside4	0.88 ± 0.03[c]	1.77 ± 0.01[a]	0.701 ± 0.003[d]	0.97 ± 0.03[b]
						Σ Total phenolics				
						Total non-anthocyanic flavonoids	31.32 ± 1.12[c]	38.60 ± 1.68[a]	25.40 ± 1.18[d]	34.77 ± 1.42[b]
						Total anthocyanic flavonoids	4.55 ± 0.12[b]	5.92 ± 0.10[a]	2.8 ± 0.1[d]	3.5 ± 0.1[c]
						Total phenolic compounds	35.87 ± 1.24[c]	42.52 ± 1.78[a]	28.20 ± 1.28[d]	38.27 ± 1.52[b]

*Standard used to viola: 1. quercetin 3-*O*-glucoside (y = 34843x – 160173; R^2 = 0.9998; LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL);2. luteolin-7-*C*-glucoside (y = 43453x – 1354.5, R^2 = 0.998; LOD = 0.40 μg/mL; LOQ = 0.88 μg/mL); 3. Pelargonidin-3-*O*-glucoside (y = 276117x − 480418; R^2 = 0.986; LOD = 0.35 μg/mL; LOQ = 0.92 μg/mL). 4. Cyanidin-3-*O*-glucoside (y = 134578x –3E + 06; R^2 = 0.9986; LOD = 0.25 μg/mL; LOQ = 0.83 μg/mL).
* Standard used to pansy: 1. quercetin 3-O-glucoside (y = 34843x – 160173; R2 = 0.9998; LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL);2. luteolin-7-C-glucoside (y = 43453x – 1354.5, R2 = 0.998; LOD = 0.40 μg/mL; LOQ = 0.88 μg/mL); 3. Pelargonidin-3-O-glucoside (y = 276117x − 480418; R2 = 0.986; LOD = 0.35 μg/mL; LOQ = 0.92 μg/mL). 4. Cyanidin-3-O-glucoside (y = 134578x –3E + 06; R2 = 0.9986; LOD = 0.25 μg/mL; LOQ = 0.83 μg/mL).

in viola and pansy samples, the presence of flavonoid classes is observed, particularly flavone (apigenin derivatives), flavonol (isorhamnetin, quercetin, and kaempferol derivatives), and anthocyanin (delphinidin and cyanidin derivatives). The presence of flavonoid glycosides in *V. tricolor* has been reported previously by several researchers (Gonçalves, Friedrich, Boligon, Piana, Beck & Athayde, 2012). Flavonoid compounds were found to be the predominant constituents in both samples, as supported by Mlcek, Plaskova, Jurikova, Sochor, Baron & Ercisli (2021), who assert that flavonoids are likely the most important class of natural phenolics, known for their diverse and widespread occurrence.

Table 4 elucidates the tentative identification of nine phenolic compounds for viola flower, of which quercetin-3-*O*-rutinoside is the major one, followed by violanthin, quercetin-3-*O*-di-rhamnosyl-glucoside and acetyl-quercetin-3-*O*-(6-*O*-rhamnosylglucoside)-7-*O*-rhamnoside. Furthermore, three different anthocyanins were tentatively identified, with delphinidin-3-(4″-p-coumaroyl)-rutinoside-5-glucoside being the predominant compound. When comparing the phenolic composition of the treatments of the four types of nutritional supplementation (-Mg-Mn (control); +Mg + Mn; +Mg; +Mn), was obtained a higher non-anthocyanic flavonoids (TNAF) for the + Mg treatment (44.54 mg/g), followed by the + Mn treatment (33.45 mg/g), +Mg + Mn (32.64 mg/g) and finally the control (namely -Mg-Mn) (27.91 mg/g). Regarding total anthocyanin flavonoids (TAF), the + Mg treatment showed the highest values (10.28 mg/g), followed by the + Mn treatment (7.34 mg/g), however, when combining the two treatments at high concentrations (+Mg + Mn), we obtained lower anthocyanins values (2.6 mg/g) compared to the -Mg-Mn (4.3 mg/g). When comparing the total phenolic compounds in viola flowers, treating with a single mineral, specifically + Mg (54.8 mg/g), resulted in a higher phenolic compound content than + Mn treatment (40.79 mg/g). The combination treatment of magnesium and manganese (+Mg + Mn) resulted in a phenolic compound content of 35.24 mg/g. This content was found to be statistically different from the -Mg-Mn (control) at 32.21 mg/g. However, the results of the combined treatments were very similar to the -Mg-Mn, suggesting that, in the case of viola, the high concentration of both minerals may have an antagonistic effect on the production of secondary metabolites, mainly phenolics.

