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Original Research Article

Determination of Phenolic Compound Profiles and Antioxidant Effect of Plant Extracts on Late-Release Soft Lozenge

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Abstract: Medicinal plants contain different bioactive compounds that have immune system stimulant, anti-inflammatory, antibacterial, antiviral, antifungal, anticancer and wound healing effects. *Echinacea purpurea* L., *Sambucus nigra* L. and *Cetraria islandica* L. are known with their high antioxidant content and health promoting properties. The main objective of the study was to determine the total phenolic content, total flavonoid content and antioxidant capacity of each plant extract with water and 75% ethanol as solvents. The extraction efficiency was significantly higher in ethanol extracts according to total phenolic content, total flavonoid content, 1-diphenyl-2-picryl-hydrazyl (DPPH) and copper reducing antioxidant capacity (CUPRAC) analysis ($p<0.05$). Among all extracts, the highest result of radical scavenging capacity was found in ethanol extract of *Sambucus nigra* L. as 901.62±24.53 mg TEAC/ g dry extract. Total flavonoid and total phenolic contents of the same extract were determined as 840.54±13.46 mg RE/ g dry extract and 339.68±1.47 mg GAE/ g dry extract, respectively. The selected phenolic compounds were quantified and phenolic profiles were determined by HPLC analysis for all extracts. By using the selected plant powders, the late-release soft lozenge product was formulated. Loss of bioactive compounds was examined by using spectrophotometric and chromatographic techniques. In lozenge product, the highest phenolic content was obtained with *Sambucus nigra* L. (2.33±0.11 mg GAE/ g dry extract. Results confirmed that the lozenge application caused a significant loss in antioxidant amount for all plant extracts. In order to obtain optimum antioxidant effectiveness from the lozenge products with functional properties, it is needed to provide a suitable recipe with optimized usage of plant extracts.

Keyword: Phenolic Content, Antioxidant Capacity, Medicinal Plant, Soft Lozenge.

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1. INTRODUCTION

Healthcare products are moving away from disease-focused products such as pharmaceuticals, into wellness-promoting products including herbs and nutraceuticals (Aziz, Sarmidi, Kumaresan, Taher, & Foo, 2004). Due to the insufficient nutrition, the oxygen free radical production and the antioxidant production mechanisms in human body can become unbalanced. Antioxidants reduce the oxidative damage of human body and they are used as protective agents (Dawidowicz, Wianowska, & Baraniak, 2006). Many antioxidants are effective in neutralization and adsorption of free radicals, degrading peroxides by quenching singlet and triplet oxygen (Zheng & Wang, 2001). The phenolic compounds are specified with their reducing function on these free radicals which determine their antioxidant effectiveness.

Herbs are known as dietary plants and they contain high phenolic content and antioxidant capacity. Dietary plant phenolic substances are known with their health promoting role in human body and due to their functional properties, they are gaining the interest of consumers in a growing manner (Rauha *et al*., 2000). Many studies proceed on characterizing compounds in extracts of herbs, which are in numerous variety. Phenolic compounds are found as a diversified collection of secondary metabolites with their known existence in most of the fruits and vegetables. The phenolic contents of the medicinal plants are specified

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mostly as flavonoids. The function of flavonoids is to prevent free radicals by inhibiting enzymes which are incorporated in the synthesis of free radicals (Stanković *et al*., 2016).

With the aim of replacing the synthetic additives with natural components rich in polyphenolics such as phenolic acids, flavonoids, tannins, *etc*.; researchers started to investigate nature. The medicinal plants which are collected from nature, extracted and applied in supplements to promote good health by preventing diseases rather than treating diseases (Agalar, 2019).

