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Effect of Drying and Steeping Temperatures on the Phenolic Content, Antioxidant Activity, Aromatic Compounds and Sensory Properties of *Cunila polyantha* Benth. Infusions

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Abstract: *Cunila polyantha* Benth. (Lamiaceae), an aromatic plant endemic to Mexico, is used in traditional medicine as tea infusions. In this study, the effects of different drying and steeping temperatures on the phenolic content and composition, antioxidant activity, volatile composition, and sensory properties of *C. polyantha* infusions were determined. Commercial green tea (*Camellia sinensis* L. Kuntze) was used as a control. The phenolic compounds identified in the *C. polyantha* infusions by high-performance liquid chromatography (HPLC) include phenolic acids such as gallic acid, chlorogenic acid, caffeic acid, and *p*-coumaric acid, flavonoids such as epigallocatechin gallate, protocatechin, quercetin, and naringenin, as well as the phenolic aldehyde vanillin. The *C. polyantha* infusions showed scavenging activity of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals as well as relevant antioxidant capacity, which was dependent on tea preparation conditions. A total of 46 volatile organic compounds (VOCs) were detected from the leaves and flowers of *C. polyantha*, while in the control group, a total of 30 VOCs were identified. Differences in consumer acceptability of *C. polyantha* infusions prepared at different conditions were observed. This research highlights the importance of linking sensory and chemical data to obtain the best sensorial quality and the optimal nutraceutical properties in *C. polyantha* infusions.

Keywords: green tea; sensory evaluation; volatile organic compounds; response surface methodology; high-performance liquid chromatography; *Cunila polyantha*

1. Introduction

Green tea is one of the most popular beverages in the world. Green tea contains many valuable compounds such as phenolics, flavonoids (catechins), amino acids, minerals, vitamins (B, C, E), xanthic bases (caffeine, theophylline), pigments (chlorophyll, carotenoids) and volatile compounds (aldehydes, alcohols, esters, lactones, hydrocarbons) [1]. Notable positive effects on human health have been observed by the consumption of green tea [2]. To produce green tea, freshly harvested leaves from the *Camellia sinensis* plant are immediately steamed or heated to destroy the enzymes responsible for breaking down the color pigments in the leaves and to allow the tea to maintain its green color

during drying. The drying process increases the shelf life by slowing or stopping microorganisms' growth and preventing certain biochemical reactions that might alter the organoleptic characteristics [3]. These processes preserve natural polyphenols with respect to their health-promoting properties [1,4]. Despite this, the temperature and time used during the drying process can affect the polyphenol content of green tea [5,6]. Moreover, another factor that may severely affect the extraction of green tea polyphenols is the steeping temperature used during infusion preparation [7,8].

The family Lamiaceae contains 236 genera and about 7173 species distributed throughout the world [9]. It is a plant family of great importance since many of the species belonging to this family have epidermal glands that secrete volatile aromatic compounds with medicinal, culinary or cosmetic uses, which makes it a rich source of essential oils of industrial interest [10]. Essential oils are the active ingredients to which the plants of the Lamiaceae family owe their characteristic aroma and flavor.

The genus *Cunila* (Lamiaceae) is an American genus that is found within the subfamily *Nepetoideae*, tribe *Mentheae* [11]. This genus consists of 22 species, 10 that are native to Mexico and 12 to southern South America. *Cunila* species are aromatic and medicinal plants commonly employed in folk medicine. *Cunila polyantha* Benth. is an endemic species of Mexico which is known as "poleo", "oregano" or "tenurite" [12]. Previous research of the *C. polyantha* species deals with the constitution of essential oils of extracts from the aerial parts of the plant [13]. *C. polyantha* leaves are used in traditional medicine, such as tea infusions to treat colds and menstrual pains, and it is indicated to function as a blood tonic [14]. Generally, people use the fresh leaves of the plant to prepare their infusions, or in some cases, dry the leaves to preserve them. However, the infusion preparation process can vary greatly, ranging from placing fresh or dried leaves in boiling water for a certain time, heating water at a certain temperature and then adding the leaves, or even placing them in cold water and heating to the boiling point, which could affect their phenolic composition and organoleptic characteristics.

The non-existent scientific research related to the phytochemical composition of *C. polyantha* results in limited knowledge regarding its use and medicinal characteristics, which drives away the possibility of sustainable management and use of the plant. Therefore, the present work aimed to investigate the effect of different drying and steeping temperatures on the phenolic content and composition, antioxidant activity, volatile composition and sensory properties of *C. polyantha* infusions.

2. Materials and Methods

2.1. Reagents and Standards

The Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS$^{\bullet+}$), potassium persulfate, ammonium molybdate, ascorbic acid, sodium carbonate, aluminum chloride, formic acid, methanol, and water (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nylon membrane filters (0.2 µm, Waters, Milford, MA, USA) were used for high-performance liquid chromatography (HPLC) analyses. Fiber coatings (50/30 µm Carboxen/Divinylbenzene/Polydimethylsiloxane) were purchased from Supelco (Bellefonte, PA, USA).

Rosmarinic acid, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, *p*-coumaric acid, ellagic acid, catechin, protocatechin, epigallocatechin gallate, naringin, rutin, hesperidin, quercetin, vanillin and naringenin (>99%) (Sigma-Aldrich, St Louis, MO, USA). Standards were prepared as stock solutions in methanol (2 mg/mL). Solutions of standards were stored in dark conditions at −20 °C.

2.2. Plant Material

C. polyantha specimens were collected during December 2017 at Zináparo, Mexican state of Michoacán (20°8′38.94′′ N, 102°1′120.14′′ W, and 2105 m altitude). The specimens were authenticated by Dr. Monserrat Vázquez Sánchez, from Colegio de Postgraduados, Campus Montecillo, México. Voucher herbarium specimens were deposited at the herbarium of Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Michoacán, with herbarium number

MVS145. Commercial green tea from leaves of *Camellia sinensis* (L.) Kuntze (Alessa Gourmet Tea®, Polanco, Mexico) were bought in a supermarket and was used as a control to validate the results.

2.3. Drying Conditions

Foliar (leaf) and reproductive tissues (flower) were separated from the *C. polyantha* plants and dried in a horizontal air flow oven (model TE-FH45DM, Terlab, Zapopan, Mexico) at different temperatures and times (30 °C for 48 h, 50 °C for 6 h, and 70 °C for 2 h). Then, the tissues were ground in a mortar until a fine powder was obtained. Subsequently, a sieve (number 60) was used in order to homogenize the particle size to 250 μm. Samples were stored at room temperature in dark conditions until they were used.

2.4. Volatile Organic Compounds (VOCs) Analysis by Headspace Solid-Phase Microextraction Coupled with Gas Chromatography–Mass Spectrometry (HS-SPME-GC-MS)

2.4.1. Extraction of VOCs

VOCs were extracted by headspace solid phase microextraction (HS-SPME) according to previous studies [15,16]. For this purpose, 50 mg of dry plant tissue or commercial green tea were placed in a 2-mL glass vial. After 90 min of incubating at 60 °C a 2 cm 50/30 μm Carboxen/Divinylbenzene/Polydimethylsiloxane SPME fiber (Supelco, Bellefonte, PA, USA) was exposed to the headspace of the vial for 3 min.

2.4.2. Gas Chromatography–Mass Spectrometry Analysis

Gas chromatography analysis was carried out using a Perkin Elmer Clarus 680 gas chromatograph (GC) equipped with a Clarus SQ-8T single quadrupole mass spectrometer (Perkin Elmer, Walham, MA, USA), a Perkin Elmer Elite 5-MS capillary column (30 m × 0.25 mm, and 0.25 μm film thickness) and Helium as the carrier gas at a flow rate of 1 mL/min. Once the adsorption/absorption was complete, the fiber was desorbed in the injector in splitless (5 min) mode with an injector temperature of 230 °C. The starting oven temperature was 35 °C. This was held for 1 min, then increased to 130 °C at a rate of 12 °C/min, increased to 160 °C at a rate of 1 °C/min. Finally, it was ramped up to 220 °C at a rate of 10 °C/min and held for 10 min with a total run time of 15 min. The temperatures of the transfer line and ionization source were 230 and 250 °C, respectively. The electron ionization mass spectra were recorded in scan mode at 70 eV with electron energy in the range of 35–400 *m*/*z*. The data were recorded with an MS ChemStation and processed using TurboMass software (version 5.4.2.1617., Perkin Elmer, Walham, MA, USA).