The phenolic composition obtained for pansy is shown at Table 4. Tentatively identified eight different phenolic compounds, the majority was quercetin-3-*O*-rutinoside followed by quercetin-3-*O*-di-rhamnosyl-glucoside, acetyl-quercetin-3-*O*-(6-*O*-rhamnosylglucoside)-7-*O*-rhamnoside and violanthin. Regarding total anthocyanins flavonoids, four different anthocyanin compounds were tentatively identified, the majority being delphinidin-3-(4″-*p*-coumaroyl)-rutinoside-5-glucoside. The dominant compounds found in pansy were NAF, with the highest values observed in the + Mg treatment (38.60 mg/g), followed by the + Mg + Mn combination (34.77 mg/g), the control (namely -Mg-Mn) group (31.32 mg/g), and + Mn treatment (25.40 mg/g). The content changes with different treatments, however, showed no statistically significant difference between + Mg treatments and the -Mg-Mn (2.19 mg/g and 2.13 mg/g, respectively) although the combined treatment with + Mg + Mn minerals (1.33 mg/g) showed the highest content compared to the treatment + Mn (0.98 mg/g). In terms of Total Phenolic Compounds (TPC), the + Mg treatment also exhibited higher values (42.52 mg/g), followed by + Mg + Mn (38.27 mg/g), control (35.87 mg/g), and + Mn (28.20 mg/g).

In general, when evaluating the TPC of the two flowers obtained in the different treatments (-Mg-Mn; +Mg + Mn; +Mg; +Mn), the behavior of the production of secondary metabolites were different in the two species of flowers being that treatment + Mn (40.79 mg/g) seems to affect more in viola, while pansy is more affected by treatment + Mg + Mn (38.27 mg/g). However, we can consider the treatment with + Mg the most effective for both flowers in increasing the content of total phenolics, 54.8 mg/g dw (viola) and 42.52 mg/g dw (pansy). Furthermore, the inclusion of high concentrations of magnesium and manganese in the nutrient solution did not result in statistically significant differences compared to the control -Mg-Mn group, which contained low concentrations of both minerals. This suggests a potential antagonistic effect of magnesium and manganese (the individual compounds for viola and pansy can be observed in the different treatments (Supplementary material - **Figure S4 and S5**, respectively), elucidated to better understand this distribution of the accumulation of a certain mineral and its influence on the flower composition).

Quercetin-3-*O*-rutinoside was identified as the majority compound for both species showed the best and highest results for the + Mg treatment. Studies conducted by Koike (2015) on viola have also identified flavonoids, particularly quercetin, as the most abundant compounds. However, the variation in total compound values could be attributed to different mineral treatments and types of treatment applied, such as direct irrigation and deficits, which can influence the production of bioactive compounds. The pro-oxidant and antioxidant

properties of flavonoids, such as quercetin and kaempferol derivatives, may also depend on the environmental conditions, chemical structure, and concentration, as highlighted by Pires et al. (2017). In contrast, Kaundal, Kumar, Kumar, Singh & Kumar (2022) reported higher total content of polyphenols and flavonoids in *Viola canescens* Wall. and *Viola pilosa* Blume flowers (51.4 mg GAE/g and 65.05 mg RE/g, and 33.26 mg GAE/g and 36.10 mg RE/g, respectively) compared to the values obtained in the present study for the different treatments analyzed.

