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Antioxidant Capacity and Physicochemical Characteristics of Carbonated *Erica Arborea* Tea Beverage

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Abstract: The current study was aimed to generate an innovative tea beverage which has water infusion of *Erica arborea*. Dehydrated *Erica arborea* leaves were extracted in boiling water and the solution was brix value-balanced to 8° with sucrose, acid, natural lemon flavor, and antimicrobial agents. Following the blending of additives, carbonation was applied. Besides some physicochemical parameters, total phenolics, bioaccessibility of total phenols, antioxidant capacity, and bioaccessible antioxidants with 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity assay (CUPRAC) methods were also investigated. The green- and yellow-tinted beverage was especially rich in potassium and calcium. The total phenolics and bioaccessible phenolics of the beverage were identified as 174.06 ± 24.53 mg Gallic Acid Equivalent (GAE) 100 mL^{-1} and 96.07 ± 3.96 mg GAE 100 mL^{-1}, correspondingly. Antioxidant capacity was 27.20 ± 1.09 µmol trolox mL^{-1}, and the bioaccessible antioxidant capacity was 0.17 ± 0.02 µmol trolox mL^{-1} in DPPH. Measured antioxidant capacity and bioaccessible antioxidant capacity with other assays were 22.41 ± 2.49 and 3.09 ± 0.44 µmol trolox mL^{-1} for FRAP; 21.09 ± 1.65 and 0.02 ± 0.00 µmol trolox mL^{-1} for CUPRAC. In addition to its nutritional and functional features, *Erica arborea* tea beverage is preferred in accordance with the panelists' sensorial decision.

Keywords: *Erica arborea*; tea beverage; antioxidant capacity; phenolics; bioaccessibility

1. Introduction

There is common understanding that daily diet has an essential role in the occurrence of many diseases. Nowadays, several aspects of research data point out that specific dietary compounds act like preventive agent against cardiovascular disorders, some types of cancer, osteoporosis, inflammatory circumstances, and obesity. Functional foods, which have specific physiological benefits, contain bioactive ingredients. These ingredients may recognize as prebiotics, probiotics, flavonoids, phenolic compounds, phytosterols, bioactive peptides, and bioactive carbohydrates [1].

The genus Erica L. (*Ericaceae*) is described by more than 700 varieties from around the world. The herbal heather (*Erica arborea*) is a tree of the *Ericaceae* family, which is generally spread in South Africa and Mediterranean and West Europe, specifically the Northern and Southern parts of Turkey [2]. Only four species of *Ericaceae* family were presented in Turkey [3]. One of these species is *Erica arborea* L. Dried leaves of *Erica arborea* contains several phytochemicals, which show high antioxidant activity, and may be the cause of several health benefits such as a hypotensor, diuretic [4]. Several parts of this herb are preferred in various regions as a urinary antiseptic, and against constipation [5]. Flavonoids, phenolics, terpenoids, coumarins, and essential oils are the principle elements isolated

from *Erica arborea* [6]. *Erica arborea* is generally consumed as a herbal tea [7]. Consumers either purchase dehydrated herbs or ready-to-use tea bags containing herbs, from the market. Extraction practices and criteria of plants vary from each other, and improper use might decrease expected advantages and might induce detrimental health effects.

This research was planned to describe a newly patented herbal tea beverage production method which can prevent inappropriate traditional applications. In order to increase functionality, the carbonation process, which adds a refreshment property, prevents microbial growth and increases digestibility, is also applied. Standardizing the herbal tea beverage process gives an opportunity to consume that product in every season and produce a microbially safe and value-added product for manufacturers.

2. Materials and Methods

2.1. Materials

Dried leaves of herb and natural lemon flavor were purchased from associated companies in Turkey. Chemicals were supplied from Fluka, Steinheim, Germany (2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) and bile salts), Sigma Aldrich, Munich, Germany (Trolox, neocuproine, DPPH, methanol, Na_2CO_3, gallic acid, oxalic acid and NaOH), and Merck, Darmstadt, Germany (pepsin, pancreatin, $FeCl_3.6H_2O$, Folin-Ciocalteu, 2,6 dichlorophenol indophenol, $CuCl_2$, $C_2H_7NO_2$ and HCl).

