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Redefining iced tea enriched with linden extract as a natural preservative

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Abstract:
Functional beverages enriched with herbal extracts are gaining popularity due to their potential health benefits. *Tilia cordata* flowers are known for their antioxidant and antimicrobial properties, making them a promising additive in food and beverage formulations. Our study aimed to develop ready-to-drink iced teas enriched with *T. cordata* flower extracts and to evaluate their antioxidant, antimicrobial, and sensory characteristics as functional food products.

Fresh *T. cordata* flowers were analyzed for metal contents. Phenolic acid profiles in ethanolic and aqueous extracts were determined using HPLC-MS. Antioxidant activity was evaluated using DPPH radical scavenging, conjugated diene, and iron ion chelation assays. Antimicrobial effects were tested against *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*. Sensory analysis was conducted using AI-based facial expression recognition to assess consumer responses.

Metal analysis revealed low concentrations of Mn, Zn, Cu, and Fe, with no detectable Pb, Cd, or Ni. Ethanolic extracts showed significantly higher levels of phenolic acids than aqueous extracts. Iced teas containing both types of extracts demonstrated strong antioxidant activity, with ethanolic formulations having the highest levels of phenols and flavonoids. Antimicrobial tests confirmed activity in both teas, with ethanolic extracts showing stronger effects. Sensory analysis indicated positive emotional responses and consumer acceptance for both formulations.

Iced teas enriched with *T. cordata* extracts exhibited significant antioxidant and antimicrobial properties, confirming their potential as functional beverages. The use of AI-driven sensory evaluation proved effective in capturing consumer preferences, supporting its application in product development. These findings suggest commercial viability for industrial production.

Keywords: medicinal iced tea, *Tillia cordata*, functional beverages, natural plant extracts, AI sensory evaluation

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INTRODUCTION

The genus *Tilia* L. (commonly known as linden or lime) from the Tiliaceae family includes approximately 44 species [1]. *Tilia cordata* (linden) has been utilized in traditional medicine for its therapeutic properties since ancient times [2]. This plant contains a variety of compounds, including hydrocarbons, esters, terpenoids, quercetin, kaempferol, phenolic compounds, condensed tannins, and scopoletin [1, 3]. Linden flowers are known for their use in treating bacterial infections and for their calming effect. Alcoholic extracts have antibacterial properties, while flower infusions are commonly used for respiratory ailments [1].

Iced tea is a popular cold beverage, often sweetened, and is commonly available in pre-packaged forms. It can be flavored with various syrups, such as lemon, raspberry, lime, passion fruit, peach, orange, strawberry, and cherry. These drinks are beneficial because they are rich in antioxidants, which help eliminate toxins from the body and promote detoxification [4]. Herbal teas made from thyme, peppermint, melissa, sage, and linden are particularly popular for their fragrance and health-promoting properties [2, 5].

The global focus on food quality and safety continues to grow, with a well-established link between food and health. Enhancing food quality and safety is crucial for improving public health, as it ensures the availability of safe food with consistent quality. In iced teas, benzoates and sorbates are among the most commonly used synthetic preservatives [6]. However, the increasing consumer demand for natural, clean-label products and the growing awareness of the potential health risks associated with synthetic additives have created a need for alternatives in the food industry.

Sodium benzoate (E211), a salt of benzoic acid, is soluble in water, tasteless, and odorless. It is used as a preservative in food due to its antifungal and antibacterial properties, though it must be added in strictly regulated amounts. When combined with vitamin C, sodium benzoate can degrade into benzene, a highly toxic, mutagenic, and teratogenic compound. It has also been shown to induce oxidative stress and negatively affect the immune system, liver, kidneys, and fertility [7, 8]. Potassium sorbate (E202) is another widely used preservative in food, cosmetics, and pharmaceuticals, with growing concerns regarding its safety. Its cytotoxic and genotoxic effects, including chromosome aberrations and DNA damage, have been reported. Long-term exposure to potassium sorbate may lead to health issues such as breathing difficulties, headaches, chest pain, airway swelling, mucosal irritation, pulmonary edema, and respiratory distress [7, 8].

Plant extracts may offer a potential alternative to sodium benzoate and potassium sorbate in iced tea production thanks to their favorable chemical composition and the presence of bioactive compounds such as phenolics and flavonoids [9, 10]. Plant extracts also present a significant advantage over synthetic additives, as they generally exhibit low toxicity, even when consumed in high doses [11]. This makes plant-based preservatives not only a safer option but also an attractive, environmentally friendly alternative that aligns with the growing trend towards sustainable food production.

Despite the promising potential of plant extracts, functional iced tea enriched with herbal extracts is not yet available on the market. Moreover, there is little research on the biological potential of functional iced tea free from synthetic additives but enriched with *T. cordata* extracts. This gap in the current literature highlights the need for further exploration of natural preservatives in the development of healthier, functional beverages. There is an increasing interest in functional foods that not only provide hydration but also offer health benefits, and iced teas enriched with plant extracts present an untapped opportunity to meet this demand.

Thus, we aimed to develop ready-to-drink iced teas enriched with *T. cordata* flower extracts, free from E211 and E202 additives, and to evaluate their antioxidant and antimicrobial potential as a functional food. Additionally, we conducted a sensory evaluation of these teas using AI algorithms to assess consumer preferences and product acceptability. Our study contributes to the growing body of knowledge on the application of natural plant extracts as preservatives in the food industry, addressing both consumer health concerns and the need for sustainable, clean-label products. By exploring the potential of *T. cordata* extracts, our research may pave the way for the development of innovative, functional iced tea products that could significantly impact the market and improve consumer health.

STUDY OBJECTS AND METHODS

Collection and preparation of linden flowers. For this study, *Tilia cordata* flowers were used as the raw material (Fig. 1). They were collected from the linden trees located in the central region of Ohrid, in the southwestern part of North Macedonia, on the shores of Lake Ohrid (695 m above sea level). To eliminate dust and other contaminants, the fresh flowers were rinsed with distilled water. The flowers were then dried in a laboratory dryer at 60°C for 4 to 5 h until a constant mass was achieved [12]. Once dried, the linden flowers were ground into a fine powder and stored in a refrigerator until further analysis.