2.4.3. VOCs Identification and Semi-Quantification

The Kovats retention indices of each compound were determined by running a series of *n*-alkanes (C8–C20) under the same chromatographic conditions and calculated according to previous reports [17]. VOCs were positively identified by comparing retention indices (RI), and the mass spectra fragmentation patterns with National Institute of Standards and Technology (NIST) spectral database data, and previously reported retention indices. The peak area of the volatile compound based on the total ion chromatograms peak area normalization method was used to quantify the VOCs. The relative peak area was obtained in relation to the peak area of the external standard 1-nonanol. The relative concentration of volatiles was expressed as μL per gram of dry tissue (μL/g DT) equivalent to 1-nonanol.

2.5. Infusion Preparation

Infusions were prepared by placing 1 g of each sample (dry plant tissue or commercial green tea) in 250 mL of sterile distilled water (previously brought to the boiling point) at different temperatures (80, 90, and 100 °C) and under continuous stirring for 5 min [18]. Infusions were then filtered

through Whatman No. 1 filter paper (pore size 11 µm) (Whatman International Ltd., Maidstone, UK). Infusions were stored at 4 °C until they were used.

2.6. Determination of Total Phenolic, Flavonoid, and Tannin Contents

2.6.1. Determination of Total Phenolic Content

The determination of total phenolic contents of the infusions was carried out according to [19] with modifications. Two hundred and fifty µL of infusion were mixed with 250 µL of distilled water, followed by 12.5 µL of 1N Folin–Ciocalteu reagent, and stirred for 5 min. Finally, 37.5 µL of 20% (*w*/*v*) Na_2CO_3 solution was added and kept up in dark conditions for 2 h at room temperature. Absorbance was read at 760 nm using a PowerWave HT Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). Total phenolic contents were estimated using a gallic acid standard curve (A_{760} = 0.0027 (gallic acid) − 0.0056, R^2 = 0.9866), obtained using eight known concentrations (8–92 µg/mL) of the compound. Total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry tissue (mg GAE/g DT).

2.6.2. Determination of Total Flavonoid Content

Total flavonoid content of each sample was determined by the $AlCl_3$ method previously reported, with slight modifications [20]. A total of 100 µL of infusion was added with 100 µL of 2% (*w*/*v*) solution of $AlCl_3 \cdot 6H_2O$. Absorbance was measured after 10 min at 430 nm using a PowerWave HT Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). Total flavonoid contents were calculated using a catechin standard curve (A_{430} = 0.0068 (catechin) − 0.0009, R^2 = 0.9928) obtained using eleven concentrations of catechin (5–100 µg/mL). Flavonoid contents were expressed as milligrams of catechin equivalents per gram dry tissue (mg CE/g DT).

2.6.3. Determination of Condensed Tannins

The vanillin-H_2SO_4 methodology was used to determine procyanidins contents in the infusions [21]. A total of 250 µL of each sample was reacted with 250 µL of 1% vanillin (*w*/*v*, dissolved in methanol) followed by 250 µL of the 25% sulfuric acid solution (*v*/*v*, dissolved in methanol) and incubated at a temperature of 30 °C for 15 min. Absorbance was read at 500 nm using a PowerWave HT Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). To estimate the concentration of tannins, a calibration curve with catechin (A_{500} = 0.0013 (catechin) − 0.001, R^2 = 0.9958) was performed using ten different concentrations (4–200 µg/mL). Condensed tannins contents were expressed as milligrams of catechin equivalents per gram dry tissue (mg CE/g DT).

2.7. Antioxidant Activity

2.7.1. DPPH• Free Radical Scavenging Assay

The determination of free radical scavenging activity was performed using the DPPH• method previously described with modifications [22]. For this method, a 24 µM ethanol solution of DPPH• was prepared. To determine the scavenging activity, 90 µL of DPPH• reagent was mixed with 10 µL of infusion (concentration gradually increased, 0.4–4 mg/mL) and they were incubated at room temperature for 10 min. After incubation, the absorbance was measured at 523 nm using a PowerWave HT Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). The scavenging effect of DPPH• was measured using the formula:

$$\text{DPPH}^{\bullet} \text{ scavenging effect } (\%) = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right] \times 100 \quad (1)$$

where $A_{control}$ is the absorbance of the control (DPPH$^{\bullet}$ solution), and A_{sample} is the absorbance of the test sample (DPPH$^{\bullet}$ solution plus 10 µL of infusion). The scavenging effect was expressed as the DPPH$^{\bullet}$ scavenging percentage.

2.7.2. ABTS$^{\bullet+}$ Radical Scavenging Assay

The antioxidant activity of infusions were evaluated using a ABTS$^{\bullet+}$ radical scavenging assay, following methodology previously described [23]. Briefly, ABTS$^{\bullet+}$ was dissolved in distilled water to a 7 mM concentration. ABTS$^{\bullet+}$ radical cations were produced by reacting 1 mL of ABTS$^{\bullet+}$ stock solution with 17.6 µL of 140 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 16 h before use. The ABTS$^{\bullet+}$ solution was diluted with deionized water to obtain an absorbance of 0.70 (±0.01) at 734 nm. After the addition of 50 µL of diluted ABTS$^{\bullet+}$ radical solution to 50 µL of the infusion, the absorbance was registered at 734 nm after 6 min using a PowerWave HT Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). The assay was performed with nine infusion concentrations (0.4–4 mg/mL, dissolved in distilled water). The scavenging effect percentage was calculated using the formula:

$$\text{ABTS}^{\bullet+}\text{ scavenging effect }(\%) = \left[\left(A_{blank} - A_{sample}\right)/A_{blank}\right] \times 100 \quad (2)$$

where A_{blank} represents the absorbance of the blank (ABTS$^{\bullet+}$ solution plus 50 µL of distilled water), A_{sample} is the absorbance of the test sample (ABTS$^{\bullet+}$ solution plus 50 µL of infusion). The infusion concentration (mg/mL) that provided a 50% inhibition of the ABTS$^{\bullet+}$ radical (IC_{50}) was calculated from a graphic built with inhibition percentage versus infusion concentration.

2.7.3. Total Antioxidant Capacity

The total antioxidant capacity was evaluated using the phosphomolybdenum method, according to [24]. In this method, the formation of a green phosphate/Mo (V) complex for the reduction of Mo (VI) to Mo (V) by an antioxidant is measured at an acidic pH. To achieve this, 50 µL of infusion was combined with 500 µL of a solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Samples were incubated in a digital dry bath (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 95 °C for 90 min. The samples were subsequently cooled down to room temperature, and the absorbance of each was measured at 695 nm using a PowerWave HT Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) against a blank formed by all components of the reaction mixture and adding distilled water instead of infusion. An ascorbic acid standard curve (A_{695} = 0.005 (ascorbic acid) − 0.0322, R^2 = 0.9603) was generated with six concentrations of ascorbic acid (0.05–0.5 mg/mL). Total antioxidant capacity values were expressed as milligrams of ascorbic acid equivalents per gram of dry tissue (AAE/g DT).

2.8. Sensory Evaluation

Aimed to establish the best dry (time–temperature) and steeping (temperature) conditions for *C. polyantha* infusions, a sensory evaluation was carried out using a consumer panel consisting of 20 consumer panelists. Consumers were recruited from tea drinkers who were willing to taste the infusions. Before the descriptive analysis, 10 mL of each tea infusion was placed in a 20 mL plastic cup and was subjected to a panelist in a room at 25 °C. The panelists were presented with a control sample (*C. sinensis* tea infusion) as a reference and instructed to evaluate odor/aroma, appearance, flavor/taste, and texture/mouthfeel [25] using a 9-point hedonic scale for each attribute ranging from 9 (like extremely) to 1 (dislike extremely). Consumers cleansed their palates between evaluations by rinsing their mouths with the mineral water used to prepare the tea infusions.

2.9. Phenolic Compounds Analysis by High-Performance Liquid Chromatography (HPLC)

2.9.1. Sample Preparation for Phenolic Compounds Determination by HPLC

Prior to qualitative analysis, the infusions (6 mL) were lyophilized using a Labconco freeze dryer, model 77,530 (Labconco, Kansas City, MO, USA) at −55 °C for 24 h and under high vacuum conditions (0.02 mBar). Each infusion was resuspended individually in 1 mL of absolute methanol. Subsequently, the infusions were taken to full dryness using a vacuum concentrator (Vacufuge plus, Eppendorf, Hamburg, Germany) at 30 °C for 1 h and 45 min. After this, 750 µL of absolute methanol was added and placed in an ultra-sonication bath (CPX 2800, Branson Ultrasonics, Danbury, CT, USA) for 1 h to completely dissolve the infusions. Then, the infusions were centrifuged (Eppendorf 5415 D, Eppendorf Corporation, Hamburg, Germany) at 13,362× *g* for 30 min at room temperature and filtered through 0.2 µm nylon membranes (Waters, Milford, MA, USA). The supernatant was transferred to a glass vial, and 250 µL of absolute methanol was added to adjust the total volume to 1 mL. Finally, the infusions were stored under refrigeration at 4 °C protected from light.

