Research article

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Improvement of Bioavailability of Sage and Mint by Ultrasonic Extraction

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ABSTRACT

Plant extracts are complex mixtures obtained from fruits, leaves, flowers, woods, resins and seeds of a fresh or dried plant by various methods. Because of the disadvantages of conventional extraction methods such as requirement of long periods and high amounts of chemicals, novel extraction methods such as ultrasound assisted systems have drawn higher attention in recent years. In this study, the ultrasound-assisted extraction (USE) conditions (temperature, time, ultrasonication power and solvent-solid ratio) were determined for the production of crude extracts from sage (*Salvia officinalis*) and mint (*Mentha piperita*). Bioavailability of the plant extracts were also compared to those obtained by classical hot-water extraction (HWE). USE parameters for the highest yield were 40 °C, 10 min, 400 W, as determined by preliminary experiments. Total phenolic contents of the mint and sage samples increased by ultrasound assisted liquid extraction method at the levels of 23.88 %and 14.97 %, respectively. The bioavailability of total phenolic contents in classic and ultrasound extraction of mint (*Mentha piperita*) extracts was 20.11 %, 32.45 % respectively. In conclusion, the results of the present study showed that ultrasound assisted extraction was more effective method for extraction of bioactive substances from sage and mint with shorter extraction time, increased bioactivity and bioavailability.

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Introduction

Turkey is one of the most important gene centers of medicinal and aromatic plants in the World, and is located in a very fertile region which hosts thousands of endemic plant species. Aromatic plants have been used as remedy in traditional medicine for ancient times, as well as in food preservation due to their antimicrobial activity and in cosmetic and pharmaceutical industry in recent years [1]. The first records of treatment of people with aromatic plants belong to Mesopotamian civilization in BC. [2]. According to the World Health Organization (WHO), 25% of the pharmaceutical drugs used today are

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produced from medicinal plants. On the other hand, Food and Agriculture Organization (FAO) states that 30% of the drugs sold worldwide contain compounds derived from plant materials [3]. This is due to the abundance of bioactive compounds such as vitamins (E and C), glutathione, enzymes and phenolic compounds in medicinal and aromatic plants [4]. Phenolic compounds which are major bioactive constituents of aromatic plants are very important due to their effects on the organoleptic properties and nutritional quality of foods such as color, taste, odor, natural colorant in foods, and have alternative use as natural antioxidant and the positive effects on health in recent years [6]. The most common crops cultivated and used in the industry are sage, anise, juniper tar, nettle, thyme, rosehip, lemon balm, chamomile, cinnamon, vanilla, lion paw and mercury [7].

Although conventional extraction techniques such as Soxhlet extraction have been used for a number of decades in order to obtain bioactive extracts from plants, they have numerous disadvantages such as being time consuming and requirement of large amounts of chemicals, which cause high energy consumption and environmental pollution [8]. Therefore novel extraction techniques such as ultrasound-assisted, microwave-assisted, supercritical and accelerated extraction systems, alternative to conventional solvent extraction from plant sources have been investigated by different researchers to overcome these disadvantages [9, 10].

Ultrasonic assisted extraction, also called sound waves-assisted liquid extraction, is considered as one of the most efficient extract recovery techniques. In this technique, extraction is performed in gas or liquid environment by the effect of cavitation which is formed on liquid-liquid or gas-liquid interfaces [11]. In this method, acoustic vibrations are applied to the sample with frequencies above 20 kHz. [12]. In the use of ultrasound in extracting, the application is in the form of mass transfer by mechanical disintegration

of the cell wall. By disintegrating the cell wall in this way, it becomes easier for the liquid extract inside the cell to exit the cell. It is faster than other extraction methods and it is an effective non-thermal alternative method since the cell wall is destroyed by the application of ultrasound [13, 14]. Ultrasonic assisted extraction is commonly applied to obtain valuable compounds from a variety of matrices mainly food and plant materials [15]. Extraction of bioactive components by ultrasound application is one of the methods that provide high level of hand in a short time, reducing heat and energy consumption, reducing solvent consumption and easy to apply [16]. In this study, it was aimed to compare the bioavailability of sage and mint extracts obtained by conventional and ultrasonic assisted methods.

Material and Methods

Materials

Fresh sage (*Salvia officinalis*) and peppermint (*Mentha piperita*) were obtained from Zeytinburnu Medicine and Aromatic Plant Garden in Istanbul, Turkey. Ethanol, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and Folin&Ciocalteu's reagent purchased from Sigma Aldrich Chemical Co. (USA). Ethanol, dialysis membrane tube and bile salts were obtained from Merck (Germany).