2.2. Production of Herbal Tea Beverage

The herbal tea beverage was prepared according to Suna et al. [8]. Basically, gravimetrically 1% *Erica arborea* was placed in a nettle cloth and soaked in boiling water for 5 min without further warming. The infusion was used when the temperature decreased to 24–25 °C. The production was made in triplicate.

Sucrose, citric acid, ascorbic acid, and flavor were added to adjust the water-soluble dry matter of the beverage to 8° ± 0.5. Na-benzoate and K-sorbate were added as antimicrobial agents. Then the mixture was filtered (plate filter 60 × 60 CFP, Zambelli Enotech, Camisano Vicentino, Italy) and then bottled into 200 mL glass containers. The beverages were carbonated with CO_2 and capped. Due to the preservation effect of both CO_2 and antimicrobial agents, pasteurization process was not applied anymore. The bottles stored at room temperature until analyzed.

2.3. Physicochemical Analysis

Moisture content, ascorbic acid, and color (*L*, *a*, *b*) were determined to indicate the physical and chemical characteristics of dehydrated *Erica arborea* leaves. Furthermore, water-soluble dry matter, titratable acidity, pH, vitamin C (ascorbic acid), color (*L*, *a*, *b*), and turbidity examinations were applied to the beverage.

Oven drying method was used to determine the moisture content of *Erica arborea* dried leaves [9], while digital refractometer (RA-500 model KEM, Kyoto, Japan) used to perform water soluble dry matter of the beverage [10]. Titratable acidity was determined by the potentiometric method, with 0.1 N NaOH and acid content given as citric acid [11]. Seven compact pH/Ion Mettler Toledo pH meters were used to determine pH. The vitamin C was determined by Shimadzu UV 1208 model spectrophotometer using a 2,6-dichlorophenol indophenol dye [12]. Color parameters were determined with a HunterLab Colour Analyzer (MSEZ4500L, Hunter Associates Laboratory, Inc., Reston, VA, USA). Afterwards, introductory calibration of white and black surface layers the *L*, *a*, and *b* values were concluded [13]. Haziness of the beverage was determined as Nephelometric Turbidity Units (NTU) with a Hach turbidimeter (2100Q, HACH, Loveland, CO, USA) [14]. All analyses were repeated three times to ensure accuracy of the results.

2.4. Determination of Minerals

Some of the minerals (Fe, Ca, Mg, K, and Na) present in leaves of herb and beverage were analyzed and quantified with the Nordic Committee on Food Analysis (NMKL) [15] method by using Agilent 7500 CX (Agilent Technologies Inc., Santa Clara, CA, USA) model Inductively coupled plasma mass spectrometry (ICP-MS). For mineral content determination of process water, Eppendorf Elex 6361 model flame photometer (Na, K and Ca) and PerkinElmer Optima 2100 DV model ICP-OES (Mg and Fe) were used [16].

2.5. Determination of Phenolic Contents and Antioxidant Capacity

Samples taken from dried herbs were extracted (chemical extract) and beverage samples were obtained directly from the bottles. Phenolics and bioaccessible phenolics were examined as reported by Vitali et al. [17], with slight adjustments made by Suna [18]. The simulation of gastrointestinal conditions using commercial digestive enzymes (pepsin and pancreatin) is commonly used to specify the potential availability of bioactives. Shortly after, 10 mL of distilled water and 0.5 mL of pepsin were combined to 1 mL of sample, pH was modified to 2 with 5 mol L^{-1} HCl, and the mixture was shaken at 37 °C in a water bath for 1 h. Mimicking of stomachic digestion was discontinued by the inclusion of 1 M $NaHCO_3$ (to adapt pH to 7.2). 2.5 mL of bile/pancreatin mixture and 2.5 mL of NaCl/KCl were supplemented to the sample, and mimicking of small intestine digestion was run for the next 2 h. Specimens were centrifuged at 3500 rpm for 10 min and the supernatant (digested beverage) was employed for the analysis.

Antioxidant capacity measurement methods aim to measure the capacity of the antioxidant substances reliably and quickly. In this study, antioxidant capacity of extractable and bioaccesible phenolics were analyzed both in raw material and beverages, employing the DPPH assay [19], FRAP [20] and CUPRAC assay [21].

