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Evaluation of the Antioxidant Capacity, Antimicrobial Effect, and *In Vitro* Digestion Process of Bioactive Compounds of Cherry Laurel Leaves Extracts

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ABSTRACT
Cherry laurel is a less known fruit species with an astringent taste and is mostly consumed as fresh fruit only in the Black Sea and Marmara regions of Turkey. Cherry laurel (*Laurocerasus officinalis* Roemer) leaves can be prepared in different forms such as infusion by steeping the dried leaf in boiled water and as an extract for its further use as a food supplement or ingredient. In this study, aqueous and ethanol extracts of cherry laurel leaves were prepared and examined in terms of total phenolic compound (TPC), total flavonoid compound (TFC), antioxidant capacities using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and the copper reducing antioxidant capacity (CUPRAC) assays after submitting to *in vitro* digestion. Additionally, the antimicrobial potential of the leaves extract was evaluated. The TPC of ethanol and aqueous extracts were found at 17.62 and 0.83 mg gallic acid equivalent GAE.g^{-1} leaves and the TFC of ethanol and aqueous extracts were determined as 11.61 and 0.47 mg catechin equivalent CE.g^{-1} leaves, respectively. In terms of antioxidant activity results, ethanol and aqueous extracts had 41.11 and 0.77 mg Trolox equivalent TE.g^{-1} leaves for the DPPH assay, and 67.05 and 1.63 mg TE.g^{-1} leaves for the CUPRAC assay. After gastric digestion post-gastric (PG), compared to the initial values significantly lower recovery of the TPC (11.2 and 41%) and TFC (5.8 and 14.9 %) was observed for ethanol and aqueous extracts. The recovery of TPC, TFC values after the intestinal fraction was lower compared to the PG fraction for ethanol extracts, whereas for aqueous extracts they were higher compared to the PG fraction. The highest inhibition zone was observed against *Listeria monocytogenes* and *Aspergillus niger* when 10% extract concentration was applied. The experimental data verified that these extracts displayed remarkable antioxidant and antimicrobial activities, and the extraction method was important in terms of the bioaccessibility of bioactive compounds.

Keywords: *Laurocerasus officinalis* Roemer, Gastrointestinal, Phenolics, Flavonoid, Bacteria, Molds

1. Introduction

Plants have been used for medicinal purposes for over a thousand years. In traditional medicine around the world, 28.187 plant species have been identified and documented (Başer 1998; Dalar et al. 2018; Antonelli et al. 2019).

Cherry laurel (*Laurocerasus officinalis* Roemer), locally called as *taflan* or *karayemis*, has an astringent taste and is primarily consumed as fresh fruit only in local markets; it can also be dried, pickled, and sometimes processed into different products such as pekmez, jam, and marmalade (Capanoglu et al. 2011). The cherry laurel tree is also an appealing ornamental plant with dark, evergreen foliage and clusters of white flowers in the spring (Macit & Demirsoy 2012). In Turkish folk medicine, the fruit, seeds, and leaves have been used for different purposes, such as decoctions of leaves have been used for the treatment of food poisoning, stomach pain, prevention of excessive oil secretion in hair; leaf infusions have been applied for stomach ache, sore throat, and hemorrhoids and the seeds have been used for the treatment of descensus ventriculi (gastric descent) (Basri 1864; Hami 1864; Yesilada et al. 1999; Uslu et al. 2018).

Only few reports have been published on the bioactive contents of cherry laurel genotypes sampled in the West Black Sea Region (Beyhan et al. 2018; Islam et al. 2020). The effects of preharvest calcium chloride ($CaCl_2$) treatment on several quality features and bioactive substances of sweet cherry fruit were examined in a study by Erbaş & Koyuncu (2022), and it was discovered that all treated kinds had lower antioxidant activity than control fruit. Compared to the fruits of cherry laurel, it was reported that the leaves

also contain a higher level of phenolic compounds (Alasalvar et al. 2005; Orhan-Erdogan & Akkol-Kupeli 2011; Karabegovic et al. 2014). A major phenolic compound identified in cherry laurel leaves was chlorogenic acid, ranging from 25.44 to 36.49 mg/g of dry extract. Other phenolic compounds determined in cherry laurel leaves were o-coumaric acid, quercetin 3-glucoside, luteolin 7-glucoside, apigenin 7-glucoside, kaempferol 3-glucoside, and naringenin (Karabegović et al. 2014). Akkol et al. (2012) also identified three phenolic compounds, hydroxyphenyl acetic acid, kaempferol, and catechin derivatives, in the cherry laurel leaf extracts. Many studies have also shown the potential use of plant extracts as antimicrobial ingredients. The use of such compounds as antimicrobial additives in mildly processed food products has also been popular (Cui et al. 2010). *Laurocerasus officinalis* Roemer has been found to have anti-inflammatory, antifungal, and anti-nociceptive effects when prepared with ethanol and water extracts (Uslu et al. 2018).