Sample preparation for extraction process

After removing impurities, the plant materials were dried using a vacuum dryer (Daihan WOV-30, Gangwon-do, South Korea) at 40 °C for 8 h. The vacuum was adjusted by a vacuum pump (EVP 2XZ-2C, Zhejiang, China) with 6 kPa ultimate pressure and 2 L/s pump seed. The dried samples were grinded and then stored in desiccator at room temperature until extraction process.

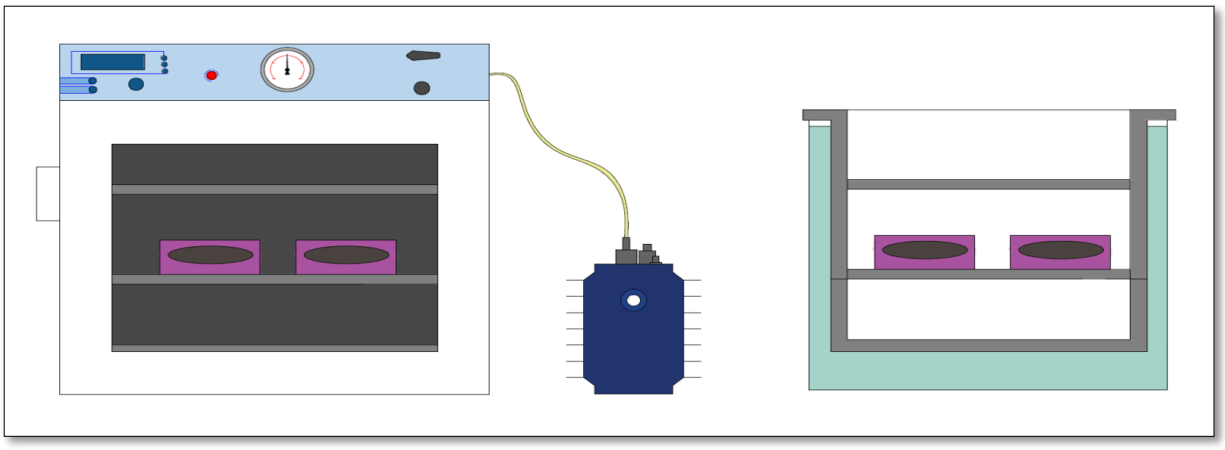

Fig 1 Vacuum drying system

Extraction Process

Classical hot water extraction (HWE)

For this purpose, 10g of the samples was incorporated with 100 mL of 80% ethanol (v/v) and mixed at 40 °C for 6 hours using a magnetic stirrer at 250 rpm. The ethanolic extracts were filtered and freeze-dried for the bioavailability assays.

Sonication-assisted liquid extraction (USE)

Ultrasound extraction process was performed using an ultrasonic processor (Hielscher UP400S, Germany) with 24 kHz frequency and 100µm amplitude values. The flow cell of the ultrasonic processor (Hielscher Flowcell D22-K) was combined with a 22 mm diameter probe (Hielscher sonotrode H22D) and the instant process volume was 15 mL. The samples were mixed with 80% ethanol (v/v) in a ratio of 1:10 (w/v) and then extracted at 40 °C for 10 min with ultrasound power of 400 W, which determined as optimum parameters with preliminary experiments to obtain maximum extraction yield. The temperature was kept at the constant value using a refrigerant during the process. After extraction, the extracts were filtered to remove impurities and freeze-dried to obtain crude powder for the bioavailability assays.

***In vitro* gastrointestinal digestion assay**

In order to simulate gastric and intestinal digestion of the extracts, the method described by McDougall et al. (2005) was performed with some modifications [17].

For stomach digestion; freeze-dried extracts were homogenously dissolved in 2.5 mL of water and completed to 20 mL of distilled water. Then 1.5 mL of pepsin solution (40 mg/ml) prepared with 0.1 M HCL was added to the mixture. The pH of the mixture was adjusted to 2.0 by adding 5 M HCl. The beaker was covered with parafilm and incubated for 2 h in a shaking incubator at 37ºC with stirring at 100 rpm. At the end of the incubation, 5 mL of PG fraction was separated and stored at -20ºC to use following analyses. For intestinal digestion, 5 mL of pancreatin (18 mg / mL), a mixture of bile (112.5 mg / mL bile salt) and 4.5 mL of $NaHCO_3$ (0.1 M) were added to the stomach digested beaker. The dialysis tube was completely immersed in the PG phase and the beaker was sealed with parafilm. It was incubated for 2 h in a shaking incubator with stirring at 100 rpm at 37ºC.