In the DPPH assay, 0.1 mL specimen was added to 3.9 mL of DPPH solution and vortexed (Vortex Mixer Classic, Velp Scientifica, Usmate, Italy) for 30 s. Test tubes were stood in the dark at room temperature for 30 mins to let the reaction occur. A trolox calibration curve (R^2 = 0.9951) was obtained by measuring the reduction in absorbance of the DPPH solution in the presence of different concentrations of trolox (10–100 µmol L^{-1}).

In FRAP assay, 3 mL of daily prepared FRAP reagent was mixed with 300 µL of distilled water and 100 µL of the sample or blank. The test samples, digested extracts, and blank were incubated at 37 °C for 60 min. At the end of the incubation, absorbance was measured immediately at 595 nm. The results were calculated from calibration curve as µmol trolox mL^{-1} for beverages (R^2 = 0.9975).

In the CUPRAC assay, antioxidant solutions were mixed with solutions of $CuCl_2$, neocuproine, and ammonium acetate, and the final absorbance was measured at 450 nm after 30 min (R^2 = 0.9947). Results were given as trolox equivalents.

2.6. Sensory Analysis

Sensory evaluation of the beverage was administrated for color, odor, appearance, and taste criteria by a panel consisting of 10 trained panelists. A 9-point structured scale, 9 being the best and 1 the worst, was preferred as the interpretation of measured criteria.

2.7. Statistical Analysis

The experimentation was managed in an entirely randomized scheme with three replicates. The analysis outcomes were statistically calculated by one-way analysis of variance (ANOVA) with the JMP software version 6.0 (SAS Institute Inc. Cary, NC, USA, 27513).

3. Results and Discussion

Based on the Turkish Standards Institution's acceptable upper limits on moisture content, which is 10 g 100 g^{-1} for dried herbs [22], dried *Erica arborea* was contained adequate amount of moisture, 7.36 ± 0.26 g 100 g^{-1}, in this study. Akis [23] also found that the moisture content of several dried herbs such as *Calluna vulgaris*, *Althaea officinalis*, *Tilia cordata*, *Urtica dioica* and other species lower than 10 g 100 g^{-1}. Even though dried leaves are generally not consumed alone and not a good source of ascorbic acid, they still had 3.54 ± 0.14 mg 100 g^{-1} in several parts of *Erica arborea.* Color values were determined as 32.77 ± 0.05; −0.60 ± 0.20; 11.33 ± 0.05 for *L*, *a* and *b* values.

Physico-chemical characteristics of carbonated *Erica arborea* tea beverage (CETB) are presented in Table 1. The titratable acidity and water soluble dry matter of the tea beverage were determined according to the marketing study, which proceeded in comparable beverages earlier than the final formulation decision. Phelan & Rees [24] determined the pH values of some herbal tea beverages commonly found in markets. They pointed out that the pH values of Lipton ice lemon tea, traditional blackcurrant tea, and raspberry mix (strawberry and loganberry), were respectively 3.26, 3.15, and 3.18.

Ascorbic acid is being recognized as an important antioxidant [25]. The ascorbic acid capacity of *Erica arborea* tea beverage was 28.39 ± 1.84 mg 100 mL^{-1}. Ascorbic acid addition during the process is led to higher levels found in herbal tea beverages compared to the raw material. Somanchi et al. [26] investigated the ascorbic acid level found in dried and brewed green tea from the US markets. They determined the <1 mg 100 g^{-1} ascorbic acid in brewed teas and <3 to 178 mg 100 g^{-1} in unbrewed leaves. Costa et al. [27] reported 21.40 ± 0.10 mg 100 mL^{-1} vitamin C in a beverage produced with 1% green tea (*Camellia sinensis*) infusion.

Color specifications of herbal tea beverage are presented in Table 1. Post-bottling storage is an important step to observe sustainable product quality in terms of clarity and color in beverages [28]. The haziness of juices is related to the irregular distribution of the pectin molecules encircling the fragments of cell walls (pulp) in a colloidal serum of macromolecules such as proteins, sugars, and organic acids [29]. The turbidity value of CETB was 0.71 NTU. Similar to our results, Koutchma et al. [30] determined 0.80 NTU in clarified apple juice.

Element concentration in the leaves, the water used in the process and in the tea beverage, is given in Table 2. Penuelas et al. [31] determined the Fe content as 100 mg kg^{-1} and Ca content 6500 mg kg^{-1} of *Erica arborea* leaves closer to our study.