The polyphenol content of *Viola* x *wittrockiana* flowers was found to be higher in the current study compared to previous findings reported by González-Barrio et al. (2018) and Vukics, Kery, & Guttman (2008). Flavonols, such as quercetin and myricetin, were identified as the main compounds in the flowers. Flavonoids are a diverse class of polyphenols known for their antioxidant properties and have been extensively studied for their health benefits (de Oliveira et al., 2023).

Kozicka & Hallmann (2023) discovered that organically grown pansy flowers had higher levels of bioactive compounds, such as polyphenols, phenolic acids, and anthocyanins, compared to conventional methods.

3.4. Bioactive properties

3.4.1. Cytotoxicity and anti-inflammatory properties

3.4.1.1. Cytotoxicity. As noted in Table 5, the results for four tumor and one non-tumor cell lines are presented. As observed for viola, the cells were more susceptible to the different extracts compared to pansy which showed lower activities in both treatments (the differences in results for cytotoxic activity in tumor and non-tumor cell lines are elucidated in Supplementary material - **Figure S9**) according to the mineral treatment submitted and the cell tested).

Of the four tumor cell lines tested for viola, the non-small cell lung carcinoma (NCI-H460) cells showed the lowest values, i.e., showed the highest proliferative activities of the tested extracts. There are, the NCI-H460 cells, were the most susceptible cells to the treatments all studied. As for the stomach tumor cells (AGS), no statistical differences were observed between the + Mg (219 μg/mL) and + Mg + Mn (238 μg/mL) treatments, with lower values for the -Mg-Mn treatment (129 μg/mL, as for colorectal adenocarcinoma cells (CaCo2) the lowest activity was for the + Mn treatment (136 μg/mL) and for breast carcinoma (MFC-7) the -Mg-Mn treatment (124 μg/mL, these lower values indicate higher proliferative activity against cells tested, so the lower the value the better is the extract tested against these tumor cells. When observed in a non-tumor African green monkey kidney epithelial (VERO) cell line, only the + Mg treatment (132 μg/mL) showed toxicity to the cells, the others showed values > 400 μg/mL, representing that within this maximum concentration tested they are not toxic to the cell.

For pansy in AGS cells, only the -Mg-Mn line treatment (257 μg/mL) showed activity with the tumor line, in CaCo2 cells no results were obtained against the tumor line within the maximum concentration tested (>400 μg/mL). For MCF-7 only the treatment showed + Mg + Mn activity (310 μg/mL), while for NCI-H460 all treatments statistically had results against the tumor line with higher susceptibility of cells for the -Mg-Mn treatment (110 μg/mL) while for non-tumor VERO cell line the -Mg-Mn treatment was the only one that showed toxicity, the others are not toxic within the maximum concentration tested (>400 μg/mL).

In this sense, we can observe that the addition of minerals and the culture conditions to which the flowers were exposed to growth contributed to reduce toxicity and not present toxic levels within the maximum concentration tested in the different treatments, presenting effective results against tumor cells for both treatments and species. When comparing the results of both floral species with the standard (ellipticin) applied as a positive control (namely -Mg-Mn) in this evaluation, the low power of inhibiting tumor cell growth in the studied samples is evident. However, most of the extracts show promising activities to be used against the toxic effects of tumor cells.

In addition, another study indicates that the entire aerial part, including the stem, flowers and leaves, is used in the treatment of cancer. Viola has been recorded as a pharmacological tool and possibly as an antitumor agent. Cycloviolacin O2 (cyo2), a cyclotide from *Viola odorata* (Violaceae), has antitumor effects and causes cell death by membrane permeabilization (Lindholm et al., 2002).