Traditional consumption of medicinal plants was preparing the infused liquid by adding the plants to the boiled water, leaving them too steep, and filtering the plants (Suna et al. 2019). However, due to advancements in the functional food and nutraceutical industries, medicinal plant extracts have been created and used in a variety of forms, including pills, tablets, liquid extracts, powders, and the development of various value-added food products in recent years. When the aqueous infusion of medicinal plants and their phenolic extracts were consumed, they would be subjected to gastrointestinal digestion. *In vitro* digestion assays have been developed as an alternative approach to *in vivo* studies since they are simple, cheap, and reproducible tools to assess the stability of different food constituents against digestion fluids. Therefore, it's crucial to figure out how digestion impacts the stability of phenolic compounds and their antioxidant properties. As a result, their bioaccessibility depends on whether the medicinal plant's aqueous infusion or ethanolic extract is taken first. There have been some studies in the literature about the determination of antioxidant potential and bioactive compounds in cherry laurel leaf extracts (Akkol-Kupeli et al. 2012; Celep et al. 2013; Karabegović et al. 2013; Karabegović et al. 2014; Uslu et al. 2018). However, to the best of our knowledge, there has been no study focusing on the antioxidant potential of leaves extracts through *in vitro* gastrointestinal digestion process and their antimicrobial potential. As a result, cherry laurel leaves were made as an ethanol extract and an infusion in this investigation (aqueous extract; dried leaves steeped in boiled water). Then subjected to *in vitro* digestion, and their antioxidant properties, total phenolic, and flavonoid contents were assessed. Additionally, the antimicrobial activity of cherry laurel leaf extract was determined.

2. Materials and Methods

2.1. Chemicals

Folin-Ciocalteu's phenol reagent, sodium carbonate, sodium nitrite, aluminum trichloride, copper (II) chloride, ammonium acetate, sodium bicarbonate, sodium hydroxide, ethanol, hydrochloric acid (37%), nutrient broth, nutrient agar, potato dextrose agar (PDA) obtained from Merck (Darmstadt, Germany). Gallic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), (±)-6- Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), catechin hydrate standard, neocuproine, pepsin (P6887), pancreatin (P7545), and all other reagents used to prepare simulated gastric fluid and simulated intestinal fluid were obtained from Sigma-Aldrich (Steinheim, Germany).

2.2. Plant material and extraction

Cherry laurel (*Laurocerasus officinalis* Roemer) leaves were obtained from Istanbul, Turkey was verified (Gul Herbarium Identification #46/4/1-1) by Prof. Dr. Hasan Ozcelik, senior botanist, Department of Biology, Suleyman Demirel University, Isparta, Turkey.

The fresh leaves were washed up with tap water and dried in the shadow at room temperature (25±2 °C) for three days and, the final moisture content of the leaves was 8.50±1.24%. The extraction conditions were based on preliminary experiments. The extracts from dried leaves were extracted by two different procedures. In the first procedure, 10 g of milled dried leaves were extracted with 100 mL ethanol mixture (80% ethanol + 20% water) for 2 h on a magnetic stirrer at room temperature. For the second procedure (aqueous extracts), the traditional method to prepare the herbal infusion was simulated by steeping 10 g of unmilled dried leaves with 100 mL of freshly boiled water for 5 min. Both ethanol and aqueous (infusion) extracts were filtered (Whatman no 1), and the clear phase was used for further analysis.

2.3. Simulated in vitro gastrointestinal (GI) digestion assay

The *in vitro* gastrointestinal digestion model of McDougall et al. (2005) and Kamiloglu et al. (2014) was applied for cherry laurel leaves extracts. The change in the antioxidant activity and release of polyphenolics of the ethanolic and aqueous extracts were examined at a

gastric stage and an intestinal stage of digestion, and the *in vitro* gastrointestinal digestion procedure is shown in Figure 1. Briefly, 2.5 mL extracts were combined with 20 mL distilled water and 5 M HCl was used to alter the pH to 1.7. 1.5 mL of pepsin solution (315 units/mL) was added and incubated for 2 hours at 37 °C in a hot water bath with 100 rpm shaking. After 2 h, 2 mL aliquots of the post-gastric (PG) digestion were collected. 4.5 mL of 4 mg/mL pancreatin solution (2 units/mL) and 25 mg/mL bile salt mixtures were added to the remainder in the 250 mL glass beaker. Segments of dialysis bags (MWCO 12,000 Da) were cut and filled with sufficient sodium $NaHCO_3$ (0.5 M) to neutralize the sample's titratable acidity (pH 7). Samples were incubated in a shaking water bath (100 rpm) at 37 °C for another 2 h to complete the intestinal phase of the *in vitro* digestion process.