LIST OF ABBREVIATIONS

SN *Sambucus nigra* L. (elderberry) dry extract powder
EP *Echinacea purpurea* L. dry extract powder
CI *Cetraria islandica* L. dry extract powder
SNE Ethanol extract of *Sambucus nigra* L.
EPE Ethanol extract of *Echinacea purpurea* L.
CIE Ethanol extract of *Cetraria islandica* L.
SNW Water extract of *Sambucus nigra* L.
EPW Water extract of *Echinacea purpurea* L.
CIW Water extract of *Cetraria islandica* L.
SNP Water extract of *Sambucus nigra* L. pastille application
EPP Water extract of *Echinacea purpurea* L. pastille application
CIP Water extract of *Cetraria islandica* L. pastille application
TPC Total Phenolic Content
TFC Total Flavonoid Content
TAC Total Antioxidant Capacity
DPPH 1,1-diphenyl-2-picrylhydrazyl
CUPRAC Copper Reducing Antioxidant Capacity
ABTS 2,2' azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
HPLC High-performance liquid chromatography
RP HPLC Reversed Phase High-performance liquid chromatography
GAE Gallic acid equivalent
RE Rutin equivalent
TEAC Trolox equivalent antioxidant capacity
DW Dry weight
FM Fresh matter
FW Fresh weight
R Correlation coefficient

2. MATERIALS AND METHODS

2.1 MATERIALS

For lozenge formulation, gum arabic (Kale Kimya, Turkey), maltitol (Sunar Mısır, Turkey), sorbitol (Sunar Mısır, Turkey), maltodextrin (Omnia Nişasta, Turkey) and modified corn starch (Cargill, Turkey) were used. Elderberry dry extract powder (fruits of *Sambucus nigra* L.; water and ethanol extract), *Echinacea purpurea* L. dry extract powder (herbs of *Echinacea purpurea* Linn. Moench; water extract) and Iceland moss dry extract powder (whole body of lichen *Cetraria islandica* L.; water extract) were purchased from Greenutra Resource Inc. (China). Ethanol, Folin-Ciocalteu's phenol reagent, methanol, Na_2CO_3, $NaNO_2$, $AlCl_3.6H_2O$, NaOH, $AlCl_3.6H_2O$, DPPH, ABTS, $CuCl_2$, neocuproine (Nc), ammonium acetate buffer (pH 7) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). For standard calibration curves, analytical grade gallic acid, rutin, trolox and HPLC-grade standards: cichoric acid, caftaric acid, caffeic acid, cyanidin 3-glucoside chloride, kaempferol-3-rutinoside, catechin, (-)-epicatechin were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

2.2 METHODS

2.2.1 Late Release Soft Lozenge Production

Gum arabic, maltitol, sorbitol, maltodextrin and modified corn starch were mixed gently and poured into the vacuum batch cooker (Bosch, Germany). The process was applied as detailed in Figure 1.3. The cooking was held for 1 h until the temperature is 110 °C and pressure is - 0.6 bar. Brix value of the solution was measured with optical refractometer and the result was recorded as 78 °Bx (Abbe 5, Xylem Analytics, Germany) after cooking. The mix was cooled until 82 °C in cooling tray and then the dough was kneaded for 5 min until the dough temperature decreased to 55 °C. 25g dry extract (DE) powder of each plant were separately added into each 1 kg of sugar dough (0.05g dry extract powder/ 2g lozenge).

2.2.2 Extraction for Analysis

For extraction of dry plant powders (SN: Elderberry dry extract powder; EP: *Echinacea purpurea* dry extract powder; CI: Iceland moss dry extract powder) and their usage in lozenges, the method of Hung & Morita (2008) was used with some modifications. Dry powder samples of SN (0.1g), EP (0.2g) and CI (0.2g) were added to 3 mL of 75% ethanol and water. The sonication was done for 15 minutes with continuous shaking in ultrasonic bath operating at 45 kHz with heating power of 600 W (USC900TH type, VWR® International Ultrasonic Cleaners, Pennsylvania, United States). The ultrasonic bath temperature was measured as 29°C after sonication. The samples were centrifuged for 10 minutes at 2500*g*. All extracts were hold at 4 °C until the analysis.

2.2.3 Total Phenolic Content (TPC)

TPC was determined by Folin-Ciocalteu method of Singleton and Rossi (1965) with some modifications. This method is based on the reduction of Folin-Ciocalteau reagent in the presence of Na_2CO_3. Absorbance of the samples were measured at 765 nm by using Shimadzu UV/VIS spectrophotometer (Shimadzu, UV–1700 PharmaSpec, Kyoto Japan). The total phenolic content of the extracts was determined by the calibration curve obtained with 10-50 mg/ml of gallic acid in 75% ethanol. The results were expressed as mg gallic acid equivalent (GAE)/g DE.