2.9.2. Qualitative Analysis of Phenolic Compounds by HPLC

A Waters Alliance e2695 HPLC-PDA system (Waters, Milford, MA, USA) equipped with a Waters e2695 separations module, a Waters 2998 photodiode array detector (PDA), an adjustable temperature oven, and Empower 3 data processing software (version 3.0, Waters, Milford, MA, USA) were used for the qualitative analysis. The chromatographic separation was performed on an analytical column Waters XSelect HSS C18 (150 mm × 4.6 mm, 5.0 µm) (Waters, Milford, MA, USA). Infusions (20 µL) were injected into the system. The column temperature was maintained at 30 °C. The separation was performed under isocratic conditions with a mobile phase consisting of methanol–water (70:30, *v*/*v*) acidified with 0.005% (*v*/*v*) acetic acid, with a flow rate of 0.3 mL/min. Phenolic compounds were detected at 280 nm and identified according to their retention time compared to analytical standards and co-elutions with pure standards. The standards used were: rosmarinic acid, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, *p*-coumaric acid, ellagic acid, catechin, protocatechin, epigallocatechin gallate, naringin, rutin, hesperidin, quercetin, vanillin and naringenin.

2.10. Statistical Analysis

All determinations were performed in triplicate. Results are reported as mean ± standard deviation of three independent replicates. Results were subjected to an analysis of variance (ANOVA). Differences between values with a $p < 0.05$ were considered significant. A Tukey's test was performed for the comparison of means for the corresponding results. Relationships between all determinations were tested using Pearson's correlation. These analyses were carried out using the SPSS software version 25.0 (SPSS Inc., Chicago, IL, USA). Three-dimensional response surface graphs were made with the drying and steeping variables against the phenolic content determinations using the statistical software STATISTICA version 7.0 (StatSoft Inc., Tulsa, OK, USA).

3. Results and Discussion

3.1. Influence of Drying Time-Temperature on Volatile Profile

In the present study a total of 46 VOCs were detected from the leaves and flowers of *C. polyantha*, while in the control group, prepared from green tea leaves, only a total of 30 VOCs were identified. The VOCs of *C. polyantha* and commercial green tea samples are summarized in Table 1. The identified VOCs were classified into five chemical classes, including 1 alkane, 5 alcohols, 4 aldehydes, 4 esters, and 32 terpenes. The major chemical classes of VOCs were terpenes, aldehydes, and esters, comprising 74%, 10% and 10%, respectively, of VOCs found in *C. polyantha* samples. The proportions of VOCs in the different drying treatments of the *C. polyantha* and *C. sinensis* samples are shown in Figure 1. The sesquiterpene (**35**) cariofilene (16%) and the racemic acyclic ester (**12**) 1-octen-3-yl acetate (13%)

were the major volatile constituents from *C. polyantha*, while (**7**) hexanal (20%), and (**2**)1-octen-3-ol (10%) were the most abundant in green tea samples. Volatile composition of *C. polyantha* samples was in agreement with previously published data [13].

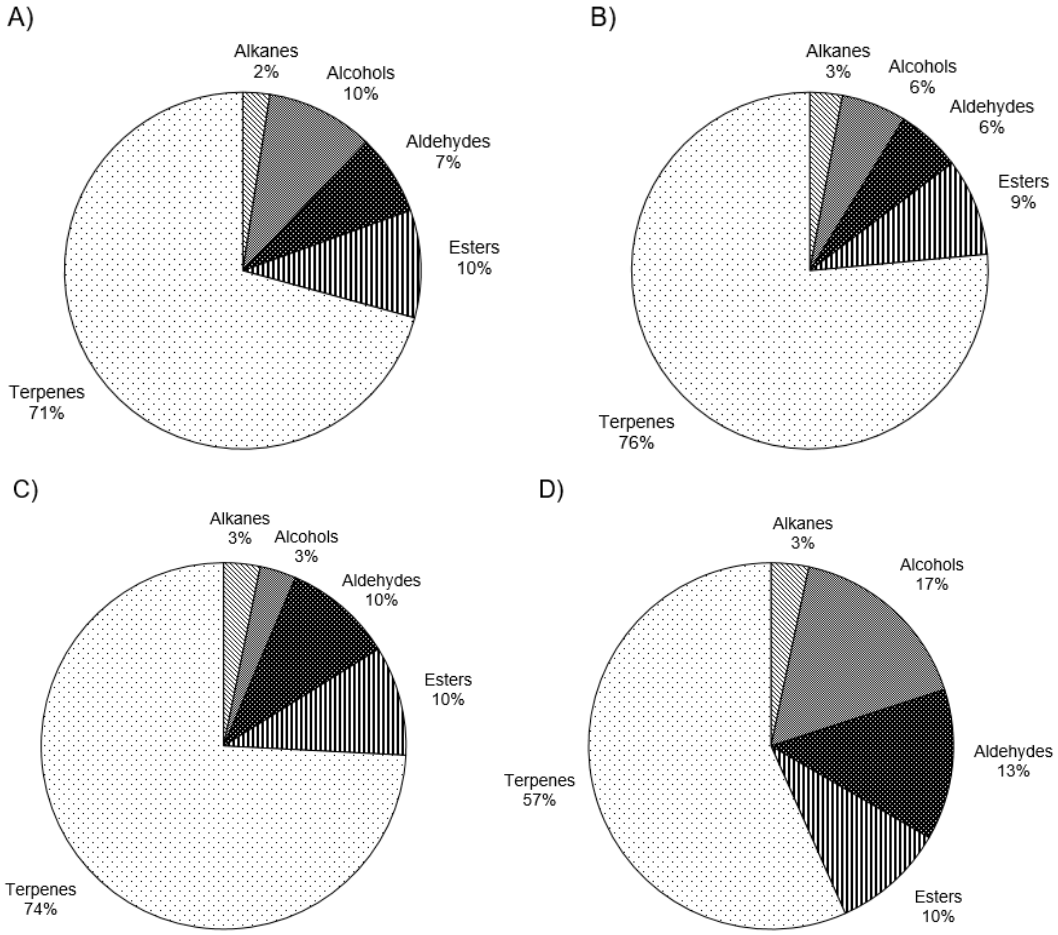

Figure 1. The amount of the different volatile organic compounds (VOCs) types of the different *Cunila polyantha* samples dried at (**A**) 30 °C for 43 h, (**B**) 50 °C for 6 h, (**C**) 70 °C for 2 h, and (**D**) commercial green tea samples (*Camellia sinensis*).

Differences in VOC profiles obtained by GC-MS analysis were observed between the drying treatments analyzed. The drying treatment at 30 °C for 48 h, generated variations in the volatile profile since it showed the presence of compounds such as (**39**) ç-muurolene and (**43**) isoespatulenol, compounds that were not detected in any other treatments, including the control group (Table 1). The VOCs in the *C. polyantha* leaves and flowers (dried at different temperatures and times) and commercial green tea leaves were compared (Table 1). The total volatile content in samples of *C. polyantha* dried at 30 °C for 48 h was significantly higher ($p > 0.05$) than in other samples, and also more individual VOCs were detected.

Table 1. Volatile organic compounds (VOCs) identified from *Cunila polyantha* samples dried at different temperatures and commercial green tea samples (*Camellia sinensis*).