At the end of the period, the IN fraction in the dialysis tube was discharged into the falcon tube. The liquid outside the tube was discharged into a separate falcon tube as the OUT fraction. It was stored at -20ºC for the following analyses.

Bioavailability is determined by dividing the amount of substance present in the IN phase to the amount of substance present in the sample, as indicated in equation 2.1. This procedure is schematized in detail in Figure 2.

$$\%\ bioavailability\ = \frac{The\ amount\ of\ matter\ in\ IN\ phase}{The\ amount\ of\ the\ matter\ in\ the\ sample} \times 100 \qquad (2.1)$$

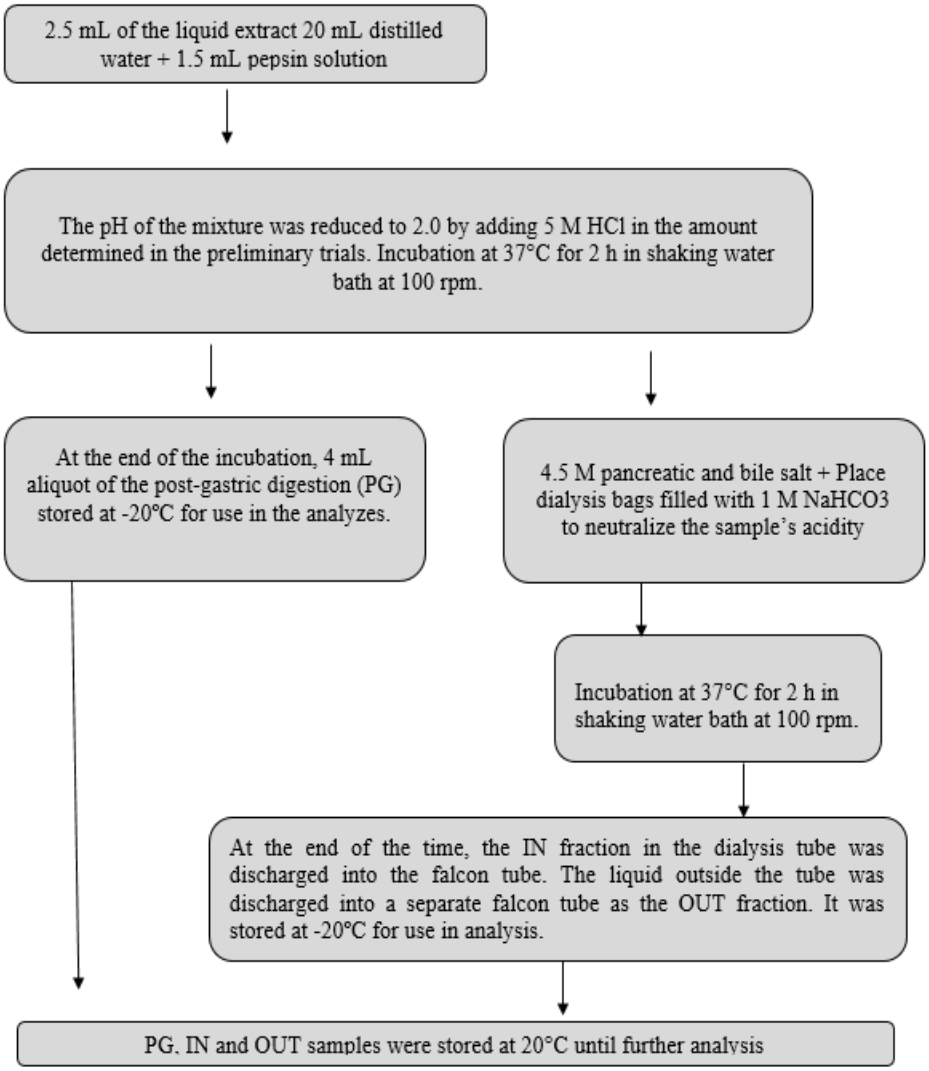

Fig 2 Flow chart of the in vitro gastrointestinal digestion method [31]