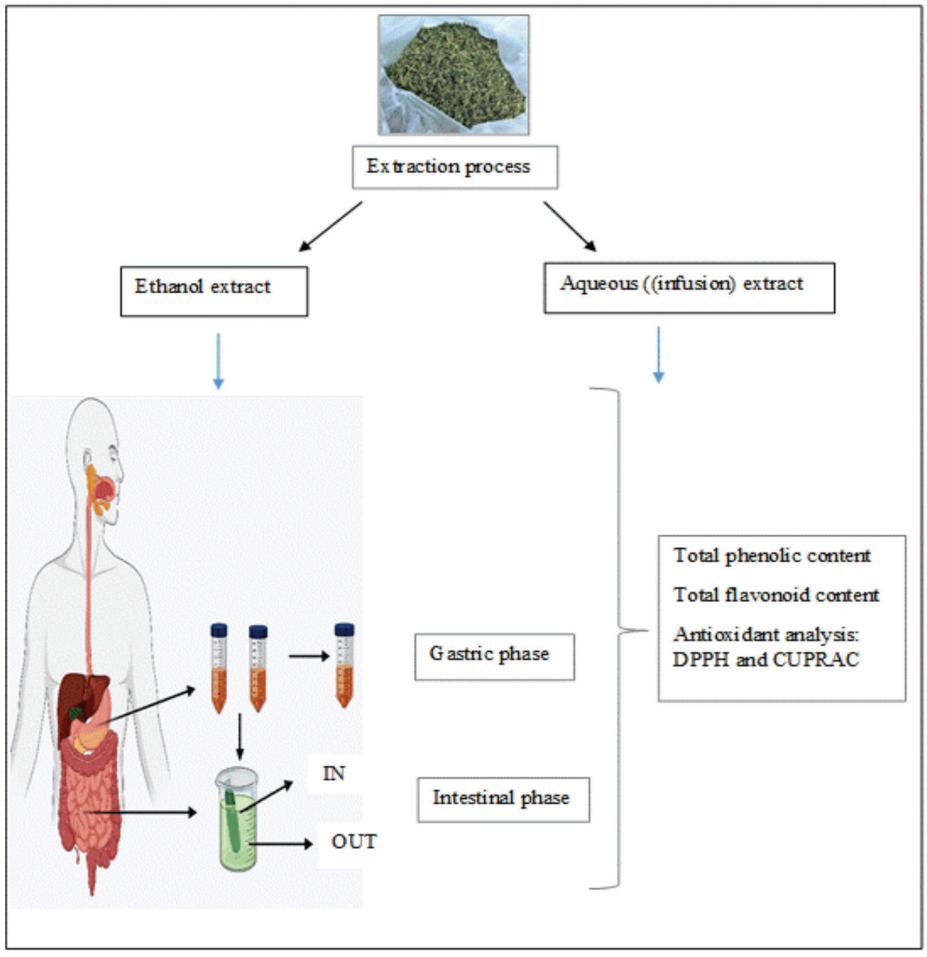

Figure 1- Flow chart outlining the steps involved in the *in vitro* gastrointestinal digestion procedure

After the intestinal phase, the content of the dialysis membrane was taken as the IN fraction, while the medium outside the membrane was referred to as the OUT fraction. The blank was also prepared with identical chemicals but without samples and underwent the same conditions. Then, the sample taken at each digestion step was centrifuged at 2268 g for 20 min and stored at -20 ºC for the analysis. Total phenolic compound (TPC), total flavonoid compound (TFC), and antioxidant activities were determined for ethanol and aqueous (infusion) extracts, as well as for each of the PG, IN and OUT fractions, using the methods described below.

2.4. Determination of total phenolic and total flavonoid content

The TPC and TFC were determined according to the method Singleton et al. (1999), Zhishen et al. (1999), respectively. The absorbances at 760 nm for TPC, 510 nm for TFC were measured using a Shimadzu 150 UV-1800 spectrophotometer (Kyoto, Japan). For TPC, the results were given as mg GAE per g dried leaves. The linear range of standard curve was from 0.01 to 0.1 $mg.mL^{-1}$ (r^2=0.993). For TFC, the results were given as mg CE per g dried leaves. The linear range of standard curve was from 0.01 to 0.35 $mg.mL^{-1}$ (r^2=0.996).

2.5. Antioxidant capacity assays

The DPPH radical scavenging capacity of cherry laurel leaves extracts was evaluated according to the method of Sanchez-Moreno (2002) and Singh et al. (2002). 0.1 mL of extract was mixed with 4.9 mL of DPPH solution (6×10^{-5} M) and incubated at room temperature for 20 min in the dark. The absorbance was measured at 517 nm, and the results were given as mg TE per g dried leaves. The linear range of standard curve was from 0.05 to 0.5 mg.mL^{-1} (r^2=0.996).