Chemical Family	Organic Volatile Compounds	#	t_R (min)	RI	Content (μL/g DT) †				CAS Number	*m/z* *
					Drying Temperature					
					30 °C for 43 h	50 °C for 6 h	70 °C for 2 h	Green Tea		
Alkanes	Tetradecane	1	13.35	1134	2.87 ± 0.44 [a]	1.15 ± 0.16 [b]	0.40 ± 0.06 [c]	0.05 ± 0.01 [d]	629-59-4	70, 87, 70
Alcohols	1-Octen-3-ol	2	8.79	893	0.99 ± 0.16 [a]	0.24 ± 0.02 [c]	0.10 ± 0.01 [d]	0.35 ± 0.02 [b]	3391-86-4	57, 72, 43
	1-Hexanol, 2-ethyl-	3	9.48	924	ND	ND	ND	0.11 ± 0.01	104-76-7	57, 41, 43
	2,5-Dimethylcyclohexanol	4	10.50	973	2.28 ± 0.45 [a]	ND	ND	0.12 ± 0.01 [b]	3809-32-3	71, 55, 95
	cis-*p*-Mentha-2,8-dien-1-ol	5	10.83	989	0.34 ± 0.01 [a]	0.29 ± 0.04 [a]	ND	0.05 ± 0.005 [b]	3886-78-0	43, 79, 109
	1-Penten-3-ol	6	20.47	1614	3.14 ± 0.78 [a]	ND	ND	0.21 ± 0.05 [b]	616-25-1	55, 56, 68
Aldehydes	Hexanal	7	4.67	747	2.38 ± 0.29 [a]	0.29 ± 0.04 [c]	0.05 ± 0.006 [d]	0.72 ± 0.12 [b]	66-25-1	44, 41, 56
	Furfural	8	5.51	775	1.17 ± 0.25 [a]	ND	0.04 ± 0.005 [b]	0.03 ± 0.004 [c]	98-01-1	96, 95, 97
	2-Hexenal, (E)-	9	6.31	802	6.73 ± 1.34 [a]	0.54 ± 0.06 [b]	0.21 ± 0.03 [c]	0.10± 0.01 [d]	6728-26-3	57, 99, 69
	Heptanal	10	7.35	840	ND	ND	ND	0.06 ± 0.01	111-71-7	44, 43, 70
Esters	Methylcyclopentyl acetate	11	7.31	838	5.91 ± 1.12 [a]	ND	ND	0.03 ± 0.005 [b]	24070-70-0	43, 84, 67
	1-octen-3-yl acetate	12	10.42	969	13.69 ± 2.99 [a]	3.90 ± 0.92 [b]	1.65 ± 0.33 [c]	0.28 ± 0.03 [d]	2442-10-6	43, 99, 54
	3-octilo acetate	13	10.56	976	1.33 ± 0.26 [a]	0.86 ± 0.12 [b]	0.46 ± 0.11 [c]	0.05 ± 0.01 [d]	4864-61-3	43, 101, 41
	Trans-Carveyl acetate	14	12.71	1094	2.05 ± 0.50 [a]	1.36 ± 0.27 [b]	0.74 ± 0.15 [c]	ND	1134-95-8	84, 43, 119
Terpenes	à-Pinene	15	8.36	877	1.58 ± 0.25 [a]	0.53 ± 0.06 [b]	0.18 ± 0.04 [c]	0.14 ± 0.02 [c]	80-56-8	93, 92, 77
	Carveol	16	9.12	907	0.86 ± 0.06 [a]	0.62 ± 0.03 [b]	0.28 ± 0.03 [c]	ND	99-48-9	84, 134, 109
	p-Mentha-1,5,8-triene	17	9.38	919	0.33 ± 0.02 [a]	0.29 ± 0.05 [a]	0.13 ± 0.02 [b]	0.01 ± 0.003 [c]	21195-59-5	91, 134, 119
	Limonene	18	9.45	922	1.22 ± 0.27 [a]	0.90 ± 0.07 [b]	0.49 ± 0.05 [c]	0.02 ± 0.003 [d]	138-86-3	93, 91, 94
	Ocimene	19	9.62	931	0.05 ± 0.01 [b]	0.08 ± 0.01 [a]	ND	0.03 ± 0.008 [c]	13877-91-3	93, 41, 79
	Linalool	20	10.38	967	1.29 ± 0.30 [a]	1.27 ± 0.22 [a]	0.51 ± 0.07 [b]	ND	78-70-6	71, 93, 55
	Verbenol	21	10.94	994	0.40 ± 0.03 [a]	ND	0.12 ± 0.01 [b]	ND	1820-09-3	94, 109, 41
	l-Menthone	22	11.10	1002	3.48 ± 0.39 [a]	2.85 ± 0.52 [b]	1.57 ± 0.29 [c]	0.06 ± 0.008 [d]	14073-97-3	112, 139, 69
	Isopulegol	23	11.33	1015	0.83 ± 0.08 [a]	0.68 ± 0.05 [b]	0.15 ± 0.02 [c]	ND	89-79-2	41, 69, 68
	à-Terpineol	24	11.48	1023	2.29 ± 0.13 [a]	1.75 ± 0.23 [b]	0.54 ± 0.10 [c]	0.07 ± 0.004 [d]	98-55-5	59, 93, 43
	8,9-deshydrothymol	25	11.78	1041	ND	ND	ND	0.13 ± 0.01	6831-16-9	105, 161, 91
	Carvenone	26	11.81	1042	5.94 ± 0.56 [a]	5.65 ± 0.99 [a]	0.66 ± 0.11 [b]	0.009 ± 0.001 [c]	499-74-1	110, 95, 67
	Pulegone	27	11.91	1048	7.53 ± 0.56 [a]	6.00 ± 0.71 [b]	2.01 ± 0.43 [c]	0.32 ± 0.05 [d]	89-82-7	81, 67, 152
	(-)-Carvone	28	12.24	1067	3.42 ± 0.81 [a]	3.50 ± 0.43 [a]	0.99 ± 0.15 [b]	0.06 ± 0.01 [c]	6485-40-1	82, 55, 108
	Thymol	29	12.55	1085	2.79 ± 0.19 [a]	2.79 ± 0.26 [a]	0.88 ± 0.14 [b]	0.05 ± 0.003 [c]	89-83-8	109, 79, 107
	Indole	30	12.64	1090	0.73 ± 0.05 [b]	0.92 ± 0.08 [a]	ND	0.03 ± 0.007 [c]	120-72-9	117, 89, 118
	Elemene	31	12.81	1099	1.51 ± 0.19 [b]	2.33 ± 0.53 [a]	0.65 ± 0.04 [c]	ND	11029-06-4	121, 136, 41
	Copaene	32	13.24	1127	0.27 ± 0.05 [a]	0.29 ± 0.02 [a]	ND	ND	3856-25-5	105, 119, 93

Table 1. Cont.

Chemical Family	Organic Volatile Compounds	#	t_R (min)	RI	Content (µL/g DT) †				CAS Number	*m/z* *
					Drying Temperature					
					30 °C for 43 h	50 °C for 6 h	70 °C for 2 h	Green Tea		
Terpenes	(-)-á-Bourbonene	33	13.32	1132	2.50 ± 0.21 [a]	1.66 ± 0.10 [b]	0.77 ± 0.09 [c]	0.02 ± 0.004 [d]	5208-59-3	81, 80, 123
	Longifolene	34	13.57	1148	ND	ND	ND	0.04 ± 0.006	475-20-7	161, 94, 91
	Cariofilene	35	13.65	1153	17.00 ± 3.97 [a]	16.25 ± 2.26 [a]	6.87 ± 0.99 [b]	0.24 ± 0.04 [c]	87-44-5	93, 133, 91
	Aromadedrene	36	13.81	1164	1.46 ± 0.34 [b]	2.29 ± 0.55 [a]	1.08 ± 0.11 [c]	ND	489-39-4	145, 159, 202
	Humulene	37	13.96	1173	1.28 ± 0.07 [b]	1.39 ± 0.34 [a]	0.51 ± 0.05 [c]	ND	6753-98-6	93, 80, 41
	á-Panasinsene	38	13.99	1176	0.57 ± 0.13 [a]	0.54 ± 0.07 [a]	0.31 ± 0.0 [b]	ND	56633-28-4	122, 107, 161
	ç-muurolene	39	14.00	1176	0.35 ± 0.02	ND	ND	ND	30021-74-0	105, 161, 41
	à-ylangene	40	14.09	1182	0.49 ± 0.11 [c]	0.91 ± 0.14 [a]	0.58 ± 0.11 [b]	0.12 ± 0.02 [d]	14912-44-8	105, 119, 93
	Trans-á-Ionone	41	14.10	1183	ND	ND	ND	0.036 ± 0.003	79-77-6	177, 43, 41
	Germacrene D	42	14.16	1187	2.84 ± 0.35 [b]	4.87 ± 0.43 [a]	1.01± 0.06 [c]	ND	23986-74-5	161, 105, 91
	Isoespatulenol	43	14.72	1224	0.14 ± 0.03	ND	ND	ND	6750-60-3	43, 41, 91
	Caryophyllene oxide	44	14.76	1227	0.45 ± 0.10 [a]	0.38 ± 0.03 [b]	0.23 ± 0.01 [c]	ND	1139-30-6	43, 41, 79
	Aromadendreno epoxide	45	15.37	1270	0.23 ± 0.03 [a]	0.23 ± 0.05 [a]	ND	ND	489-39-4	41, 61, 91
	(-)-Spathulenol	46	15.39	1271	0.52 ± 0.08 [a]	0.18 ± 0.01 [c]	0.59 ± 0.09 [a]	ND	77171-55-2	43, 41, 91

Abbreviations: t_R, retention time; RI, retention indices; ND, not determined; DT, dry tissue. † Relative concentration of compound calculated, equivalent to 1-nonanol. * *m/z* Top Peak, *m/z* 2nd Highest, *m/z* 3rd Highest. Values are expressed as mean ± standard deviation of three repetitions. [a, b, c, d] Values with different letters indicate significant differences (Tukey, $p < 0.05$).