Determination of metal content. The procedure for determining the metals Mn, Zn, Cu, Fe, Pb, Cd, and Ni in linden flowers involves wet digestion of the air-dried, ground plant material, followed by direct reading using an atomic absorption spectrophotometer [13]. Between 0.5 to 1 g of the material was weighed and transferred to a flask, then 5 mL of a 1:1 mixture of H_2SO_4 and $HClO_4$ was added. The flasks were placed in a digestion block, and 5 mL of H_2O_2 was subsequently added. The reaction is highly exothermic, so the temperature should not exceed 400°C. The ignition process takes approximately 30 min, or lasts until a clear liquid with no remaining organic residue is obtained. After cooling, 50 mL of deionized water was added to the flask, and the mixture was filtered into a 100-mL volumetric flask. The metal concentrations were then measured using an atomic absorption spectrophotometer. The analysis was performed in triplicate.

Preparation of aqueous extract. The aqueous extract was prepared by the methods of Sławińska *et al.* [14] and Ribeiro *et al.* [15]. For this, 10 g of the dried and

Figure 1 A linden tree in Ohrid (photo by M. Stojanova)

powdered linden flowers were combined with 200 mL of distilled water and then extracted in a boiling water bath for 1 h. The extract was filtered through filter paper, rinsed once more with boiling water, and then filtered again. The resulting supernatant was collected and evaporated using a vacuum evaporator. The remaining extract was further dried under a stream of warm air (40°C) until a constant mass was achieved. The extraction procedure was performed in triplicate for each sample (Fig. 2).

Figure 2 Aqueous linden extract (photo by M. Stojanova)

Preparation of ethanolic extract. The ethanolic extract was prepared by the method of Vidović *et al.* [16]. For this, 10 g of the dried and finely powdered linden flower samples were mixed with 100 mL of 70% ethanol, and the extract was sonicated for 40 min in an ultrasonic bath at 45°C. The sample was then filtered through filter paper. The resulting supernatant extract was evaporated at 60°C until a constant mass was achieved. The extraction procedure was performed in triplicate for each sample (Fig. 3).

Figure 3 Ethanolic linden extract (photo by M. Stojanova)

Determination of total phenolic compounds. The total phenol content in the extracts was determined using the method of Singleton *et al.* [17] modified by Stojanova *et al.* [10] and adapted for microplates. This method is based on the reaction between phenols and Folin-Ciocalteu's reagent, resulting in the formation of a colored complex. The phenol content was calculated using a calibration curve (concentration-dependent absorbance function) derived from a standard gallic acid solution.

Equivalent gallic acid (GAE) values, mg EQ GAE/g dry matter, were obtained according to the following Eq. (1):

$$\text{GAE} = \frac{C_{read}}{C_{working}} \times 1000 \qquad (1)$$

where C_{read} is read concentration GAE, µg/mL; $C_{working}$ is the working concentration, µg/mL. The results were presented as a mean of three measurements.

Determination of total flavonoid content. The flavonoid content in the extracts was determined using the method of Chang *et al.* [18] modified by Stojanova *et al.* [10] and adapted for microplates. This method is based on the ability of flavonoids and flavoglycosides to form complexes with metal ions, particularly the aluminum (Al) complex. The method involves the formation of a colored complex, which has an absorption maximum at 430 nm.

The flavonoid content was calculated using a calibration curve (concentration-dependent absorption function) derived from a standard quercetin solution.

Equivalent quercetin (QE), mg EQ QE/g dry matter, were obtained according to the following Eq. (2):

$$\text{QE} = \frac{C_{read}}{C_{working}} \times 1000 \qquad (2)$$

where C_{read} is the read concentration QE, µg/mL. The results were presented as a mean of three measurements.

HPLC-MS analysis of phenolic acids. HPLC-MS analysis was performed to determine phenolic compounds in the linden extracts [19]. For the analysis, we used an Agilent LC/MS system 1260/6130, with ChemStation software, a UV detector (DAD) (G4212B), a single quadrupole API-ESI mass detector, and a Zorbax SB-Aq column (150×3.0 mm; 3.5 µm particle size, Agilent Technologies). Acetonitrile of the gradient grade was used as the mobile phase with bidistilled water mixed with 0.5% formic acid in gradient separations. The flow rate was 1 mL/min, the injection volume was 20 µL, and the temperature of the column was set at 25°C. The gradient program was optimized as follows: 0 min (10% acetonitrile in formic acid); 25 min (20% acetonitrile in formic acid); 45 min (30% acetonitrile in formic acid); and 47 min (100% acetonitrile). Identification was performed by comparing the retention times (τ_r) and the spectra with those from the appropriate standards. The qualitative and quantitative determination was performed at the following wavelengths: λ = 280, 320 and 350 nm. The content of phenolic compounds in the extracts was determined by using calibration plots constructed for every standard. Standard solutions were prepared under the same conditions as the samples. The analysis was performed in triplicate.

Preparation of linden ready-to-drink iced tea enriched with extracts from linden flowers. 1.5 g of dried linden flowers was put in a cup and immersed with 200 mL of boiled water. It was left to stand for 15 min and then strained. Further, the tea was cooled to 40°C, and then 1 g of the flower extract was added, separately aqueous and ethanolic.

The prepared tea was transferred to sterile and heated glass bottles and then pasteurized at 80°C for 10–15 min. After that, the tea was cooled to 10°C and was ready for storage and further distribution.

Determination of antioxidant potential of iced tea enriched with linden extracts. *Ability to capture DPPH radicals.* The ability to capture DPPH radicals was determined using the method of Stojanova *et al.* [20]. For this, 200 μL of a tea sample was added to each test tube, followed by filling the tube with 4 mL of 0.1 mM DPPH solution. A control test was prepared in the same way, but with 200 μL of water instead of the tea sample. The tubes were left to stand for 10 min, after which the absorbance was measured at 517 nm using a UV/VIS spectrophotometer. Calibration was performed using 96% ethanol. The analysis was conducted in triplicate. The ability to capture DPPH radicals, %, was calculated using the following Eq. (3):

$$\text{DPPH radicals} = \left[\frac{(A_{blank} - A_{sample})}{A_{blank}}\right] \times 100 \quad (3)$$

where A_{blank} is the absorbance of blank sample; A_{sample} is the absorbance of sample.