The copper reducing antioxidant capacity (CUPRAC) assay was carried out according to the method of Apak et al. (2004). One mL of each $CuCI_2$ solution (0.01 M), neocuproine (7.5 mM), and 1 M of ammonium acetate buffer (pH 7.0) solutions were added to a test tube. After the addition of 0.1 mL of extract, 1 mL of distilled water was added. All samples were incubated at room temperature for 1 h in the dark. The absorbance was measured at 450 nm, and the results were given as mg TE per g dried leaves. The linear range of the standard curve was from 0.05 to 1 mg.mL^{-1} (r^2=0.991).

2.6. Antimicrobial activity assays

Antimicrobial activity of the extracts was determined by using the agar diffusion method against five bacteria (*Bacillus cereus* FMC19, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19118, *Salmonella* Typhimurium ATCC 14028, *Escherichia coli* O157: H7 ATCC 33150) and three molds (*Penicillium carneum, Aspergillus flavus, Aspergillus niger*. Bacteria were inoculated in nutrient broth and incubated at 37 °*C for 24 h, while the* molds were activated in PDA solid medium at 27 °C for 3 days. Then 1% of bacteria were added in nutrient broth and re-incubated for 18 h. Final cell concentrations were measured as 10^6-10^7 *colony-forming unit/*mL. One hundred *µL of microbial cultures* were pipetted, sowed by the spread plate method, and left for 20 min. Then, 4 equidistant wells were bored by sterile cork borers (*Ø*=5 mm) (Sagdic et al. 2013). According to our preliminary study, extract with 80% ethanol of leaves was found most effective against bacteria and mold whereas the aqueous (infusion) extract of leaves had no or limited inhibiting effect on any of the tested bacteria and mold. This might be owing to the excessive heat generated by the high boiling temperature during water extraction, as well as the chemical changes that resulted. That's why ethanol extract of cherry laurel leaf only was used. Dried leaves (6 g) were extracted successively with 60 mL of ethanol mixture (80% ethanol 20% water) by using a Soxhlet extractor (Buchi Extraction Unit E-816) for 4.5 h at 60 °C temperature, and then concentrated under vacuum at 40 °C. The dried plant extracts were dissolved in ethanol as a final concentration of 1%, 5%, 10% (w:v), and 20 µL of extract and negative control was prepared using ethanol. The petri dishes were incubated at 27 ºC for 3-4 days for the molds, and at 37 ºC for 18-24 h for the bacteria. After the incubations, the zones were measured as mm and the analyzes performed in triplicate.

2.7. Statistical analysis

The statistical analysis was carried out by means of SPSS Statistics (IBM SPSS 17.0, USA). All data were presented as a mean of at least three measurements, i.e. ± standard deviation for each extract. The differences of bioactive compounds or antioxidant capacity between cherry laurel leaves obtained by ethanol and aqueous (infusion) extracts were evaluated by t-test, the differences among value of bioactive compounds or antioxidant activity that obtained in different steps of the *in vitro* digestion assay and the differences among concentration of antimicrobial effects were analyzed by one-way ANOVA combined with the Duncan comparison test at $p<0.05$ significance level.

3. Results and Discussion

3.1. Total phenolic, total flavonoid contents, and antioxidant capacity of cherry laurel leaves extracts

The TPC, TFC, DPPH radical scavenging capacity, and CUPRAC of ethanol and aqueous extracts (infusion) of cherry laurel leaves were given in Table 1. The TPC and TFC of ethanol and aqueous extracts were determined as 17.62 and 0.83 mg GAE g^{-1} of leaves, and 11.61 and 0.47 mg CE g^{-1} of leaves, respectively. In terms of antioxidant capacity results, ethanol and aqueous extracts had 41.11 and 0.77 mg TE g^{-1} leaves for DPPH assay, and 67.05 and 1.63 mg TE g^{-1} leaves for CUPRAC assay (Table 1). In a study by Karabegović et al. (2014), the effects of different extraction techniques on the composition and antioxidant capacity of cherry laurel fruits and leaves were studied. TPC and TFC content of dried cherry laurel leaves were found as 36.65±0.6 mg of GAE.g^{-1} leaf and 26.31±0.72 mg rutin/g leaf when the soxhlet extraction technique was applied with methanol for 2 hours. Orhan-Erdogan & Akkol-Kupeli (2011), found the TPC content of dried cherry laurel leaves as 10.04±0.06 and 14.93±0.98 mg of GAE.g^{-1} leaves when methanol and distilled water (not boiling water) were used as the extraction solvents. The TPC result of ethanol extract (17.62±0.14