2.2.4 Determination of Total Flavonoid Content (TFC)

TFC was determined according to the method of Dewanto *et al*., (2002). 0.25 mL rutin was added to 75 µL $NaNO_2$ (5%). After 6 min, 150 µl 10% $AlCl_3.6H_2O$ was added and then after 5 min, 0.5 ml 1 M NaOH was added. The volume was completed to 2.5 mL with distilled water. The mixture was gently mixed for 10 seconds at ambient temperature. The absorbance was measured against a blank sample with 75% ethanol at 510 nm with UV/VIS spectrophotometer. Results were expressed as mg rutin equivalent (RE)/g DE using a standard calibration curve obtained with 10-50 mg/ml rutin in ethanol.

2.2.5 Determination of Total Antioxidant Capacity (TAC)

TAA of the samples were analyzed according to three different methods: DPPH, CUPRAC and ABTS assays. Trolox (0.01-0.10 mg /ml in 75% methanol) was used as standard.

2.2.5.1 DPPH Free Radical Scavenging Capacity

DPPH radical in methanol is reduced in the presence of a hydrogen donating antioxidants. The non-radical form of DPPH-H is formed during the reaction. DPPH method was applied based on the study of Kumaran et al. (2006) and Rai *et al*., (2006) with some modifications. 1 mL of 0.1 mM DPPH in methanol was added to 100 mL of extract sample. Samples were stored in a place away from light for 30 min at ambient temperature. Absorbance was measured with UV/VIS Spectrophotometer at 517 nm wavelength against blank with 75% ethanol.

2.2.5.2 Cupric Reducing Antioxidant Capacity (CUPRAC) method

This assay is based on the reduction of the cupric ion (Cu^{2+}) in copper (II) neocuproine [Cu (II)-Nc] reagent that the structure is known to be stable and both hydrophilic and lipophilic antioxidants can be detected by this rapid analysis method (Apak *et al*., 2004). 1 ml of 10 mM $CuCl_2.2H_2O$ in water, 1 ml neocuproine alcoholic solution (7.5 mM) and 1 ml ammonium acetate (NH_4Ac) buffer (1M, pH 7.0) solutions are mixed and added to 100 µl of extract and then 1 ml water was added. The solution was kept at ambient temperature for 30 min. Absorbance was measured with UV/VIS Spectrophotometer at 450 nm wavelength against blank with 75% ethanol.

2.2.5.3 Total Antioxidant Capacity by ABTS method

ABTS method was carried out according to the method of Miller and Rice-Evans (1997) with some modifications. 220 mg of ABTS was dissolved in 200 mL of distilled water and 38 mg of $K_2S_2O_8$ was dissolved in 2 mL of distilled water. The solutions were mixed and stored overnight in the dark to complete radicalization. The solution was diluted with 0.05 M phosphate buffered saline (PBS) (pH 7.4) until its absorbance reaches 0.9±0.2. Then, 1 mL of ABTS solution with 0.05M PBS was added to the 100 µL of extract and the mixture was vortexed for 10 seconds at ambient temperature. Absorbance was measured with UV/VIS Spectrophotometer at 734 nm after 1 minute against 75% ethanol blank sample. The result was expressed as mg Trolox Equivalent Antioxidant Capacity (TEAC)/g DE.

2.2.6 HPLC analysis of phenolic compounds

For the quantification of phenolic compounds, the method of Bakir *et al*., (2016) was applied in Waters W600 HPLC system (Milford, MA, USA) with PDA (photodiode array-Waters 996) detector and Luna - C18 (250×4.6 mm, 5 µm; Supelco Analytical, Bellefonte, PA, USA) column. Column temperature was set to 40 °C and autosampler temperature was 10 ± 5 °C. PDA detector scan interval was 200-600 nm.

The flow rate was 1 ml/min and the injection volume of 10 µL was used over a period of 50 minutes for separation. Standard calibration curves were prepared with caftaric acid, caffeic acid, chicoric acid, catechin, epicatechin, cyanidin 3-O-glucoside chloride, kaempferol 3- rutinoside. All of the standard solutions and the samples were filtered through a 0.45 µm membrane filter. 1 ml of each filtered sample was put into the vials and analyzed.