Ability to chelate iron ions. The chelating ability of iron was determined using the method of Dinis *et al.* [21] modified by Stojanova *et al.* [20]. For this, 3.7 mL of MQ water and 0.1 mL of 2 mM ferrochloride were added to the tea samples in MQ water. The reaction was initiated by adding 0.2 mL of 5 mM ferrosine. After incubating the solution at room temperature for 10 minutes, the absorbance was measured at 562 nm using a UV/VIS spectrophotometer. The blank sample did not contain ferrochloride or ferrosine, which are involved in the formation of the complex. A lower absorbance indicated a stronger chelating ability. The analysis was performed in triplicate. The chelating ability of iron ions, %, was calculated using the following Eq. (4):

$$\text{Ability to chelate iron} = \left[\frac{(A_{blank} - A_{sample})}{A_{blank}}\right] \times 100 \quad (4)$$

Conjugated diene method. Antioxidant activity was determined using the conjugated diene method [22]. For this, tea samples were dissolved in MQ water, and 2 mL of a 10 mM linoleic acid emulsion in 0.2 M sodium phosphate buffer was added. The stability of the emulsion was maintained by adding 6.5 mM Tween 20, and the solution was incubated with stirring for 15 h in the dark at 37°C to accelerate oxidation. After incubation, 0.2 mL of the solution was mixed with 6 mL of absolute methanol. The transparency of the solution was achieved through centrifugation. The supernatant was collected for measurement. The absorbance of the supernatant was measured at 234 nm using a UV/VIS spectrophotometer. The blank sample contained all the components except the extract. The analysis was performed in triplicate. Antioxidant activity, %, was calculated using the following Eq. (5):

$$\text{Antioxidant activity} = \left[\frac{(A_{blank} - A_{sample})}{A_{blank}}\right] \times 100 \quad (5)$$

Determination of antimicrobial potential of iced tea enriched with linden extracts. Antimicrobial potential was determined by the disk-diffusion method. For this, 9 pathogenic bacteria were used, of which 4 were Gram-positive and 5 were Gram-negative. The tested bacteria were stored on suitable oblique agar at +4°C.

Disc-diffusion method. The disc diffusion analysis was performed following the method of Klaus *et al.* [23]. The microorganisms to be tested were prepared in the appropriate broth, sieved twice for 24 h, resulting in concentrations of approximately $1{\times}10^6$ to $1{\times}10^8$ CFU/mL. A 100 μL suspension of each microorganism culture was then spread onto the appropriate agar. Three sterile filter discs (6 mm in diameter) were placed on the agar surface, each soaked with 50 μL of the suspension of one of the extracts. After standing for 2 h at 25°C, the Petri dishes were incubated for 24 h at 37°C. Following incubation, the zone of inhibition was measured. The analysis was performed in triplicate.

Consumer preference and facial expression analysis of iced teas. The sensory evaluation was conducted in a group of 50 participants, comprising 25 women (50%) and 25 men (50%), aged between 21 and 60 years. Each participant was asked to assess the new iced teas, each served in a plastic cup with approximately 30 g of the product at 7 ± 2°C, as described by Gupta *et al.* [24]. To capture the participants' facial expressions during the evaluation, a video camera equipped with PsychoPy software was mounted on a tablet. The video recordings were made at a resolution of 1080×720 pixels and 30 frames per second, with consistent overhead lighting provided by a direct current light source to maintain uniform illumination throughout the process.

The facial expressions were later analyzed using the iMotions Biometric Research Platform 6.2 [25], with the Affectiva facial expression recognition engine. To ensure proper alignment, the participants were instructed to place their faces between two adjustable bars of a headrest, keeping their faces centered within the camera's field of view. The participants were then asked to replicate the emotions portrayed in 60 frontal portrait images (20 images for each emotion category), as described by Kulke *et al.* [26]. The goal was to assess the quality and overall acceptability of the new iced teas as perceived by the participants.

Statistical analysis. The collected data were subjected to rigorous statistical analysis using the SPSS 20 software package. To identify statistically significant differences among the measured values, an Independent Sample *t*-test was conducted at a significance level of $p = 0.05$. Furthermore, one-way analysis of variance (ANOVA) was performed, followed by Tukey's post-hoc test ($p = 0.05$) to determine specific group differences. The strength and direction of linear relationships between the variables were evaluated using Pearson's correlation coefficient. Additionally, multivariate statistical techniques were employed, including principal component analysis, to explore the underlying patterns, associations, and interrelationships among the analyzed samples and the sensory parameters.

RESULTS AND DISCUSSION

In phytotherapy, *Tilia* flowers are widely used for a variety of conditions, including the treatment of the common cold, nervous tension, migraines, liver and gallbladder disorders, as well as for their expectorant, diuretic, antispasmodic, sedative, stomachic, and diaphoretic properties. The European Medicines Agency has recognized the traditional use of *Tilia cordata* and *Tilia platyphyllos* in alleviating symptoms of the common cold, chronic cough, and mental stress in several countries [27].

Metal content in the *T. cordata* flowers. The European Pharmacopoeia underscores the importance of assessing the metal content in plant samples intended for medicinal use. Yet, it does not provide explicit thresholds for permissible metal concentrations [28]. This lack of regulatory guidelines presents a challenge, especially since metals such as chromium (Cr), mercury (Hg), arsenic (As), lead (Pb), and cadmium (Cd) can be highly toxic to plants and pose a significant risk to human health at elevated concentrations [29]. Consequently, this issue becomes even more critical in the context of herbal products, where safety and quality assurance are paramount. Given the increasing demand for natural remedies, regulatory bodies might need to consider more stringent criteria to mitigate potential health hazards.

In this study, the metal analysis of *T. cordata* flowers revealed relatively low concentrations of essential metals such as manganese (Mn), zinc (Zn), copper (Cu), and iron (Fe), while no traces of Pb, Cd, or nickel (Ni) were detected (Table 1). These findings align with the European Law Regulation no. 1881/2006, which sets guidelines for the metal content in food and medicinal plants. The absence of toxic heavy metals such as Pb, Cd, and Ni is particularly significant, as it supports the idea that *T. cordata* flowers, specifically, can be considered a safe food source free from contamination by harmful substances. This is an important result, as it could reassure both consumers and regulators regarding the safety of linden flowers in therapeutic and culinary applications.