Exposure to high temperatures can modify the volatile compounds in tea, and thus the formation of aromas [26]. Based on the differences between *C. polyantha* samples volatile composition, it is possible to infer that drying time–temperature affected the volatile profile. As can be seen in Table 1, most of the volatile compounds identified in the *C. polyantha* samples decreased in concentration as the drying temperature increased. Some of the other volatile constituents identified in samples dried with low-temperature and long-time were not identified in samples dried with high-temperature and short-time, which resulted in an increase in the percentage of other volatile constituents. In the case of terpenes, such as (**28**) (-)-carvone, (**30**) indole, (**31**) elemene, (**32**) copaene, (**36**) aromadedrene, (**37**) humulene, (**40**) à-ylangene, and (**42**) germacrene D were found to be more abundant in the samples dried at 50 °C for 6 h.

When herbs are dried using convective air drying, which is characterized by a low heat and mass transfer efficiency, a prolonged drying time may lead to high-quality deterioration in the final dried product [27]. The variation in the volatile components from *C. polyantha* samples dried with prolonged times can be related to the degradation of heat-sensitive volatile constituents. Moreover, the high temperature environment of drying can promote the hydrolysis, reduction, dehydration, isomerization, and other reactions of bonded terpenes to produce terpenes [28] which could explain the increase in concentration of terpenes in the *C. polyantha* samples dried with high-temperature and moderate-time.

3.2. Effects of Drying (Time–Temperature) and Steeping (Temperature) on Phenolic Contents and Antioxidant Activity of Infusions

In this study, we investigated the effect of different drying and steeping temperatures and their interaction on the total phenolic, flavonoid, and tannin contents, free radical scavenging activity, and total antioxidant capacity of *C. polyantha* infusions.

The analysis showed that the combination of different drying and steeping treatments showed an effect on the total phenolic content of the *C. polyantha* infusions. A higher concentration of total phenolics in the infusions of *C. polyantha* was obtained when low drying temperatures and high steeping temperatures were used (Figure 2A). The treatment CP-D30S100, that consisted of drying at 30 °C for 43 h and steeping temperature at 100 °C, was the most effective since it obtained the highest total phenolic content (25.62 ± 1.46 mg GAE/g DT) in the *C. polyantha* infusions (Table 2). Despite the above, the concentration of total phenolics in the *C. polyantha* infusions was lower than green tea infusions (CS-S80, CS-S90, and CS-S100), since the control group showed higher total phenolic contents in all the steeping temperatures analyzed (80, 90, and 100 °C), which were significant ($p < 0.05$) according to Table 2. Regarding the control group, the different steeping temperatures used also showed an effect on the total phenolic content of the commercial green tea infusions, and as before, higher phenolic contents were observed in the green tea infusions with high steeping temperatures.

As can be seen in Table 2, the lowest value of total flavonoid content of the *C. polyantha* infusions was found in the treatment that combined drying temperature of 50 °C for 6 h and steeping at 90 °C (CP-D50S90). On the other hand, the highest flavonoids concentration (12.82 ± 1.61 mg CE/g DT) was determined in the treatment that combined drying at 70 °C for 2 h and steeping at 100 °C (CP-D70S100). The total flavonoids content of *C. polyantha* infusions were not significantly different ($p > 0.05$) from green tea infusions (Table 2). According to Figure 2B, higher total flavonoid content values were obtained when short periods of drying time (2 h), combined with high steeping temperatures (100 °C) were used. The increase in the concentration of total flavonoids can be attributed to the increase in steeping temperature, since as the steeping temperature increased (to 90 and 100 °C) the total flavonoid concentrations increased, until reaching the maximum values in infusions with steeping temperatures of 100 °C. The obtained results support previously published data [29] that water at a higher temperature (100 °C) extracted higher total phenolic and total flavonoid contents.

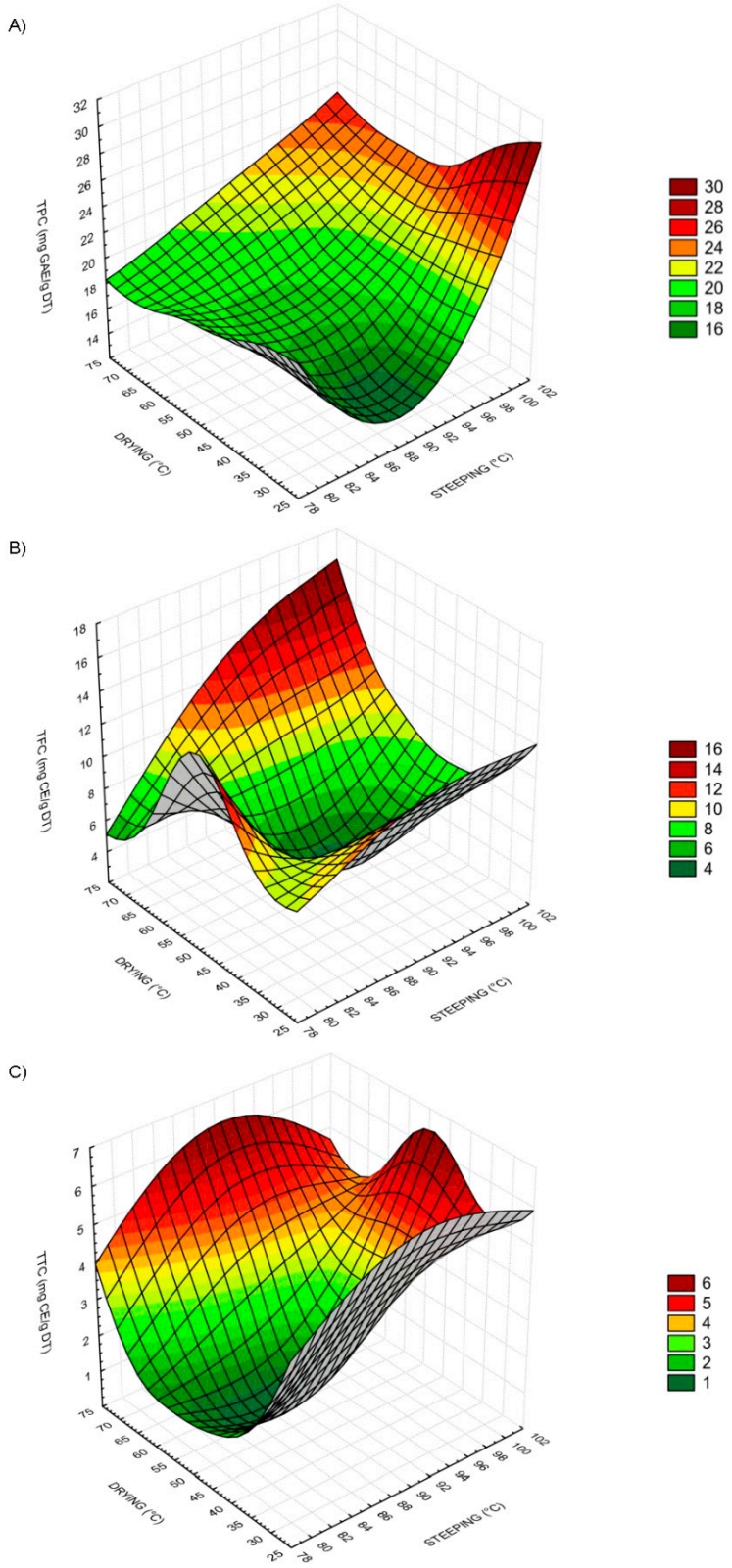

Figure 2. Interaction effect of different drying and steeping temperatures on (**A**) total phenolic content (TPC), (**B**) total flavonoid content, and (**C**) total tannins content of infusions obtained from flowers and leaves of *Cunila polyantha*.