The mobile phase was distilled water with 0.1% (v/v) trifluoric acid (solvent A) and acetonitrile with 0.1% (v/v) trifluoric acid (solvent B). A linear gradient was used as in the followings: (1) 95% solvent A and 5% solvent B (at the beginning, time = 0); (2) 65% solvent A and 35% solvent B (at time = 45 min);(3) 25% solvent A and 75% solvent B (at time = 47 min).

The time to return initial conditions is 54 min. Chromatograms were recorded at 280, 330, 340 and 520 nm wavelengths. Based on the retention times and UV spectra, each characteristic phenolic identification was done. The standard curves presented in Appendix C were used for quantification of phenolic compounds.

2.3 Statistical Analyses

Each analysis was performed in triplicate and the results were reported as mean value ± standard deviation. The data were analyzed by Minitab Statistical Software (18th version, Minitab Ltd., UK) by using one way analysis of variance (ANOVA) at 0.05 significance level, and Tukey's New Multiple Range Test was applied to analyze the results of the experimental data. The Range Test was applied to exact values to identify the differences between TPC, TFC and TAC which is specifically evaluated by DPPH, CUPRAC and ABTS tests ($p<0.05$). The results were evaluated statistically and the analysis tables were presented at Appendix B.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content, Total Flavonoid Content and Total Antioxidant Capacity

3.1.1 Total Phenolic Content (TPC)

Significantly highest TPC values were obtained in SN samples and they were followed by TPC values in EP and CI samples in decreasing order. TPC values were significantly decreased during lozenge production from plant extract powders. The decrease in TPC amount from plant extract powder to lozenge form was determined to be the highest in SN, since TPC value decreased from 178.42±11.48 to 2.33±0.11 mg GAE/g DW. In lozenges, SNP, EPP and CIP phenolic amounts were found statistically to be same ($p>0.05$).

Table 3.1: Total phenolic content of samples

Samples	Total phenolic content (mg GAE/g DW)
SNE	$339.68\pm1.47^{Aa*}$
EPE	38.48 ± 8.48^{Ad}
CIE	2.62 ± 0.82^{Ae}
SNW	178.42 ± 11.48^{Bb}
EPW	60.77 ± 0.17^{Bc}
CIW	8.02 ± 0.12^{Be}
SNP	2.33 ± 0.11^{Ce}
EPP	1.23 ± 0.05^{Ce}
CIP	1.16 ± 0.02^{Ce}

*Data represent a mean ± SD (*n=3*); different letters within the same column shows significance difference ($p \leq 0.05$). Comparison within the same plant is expressed by uppercase letters, while comparison within all the samples is expressed by lowercase letters.

3.1.2 Total Flavonoid Content (TFC)

Table 3.2: Total flavonoid content of samples

Samples	Total flavonoid content (mg RE/g DW)
SNE	$840.54\pm13.46^{Aa*}$
EPE	112.00 ± 4.00^{Ad}
CIE	2.80 ± 1.26^{Ae}
SNW	527.02 ± 11.26^{Bb}
EPW	329.54 ± 8.04^{Bc}
CIW	3.78 ± 0.38^{ABe}
SNP	5.24 ± 0.23^{Ce}
EPP	1.93 ± 0.27^{Ce}
CIP	1.12 ± 0.27^{Be}

*Data represent a mean ± SD (*n=3*); different letters within the same column shows significance difference ($p \leq 0.05$). Comparison within the same plant is expressed by uppercase letters, while comparison within all the samples is expressed by lowercase letters.

Ethanol extract of SNE has higher TFC than the water extract of the same plant (SNW), but the other two plants showed the reverse results. Total flavonoid content in EP and CI water extracts showed higher results when compared to ethanol extracts. When the TFC values of samples were compared, the highest results were found in all extract forms of SN, then EP and CI. Significant decrease in TFC in pastille form was determined that the greatest loss was obtained for SN product. In lozenge samples, TFC of SNP (5.24±0.23e mg RE/g DW), EPP (1.93±0.27e mg RE/g DW) and CIP (1.12±0.27e mg RE/g DW) results were similar statistically in comparison within all the samples ($p<0.05$).