mg GAE.g^{-1} leaves) determined in our study was higher than those determined by Orhan-Erdogan & Akkol-Kupeli (2011), but lower than Karabegović et al. (2014). The differences in the results can be explained with differences in the extraction conditions, type of extraction solvent, as well as with environmental factors, plant varieties, and age of the trees, post-harvesting conditions, or storage (Kolayli et al., 2003). In our study, the TPC and TFC of the infusion (aqueous extract) were lower than the ethanol extract as expected due to the procedure applied for its preparation. The decrease in phenolic acids and flavonoids found in water extraction (infusion) could be due to oxidation of this chemical in the presence of water at high temperatures. Flavonoids are known to breakdown in the water at temperatures of 100 °C and above. The thermal stability of flavonoids is affected by the quantity and type of substituents as well as the position of the hydroxyl group, with compounds with fewer substituents being less stable at high temperatures (Kasmi et al. 2021). As a result, the higher bioactive compounds in the ethanol extract than in the infusion extract can be explained by the fact that the leaves are not ground and boiled water is used in the extraction process. Similarly, Martins et al. (2014) discovered that the hydroalcoholic extract of *Origanum vulgare* has higher antioxidant activity than the aqueus extract (infusion). In the same way, Ozkan (2009) found that ethanol extracts of *Sideritis* species had higher amount of phenolics as compared to aqueous infusions. In theory, the bigger the particle size of leaves the lower the yield of extraction will be due to the lower contact surface area of the plant with the water. Also, 100 °C treatments produce lower extraction yield and bioactive compounds (Vuong et al. 2011; Geoffroy et al. 2017).

Table 1- The TPC, TFC, and antioxidant capacities of ethanol and aqueous (infusion) extract of cherry laurel leaves

	Unit	***Ethanol extract***	***Aqueous extract (infusion)***
TPC	mg GAE.g^{-1}	17.62±0.14^{a}	0.83±0.10^{b}
TFC	mg CE.g^{-1}	11.61±0.30^{a}	0.47±0.16^{b}
DPPH	mg TE.g^{-1}	41.11±0.57^{a}	0.77±0.70^{b}
CUPRAC	mg TE.g^{-1}	67.05±1.30^{a}	1.63±0.02^{b}

The results are given as mean ± standard deviation of triplicate measurements. Means with different letters in the same row are significantly different ($p<0.05$). The results are expressed for per g dried leaves, *TPC* total phenolic content, *TFC* total flavonoid content

In terms of antioxidant capacity, Orhan-Erdogan & Akkol-Kupeli (2011) found that at 2000 g.mL^{-1} of extract, methanol and aqueous (not infusion) extracts of cherry laurel leaves had 64% and 31% of DPPH radical scavenging capacity, respectively. The aqueous extract likewise had a lesser antioxidant capacity than the ethanol extract in our study. The ethanol is frequently used to extract tannins, polyphenols, and flavonols, and ethanolic extracts have high antioxidant activity. With only a tiny amount of flavonols leached into the polar solvent during water infusion, such preparations have the inferior antiradical-scavenging capability (Kobus et al. 2009). The higher antioxidant capacity of leaves compared to the fruits of cherry laurel had been determined in previous studies, Karabegović et al. (2014) determined EC_{50} values of the extracts of cherry laurel fruits and leaves found that leaf extracts showed higher antioxidant capacity than those of the fruit extracts. Antioxidant capacity values of any compound would differ depending on the measurement method. In our study, the CUPRAC values of both extracts were higher than the values obtained by the DPPH method. Cu^{2+} ion takes part in the formation of free radicals; the reduction of cupric ion indicates another mechanism than that of the DPPH method reflecting the antioxidant potential. This could be because the CUPRAC assay can detect both the hydrophilic and lipophilic antioxidant capacity of extracts because the reagent is soluble in both aqueous and organic solvents. DPPH assay with hydrophobic systems because it uses a radical that is only dissolved in the organic solvent (Capanoglu et al. 2018).

3.2. The TPC, TFC, DPPH and CUPRAC of cherry laurel leaves extracts obtained after simulation in vitro gastrointestinal digestion

In Figure 2, the recovery (%) of the TPC, TFC, and antioxidant activities of the extracts were given at each step of simulated gastrointestinal digestion, namely PG (after stomach digestion), IN (the material entered the serum), and OUT (the material remained in the GI tract) after intestinal digestion.