However, it is essential to contextualize these findings within the broader spectrum of research on metal contamination in plants. For example, Celechovská *et al.* [30] quantified zinc concentrations in *Tiliae flos cum bracteis*, reporting levels ranging from 13.8 to 32.5 μg/g. While these values fall within the safe limits outlined in many regulatory frameworks, they highlight the variable nature of metal content depending on environmental conditions and the specific part of the plant being analyzed. On the other hand, Tomašević *et al.* [31] studied linden leaves and bark from various geographical regions and observed a significant correlation between elevated metal concentrations and pollution levels in the surrounding air or soil. This suggests that environmental factors, particularly pollution, could contribute to higher metal uptake in plant tissues, underscoring the need for ongoing monitoring of plant products, especially those sourced from urban or industrialized regions.

Table 1 Metal content in dried *Tilia cordata* flowers

Metal	n	Content, mg/100 g dry matter
		$\bar{x}$ ± SD
Manganese	3	2.95 ± 0.05
Zinc	3	3.20 ± 0.01
Copper	3	0.81 ± 0.22
Iron	3	3.50 ± 0.01
Lead	3	n.d.
Cadmium	3	n.d.
Nickel	3	n.d.

n.d. – not determined

Mircea *et al.* [28] found a similar pattern of metal presence in *Tiliae flos cum bracteis*, ranging from 3.17 to 10.35 mg/100 g for Mn, 4.56 to 22.66 mg/100 g for Zn, 0.79 to 1.36 mg/100 g for Cu, and 3.76 to 9.92 mg/100 g for Fe, with Pb detected at levels between 0.35 and 9.15 mg/100 g. While these findings are broadly consistent with our results, it is worth noting that the presence of Pb, even at low levels, raises concerns about the potential cumulative exposure to toxic metals in the populations consuming medicinal herbs on a regular basis. The detection of Pb in *Tiliae flos cum bracteis* samples highlights the variability in contamination across different studies and regions, suggesting that environmental monitoring and quality control are crucial for ensuring the safety of herbal products.

The varying concentrations of metals in different studies underscore the need for standardized methodologies in plant metal analysis, as discrepancies between the studies may arise from the differences in sample handling, geographic factors, and analytical techniques. Furthermore, while *T. cordata* may appear to be a relatively low-risk herb in terms of metal contamination, future research should continue to explore the long-term effects of consuming plant-based products with trace metal content, especially for vulnerable populations.

Content of bioactive compounds in linden flower extracts. The study of bioactive compounds in plants has garnered increasing attention due to their potential therapeutic benefits to human health [10]. We evaluated the bioactive profile of *T. cordata* flower extracts, comparing both ethanolic and aqueous extraction methods (Table 2).

As indicated in Table 2, the ethanolic extracts from *T. cordata* flowers exhibited significantly higher ($p < 0.05$) concentrations of both total phenols (61.07 mg/g) and flavonoids (23.14 mg/g) when compared to the aqueous extracts, which showed 56.79 mg·g for phenols and 19.75 mg/g for flavonoids. These findings are consistent with the previous studies that suggest a superior extraction efficiency of phenolic compounds using ethanol as a solvent. Notably, the total phenolic content exceeded the flavonoid content in both extracts, underlining the greater abundance of phenolic acids in *T. cordata* flowers.

Table 2 Total phenols and flavonoids content in linden flower extracts ($\bar{x}$ ± SD)

Tilia cordata flower extracts	n	Content, mg/g dry matter	
		Total phenols	Total flavonoids
Aqueous extract	3	$56.79 \pm 3.17^{a, A}$	$19.75 \pm 2.30^{b, A}$
Ethanolic extract	3	$61.07 \pm 2.98^{a, B}$	$23.14 \pm 1.25^{b, B}$

a, b – values for the different parameter of the same extract marked with different letters have statistically significant difference ($p < 0.05$), *t*-test

A, B – values for the same parameter of the different extract marked with different letters have statistically significant difference ($p < 0.05$), *t*-test

Table 3 HPLC-MS analysis of phenolic acids in *Tilia cordata* flower extracts ($\bar{x}$ ± SD)

Phenolic acids	n	t_r, min	Content, µg/g dry matter	
			Aqueous extract	Ethanolic extract
Gallic acid	3	2.43	451.07 ± 0.05^a	586.29 ± 0.06^b
Protocatechuic acid	3	3.90	1684.61 ± 0.09^a	1738.77 ± 0.01^b
p-Hydroxybenzoic acid	3	0.56	n.d.	n.d.
Catechin	3	5.25	9.65 ± 0.13^a	10.89 ± 0.01^b
Rutin	3	6.37	359.47 ± 0.05^a	489.36 ± 0.06^b
Quercetin	3	9.63	191.10 ± 0.02^a	213.95 ± 0.02^b
Isoquercetrin	3	6.67	89.26 ± 0.02^a	97.17 ± 0.01^b
Vanillic acid	3	12.35	7.31 ± 0.04^a	6.05 ± 0.01^a
Abscisic acid	3	9.88	n.d.	n.d.

a, b – values for the same parameter of the different extract marked with different letters have statistically significant difference ($p < 0.05$), *t*-test

n.d. – not determined

Cittan *et al*. [32] emphasized the significant role of the extraction method and solvent polarity in the efficiency of phenolic compound extraction. Their study found that ultrasound-assisted extraction with methanol resulted in a total phenolic content of 111.84 mg GAE/g in *T. cordata* fruit extracts, compared to 58.86 mg GAE/g when using water. Similarly, Ziarno *et al.* [33] reported a total phenolic content of 104.72 mg GAE/g in the aqueous extracts of *Tilia* sp. blossoms. This aligns with our findings, although they are slightly lower than those from the ethanolic extractions. Akyuz *et al.* [34] observed much lower values (17.372 mg/g) for phenolic compounds in *Tilia rubra* subsp. caucasica, highlighting the variability in phenolic concentrations across the species and extraction techniques.

In line with the results from Pavlović *et al.* [35], which reported a range of 2.78–24.32 mg GAE/g in 80% ethanolic extracts, the ethanolic extract in our study had a higher phenolic content than the aqueous extract. This further corroborates the influence of a solvent on the extraction efficiency.