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of the samples was determined using Folin-Ciocalteu method as described by Singleton and Rossi (1965). Briefly, 0.5 ml of the ethanolic extract was mixed with 2.5 mL of 0.2 N Folin-Ciocalteau's reagent and after the incubation for 3 min, 2 mL of 7.5% Na_2CO_3 solution was added to the mixture. After keeping at the room temperature for 30 min, the absorbance of the samples was measured at 760 nm using a UV-vis spectrometer (Shimadzu, UV-1800, Japan). The results were

calculated using the following equation and were expressed as mg gallic acid equivalents in liters (mg GAE/L) [18].

$$TPC(mg\ GAE/L) = \frac{absorbance - 0.0791}{0.0103} \times dilution\ factor \quad (2.2)$$

Determination of total flavonoid content (TFC)

Determination of total flavonoid content (TFC) was performed according to the method described by Zhinsen et al. (1999). For this purpose, 1 mL of extract was mixed with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ solution (w/v). After 5 min, it was incorporated with 0.3 mL of 10% $AlCl_3$ solution (w/v) and mixed for 6 min. Following the addition of 2 mL of 1M NaOH, the volume was completed to 10 mL with distilled water. Then the absorbance values of the samples were measured at 510 nm with UV-VIS spectrophotometer (Shimadzu UV-1800, Japan). All the results were expressed as mg catechin equivalents (CAE)/100g of sample [19]. The formula used to calculate the total flavonoid content was given in Equation 2.3.

$$TFC\ \left(mg\frac{CAE}{L}\right) = [(151.6 \times absorbance) - 0.05454] \times dilution\ factor \quad (2.3)$$

Determination of antioxidant activity by DPPH radical scavenging assay

The antioxidant activity of the samples was determined according to the method described by Sanchez Moreno et al. (2002). For this purpose, 0.1 mL of the extract was mixed with 4.9 mL of 0.1 mM DPPH solution and the mixture was kept at 27 °C for 20 min. Then, the absorbance of the samples was measured at 517 nm using a UV-vis spectrometer (Shimadzu, UV-1800, Japan). The results were presented as mg Trolox equivalent (TEAC)/ 100 g of the sample [20].

Statistical analysis

Mean values and standard deviations of the data were evaluated using Excel software (Microsoft Office, 2017). Statistical analysis was performed by using a statistical software of SPSS 20.0 (SPSS, Inc., Chicago) with one way analysis of variance (ANOVA). Differences between the data were determined by Duncan's multiple comparison test with 95% confidence level.

Results and Discussion

Changes in bioactive components in extracts

Bioactive properties of sage and mint extracts are shown in Table 1. As seen in the table, ultrasonication process significantly increased the extraction yield of both sage and mint. Total phenolic contents (TPCs), total flavonoid contents (TFCs) and DPPH scavenging activities of the sage extracts were significantly higher than those of mint. Considering the effect of extraction technique on bioactive properties, ultrasonication enabled higher values than classical extraction, for instance, TPCs of mint and sage samples increased by 23.88 % and 14.97 %.by ultrasound assisted liquid extraction method, respectively.

Table 1 Bioactive properties and yields of sage and mint extracts

Sample	Extraction method	TPC (mg GAE/L)	TFC (mg CAE/L)	DPPH (mg TE/L)	Yield (%)
Sage	HWE	4066.24±4.01^{a}	356.53±0.92^{a}	66.41 ±0.96 ^{a}	3.36± 0.11^{a}
	USE	4491.14±2.11^{b}	378.45±1.83^{c}	101.6±4.34^{d}	5.28±0.23^{c}
Mint	HWE	198.35±3.67^{c}	65.67±1.23^{b}	72.09±1.04^{b}	3.82±0.19^{b}
	USE	245.73±5.45^{d}	87.35±1.56^{d}	83.67±1.62^{c}	5.79±0.13^{d}

*Different lowercase letters indicate the significance of the statistical difference between the data on the same row according to the ANOVA Duncan test ($p<0.05$)

Changes in post-digestive bioactive properties

Change in bioactive properties (TPCs, TFCs and DPPH scavenging activities) of sage and mint extracts obtained by classical or ultrasound assisted liquid extraction method after *in vitro* gastric and intestinal digestion (intestinal digestion, post-gastric (PG), IN (absorbed through the small intestine) and OUT (not absorbed from the small intestine)) are given in Figures 3, 4 and 5, respectively.