Koike (2015) evaluated the cytotoxic activity of *Viola tricolor* in tumor and non-tumor cell lines. The results showed low activity, with no toxicity observed in non-tumor pig liver cells. The inhibition of tumor cell growth was also low, as the values obtained were close to or greater than > 400 g/mL, indicating no significant antitumor activity. However, other studies have shown that certain flower species have beneficial compounds with potential preventive effects against diseases such as cardiovascular diseases and cancer, as well as possessing anti-inflammatory, antibacterial, antidiabetic, diuretic, and antifungal properties (Pires et al., 2017).

Thus, it is understood that some flower species may be promoters in reducing the risk of diseases such as cancer. In the case of the flowers analyzed in this research, the results showed no action in inhibiting the growth of cancer cells, that is, antitumor activity; however, the results of the antioxidant activity and phenolic compounds assays demonstrated the high potential as sources of oxidative stress reducing substances, such as flavonoids and anthocyanins.

3.4.1.2. Anti-inflammatory. Table 5 shows the values for anti-inflammatory activity in murine macrophage cells (RAW 264.7). For viola, we obtained activity for the individual treatments of + Mg (346 μg/mL) and Mn (319 μg/mL), with Mn having a more efficient activity exhibiting a higher capacity to inhibit the pro-inflammatory mediator

Table 5
Cytotoxicity, anti-inflammatory properties EC_{50} Values, antioxidant activity for TBARS IC_{50} values and CAA assays for Viola and Pansy.

Samples	Cytotoxicity					Anti-inflammatory	Antioxidant	
	AGS μg/mL	Caco2 μg/mL	MCF-7 μg/mL	NCI-H460 μg/mL	VERO μg/mL	RAW 264.7 μg/mL	TBARS μg/mL	CAA %
Viola -Mg-Mn (control)	129 ± 12^{c}	$>400^{c}$	124 ± 12^{c}	65 ± 7^{b}	$>400^{b}$	$>400^{c}$	82 ± 2^{c}	40 %
Viola + Mg	219 ± 21^{a}	$>400^{c}$	298 ± 19^{a}	56 ± 5^{b}	132 ± 6^{a}	346 ± 2^{a}	81 ± 1^{d}	33 %
Viola + Mn	184 ± 13^{b}	136 ± 3^{b}	261 ± 16^{b}	76 ± 3^{a}	$>400^{b}$	319 ± 27^{b}	86 ± 3^{a}	>2000
Viola + Mg + Mn	238 ± 14^{a}	274 ± 13^{a}	279 ± 10^{ab}	56 ± 4^{b}	$>400^{b}$	$>400^{c}$	84 ± 2^{b}	>2000
Pansy -Mg-Mn (control)	257 ± 17^{a}	$>400^{a}$	$>400^{b}$	50 ± 3^{c}	110 ± 6^{a}	$>400^{a}$	118 ± 7^{c}	>2000
Pansy + Mg	$>400^{b}$	$>400^{a}$	$>400^{b}$	169 ± 13^{a}	$>400^{b}$	$>400^{a}$	180 ± 4^{a}	>2000
Pansy + Mn	$>400^{b}$	$>400^{a}$	$>400^{b}$	95 ± 1^{b}	$>400^{b}$	$>400^{a}$	169 ± 4^{b}	>2000
Pansy + Mg + Mn	$>400^{b}$	$>400^{a}$	310 ± 1^{a}	86 ± 6^{b}	$>400^{b}$	$>400^{a}$	93 ± 3^{d}	>2000

* Positive control for cytotoxicity: Ellipticine: AGS: 1.23 ± 0.03 μg/mL; Caco2: 1.21 ± 0.02 μg/mL; MCF-7: 1.02 ± 0.02 μg/mL; NCI-H460: 1.01 ± 0.01 μg/mL; VERO: 1.41 ± 0.06 μg/mL. For the anti-inflammatory activity Dexamethasone: 6.3 ± 0.4 μg/mL. MCF-7 (breast carcinoma), NCI-H460 (non-small cell lung carcinoma), AGS (gastric adenocarcinoma), CaCo-2 (colorectal adenocarcinoma), VERO (a renal epithelial cell line from an African green monkey), RAW 264.7 (murine macrophage cell line).
*Positive control for TBARS Trolox 139 ± 5 μg/mL; Positive control for CAA: Quercetin = 95 ± 5 % oxidation inhibition at 0.3 μg/mL.