3.1.3 Total Antioxidant Capacity (TAC)

3.1.3.1 Total antioxidant capacity: DPPH (2,2-diphenyl-1-picrylhydrazyl) method

Table 3.3: Total antioxidant capacity: DPPH method

Samples	capacity (mg TEAC/g DW)
SNE	$43.48\pm6.85^{a*}$
EPE	8.89 ± 2.12^{b}
CIE	0.18 ± 0.05^{b}

*Data represent a mean ± SD (*n=3*); different letters within the same column shows significance difference ($p \leq 0.05$)

According to the data presented in Table 3.3, the antioxidant capacity of SNE (43.48±6.85[a] mg TEAC/g DW) was statistically different from the antioxidant capacities of EPE (8.89±2.12[b] mg TEAC/g DW) and CIE (0.18±0.05[b] mg TEAC/g DW**).** There was no significant difference between EPE and CIE capacities. Among the data collected from the literature, the result obtained for antioxidant capacity for SNE (43.48±6.85 mg TEAC/g DW) has the lowest value. This may be related with selected berry type, geographical and altitudinal differences and also part of the plant used in the analysis (Atkinson et al., 2002; Cejpek et al., 2009).

3.1.3.2 Total antioxidant capacity: Cupric Reducing Antioxidant Capacity (CUPRAC) method

Table 3.4 Total antioxidant capacity: CUPRAC method

Samples	Antioxidant capacity (mg TEAC/g DW)
SNE	901.62±24.53^{Aa*}
EPE	91.83±4.44^{Bd}
CIE	3.42±0.17^{Be}
SNW	472.08±18.10^{Bb}
EPW	242.01±50.50^{Ac}
CIW	16.31±0.68^{Ae}
SNP	4.08±0.05^{Ce}
EPP	1.65±0.07^{Ce}
CIP	1.16±0.06^{Ce}

*Data represent a mean ± SD (*n=3*); different letters within the same column shows significance difference ($p \leq 0.05$). Comparison within the same plant is expressed by uppercase letters, while comparison within all the samples is expressed by lowercase letters.

According to the CUPRAC analysis results, the highest antioxidant capacity was found in SNE (901.62±24.53[a] mg TEAC/g DW) and it was followed by SNW (472.08±18.10[b] mg TEAC/g DW). The water extracts of EP and CI were found higher when compared to their ethanol extracts. Statistically similar results were observed in the study for CIE and CIW; 3.42±0.17[e] and 16.31±0.68[e] mg TEAC/g DW respectively. The antioxidant capacity in SN decreased from 901.62±24.53 (SNE) to 472.08±18.10 (SNW) mg TEAC/g DW, when water solvent is used in extraction instead of ethanol.

3.1.3.3 Total antioxidant capacity: ABTS method

Table 3.5: Total antioxidant capacity: ABTS method

Samples	Antioxidant capacity (mg TEAC/g DW)
SNE	299.39±15.57^{Aa*}
EPE	77.40±0.62^{Ab}
CIE	14.70±0.51^{Ac}
SNW	302.44±5.31^{Aa}
EPW	74.72±8.29^{Ab}
CIW	8.93±1.18^{Bc}
SNP	0.82±0.04^{Bc}
EPP	0.75±0.02^{Bc}
CIP	0.72±0.02^{Cc}

* Data represent a mean ± SD (*n=3*); different letters within the same column shows significance difference ($p \leq 0.05$). Comparison within the same plant is expressed by uppercase letters, while comparison within all the samples is expressed by lowercase letters.

There was no significant difference in antioxidant capacities among different plant type lozenge products; SNP, EPP and CIP. In CI samples, extraction solvent type did not affect the antioxidant capacity, statistically (CIE: 14.70±0.51; CIW: 8.93±1.18 mg TEAC/g DW). Also, the loss in antioxidant capacity due to processing can be ignored since the result obtained for lozenge form of *C.islandica* (CIP: 0.72±0.02 mg TEAC/g DW) is statistically similar with the powder extracts.