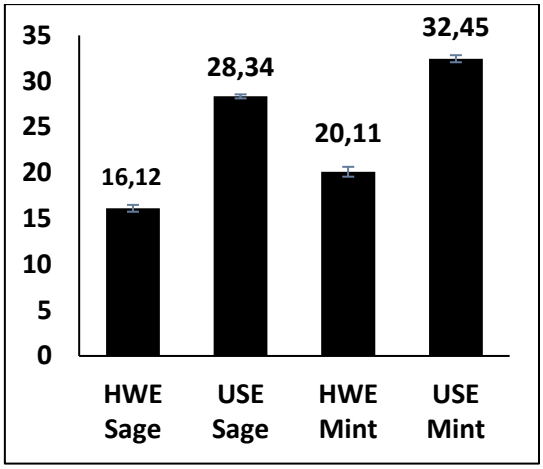

Figure 3 % Recovery of TPC

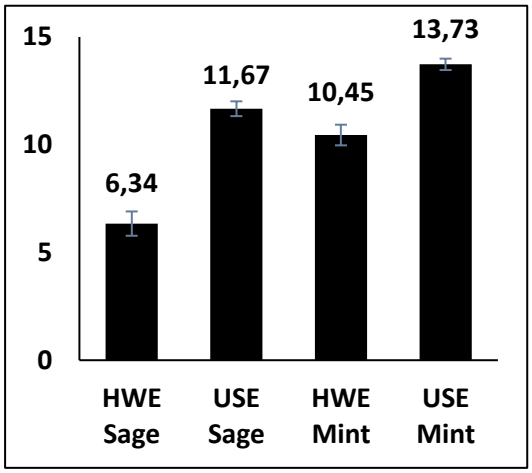

Figure 4 % Recovery of TFC

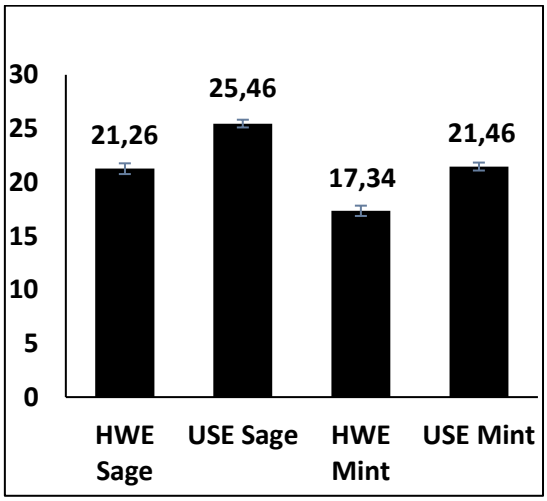

Fig 5 % Recovery of DPPH scavenging activity

Ultrasound was shown to improve the bioavailability of medicinal plants after gastrointestinal digestion. The bioavailability of TPCs in classic and ultrasound extraction

of sage extracts was 16.12% and 28.34% while it was 6.34% and 11.67% in the case of TFC, respectively. The bioavailability of DPPH scavenging activity was determined as 21.26 % and 25.46 % (Figure 6).