According to the 9-point structured scale, values of color, odor, appearance, and taste were determined as 6.8 ± 1.8; 6.8 ± 1.5; 6.2 ± 1.8, and 6.0 ± 1.4, respectively. CETB was generally accepted by the panelists.

Polyphenols are found in almost all plant-originated foods and affected by the food matrix, and background diet may affect their absorption and bioavailability [32,33]. Dried leaves of *Erica arborea* were evaluated based on their phenolic content and antioxidant capacity to understand the phytochemical potential. Phenolic content of dried leaves of *Erica arborea* was 749.48 ± 34.46 mg GAE 100 g^{-1} with chemical extract, and 249.50 ± 18.10 mg GAE 100 g^{-1} with physiological extract (bioaccessible phenols). Some researchers investigated the phenolic content of *Erica arborea* in several parts of the herb. Guendouze-Bouchefa et al. [34] studied dried herbs with methanol extract and found 70.8 ± 2.5 mg GAE g^{-1} DW total phenolic content. Jimenez-Zamora et al. [35] investigated the total phenolic content and antioxidant activity of several herbs at room temperature and 50 °C storage for six months. They concluded that 44 ± 7 to 119 ± 5 mg GAE L^{-1} was present in heather at different storage conditions. The results may vary in studies because of the environmental differences, choice of parts tested, time of taking samples, and determination methods [36]. In another study, researchers investigated the phenolic content of water and ethanol extracts of *Erica herbacea* L. They concluded that there was 119.88 ± 0.50 mg GAE g^{-1} sample and 39.26 ± 0.94 mg GAE g^{-1} sample in water and ethanol extracts, respectively [37].

Dried *Erica arborea* leaves had 93.81 ± 0.72, 154.73 ± 1.59 and 75.26 ± 1.81 µmol trolox g^{-1} dry weight antioxidant capacity in chemical extract with DPPH, FRAP, and CUPRAC assays.

In physiological extract the results were lower than chemical extracts as expected. The antioxidant capacity with DPPH, FRAP, and CUPRAC assays were 3.83 ± 0.01, 20.85 ± 0.29 and 9.87 ± 0.15 μmol trolox g^{-1} dry weight, consecutively. Some researchers [38] investigated the antioxidant capacity of *Erica arborea* grown in Morocco. According to their results, antioxidant activity of *Erica arborea* samples extracted with ethanol, were 9.48 ± 0.05 mg Vitamin E equivalent g^{-1} for the FRAP assay and 86.5 ± 0.01% for the DPPH assay. Ay et al. [5] determined the antioxidant activity of mixed parts of *Erica arborea* and they stated (IC_{50}) 23.06 ± 0.36 μg mL^{-1} antioxidant capacity in the DPPH assay.

Mathivha and Mudaubush [39] studied total phenolic content and antioxidant capacity of *Athrixia phylicoides* DC tea (bush tea) and *Monsonia burkeana* tea (special tea) using different extraction methods. Total amount of phenolic compounds of 5 min extracted (hot water) bush tea and special tea was determined 4.23 ± 0.08 and 2.89 ± 0.23 mg GAE/100 g respectively. Antioxidant activity of these teas were also 35.66 ± 2.45 and 99.57 ± 1.88 umol/g trolox eq for the ABTS method, and 62.60 ± 4.76 and 102.72 ± 2.25 umol/g trolox eq for the DPPH method. In comparison with our study, the botanical origin of raw material is one of the main reasons to cause differences in the measured parameters. Kiliç et al. [40] studied antioxidant properties of green tea, senna, corn silk, and rosemary tea infused in water at various temperatures, and senna tea had 487.2 ± 0.005 mg GAE/100 g total phenolic content brewed at 100 °C.

Bioaccessibility is defined as the accessible amount of food components, which are delivered from ingested food, in the gut media. Phenolic compounds are released from the food through enzymatic pathways through the small intestine (digestive enzymes) and large intestine (bacterial micro-flora). In consequence of this release they become potentially bioavailable [41]. The results of extractable and bioaccessible phenolics and antioxidant capacity of the CETB are presented in Table 3.