NO production.

For pansy, there was no anti-inflammatory activity detected in any of the extracts tested within the maximum concentration tested (>400 μg/mL). When comparing the results of both species with the control dexamethasone (6.3 μg/mL), the low power of anti-inflammatory activity of the studied extracts is evident (Supplementary material - **Figure S10**) it is possible to observe more clearly the distribution of the anti-inflammatory activity according to the mineral treatment in which the cell was subjected).

3.4.2. Antioxidant activity

The antioxidant activity was determined using two different *in vitro* methodologies: TBARS and CAA (Table 6). For the TBARS assay, the results were presented as EC_{50} values, that correspond to the extract concentration responsible for inhibiting 50 % of the thiobarbituric acid reactive substances formation. Therefore, lower EC_{50} values are indicative of high antioxidant activity (Fernandes et al., 2019). Furthermore, for the CAA assay, the results were expressed as percentage inhibition of the oxidation at the maximum concentration tested (2000 μg/mL). We can observe that for the TBARS results in viola flowers, the best antioxidant activity was for the treatment with magnesium, which was already expected due to its potential profile of phenolic compounds mentioned previously. The treatments with Mn and combined Mg + Mn obtained lower activities than the control (namely -Mg-Mn) treatment. As for pansy, we can observe that the best antioxidant activity was for the combined treatment of the two different minerals that showed the lowest values, i.e., we need a smaller number of samples to obtain a better activity rate. Regarding the cellular antioxidant activity, for pansy, we did not obtain results within the maximum concentration tested (2000 μM). This difference in the antioxidant profile of the compounds can be explained due to the different treatment profiles as well as the irrigation processes used during the development of the flowers, which can have a direct relationship with the development of their biological and antioxidant properties (Supplementary material - **Figure S8**), according to the mineral treatment submitted.

Antioxidant activity in flowers varies widely due to differences in methods, standards, and units of measurement used by different authors. Some studies only evaluated specific fractions of extracts, making comparisons challenging. Within studies that analyzed multiple flowers, a high range of antioxidant activity was observed. Antioxidants can have multiple mechanisms of action, necessitating the use of different methods to assess their activity (Xiong et al., 2014).

Fernandes et al. (2019) evaluated the antioxidant properties of candied pansies (*Viola* x *wittrockiana*) and found that fresh pansies had higher antioxidant activity compared to candied pansies. Over a period of 90 days, there was a slight decrease in the EC_{50} DPPH value, indicating a decrease in antioxidant power. However, the EC_{50} reducing power values showed a slight decrease, suggesting increased antioxidant activity.

Most studies have shown that there is a high correlation between antioxidant capacity and total polyphenol content, indicating that phenolic compounds may be the main contributors to antioxidant capacity (da Silva et al., 2020). Among them, the antioxidant activity of flowers seems to be mainly due to the presence of flavonoids, phenolic acids, anthocyanins and alkaloids, making their individual quantification essential to understand the true bioactivity potential (Fernandes et al., 2017).

3.4.3. Antimicrobial activity

3.4.3.1. Antibacterial activity. Antimicrobial activity is sometimes associated with various edible flower species due to the presence of substances inhibiting certain microorganisms. In this study when comparing the different treatments control (namely -Mg-Mn), Mg, Mn, +Mg + Mn on a hydroethanolic extraction (80:20, *v:v*) for viola and

Table 6
Antibacterial and antifungal activity for Viola and Pansy.