The TPC, TFC, and antioxidant capacity recovery (%) was calculated by dividing the values obtained for the PG, IN, and OUT fractions by the initial values of undigested extracts. For a better evaluation of the change, the initial values of ethanol and aqueous (infusion) extract were considered as 100%. After gastric digestion (PG), compared to the initial values a significantly lower ($p<0.05$) amount of the TPC (11.2 and 41%) and the TFC (5.8 and 14.9%) were recovered from ethanol and aqueous extract, respectively. When DPPH radical scavenging capacity and CUPRAC values of initial values and those obtained after gastric digestion (PG) were compared, significantly lower recovery values were observed for ethanol extract (71% and 30%, respectively). The trend was similar for CUPRAC

values of aqueous extract (32% recovery), whereas it was different for the aqueous extract in the DPPH values. The initial and PG values for DPPH recovery were not significantly different for aqueous extract.

After the intestinal digestion, for the IN fractions of both ethanol and aqueous extracts, the recovery (%) values for the TPC (3.9 and 15.7%) and the TFC (0.4 and 6.4%), respectively, significantly lower than those obtained at the initial values. Except for the recovery (%) of the antioxidant capacity value of aqueous extract attained by the CUPRAC assay, the antioxidant capacity values of the IN fractions were reduced (6.5-19.5%) compared to the values obtained after the initial values.

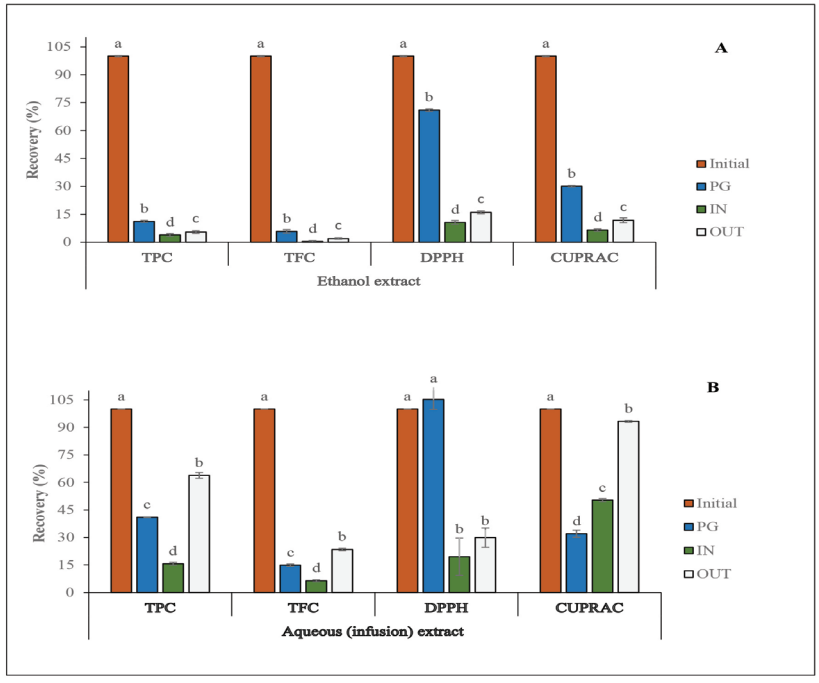

Figure 2- Changes in the TPC, TFC, and antioxidant capacity (determined by DPPH and CUPRAC assays) of cherry laurel leaves ethanol extract (A) and aqueous (infusion) extract (B) during *in vitro* gastrointestinal digestion, expressed as recovery percentage. The terms represent; initial, undigested sample (100%); PG (post-gastric), recovered after gastric digestion; IN, dialyzed fraction; OUT, non-dialyzed fraction recovered after intestinal digestion. Data represent the average values±standard deviation of four independent samples. Different letters above the bars represent the statistically significant differences ($p<0.05$)

The recoveries of the TPC and TFC of OUT fractions were significantly higher than those of the IN fractions. Non-dialyzed phenolic fraction (OUT) for ethanol extract accounted for 5.4 and 2% of the initial TPC and TFC, respectively. For aqueous extracts, the OUT fraction was 63.9 and 23.5% of the initial TPC and TFC. Similar to the outcomes of TPC and TFC, for all samples, the recovery values for antioxidant capacity obtained for OUT samples were higher than the IN values.

In our study, after gastric digestion (PG) 88.2 and 94.2% of the TPC and TFC in ethanol extract was lost, and correspondingly the antioxidant capacity values were decreased by 30 and 70% obtained by the DPPH and CUPRAC assays compare to the initial values. Whereas for aqueous extract, although the initial TPC and TFC were lower compared to the ethanol extract, the loss of TPC and TFC (59 and 85.11%) was lower at the PG stage. Compared to the initial values, the antioxidant capacity value was reduced to 32% when determined by the CUPRAC assay, whereas it wasn't statistically significant difference when determined by the DPPH assay for the aqueous extract. This difference could be related to the difference in the mechanisms related to the methods of determination, which means employing a method depending on one mechanism may not reflect the true antioxidant capacity. Furthermore, the accessibility and metabolism of dietary antioxidants influence their effect in biological systems. As a result, the differences in the conditions of antioxidant activity/capacity models applied to biological systems, as well as potential interactions between food-derived and endogenous antioxidants, must be considered (Capanoglu et al. 2018).