Total phenolic content and antioxidant capacity of CETB were low as a consequence of the usage of 1% of herbal extract. Also, the bioavailable phenolic content of herbal tea beverage was 55.20% lower than measured phenolic content without extraction. NETO et al. [42] investigated the phenolic bioavailability in *Triplaris gardneriana* seed ethanolic extract. They found that the total in vitro phenolic bioavailability was 32.58%.

When antioxidant capacity results were compared, even if their mechanism of action differed, the DPPH showed the best results. Bioaccessible antioxidants of herbal tea beverage was the highest in the FRAP assay. Besides, the FRAP assay had the highest (13.79%) bioaccesibility in comparison with DPPH (0.625%) and CUPRAC (0.095%). Suna [18] investigated the bioavailable phenolic and antioxidant capacity of rooibos tea. Differing from this study, the researchers identified higher bioaccesible antioxidant capacity in the CUPRAC assay. Phenolic compounds may not completely absorb in the small intestine. The bioavailability of phenolic contents after digestion may be related with molecular mass, number and position replacement, and glycoside-forming reactions of flavonoids [43].

Table 1. Physico-chemical properties of CETB.

Analyses	Water Soluble Dry Matter (g 100 g^{-1})	Titratable Acidity (g 100 mL^{-1}) ~	pH	Ascorbic Acid (mg 100 mL^{-1})	Color			NTU
					L	*a*	*b*	
CETB	8.10 ± 0.00	0.17 ± 0.00	3.21 ± 0.00	28.39 ± 1.84	19.26 ± 0.20	−9.93 ± 0.83	3.70 ± 0.45	0.71 ± 0.06

~: Citric acid.

Table 2. Concentration of elements in *Erica arborea* leaves and tea beverage.

	Fe	Ca	Mg	K	Na
Dried leaves (mg kg^{-1})	108.65 ± 0.27	5900 ± 0.01	2274.75 ± 54.46	3800 ± 0.01	358.59 ± 7.89
Water used in process (mg L^{-1})	0.03	13.0	1.72	0.51	14.3
Beverage (mg L^{-1})	0.20 ± 0.02	58.62 ± 0.54	22.82 ± 0.52	53.74 ± 1.46	40.99 ± 0.89

Table 3. The phenolics, bioaccessible phenolics and antioxidant capacity of carbonated Erica arborea tea beverage (CETB).

Phenolic Content		Antioxidant Capacity					
Phenolics (mg GAE * 100 mL^{-1})	Bioaccessible Phenolics (mg GAE * 100 mL^{-1})	DPPH (μmol trolox mL^{-1} *)	Bioaccessible DPPH (μmol trolox mL^{-1} *)	FRAP (μmol trolox mL^{-1} *)	Bioaccessible FRAP (μmol trolox mL^{-1*})	CUPRAC (μmol trolox mL^{-1} *)	Bioaccessible CUPRAC (μmol trolox mL^{-1} *)
174.06 ± 24.53	96.07 ± 3.96	27.20 ± 1.09	0.17 ± 0.02	22.41 ± 2.49	3.09 ± 0.44	21.09 ± 1.65	0.02 ± 0.00

* GAE: Gallic acid equivalent, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: ferric reducing antioxidant power, CUPRAC: cupric ion reducing antioxidant capacity assay.

4. Conclusions

The production of *Erica arborea* tea beverage allowed for the development of new functional beverages which have high amounts of bioavailable bioactive compounds and mineral contents compared to industrialized products on the market. Tea beverages have 0.02–22.41 µmol trolox mL^{-1} antioxidant capacity as measured by different assays, and 174.06–96.07 mg GAE 100 mL^{-1} total phenolics and bioaccessible phenolics. The elemental content of tea beverage was found to be 0.20 mg L^{-1} of iron, 58.62 mg L^{-1} of calcium, 22.82 mg L^{-1} of magnesium, 53.74 mg L^{-1} of potassium, and 40.99 mg L^{-1} of sodium.

Nowadays, consumer demands on functional foods, especially on functional drinks, are growing quickly. As a result of this trend, natural herbal teas become a primary outcome because of their bioactive potential. Herbs have great contribution in the functional activity of a beverage. When considering *Erica arborea* as a tea beverage, there are not enough studies to understand its bioactive substances and their bioaccessibilities. As a final point, the consequences of this study will be a source for other studies.

5. Patent

This product was patented by Turkish Patent Institute (2012/09534).

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