Samples	Antibacterial activity (mg/mL)														Antifungal activity (mg/mL)			
	Gram +						*Gram* -											
	Bacillus cereus		*Listeria monocytogenes*		*Staphylococcus aureus*		*Enterobacter cloacae*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Salmonella Typhimurium*		*Yersinia enterocolitica*		*Aspergillus brasiliensis*		*Aspergillus fumigatus*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Viola -Mg-Mn (control)	2.5	>10	>10	>10	2.5	>10	>10	>10	10	>10	>10	>10	5	>10	1.25	>10	5	>10	10	>10
Viola + Mg	0.3	>10	>10	>10	10	>10	>10	>10	5	>10	>10	>10	10	>10	0.6	>10	5	>10	10	>10
Viola + Mn	0.6	>10	>10	>10	10	>10	>10	>10	10	>10	>10	>10	2.5	>10	0.6	>10	2.5	>10	>10	>10
Viola + Mg + Mn	0.6	>10	>10	>10	10	>10	>10	>10	5	>10	>10	>10	2.5	>10	0.6	>10	5	>10	>10	>10
Pansy -Mg-Mn (control)	1.25	>10	10	>10	5	>10	10	>10	10	>10	>10	>10	2.5	>10	2.5	>10	5	>10	5	>10
Pansy + Mg	2.5	>10	>10	>10	10	>10	>10	>10	5	>10	>10	>10	2.5	>10	10	>10	5	>10	>10	>10
Pansy + Mn	2.5	>10	>10	>10	10	>10	10	>10	10	>10	>10	>10	5	>10	0.6	>10	5	>10	>10	>10
Pansy + Mg + Mn	1.25	>10	>10	>10	10	>10	10	>10	5	>10	>10	>10	5	>10	0.6	>10	2.5	>10	>10	>10
Positive Control																				
Streptomicin (1 mg/mL)	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.01	0.01	0.06	0.06	0.007	0.007	0.007	0.007	–	–	–	–
Methicilin (1 mg/mL)	n.d.	n.d.	n.d.	n.d.	0.007	0.007	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.007	n.d.	n.d.	–	–	–	–
Ampicillin (20 mg/mL)	n.d.	n.d.	0.15	0.15	1.15	0.15	0.15	0.15	1.15	0.15	0.63	0.63	0.15	0.15	1.15	0.15	–	–	–	–
Ketoconazole	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.06	0.125	0.5	1.0

*MIC- minimal inhibitory concentration; MBC – minimal bactericidal concentration. n.d. not detected.
*MIC- minimal inhibitory concentration; MFC – minimal fungicidal concentration.

pansy flowers against eight different food strains we verified that only for *Escherichia coli, Enterobacter cloacae* and *Listeria monocytogenes* we obtained inhibition values at the maximum concentration tested (10 mg/mL) (Table 6). In the other strains we can verify low concentrations of inhibition of bacterial growth emphasizing the good activity of these extracts against the inhibition of bacterial growth, however when evaluating values of bactericidal capacity none of the extracts for both viola and pansy obtained activity in the maximum concentrations tested (10 mg/mL). Also, the best inhibition concentrations for gram-positive bacteria were for *Bacillus cereus* in the different treatments, while for gram-negative bacteria we obtained better inhibition results for *Salmonella Typhimurium* and *Yersinia enterocolitica* with low inhibition values, proving the effectiveness of these extracts in the different mineral treatments (+Mg, +Mn, and + Mg + Mn) of the two different species of edible flowers (viola and pansy) against the different food strains.

Fernandes et al. (2019) studied candied pansies and found that the microbial load in the candied flowers was lower than in fresh samples, decreasing further during storage. Crystallized samples stored for seven days had higher counts of aerobic microorganisms compared to those stored for 90 days. The addition of sugar in the candied pansies retained water and limited microbial growth. Previous studies support the notion that sugar inhibits microbial growth. Consequently, candied pansies remained safe for consumption during the three-month storage period (Muzzaffar, Baba, Nazir, Masoodi, Bhat & Bazaz, 2016).