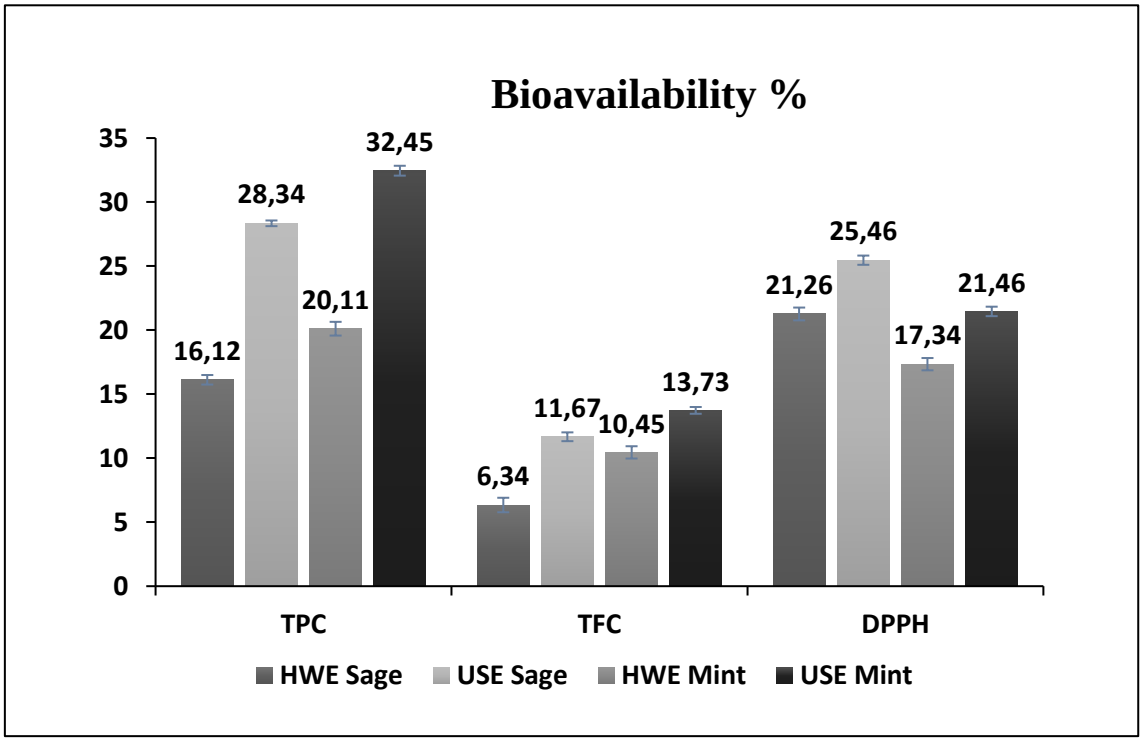

Fig 6 Recovery (%) of TPCs, TFCs and DPPH radical scavenging activities of sage and mint extracts after *in vitro* gastrointestinal digestion

The bioavailability of TPC of the mint extracts obtained by classical or ultrasound extraction was 20.11 % and 32.45 % while it was 10.45 % and 13.73 % for TFC while the bioavailability of the extract as measured by DPPH scavenging activity was determined as 17.34 % and 21.46 %, respectively.

Dahmoune et al. (2013) investigated effect of EU using solvents with different ethanol concentrations varying from 30% to 70% on bioactive properties of lemon peel and found that TPC values increased at higher ethanol ratios up to 62.93%. [21]. In another study, Wang et al. (2008) extracted the phenolic compounds from wheat bran by ultrasound-

assisted extraction technology at different processing conditions for extraction optimization. They found that extraction time was the most significant parameter for optimization and at the optimum extraction conditions (ethanol concentration, 64%; extraction temperature, 60 C; and extraction time, 25 min) TPC was 3.12 mg GAE/g of wheat bran [22].

Plant extracts with high antioxidant capacity, rich in phenolic acids, act as free radical terminators and reduce the effect of oxidative damage on dsDNA [23]. In one study, Skeva and Girousi investigated the antioxidant ability of *Camelia sinensis* (black and green tea) plant extracts *in vitro* by using a dsDNA biosensor to achieve oxidative damage on dsDNA. (Compared to Gallic acid, caffeic acid and trolox standard.)[23]. Putnik et al. (2018) used microwave assisted extraction (MAE) technique to obtain phenolic compounds from sage. They tested three different solvents (30% ethanol, 30% acetone and water), five different times (3, 5, 7, 9 and 10 min) and five different temperatures (30, 50, 60 and 80 °C) for extraction. The best result for the total polyphenols was obtained at 30% acetone as solvent and 80 °C for 10 minutes [24]. Bender et al., reported that *in vitro* oxygen radical absorbance capacity (ORAC) and antioxidant capacity (CAA) of peppermint extract were 1438 mmol trolox eq/g and s 27.9 mmol quercetin eq/g while Those values were 1351 mmol trolox eq/g and 35.3 mmol quercetin eq/g for sage, respectively [25]. Our results were in accordance with these findings. On the other hand, industrial microwaving of black pepper, sage and basil did not change their antioxidant properties [26].

Advantages of ultrasound assisted extraction are; inexpensive equipment, ease of use, environmentally friendly, maximization of the extract yield of the targeted component with minimum degradation and achieving high efficiency. Ultrasound-assisted extraction

accelerates mass transfer, thus providing more processing time and less solvent consumption compared to the classical extraction method [27, 28, 29] and increases penetration by breaking down cells [30].

Conclusion

In this study, separation of bioactive extracts from sage and mint was carried out by classical solvent extraction and ultrasound assisted extraction techniques. Although the classical extraction process was performed at higher temperatures than the ultrasound assisted extraction, TPC, TFC and DPPH scavenging ability of the extracts remained below the amount of phenolic material obtained by ultrasound assisted extraction.

When ultrasound assisted extraction method was examined, it was found that ultrasound assisted extraction was the most effective method for extracting of bioactive substances from sage and mint in a much shorter time compared to classical method. While increasing the amount of bioactive properties, it also increased the bioavailability of the extracts.

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