Table 2. Total phenolic, flavonoids, and tannins contents, free radical scavenging activity, and total antioxidant capacity of *Cunila polyantha* and *Camellia sinensis* infusions obtained with different drying and steeping temperatures.

Infusion	TPC (mg GAE/g DT)	TFC (mg CE/g DT)	CTC (mg CE/g DT)	DPPH	ABTS•+	TAC (mg AAE/g DT)
				%	IC_{50} (mg/mL)	
CP-D30S80	19.53 ± 0.95 ^{c,d,e}	8.73 ± 1.69 ^{b,c,d}	3.78 ± 0.29 ^{e,f}	23.30 ± 3.69 ^{b,c}	0.05 ± 0.001 ^{a}	131.91 ± 20.43 ^{e}
CP-D30S90	14.42 ± 0.53 ^{e}	10.36 ± 1.30 ^{a,b}	5.38 ± 0.38 ^{c}	30.67 ± 3.61 ^{b}	0.18 ± 0.04 ^{b}	149.58 ± 12.08 ^{e}
CP-D30S100	25.62 ± 1.46 ^{c}	9.95 ± 0.49 ^{a,b,c}	5.25 ± 0.11 ^{c,d}	24.09 ± 1.92 ^{b,c}	0.18 ± 0.03 ^{b}	163.417± 0.28 ^{d,e}
CP-D50S80	18.77 ± 1.69 ^{e,d}	12.00 ± 0.88 ^{a,b}	1.15 ± 0.19 ^{g}	24.04 ± 3.20 ^{b,c}	0.69 ± 0.06 ^{e}	126.25 ± 11.35 ^{e}
CP-D50S90	17.68 ± 0.66 ^{e,d}	3.53 ± 0.82 ^{e}	1.02 ± 0.29 ^{g}	15.84 ± 7.73 ^{c}	0.40 ± 0.03 ^{d}	198.41 ± 9.87 ^{d}
CP-D50S100	22.35 ± 0.17 ^{c,d}	6.54 ± 0.41 ^{c,d,e}	5.44 ± 0.58 ^{c}	28.88 ± 6.21 ^{b}	0.01 ± 0.001 ^{a}	243.58 ± 12.41 ^{c}
CP-D70S80	18.31 ± 0.27 ^{e,d}	6.40 ± 0.98 ^{d,e}	3.14 ± 0.11 ^{f}	21.68 ± 2.08 ^{b,c}	0.41 ± 0.04 ^{d}	169.08 ± 11.25 ^{d,e}
CP-D70S90	20.90 ± 0.82 ^{c,d}	11.18 ± 0.92 ^{a,b}	5.19 ± 0.19 ^{c,d}	22.59 ± 2.84 ^{b,c}	0.22 ± 0.04 ^{b,c}	160.583± 15.76 ^{d,e}
CP-D70S100	23.73 ± 1.45 ^{c,d}	12.82 ± 1.61 ^{a}	4.42 ± 0.38 ^{d,e}	20.73 ± 5.22 ^{b,c}	0.31 ± 0.04 ^{c,d}	192.91 ± 23.02 ^{d}
CS-S80	57.19 ± 0.69 ^{b}	10.77 ± 0.80 ^{a,b}	5.96 ± 0.19 ^{c}	88.45 ± 0.81 ^{a}	0.05 ± 0.003 ^{a}	337.83 ± 12.85 ^{b}
CS-S90	70.77 ± 1.73 ^{a}	10.36 ± 1.69 ^{a,b}	10.38 ± 0.19 ^{a}	88.99 ± 1.67 ^{a}	0.05 ± 0.009 ^{a}	364.50 ± 4.00 ^{a,b}
CS-S100	71.92 ± 6.20 ^{a}	12.41 ± 1.61 ^{a}	9.23 ± 0.38 ^{b}	88.75 ± 0.33 ^{a}	0.04 ± 0.008 ^{a}	397.83 ± 23.02 ^{a}

Abbreviations: TPC, total phenolic content; TFC, total flavonoid content; CTC, condensed tannin content; CP, *Cunila polyantha*; CS, *Camellia sinensis*; D, drying temperature; S, steeping temperature; GAE, gallic acid equivalents; CE, catechin equivalents; AAE, ascorbic acid equivalents; DT, dry tissue; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); IC_{50}, median inhibitory concentration. Values are expressed as mean ± standard deviation of three repetitions. Values with different letters indicate significant differences (Tukey, $p < 0.05$).

Condensed tannins are compounds of greater molecular weight, or bound to fiber or protein [30], this complex chemical structure can explain the resistance to thermal degradation since as can be observed in Figure 2C, no degradation of these compounds was shown in the *C. polyantha* tissues dried at the highest temperature (70 °C). Regarding the total tannins content, most treatments were effective in tannins extraction (Figure 2C), however their tannin concentrations were lower than the control group (*C. sinensis*), since all treatments showed total tannin contents in the *C. polyantha* infusions to be lower than the commercial green tea infusions prepared at steeping temperatures of 90 and 100 °C (CS-S90 and CS-S100), as seen in Table 2. The thermal destruction of cell walls and sub-cellular compartments during the steeping process can favor the release of phenolic compounds [31]. The increased concentration of condensed tannins in the infusions of *C. polyantha* treated with high steeping temperatures (90 and 100 °C), could be due to the fact that at a higher steeping temperature a better degradation of the cell walls and organelles of *C. polyantha* tissues was achieved, and therefore a greater release of tannins was obtained. The correlation analysis showed a significant positive correlation between the total phenolic with the condensed tannins contents ($R = 0.820$, $p < 0.01$).

Phenolic compounds have shown to possess antioxidant properties. In this work, *C. polyantha* infusions showed scavenging activity of DPPH• and ABTS•+ radicals, as well as relevant antioxidant capacity, which was dependent on tea preparation conditions. As seen in Table 2, a significant difference between antioxidant profiles of the infusions was observed. According to the analysis, the maximum DPPH• scavenging values of the *C. polyantha* infusions were in the treatments CP-D50S100 and CP-D30S90 (28.88 and 30.67%, respectively), while the green tea infusions CS-S80, CS-S90, and CS-S100, showed the highest DPPH• scavenging percentages (88.45%, 88.99%, and 88.75%, respectively). Horžić and co-workers [29] report inhibition percentages for commercial teas infusions with different steeping temperatures, ranging from 85.22% for white tea (at 60 °C), 85.77% for white tea (at 80 °C), 88.32% for white tea (100 °C), 86.13% for green tea (at 60 °C), 88.50% for green tea (at 80 °C), 89.23% for green tea (at 100 °C), showing that the steeping temperature of the infusions were decisive in the DPPH radical inhibition percentages shown. According to the analysis, a clear correlation was observed between the total phenolic content with the DPPH• scavenging activity ($R = 0.959$, $p < 0.01$) of the *C. polyantha* infusions. Additionally, a moderate correlation was shown between condensed tannins content and the DPPH• scavenging activity ($R = 0.788$, $p < 0.01$).

The results of this study show a significant highest scavenging activity of $ABTS^{\bullet+}$ by the treatments CP-D50S100 and CP-D30S80 (0.01 ± 0.001 and 0.05 ± 0.001 mg/mL, respectively), which were not significantly different from the control ($p > 0.05$). In this study also, a negative correlation was observed between the condensed tannins content with the IC_{50} values of $ABTS^{\bullet+}$ radical ($R = -0.731$, $p < 0.01$) indicating that increasing the quantity of condensed tannins leads to a quantitative decrease in the IC_{50} values of the infusions and thus, significantly higher scavenging activity of $ABTS^{\bullet+}$ radicals. According to the above, the presence of phenolic compounds in *C. polyantha* may be responsible for their radical scavenging activity.

The analysis of the total antioxidant capacity of the infusions showed values in the range of 131.91 ± 20.43 to 397.83 ± 23.02 mg AAE/g DT (Table 2). The correlation analysis indicated a positive correlation between the total phenolic and condensed tannin contents with the total antioxidant capacity ($R = 0.932$, $p < 0.01$ and $R = 0.775$, $p < 0.01$, respectively).