3.4.3.2. Antifungal activity. Using the microdilution method, different treatments (-Mg-Mn (control), +Mg, +Mn, +Mg + Mn) for viola and pansy were analyzed with a hydroethanolic extraction solution (80:20; *v:v*) to obtain the extract, which was tested on two food strains. From these, maximum concentrations of 10 mg/mL were tested for both, where, for *Aspergillus fumigatus* it was obtained for the pansy extract inhibition of fungal growth only for the control (namely -Mg-Mn) treatment (5 mg/mL) while viola obtained minimum inhibition concentration for the control (namely -Mg-Mn) treatment and the treatment with magnesium addition (10 mg/mL) (Table 6). As for *Aspergillus brasilienses* all treatments for both viola and pansy showed fungal inhibition concentrations with lowest values found at the concentration of 2.5 mg/mL for pansy + Mg + Mn and viola + Mn treatments, the other treatments showed minimum inhibitory concentration of 5 mg/mL. We can observe that for both fungi tested for the different treatments we did not obtain fungicidal activity; however, we obtained good growth inhibition values which emphasizes that these extracts have potential to be used as natural fungicides against the fungal strains tested.

4. Conclusion

The research was conducted on the edible flowers of pansy and viola, which were hydroponically grown with increased levels of + Mg, +Mn or a combination of both + Mg + Mn or reduced levels of the combination of both -Mg-Mn (namely control treatment). The nutritional and chemical composition of the flowers, as well as various *in vitro* bioactivities, were evaluated. It is observed that the flowers of viola and pansy have an excellent mineral, nutritional, phenolic compounds and bioactive profile that prospect excellent alternative nutritional sources for the increment in the food industry for different preparations or consumption in natura and or processed. In addition, these flowers, due to their appearance and excellent nutritional content, can contribute to make dishes/drinks more attractive and eye-catching, and can be a probable source of essential nutrients. This fact may be useful for consumers, chefs' cuisine, producers, and the pharmaceutical and food industry to improve the production of edible flowers as new products. All edible flowers are considered natural sources of phytochemical compounds that exhibit biological activities, and the amount of these compounds is associated with antioxidant activity. On the other hand, the variety of colors of flowers reflects the different types of natural pigments present, namely carotenoids and anthocyanins. The anthocyanin content is associated with the total flavonoid content and, therefore, with antioxidant activity, a fact that allows classifying all edible flowers as a source of nutraceuticals in human nutrition.

This study showed that the edible flowers are still considered a novelty in the market and the analyses of their nutritional, chemical and bioactive potentials demonstrate the importance of their introduction in the food industry as well as demystify their potential use. Overall, this study on edible Pansy and Viola flowers, influenced by varying levels of Mg and Mn, not only highlights the complexity of these element interactions but also underscores their significant relevance and correlations between the investigated parameters. The findings not only provide crucial insights for optimizing hydroponic cultivation but also emphasize the importance of understanding the nutritional and biochemical specifities of these flowers.

CRediT authorship contribution statement

Izamara de Oliveira: Methodology, Investigation, Writing – original draft. **Antonios Chrysargyris:** Methodology, Investigation, Writing – original draft. **Tiane C. Finimundy:** Methodology, Investigation. **Márcio Carocho:** Methodology, Investigation. **Celestino Santos-Buelga:** Methodology, Writing – review & editing. **Ricardo C. Calhelha:** Methodology, Investigation. **Nikolaos Tzortzakis:** Conceptualization, Project administration, Methodology, Writing – review & editing. **Lillian Barros:** Conceptualization, Project administration, Methodology, Writing – review & editing. **Sandrina A. Heleno:** Methodology, Investigation, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

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