The loss of TPC and TFC in the digested samples were in agreement with the results of other authors. Ortega-Vidal et al. (2019) studied the effects of *in vitro* digestion on the phenolic profile and antioxidant capacity of *Jasonia glutinosa* herbal tea infusion and found that

the TPC content was reduced approximately 86% after *in vitro* digestion and a similar trend was observed for antioxidant capacity values obtained by the DPPH and ABTS, approximately 57 and 86% reduction compared to initial values obtained before digestion.

Donlao & Ogawa (2018) showed that *in vitro* digestion reduced antioxidant activity in all tea infusion samples. They also revealed that whereas polyphenols in tea infusions were reasonably steady during digestion, the DPPH values were reduced by 16.0 to 25.7 percent after digestion.

The sum of IN and OUT fractions was representing the total amount of TPC and TFC at the intestinal stage, and IN (dialyzed fraction) was taken as serum available, and OUT (non-dialyzed fraction) was referred to as colon available. When TPC and TFC content was analysed after the intestinal stage (IN + OUT), there was a decrease in TPC compared to the PG stage (from 1.97±0.01 to 1.64±0.01 mg GAE. g^{-1} leaves) and TFC values (from 0.67±0.03 to 0.27±0.01 mg CE.g^{-1} leaves) for ethanol extracts, interestingly whereas for aqueous extracts there was an increase in both TPC (from 0.34±0.02 to 0.66±0.01 mg GAE.g^{-1} leaves) and TFC values (from 0.07±0.01 to 0.14±0.04 mg CE.g^{-1} leaves).

For both ethanol and aqueous extracts, the TPC and TFC of OUT fractions were significantly higher than of the IN fractions representing those larger amounts of compounds could be metabolized by the microflora in the colon. The recovery (%) of TPC and TFC in the OUT fraction in the aqueous extract (64 and 23%) was significantly higher than the values obtained by ethanol extract (5.44 and 2%). Similar to the outcomes of TPC and TFC, the recovery values for antioxidant capacity obtained for the OUT samples were higher than of the IN fractions.

The recovery (%) of TPC, TFC, and antioxidant capacity at the intestinal stage (IN + OUT fraction) were lower compared to the PG fraction for ethanol extracts. Whereas for aqueous extracts, at the intestinal stage, the recovery (%) of TPC, TFC, and CUPRAC were higher compared to the PG fraction. Except for the recovery value of DPPH, it is thought that the transition from an acidic to an alkaline environment improves the ability of phenolics to donate protons present on their aromatic rings, enhancing their antioxidant properties (Ucar & Karadag, 2019). This was only observed for aqueous extracts. The differences in the recovery of antioxidant capacity at the intestinal stage might be related to possibly different individual phenolics obtained in ethanol and aqueous extract. Karabegović et al. (2014) investigated the phenolic profile of methanolic from cherry laurel leaves and fruit Regardless of plant materials or extraction techniques, chlorogenic acid was the most common component in cherry laurel leaf and fruit extracts, ranging from 25.44 to 36.49 mg.g^{-1} dry extract, although quercetin 3-glucoside, o-coumaric acid, luteolin 7-glucoside, kaempferol 3-glucoside, apigenin 7-glucoside, and naringenin were present in the leaf extracts, while rutin, caffeic acid, and vanillic acid were detected in fruit extracts.

Phenolics are highly sensitive to pH changes and, thus differences in the TPC and TFC, and antioxidant capacity after digestion could be due to the stability of each type of phenolic compound present in the sample matrix. In our study, the extraction solvent and preparation methods were different, the individual phenolics would be different in ethanol and aqueous extract of cherry laurel leaves, therefore this difference obtained *in vitro* digestion study could be related to the effects of food matrix (such as particle size, food components) and the presence of individual compounds in the extract. A similar trend was reported by Siracusa et al. (2011) who evaluated the antioxidant capacities and phenolic composition of *Capparis spinosa* L. and *Crithmum maritimum* L. aqueous extracts before and after the submission to an *in vitro* digestion process. They found that the amount of total phenol in digested infusions significantly decreased (<1%) in both samples, suggesting that the dominant phenolic components detected in original samples are not stable under the gastrointestinal tract. Total loss of chlorogenic acid after intestinal digestion was only 33% in the caper extract whereas it was 81.7 and 95.7% for the standard mixture and sea fennel extract. Although the initial chlorogenic acid amount was the lowest in the caper extract, after digestion its recovery was the highest in the caper extract, indicating that the extract composition, the presence of other constituents may strongly influence the behavior of a single compound. Similarly, when rutin was submitted to gastric digestion as a part of the standard mixture, at PG the loss was 88%, whereas it was negligible (1.7%) when it was in the composition of the caper extract. Even for the same particular phenolic component, the extract composition had an effect on its stability after digestion, according to their study. When the standard combination, aqueous extract of caper, and sea funnel were subjected to gastric digestion (PG), the loss of chlorogenic acid was 58, 5.8, and 66%, respectively.