According to the HPLC-MS analysis (Table 3), several phenolic acids showed the highest values in the *T. cordata* flower extracts (aqueous and ethanolic), namely protocatechuic acid (1684.61 and 1738.77 µg/g), gallic acid (451.07 and 586.29 µg/g), rutin (359.47 and 489.36 µg/g), and quercetin (191.10 and 213.95 µg/g). It is also evident that the ethanolic extracts had significantly higher ($p < 0.05$) concentrations of phenolic acids than the aqueous extracts.

These results align with the findings from Akyuz *et al.* [34], who identified gallic acid (246.54–346.02 µg/g), protocatechuic acid (1054.23–1369.34 µg/g), p-hydroxybenzoic acid (45.10–92.57 µg/g), abscisic acid (2.00–3.67 µg/g), and quercetin (31.81–127.45 µg/g dry sample) in *T. rubra* subsp. caucasica flowers. Additionally, catechin (341.82 µg/g dry sample) and chlorogenic acid (6.46 µg/g) were only detected through acidic hydrolysis.

Furthermore, our findings were consistent with those of Cittan *et al.* [32], who identified gallic acid (356.92–583.95 µg/g), protocatechuic acid (1723.08–2183.52 µg/g), and vanillin (7.11–7.94 µg/g) in *T. cordata* extracts. Interestingly, the ethanolic extracts in that study exhibited higher ($p < 0.05$) concentrations of phenolic acids compared to the aqueous extracts, confirming the greater extraction efficiency of ethanol for these compounds.

Antioxidant activity of ready-to-drink iced tea enriched with *T. cordata* extracts. Phenolic compounds are among the most widely used plant by-products due to their antioxidant properties, acting as reducing agents and hydrogen donors. Antioxidants help protect the cells from damage caused by free radicals, which are unstable molecules that can harm cells [36].

As presented in Fig. 4, the iced teas enriched with both aqueous and ethanolic extracts demonstrated higher ($p < 0.05$) antioxidant activity compared to the control sample (conventional linden tea). Additionally, the ethanolic extracts exhibited higher antioxidant values across all the three tests, compared to the aqueous extracts. The iced teas with the ethanolic extracts showed the highest ability to capture free DPPH radicals (32.75%), followed by the iced teas with the aqueous extract (30.61%). The control sample had a significantly lower ability, namely 15.21%.

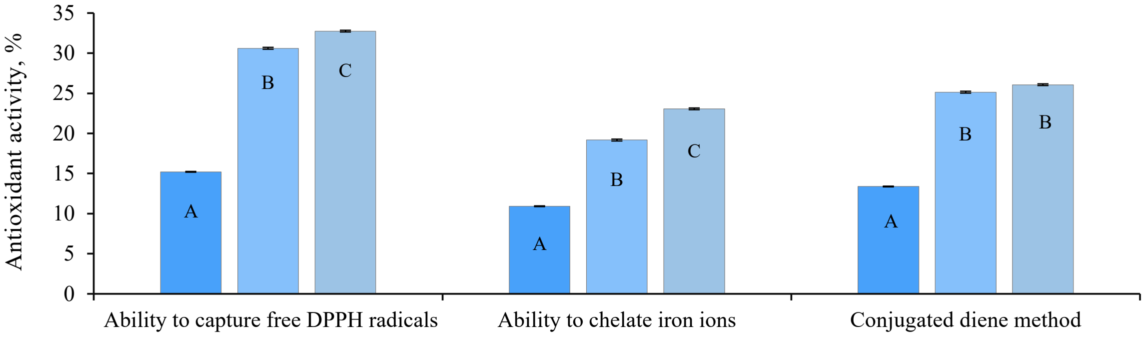


Figure 4 Antioxidant activity of ready-to-drink iced tea enriched with *Tilia cordata* extracts ($\bar{x}$ ± SD)

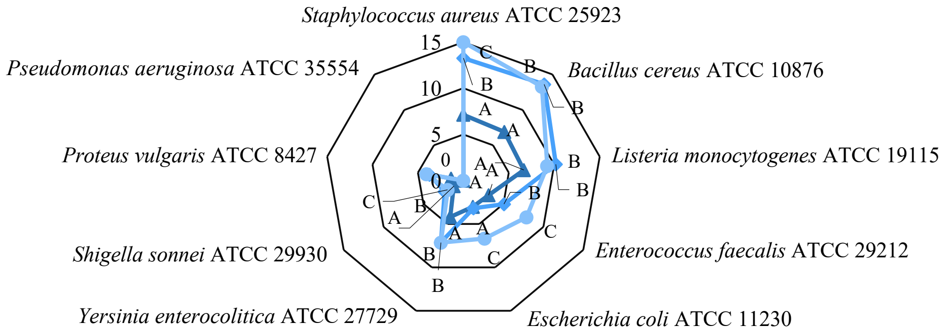


Figure 5 Antimicrobial activity (mm) of ready-to-drink iced tea enriched with *Tilia cordata* extracts ($\bar{x}$ ± SD)

Slightly lower values were obtained by the conjugated diene method, namely 26.07, 25.14, and 13.38% for the iced teas with the ethanolic extract, aqueous extract, and the control, respectively. The order of antioxidant activity was as follows: ability to capture DPPH radicals > conjugated diene method > ability to chelate iron ions.

By comparing these results with the data in Tables 2 and 3, we can conclude that the highest antioxidant activity was observed in the iced teas enriched with the ethanolic extract. Furthermore, the ethanolic extracts were characterized by higher ($p < 0.05$) contents of phenols and flavonoids, compared to the aqueous extracts. The antioxidant activity of the plant extracts can largely be attributed to their phenolic content. Therefore, selecting the correct extraction method is of critical importance, as the biological activity of the resulting extracts directly depends on this method. Given that phenolic compounds are polar, they are most effectively extracted using polar solvents such as water or ethanol [10].

In a study by Alaşalvar & Çam [2], the EC_{50} (g/g DPPH) for microencapsulated linden extract was found to be 1.38, while the EC_{50} (mL/g DPPH) for the iced tea was 358.21. In contrast, Ziarno *et al.* [33] reported the ability to capture DPPH radicals in linden blossom aqueous extracts as 0.161 mmol TE/g extract. Thus, there is a lack of comprehensive data on the antioxidant potential of iced teas enriched with linden extracts.