According to ABTS antioxidant capacity measurement, SNW and SNE results were statistically estimated similar (SNE: 299.39±15.57; SNW: 302.44±5.31 mg TEAC/g DW). Also the antioxidant capacities of EP extracts (EPE: 77.40±0.62 and EPW: 74.72±8.29 mg TEAC/g DW) and CI extracts (CIE: 14.70±0.51 and CIW: 8.93±1.18 mg TEAC/g DW) are statistically related. The decrease in the antioxidant capacity in lozenge processing, is consistent with the ABTS assay results.

3.1.4 **Correlation among** TPC, TFC and TAC results

The correlations among all TPC, TFC and TAC results were analyzed by Pearson's correlation analysis method and computed as Pearson's correlation coefficients (R) that were given in Table 3.6. All relations were found as statistically significant ($p<0.01$). A significant positive relation was observed between TFC and TPC (R=0.977). This result shows that phenolic groups are dominating flavonoids in all extracts. CUPRAC method shows less relation with TFC (R=0.990) than TPC (R=0.996). ABTS method shows less close relationship among other methods. According to Table 3.7, for ethanol extracts of the plants, both DPPH and ABTS assays did not show significant correlations with TPC ($p>0.05$). However, TFC or CUPRAC can be used to predict TPC of all plant extracts due to their good correlation (1.00, $p<0.05$).

According to the values indicated in Table 3.7, ABTS and DPPH antioxidant capacity methods have also a significant relation (1.00, $p<0.05$). This argument is consistent with the correlation analysis results obtained from a previous study on elderberries. The correlation coefficients (R) of total polyphenols (R=0.98, $p<0.001$; R=0.98, $p<0.001$), total anthocyanins (R=0.93, $p<0.001$; R=0.90, $p<0.001$) and total flavonols (R=0.79, $p<0.01$; R=0.73, $p<0.05$) over DPPH and ABTS assays (consequently) were evaluated in elderberries.

Table 3.6: Pearson's correlation analysis for TPC, TFC, CUPRAC and ABTS methods.

Methods	TPC	TFC	CUPRAC
TFC	0.977	-	-
CUPRAC	0.996	0.990	-
ABTS	0.932	0.944	0.927

The regression variance analysis is statistically significant for all values ($p<0.01$).

Table 3.7: Pearson's correlation analysis for TPC, TFC, DPPH, CUPRAC and ABTS methods for ethanol extracts of the plants (SNE, EPE, CIE)

Methods	TPC	TFC	DPPH	CUPRAC
TFC	1.000*	-	-	-
DPPH	0.996	0.998*	-	-
CUPRAC	1.000*	1.000*	0.995	-
ABTS	0.993	0.996	1.000	0.993

*****The regression variance analysis is statistically significant for values ($p<0.05$).

3.2 Determination of Phenolic Compounds by HPLC-PDA

3.2.1 ***Cetraria islandica*** **L.**

Island moss extract samples were analyzed by HPLC at the highest absorbance 280 nm and the phenolic amount was given in Table 3.8. Any phenolic type was not identified in the analyses, but the most near spectrum was defined as term of 4-hydroxy benzoic acid.

CIE showed a peak in minute 3, where it can be defined as protocetraric acid according to HPLC chromatogram of the natural tallus of *Cetraria islandica* L., evaluated by Yoshimura *et al*., (1994). In CIW and CIP, unidentified substances were detected, thus the peaks could only be quantified as indicated in Table 3.8.

Table 3.8: *Cetraria islandica* L. phenolic amount

Sample	Phenolic amount (µg/g DW)
CIE	887
CIW	1404
CIP	16

3.2.2 *Echinacea purpurea* L.

EP samples were analyzed by HPLC at the highest absorbance, 330 nm. Caftaric acid and chicoric acid were the dominant phenolics in EPE and EPW, specifically chicoric acid was only identified in EPE and EPP.