3.3. Antimicrobial activities of cherry laurel leaf extract

The antimicrobial activity of ethanol extract of cherry laurel leaves at different concentrations was given in Table 2. Generally, upon increasing the ethanol extract concentration, the inhibition zone was also increased, and the highest inhibition zone was observed at 10% extract concentration applied against *Listeria monocytogenes.* Between 5 and 10% of extract concentration, the differences

in the inhibition against *Bacillus cereus* and *Escherichia coli* O157: H7 were not significant ($p>0.05$). The leaves extract showed good antimicrobial activity and can be used to explore novel antimicrobial activities. In terms of antifungal properties, the inhibition zone was the highest against *Aspergillus niger*, and it was increased by increasing the extract concentration from 1 to 10%. Against *Penicillium carneum*, at 1% extract concentration no inhibition was observed, whereas the inhibition was increased with the higher concentrations applied. The ethanol extract of cherry laurel leaves was not effective against *Aspergillus flavus* at any concentration applied in this study.

Table 2- Antimicrobial activity of the cherry laurel leaves extract (inhibition zone, mm)

		Extract concentration (%)		
Bacteria	***Negative control (Ethanol)***	***1***	***5***	***10***
B. cereus	nz	8.33±1.15^{b}	13.00±1.00^{a}	13.33±0.58^{a}
S. aureus		10.33±1.15^{b}	10.67±0.58^{b}	12.67±0.58^{a}
L. monocytogenes		9.00±1.00^{b}	8.33±1.15^{b}	18.33±3.06^{a}
S. Typhimurium		7.67±1.15^{b}	8.00±1.00^{b}	11.67±1.15^{a}
E. coli O157:H7		8.33±1.15^{b}	10.00±1.00^{a}	10.83±0.76^{a}
Molds				
P. carneum	nz	nz	11.00±1.00^{b}	17.33±2.52^{a}
A. flavus		nz	nz	nz
A. niger		6.33 ± 0.58^{c}	12.00±1.00^{b}	15.33±1.53^{a}

The results are given as mean ± standard deviation of triplicate measurements. Means with different letters in the same row are significantly different ($p<0.05$). nz: no inhibition zone

In the study of Ayla et al. (2019), the antimicrobial activity of methanol extracts of the fruit part of *L. officinalis* against *E. coli*, *S. aureus*, *B. cereus* was determined by the agar well diffusion technique. While the strongest antimicrobial activity was found for *S. aureus* (34 mm), the weakest antimicrobial activity was found for *E. coli* (23 mm) when the extract concentration was 25%, in our study, when the leaves the part of *L. officinalis* was used at 10% of extract concentration, inhibition zones were obtained, 12.67±0.58 and 10.33±0.58 against *S. aureus* and *E. coli*. In the study of Sahan (2011), among all the extracts (methanol, ethanol, acetone, chloroform), the ethanol extract of *Prunus laurocerasus* L. leaves was found most effective against *Fusarium oxysporum, Penicillium solitum,* and *Rhizopus oligosporus*. The antibacterial activity of different concentrations of *Laurus nobilis* leaves extract (1, 2.5, 5, 7.5, 10, and 20%) against *S. aureus* was investigated in another study utilizing a Vernier Calliper to measure the zone of growth inhibition. *S. aureus* growth was inhibited at doses of *L. nobilis* leaves extract ranging from 2.5 to 20 (v/v) (Hamdan & Masoud 2020).

4. Conclusions

The innovation of this study is the evaluation of change in total phenol, total flavonoid content, and antioxidant capacities of cherry laurel leaves subjected to a simulated *in vitro* digestion model. Although the initial levels of TPC, TFC, and antioxidant capacities were higher for ethanol extracts, the highest recovery (%) for PG, IN, and OUT fractions was obtained by the aqueous extract, after digestion. As a result, when medical plants and their extracts were consumed, the fate of desirable phenolic components after digestion would be critical in determining which medicinal plant consumption method, such as infusion, to use. Additionally, the growth inhibition of the tested molds and bacteria by the cherry laurel leaf extract indicated its potential as a novel antimicrobial food ingredient. Further studies should include the determination of individual phenolic compounds in the cherry laurel leaf extracts depending on the method of preparation either by aqueous infusion or organic solvent extraction, and how the composition of the extract would affect their stability during *in vitro* digestion.

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