Antimicrobial activity of ready-to-drink iced tea enriched with *T. cordata* extracts. The emergence of resistance in a growing number of pathogenic microorganisms to existing antibiotics is becoming a major concern in the pharmaceutical industry. As a result, it is increasingly important to identify components from natural sources that exhibit strong antimicrobial properties. These components, when properly extracted, could serve as a partial substitute for synthetic antibiotics. Additionally, such natural compounds have the potential to serve as a foundation for the development of new functional food products. This approach not only enhances the effectiveness against pathogenic microorganisms but also ensures that the products remain completely safe for consumer health. Plants, as rich sources of bioactive compounds, hold significant promise in this regard.

As shown by Fig. 5, the iced teas enriched with both linden extracts (aqueous and the ethanolic) showed higher ($p < 0.05$) antimicrobial potential against the tested pathogen strains when compared to the control. Notably, the tea with the ethanolic extract exhibited slightly higher antimicrobial potential compared to the tea with the aqueous extract. The highest values were determined against *Staphylococcus aureus* (15.00 mm for ethanolic extract, 13.27 mm for aqueous extract, and 7.13 mm for the control), *Bacillus cereus* (13.26 mm for ethanolic extract, 13.61 mm for aqueous extract, and 6.90 mm for the control), and *Listeria monocytogenes* (9.23 mm for ethanolic extract, 10.17 mm for aqueous extract, and 6.65 mm for the control). Neither the enriched iced teas nor the control showed antimicrobial activity against *Pseudomonas aeruginosa*.

In their study of linden extracts from Serbia, Pavlović *et al*. [35] found that the most sensitive strains were Gram-positive isolates of *Streptococcus mutans*, *Streptococcus pyogenes*, *Enterococcus faecalis* (ATCC 29,212 and 89), and particularly *S. aureus*, with clear zones of inhibition ranging from 12 to 15 mm. However, the authors concluded that compared to *Tilia europaea* and *T. platyphyllos*, the tea extracts from *T. cordata* showed more activity in inhibiting the growth of *P. aeruginosa* 589/1 and *Pseudomonas syringae* P7/16_2. Also, the antimicrobial activity of linden tea extracts against *S. aureus* and *Escherichia coli*, as well as the diameter of the zones, depended on the plant part used in the extraction process and the localities from which the samples were collected [37].

Correlation between bioactive compounds and biological potential of iced teas with *T. cordata* extracts. The increasing body of research linking plant-based foods to the prevention of degenerative diseases has sparked significant interest in medicinal herbs and their bioactive constituents. These compounds are considered to be safer alternatives to synthetic drugs, with the added benefit of being cost-effective and more widely available [1]. The *T. cordata* extracts in our study demonstrated promising biological activities, and a closer look at the correlations between the bioactive compounds and the observed biological potential reveals crucial insights into the mechanisms behind these effects.

The results presented in Table 4 highlight a strong positive correlation between the phenolic content of the extracts and their antioxidant activity, as measured by the DPPH test for both aqueous ($r = + 0.98$) and ethanolic extracts ($r = + 0.92$). This finding is consistent with previous research, which has established phenolic compounds, especially flavonoids and phenolic acids, as the main contributors to antioxidant activity in plant extracts. The DPPH radical scavenging assay is widely used as a reliable method to evaluate the antioxidant potential of natural products because it directly measures the capacity of a compound to donate electrons or hydrogen atoms to neutralize free radicals [38]. In this study, the exceptionally high correlation coefficient for both extracts (aqueous and ethanolic) suggests that the phenolic compounds in *T. cordata* are highly effective in scavenging free radicals. This underscores their role as the primary antioxidants in the extracts.

The conjugated dienes method, which measures the inhibition of lipid peroxidation, also revealed a strong positive correlation with the phenolic content ($r = + 0.95$) for both the aqueous and the ethanolic extracts. Lipid peroxidation is a process in which free radicals attack the lipids in cell membranes, leading to cell damage. By inhibiting this process, the phenolic compounds present in *T. cordata* flower extracts provide an additional layer of protection against oxidative stress. The fact that the phenolic content correlates so strongly with both the DPPH radical scavenging ability and lipid peroxidation inhibition suggests that these compounds have a broad-spectrum antioxidant action, effectively neutralizing free radicals and preventing oxidative damage at various levels.

Flavonoids, another key class of bioactive compounds found in *T. cordata* extracts, also showed strong correlations with antioxidant activity, especially in the DPPH test and the conjugated dienes method. The positive correlation between the flavonoid content and the DPPH radical scavenging activity ($r = + 0.88$ for aqueous extract and $r = + 0.76$ for ethanolic extract) further confirms that flavonoids contribute significantly to the antioxidant properties of these extracts. Flavonoids are known to possess potent antioxidant properties, partly due to their ability to chelate metal ions, donate hydrogen atoms, and act as free radical scavengers [39]. In addition, the correlation between the flavonoid content and the conjugated dienes method ($r = + 0.91$ for aqueous and $r = + 0.87$ for ethanolic extracts) reinforces the idea that flavonoids are involved in protecting lipids from oxidative damage, enhancing the overall antioxidant effectiveness of *T. cordata* extracts.

Table 4 Correlation between phenols and flavonoids contents and antioxidant potential (r^{+})

Correlated parameters	Iced tea enriched with *Tilia cordata* aqueous extract	Iced tea enriched with *Tilia cordata* ethanolic extract
Phenols – DPPH	0.98	0.92
Phenols – chelating ability	n.d.	0.29
Phenols – conjugated dienes method	0.95	0.95
Flavonoids – DPPH	0.88	0.76
Flavonoids – chelating ability	n.d.	0.45
Flavonoids – conjugated dienes method	0.90	0.91

Correlation is significant at the 0.01 level (two-tailed)

n.d. – not determined

Interestingly, the observed correlations suggest that phenolic compounds contribute more than flavonoids to the antioxidant activity of *T. cordata* extracts. This could be attributed to the higher concentration of phenolic acids (such as gallic and protocatechuic acids), which are known for their robust antioxidant capabilities [40]. Flavonoids, while important, may contribute to antioxidant activity in a complementary manner, enhancing the overall effectiveness of the extracts. This synergistic relationship between phenolic acids and flavonoids is a characteristic feature of plant extracts and is crucial for their efficacy in protecting against oxidative stress.