Considering our results, we can deduce that the drying conditions and steeping temperature affected the phenolic composition and antioxidant properties of the *C. polyantha* infusions. In general, in terms of antioxidant activity, prolonged drying times combined with higher steeping temperatures showed the highest antioxidant activities compared to the other drying and steeping conditions analyzed.

3.3. Sensory Analysis

A sensory analysis was carried out by a panel of consumer judges, made up of 14 women and six men, who said they consumed infusions between three and 14 times per week, to determine the preference of infusions of leaves and flowers of *C. polyantha*. The analysis showed variations in the panelists' preferences for certain infusions. The results of the acceptance of infusions for each of the three different drying and steeping treatments analyzed are described below.

In Figure 3A, which corresponds to the drying treatment at 30 °C, it was observed that the judges, for the most part, preferred the odor/aroma and flavor/taste of the CP-D30S80 infusion, which was prepared at a steeping temperature of 80 °C. According to the panelists, the appearance and texture/mouthfeel of the infusions CP-D30S80 and CP-D30S100, was very similar, although its odor/aroma and flavor/taste were different. On the other hand, the least preferred infusion of this treatment was that prepared with a steeping temperature of 90 °C (CP-D30S90), which was rated by the judges with low preference values of odor/aroma, flavor/taste, and texture/mouthfeel, but with similar appearance to the treatments with steeping temperatures of 80 and 100 °C.

The preferred behavior of *C. polyantha* infusions of the 50 °C drying treatment, determined by the judges, is shown in Figure 3B. The judges preferred the odor/aroma, flavor/taste, and texture/mouthfeel of the CP-D50S100 infusion, while, on the other hand, they preferred the appearance of the CP-D50S90 infusion, prepared with a steeping temperature of 100 and 90 °C, respectively. On the contrary, the infusion that showed the least flavor/taste and appearance preference was the CP-D50S80 infusion, prepared with a steeping temperature of 80 °C.

Regarding the drying treatment of the leaves and flowers of *C. polyantha* at 70 °C, the judges preferred the appearance and odor/aroma of the infusions of the CP-D70S100 treatment whose steeping temperature was 100 °C, while the most preferred flavor/taste and texture/mouthfeel was the CP-D70S90 treatment, with steeping temperature of 90 °C. The infusion that showed the least preference in all the criteria was the CP-D70S80 infusion. The preferred behavior of *C. polyantha* infusions of the 70 °C drying treatment is shown in Figure 3C.

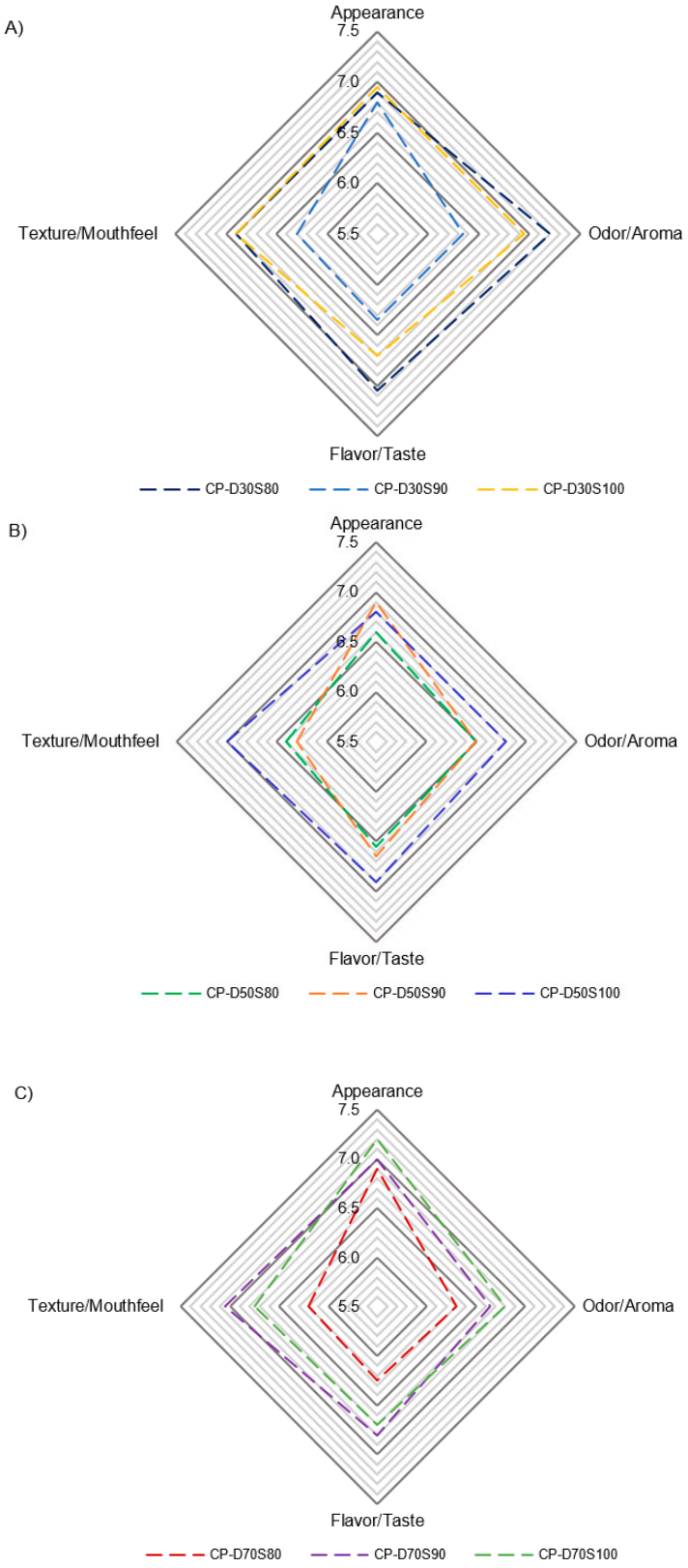

Figure 3. Effects of steeping temperature (80, 90, and 100 °C) on sensory properties of infusions obtained from flowers and leaves of *Cunila polyantha* dried at (**A**) 30 °C for 43 h, (**B**) 50 °C for 6 h, and (**C**) 70 °C for 2 h.

Overall, the best perception of odor/aroma and flavor/taste, according to the judges, was obtained by the infusion of leaves and flowers of *C. polyantha* dried at 30 °C and prepared with a steeping temperature of 80 °C (CP-D30S80). The results of sensory analysis were consistent with the results of GC-MS analysis, since these infusions showed different aroma characteristics from the others, since—as mentioned earlier—they showed the presence of compounds such as 1-octen-3-ol, 1,3,8-*p*-menthatriene, and ocimene that were not detected in any other treatments, including the control group. These odorants provide mushroom-like, metallic, woody, pine, tropical, green aroma notes to the *C. polyantha* infusions [32–34]. Therefore, the above-mentioned aroma-active compounds could have contributed in the particular odor constitution of these infusions.

3.4. Drying-Steeping Temperatures Relation with Phenolic Compounds Extraction of Infusions

The qualitatively analysis by HPLC-PAD revealed the presence of nine phenolic compounds identified in the infusions of *C. polyantha*. Four of the identified phenolic compounds belong to phenolic acids, four to flavonoids, and one to phenolic aldehydes. The phenolic acids identified in the infusions of *C. polyantha* were: gallic acid (**2**), chlorogenic acid (**3**), caffeic acid (**5**), and *p*-coumaric acid (**6**). The flavonoids identified were: epigallocatechin gallate (**1**), protocatechin (**4**), quercetin (**8**) and naringenin (**9**). Finally, the phenolic aldehyde identified was vanillin (**7**). The phenolic compounds identified in the *C. polyantha* infusions are summarized in Tables 3–5. The compounds identified in the infusions of the control group were: epigallocatechin gallate (**1**), caffeic acid (**5**) and naringenin (**9**), which are shown in Table 6. The HPLC-PDA chromatograms of phenolic compounds detected in the infusions are summarized in Figure S1.

Differences between treatments in the phenolic profiles of the *C. polyantha* infusions were observed. Each combination of drying and steeping temperature used in the preparation of the *C. polyantha* infusions generated a unique and different phenolic profile. In addition, a tendency to increase the peak area of the compounds was observed as the steeping temperature increased. As indicated in Table 6, no differences were observed in the phenolic profiles of the commercial green tea infusions, nevertheless, the same tendency to increase the peak area of the individual phenolic compounds with increasing steeping temperature was observed. Comparing the peak area of the epigallocatechin gallate (**1**) and caffeic acid (**5**) present in the infusions of green tea, it can be seen that they are superior to those obtained in the infusions of *C. polyantha*, but not in the case of the flavonoid naringenin (**9**).