Table 3.9: *Echinacea purpurea* L. ethanol extract phenolic compounds

Compound	Area	Retention time	Factor	Amount (µg/g DW)
Caftaric acid	180072	10.167	$1x10^7$	450
Caffeic acid	46406	13.482	$4x10^7$	30
Chicoric acid	345277	24.117	$4x10^7$	220

Table 3.10: *Echinacea purpurea* L. water extract phenolic compounds

Compound	Area	Retention time	Factor	Amount (µg/g DW)
Caftaric acid	17929	10.261	$1x10^7$	17.93
Chicoric acid	26271	24.186	$4x10^7$	6.57

Table 3.11: *Echinacea purpurea* L. lozenge form water extract phenolic compounds

Compound	Area	Retention time	Factor	Amount (µg/g DW)
Caftaric acid	1229476	10.305	$1x10^7$	3070
Caffeic acid	72487	13.623	$4x10^7$	50
Chicoric acid	2364838	24.192	$4x10^7$	1480

3.2.3 *Sambucus nigra* L.

SN samples were analyzed in the highest absorbance 280 nm.Most dominant phenolics in elderberry were identified as catechin, epicatechin, cyanidin 3-glucoside and kaempferol 3-rutinoside.

Table 3.12: *Sambucus nigra* L. ethanol extract phenolic compounds

Compound	Area	Retention time	Factor	Amount (µg/g DW)
Catechin	545203	10.934		380
Epicatechin	66186	13.974		470
Epicatechin	404354	14.761		2800
Cyanidin 3-glucoside	1655540	18.974		2760
Kaempferol 3- rutinoside	27840	19.889		150

Table 3.13: *Sambucus nigra* L. water extract phenolic compounds

Compound	Area	Retention time	Factor	Amount (µg/g DW)
Catechin	32695	11.389	$7x10^7$	4
Epicatechin	13787	15.188	$7x10^6$	10
Cyanidin 3-glucoside	62455	18.414	$3x10^7$	12

Table 3.14: *Sambucus nigra* L. lozenge form water extract phenolic compounds

Compound	Area	Retention time	Factor	Amount (µg/g DW)
Catechin	93581	10.923	$7x10^7$	670
Epicatechin	17472	12.968	$7x10^6$	1250
Epicatechin	57047	14.772	$7x10^6$	4070
Cyanidin 3-glucoside	79121	19.010	$3x10^7$	1840

4. CONCLUSION AND RECOMMENDATIONS

In this study, the bioactive compounds in the powder extracts of *Cetraria islandica* L., *Echinacea purpurea* L. and *Sambucus nigra* L. were investigated by spectrophotometric methods: TPC, TFC and TAC assays with two different solvent in extraction: water and 75% ethanol. All analyses were also applied to the lozenge formulations containing plant extract powders to investigate the loss of bioactive compounds in the final product. The correlation analysis was performed for comparing the efficiency and relationships of each spectrophotometric analysis method on identifying the bioactive compounds. HPLC analysis was conducted to identify and quantify the phenolic components in each sample.

The solvent efficiency of 75% ethanol extracts was found to be significantly higher than water extracts in all methods of TPC, TFC, DPPH and CUPRAC, however the solvent type used in the extraction did not demonstrate a significant change in ABTS antioxidant activity method ($p>0.05$). According to all antioxidant activity analysis methods, the lozenge processing were found to lead antioxidant loss.

All the plant powders showed a similar trend in lozenge applications, since the statistical results exhibited no significant difference ($p<0.05$) in SNP, EPP and CIP samples. Among the antioxidant activity analysis methods, the radical scavenging potentials of DPPH and ABTS were evaluated. ABTS method of antioxidant activity showed higher results than ABTS in ethanol solvent extracted samples. CUPRAC method was found to be most efficient among others, due to highest content of bioactive compounds. The correlation analysis showed that, the total phenolic and flavonoid contents exhibited a meaningful association with CUPRAC analysis method results ($p<0.05$).

In future studies, regarding the changes in the bioactive compounds in lozenge processing, different formulations can be studied. The present findings are the data that contribute a baseline for different studies on new lozenge formulations that deal with many diseases. All of the extracts can be applied together in a lozenge formulation in different ratios and the usage ratios can be determined with optimization studies with Response Surface Methodology (RSM). The phenolic content of different lozenge formulations with different amount of medicinal plants can be compared with spectrophotometric analysis methods and HPLC analysis method to investigate possible health promotion. For further studies, *in vitro* bioavailability of phenolic compounds in the late release soft lozenge with this invaluable medicinal plants *Cetraria islandica*

L., *Echinacea purpurea* L. and *Sambucus nigra* L. can be investigated.

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