Furthermore, these findings underscore the importance of the extraction method. The ethanolic extract, which consistently exhibited higher concentrations of both phenols and flavonoids, showed stronger antioxidant activity across all the assays, compared to the aqueous extract. This suggests that ethanol, as a solvent, may extract a broader range of bioactive compounds with superior antioxidant properties, compared to water. The high correlation coefficients for both extracts suggest that the solvent choice does not diminish the biological relevance of the phenolic and flavonoid contents but, instead, magnifies their bioactive potential.

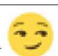
In terms of practical applications, these strong correlations indicate that phenolic compounds and flavonoids can serve as reliable biomarkers for the antioxidant potential of *T. cordata* extracts. By targeting these bioactive compounds, it is possible to predict and optimize the antioxidant capacity of products such as iced teas or other functional foods enriched with *T. cordata* extracts. Additionally, the correlations suggest that increasing the concentration of phenolic compounds, particularly in ethanolic extracts, could further enhance the biological effectiveness of such products.

The correlations observed between phenolic and flavonoid contents and antioxidant activity provide valuable insights into the bioactive profile of *T. cordata* extracts. The strong positive relationships, particularly in the DPPH and conjugated dienes assays, confirm the role of these compounds in neutralizing free radicals and protecting cells from oxidative damage. These findings not only validate the therapeutic potential of *T. cordata* as an antioxidant-rich source but also emphasize the importance of extraction methods in maximizing the bioactive properties of plant-based products.

Consumer preference and facial expression analysis of iced tea products. The sensory evaluation results, as reflected in the facial expressions recorded by the participants (Table 5), provide valuable insights into the emotional and physiological responses to the iced teas enriched with aqueous and ethanolic *T. cordata* extracts. Notably, the iced tea enriched with the aqueous extract elicited significantly higher ($p < 0.05$) facial expressions such as lip press (11.25), surprise (3.82), joy (10.61), smiley (18.75), and smirk (5.99), compared to the other tested variants. These expressions are commonly associated with positive emotional responses, which suggests that the participants found the product enjoyable and highly acceptable.

Interestingly, despite being a new product for the participants, the iced teas, both those enriched with

Table 5 Consumer emotional responses to iced tea samples ($\bar{x}$ ± SD)

Type	Parameter	Conventional iced tea	Iced tea with aqueous extract	Iced tea with ethanolic extract
Facial Expression	Lip Press	5.21^{a} ± 1.27	11.25^{b} ± 1.67	9.62^{c} ± 0.29
	Lip Suck	2.08^{a} ± 3.02	2.93^{b} ± 1.15	3.15^{b} ± 1.01
	Mouth Open	0.29^{a} ± 2.36	1.86^{b} ± 1.27	2.07^{b} ± 1.62
Head Orientation	Yaw 	–2.97^{a} ± 1.44	–1.02^{b} ± 1.69	–0.57^{b} ± 0.58
	Roll 	–1.75^{a} ± 1.76	–0.41^{b} ± 1.51	–0.98^{b} ± 1.67
Emotion	Surprise	2.01^{a} ± 2.03	3.82^{b} ± 2.10	3.09^{c} ± 1.96
	Joy	8.37^{a} ± 1.55	10.61^{b} ± 1.37	9.26^{c} ± 1.20
	Disgust	0.14^{a} ± 2.68	0.30^{b} ± 2.91	0.78^{c} ± 2.03
	Relaxed	3.67^{a} ± 1.71	1.48^{b} ± 2.36	2.13^{c} ± 0.81
Emoji	Smiley 	10.82^{a} ± 1.69	18.75^{b} ± 1.16	17.54^{c} ± 1.13
	Stuck Out Tongue 	–4.19^{a} ± 1.26	–3.38^{b} ± 1.17	–3.70^{b} ± 1.14
	Stuck Out Tongue Winking Eye 	–3.57^{a} ± 1.07	–3.21^{b} ± 1.60	–3.10^{b} ± 1.24
	Smirk 	4.09^{a} ± 1.89	5.99^{b} ± 1.67	5.36^{b} ± 2.31

$^{a, b, c}$ – values for the different samples and the same parameter marked with different letters are statistically significantly different, ANOVA, post-hoc Tukey's test ($p < 0.05$); (n = 50) aqueous and ethanolic extracts, were met with relatively little skepticism. This is an important finding, as the novelty of the product could have potentially led to hesitation or negative reactions. Instead, the participants demonstrated positive emotional expressions and an overall favorable reception. This can be interpreted as an indication that the taste, appearance, and overall experience of the iced teas were appealing to the participants, making them open to trying new, functional beverages. These results are consistent with a study by Gupta *et al.* [24], which showed that food stimuli can strongly influence physiological responses and that these responses are often reflective of the participants' overall acceptance of a product.

The reliability and accuracy of the facial expression recognition software (Affectiva) further validate the robustness of the results. Previous research by Kulke *et al.* [26] confirmed that the participants' facial expressions were often more pronounced and quicker when encountering emotionally charged stimuli, such as tasting food. This suggests that the facial expressions recorded in our study are not only accurate but they also capture the immediate and genuine reactions to the iced teas. The higher occurrence of joy-related expressions (such as smiley and smirk) highlights the participants' enjoyment, indicating not only their acceptance but also appreciation.

These findings are promising in the context of the iced tea market. While plant-based and functional beverages are still relatively new to many consumers, the positive emotional reactions to these iced teas suggest a market potential for such products. The ability of both aqueous and ethanolic extracts to elicit strong positive reactions reinforces their appeal and the successful integration of bioactive compounds in an appealing, consumable format. Future product development could build upon this foundation, exploring different formulations and further enhancing the sensory characteristics of the teas to optimize consumer enjoyment.

Thus, the results demonstrate that both types of iced tea, although new to the market, are perceived positively and hold promise as functional beverages. The consumers' willingness to accept and even enjoy these novel products provides a solid base for further exploration of their potential benefits, both in terms of sensory appeal and health impact.

Principal component analysis of AI-supported sensory evaluation. The Principal Component Analysis results provide a detailed insight into the sensory differences among the iced tea formulations, with PC_1 explaining 97.50% of the total variance. This dominant principal component suggests that the primary distinguishing factor among the samples is strongly represented in this axis. This might be due to the fundamental differences between conventional and linden-enriched iced teas. PC_2 contributes 2.39% to the total variance, while PC_3 explains only 0.11%, confirming that most of the variability is captured in the first two principal components (Fig. 6).