Table 3. Phenolic composition of infusions obtained from flowers and leaves of *Cunila polyantha* dried at 30 °C for 48 h and prepared at different steeping temperatures (80, 90, and 100 °C) identified by high-performance liquid chromatography (HPLC-PDA).

Number	Compound	Steeping Temperature					
		80 °C		90 °C		100 °C	
		t_R (min)	Peak Area	t_R (min)	Peak Area	t_R (min)	Peak Area
1	Epigallocatechin gallate	4.373	106,181	4.371	286,415	4.370	541,991
2	Gallic acid	4.730	1,136,667	4.738	1,747,984	4.736	2,180,084
3	Chlorogenic acid	ND	ND	5.347	1,866,047	5.003	358,503
4	Protocatechin	ND	ND	ND	ND	5.34	3,227,782
5	Caffeic acid	5.363	1,301,061	5.857	436,420	6.087	404,754
6	*p*-coumaric acid	6.131	98,752	6.369	310,214	ND	ND
7	Vanillin	6.395	283,691	ND	ND	6.623	118,805
8	Quercetin	7.218	416,209	7.193	734,569	7.171	989,376
9	Naringenin	ND	ND	8.078	78,809	8.038	31,122

Abbreviations: t_R, retention time; ND, not determined.

Table 4. Phenolic composition of infusions obtained from flowers and leaves of *Cunila polyantha* dried at 50 °C for 6 h and prepared at different steeping temperatures (80, 90, and 100 °C) identified by high-performance liquid chromatography (HPLC-PDA).

Number	Compound	Steeping Temperature					
		80 °C		90 °C		100 °C	
		t_R (min)	Peak Area	t_R (min)	Peak Area	t_R (min)	Peak Area
1	Epigallocatechin gallate	ND	ND	4.355	486,289	4.357	404,959
2	Gallic acid	4.797	98,398	4.728	1,876,394	4.731	1,465,993
3	Chlorogenic acid	ND	ND	ND	ND	4.960	757,494
4	Protocatechin	5.021	110,616	5.003	304,986	ND	ND
5	Caffeic acid	5.375	206,102	5.323	2,276,951	5.326	2,217,151
6	*p*-coumaric acid	ND	ND	6.075	302,784	6.076	180,639
7	Vanillin	6.102	230,575	6.609	94,170	6.344	322,764
8	Quercetin	7.187	19,769	7.151	423,062	7.148	462,929
9	Naringenin	8.087	12,806	8.030	46,773	8.033	41,433

Abbreviations: t_R, retention time; ND, not determined.

Table 5. Phenolic composition of infusions obtained from flowers and leaves of *Cunila polyantha* dried at 70 °C for 2 h and prepared at different steeping temperatures (80, 90, and 100 °C) identified by high-performance liquid chromatography (HPLC-PDA).

Number	Compound	Steeping Temperature					
		80 °C		90 °C		100 °C	
		t_R (min)	Peak Area	t_R (min)	Peak Area	t_R (min)	Peak Area
1	Epigallocatechin gallate	4.346	194,871	4.356	436,664	4.354	532,883
2	Gallic acid	4.731	1,025,337	4.739	1,572,073	4.729	1,820,868
3	Chlorogenic acid	4.964	495,376	4.971	729,126	4.973	772,322
4	Protocatechin	ND	ND	ND	ND	ND	ND
5	Caffeic acid	5.330	1,828,991	5.327	2,402,805	5.328	3,109,359
6	*p*-coumaric acid	ND	ND	6.077	294,241	ND	ND
7	Vanillin	ND	ND	6.604	95,805	6.602	119,970
8	Quercetin	7.152	239,646	7.158	350,394	7.153	513,438
9	Naringenin	8.028	33,211	8.037	40,611	8.035	60,627

Abbreviations: t_R, retention time; ND, not determined.

Table 6. Phenolic composition of infusions obtained from commercial green tea from leaves of *Camellia sinensis* prepared at different steeping temperatures (80, 90, and 100 °C) identified by high-performance liquid chromatography (HPLC-PDA).

Number	Compound	Steeping Temperature					
		80 °C		90 °C		100 °C	
		t_R (min)	Peak Area	t_R (min)	Peak Area	t_R (min)	Peak Area
1	Epigallocatechin gallate	4.652	36,322,211	4.655	48,547,546	4.651	48,377,742
2	Gallic acid	ND	ND	ND	ND	ND	ND
3	Chlorogenic acid	ND	ND	ND	ND	ND	ND
4	Protocatechin	ND	ND	ND	ND	ND	ND
5	Caffeic acid	5.553	13,670,685	5.556	17,168,486	5.552	17,909,559
6	*p*-coumaric acid	ND	ND	ND	ND	ND	ND
7	Vanillin	ND	ND	ND	ND	ND	ND
8	Quercetin	ND	ND	ND	ND	ND	ND
9	Naringenin	7.837	11,910	7.822	6550	7.817	8534

Abbreviations: t_R, retention time; ND, not determined.

It has been reported that both time and temperature of tea infusion have pronounced effects on the extraction of catechins [35–37]. Besides, if infusion time is prolonged, tea epicatechins (epicatechin,

epicatechin gallate, epigallocatechin and epigallocatechin gallate) may undergo epimerization, where tea catechins are converted to their corresponding non-epi isomers (gallocatechin gallate, catechins gallate, gallocatechin, and catechin) [38,39] that show lower antioxidant capacity [40]. In this sense, other authors [41] studied the effect of both time (1, 2, 3, 5, 10, 20, 30 and 45 min) and temperature (75, 85 and 95 °C) on the extraction and epimerization of catechins in green tea, and they found that steeping at 85 °C for 3 min was the optimal condition for the extraction of epigallocatechin gallate. Although in this investigation we did not analyze the effect of steeping time (since it remained constant at 5 min for all treatments), we observed that the steeping temperature affected the amount (peak area) of epigallocatechin gallate (**1**) of the infusions, finding the greatest amount (peak area) of this compound in the infusions obtained from flowers and leaves of *C. polyantha* dried at 30 °C for 48 h and prepared at 100 °C (Table 3), while in green tea it was shown in the infusions prepared at a steeping temperature of 90 °C (Table 6). The above might be because the steeping temperature increases the solubility of tea catechins [42]. Analysis of the correlation was performed on the abundance (peak area) of epigallocatechin gallate of infusions and its mutual relationship with the investigated antioxidant properties. A statistically significant relationship is presented below. Statistically significant positive correlations between the peak area of epigallocatechin gallate with the DPPH$^{\bullet}$ scavenging activity, and with the total antioxidant capacity were found ($R = 0.975$, $p < 0.01$ and $R = 0.935$, $p < 0.01$, respectively).

Based on the results, it can be concluded that the drying and steeping temperature of flowers and leaves of *C. polyantha* affected the phenolic profile and the antioxidant properties of the infusions.

4. Conclusions

This is the first investigation of phenolic composition, antioxidant activity, volatile composition and sensory properties of *C. polyantha* to be conducted simultaneously. The phenolic composition of *C. polyantha* infusions showed that the main compounds were flavonoids and phenolic acids. The recognized antioxidant flavonoids, epigallocatechin gallate, protocatechin, quercetin and naringenin were identified in the infusions. In addition, the infusions showed a high radical scavenging activity and high total antioxidant capacity values. Based on the GC-MS data, a total of 46 VOCs were detected and variations in the concentrations of these volatiles were observed in each of the *C. polyantha* samples, demonstrating that the samples differed in aroma quality. Current work demonstrates the differences in consumer acceptability of *C. polyantha* infusions prepared at different drying and steeping temperatures. According to the results of sensory analysis, the phenolic content and composition did not negatively influence consumer acceptance. However, the volatile profile did show an influence on the sensory properties of the *C. polyantha* infusions. This research highlights the importance of linking sensory and chemical data to obtain the best sensorial quality and the optimal nutraceuticals properties in *C. polyantha* infusions.

Supplementary Materials: The following are available online at http://www.mdpi.com/2227-9717/8/11/1378/s1, Figure S1. HPLC-PDA chromatograms of phenolic standards and infusions from flowers and leaves of *Cunila polyantha* (dried at 30 °C for 43 h) obtained at a steeping temperature of 90 °C and 100 °C, respectively.

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