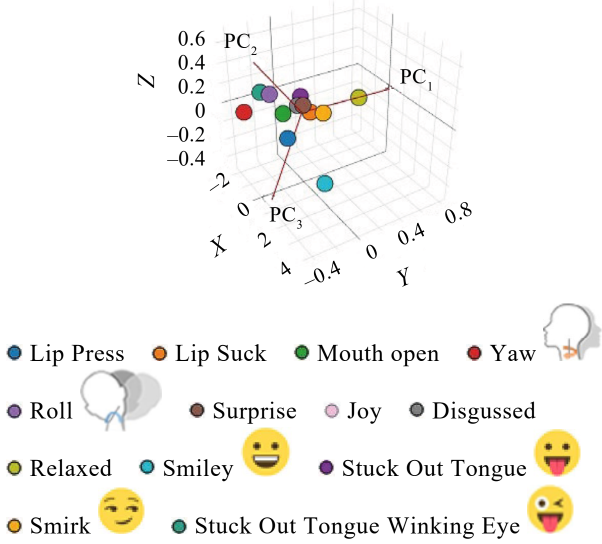


Figure 6 Principal component analysis of AI-supported sensory evaluation

The Principal Component Analysis plot illustrates a clear separation between the conventional iced tea (PC_1) and the linden-enriched variants (PC_2 and PC_3), suggesting a significant impact of linden extracts on sensory perception. Lip press, lip suck, and Mouth open appear prominently along PC_1 and PC_2, indicating that these expressions are significantly influenced by the formulation. This suggests notable differences in the mouthfeel and taste perception across the samples. Yaw and roll are clustered near the enriched tea formulations, particularly PC_2, suggesting that linden aqueous extract might induce a distinct sensory reaction, possibly linked to aroma or bitterness. Joy and smiley are observed near PC_3 (ethanolic extract), indicating a potential preference or positive response to this formulation. Conversely, disgusted is positioned further from PC_3, suggesting that ethanolic enrichment may mitigate negative sensory experiences. Meanwhile, expressions like smirk and stuck out tongue winking eye are more aligned with PC_2 and PC_3, potentially reflecting the modified sensory characteristics introduced by the aqueous and ethanolic linden extracts.

The separation of PC_2 and PC_3 from PC_1 suggests that the addition of linden extracts significantly alters the sensory profile of iced tea. The closer positioning of PC_3 (ethanolic extract) to positive expressions like smirk, smiley, and Joy suggests that this variant may be more appealing in terms of flavor or aroma. Conversely, the presence of yaw and roll in PC_2 indicates that the aqueous extract formulation may evoke stronger or more unexpected reactions, potentially due to the differences in solubility and the extraction of bioactive compounds.

From a product development perspective, the clustering of sensory responses along PC_2 and PC_3 suggests that while both enriched formulations differ from the conventional variant, they also exhibit distinct sensory signatures. The ethanolic extract (PC_3) appears to introduce greater variation in specific facial expressions compared to the aqueous extract (PC_2), which could imply a more pronounced influence on attributes such as bitterness, herbal intensity, or aftertaste persistence. These findings are particularly relevant given the growing consumer interest in functional beverages. The ability to modify sensory profiles through linden enrichment could be leveraged to enhance consumer acceptance and differentiate products in the market.

Furthermore, our study highlights the potential of AI-supported sensory evaluation as a valuable tool for product optimization. By understanding which formulations elicit more favorable sensory responses, manufacturers can refine ingredient compositions to enhance consumer satisfaction. Future research could expand the dataset with a larger sample size or incorporate additional sensory attributes such as bitterness intensity, sweetness perception, and aftertaste duration. This could provide a more comprehensive understanding of the sensory impact of linden extracts in iced tea.

CONCLUSION

The results of our study offer strong evidence supporting the safety and health benefits of iced teas enriched with *Tilia cordata* extracts. The analysis of fresh *T. cordata* flowers confirmed that they are free from detectable levels of heavy metals, reinforcing their suitability for human consumption. The ethanolic extraction method proved to be significantly more effective ($p < 0.05$) in extracting bioactive compounds, as it yielded higher concentrations of phenols and flavonoids compared to aqueous extracts. This highlights the enhanced bioactive potential of ethanolic extracts. They are not only beneficial for their antioxidant and antimicrobial properties but are also fully soluble, which makes them ideal for use in ready-to-drink iced tea formulations.

Both ethanolic and aqueous extracts exhibited significantly higher ($p < 0.05$) antioxidant and antimicrobial activities compared to conventional linden tea, which establishes them as functional food ingredients. Given the absence of synthetic additives, such as artificial colors or preservatives, these iced teas can be classified as natural health-promoting products with promising consumer appeal. Furthermore, the industrial production of these functional iced teas does not require special processing conditions. This distinct advantage over traditionally available linden teas enhances their market competitiveness.

The use of AI-driven sensory analysis, particularly through facial expression recognition, represents a significant advancement in food technology, enabling more precise and real-time consumer feedback. The data obtained from this innovative approach showed that both types of iced teas were well-received by the consumers, with positive emotional responses recorded during the sensory evaluation. This underscores the value of AI-based methods in the food industry, as they provide deeper insights into consumer preferences and acceptance, helping refine and improve product formulations. Thus, iced teas enriched with *T. cordata* extracts are highly likely to gain acceptance in the market due to their health benefits and consumer appeal. As a result, their development is a promising avenue for functional beverage innovation.

Future research will focus on *in vivo* studies to explore the biological effects and long-term health benefits of these enriched iced teas, providing further insights into their potential for improving consumer health. This research may ultimately lead to the creation of novel functional beverages that maximize the bioactive potential of *T. cordata* and other natural plant sources.

CONTRIBUTION

All the authors were equally involved in the manuscript and are equally responsible for any potential plagiarism.

CONFLICT OF INTEREST

The authors declare that there is no competing interest regarding this publication.

DATA AVAILABILITY STATEMENT

The data supporting this article are included in the article.

DECLARATION OF AI

The authors declare that no AI algorithms or programs were used to write the text. AI algorithms were only used for the sensory analysis of the product, which is described in detail in the paper.

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