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A Comparative Study on the Phenolic Extraction of Total Hydro-Alcoholic Extract of *Satureja Hortensis L.* for Bioactive Properties

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ABSTRACT

In Türkiye, *S. hortensis* species are naturally found especially in the Thrace region. This plant, which has been used as a spice since ancient times and benefited from its medicinal properties, is the only annual member of the *Lamiaceae* family. Natural products derived from the *Lamiaceae* family are predominantly characterized by polyphenols and flavonoids, responsible for antioxidant properties among others. In the research, extracts of plant samples were obtained with different concentrations of ethanol (EtOH) and three different extraction methods. The plant material (*Satureja hortensis L.*) was sourced from the province of Kırklareli. Three different extraction methods, namely traditional, ultrasound, and microwave, were employed along with three different ethanol solvent ratios (40%, 50%, and 60%, v:v) for phenolic compound extraction. The total phenolic content and total antioxidant activity (DPPH) were compared for all samples. Among the samples, the highest total phenolic content (TPC) value was determined for extracts obtained with ethanol (60%, v:v) using traditional extraction (TE), ultrasound extraction (15 minutes) (US-15), and microwave extraction (2.5 minutes) (MW-2.5), respectively. Other extracts obtained with ultrasound extraction (30 minutes) (US-30) and microwave extraction (5 minutes) (MW-5) resulted in the highest TPC value when applied with ethanol (50%, v:v). Some Satureja species known to have therapeutic properties for diabetes also possess antioxidant characteristics. However, there is no research available for *S. hortensis L.* This study, by presenting the characteristics for the first time, investigated the potential anti-hyperglycemic and anti-obesity effects of *S. hortensis L.* extracts. For this purpose, the enzyme activities of amylase and lipase were determined for all samples. Thus, the data obtained for *S. hortensis L.* holds significance for future research and industrial applications.

Biyoaktif Özellikler Açısından *Satureja Hortensis* L.'nin Toplam Hidro-Alkolik Ekstraktının Fenolik Ekstraksiyonu Üzerine Karşılaştırmalı Bir Çalışma

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ÖZ

Türkiye'de *S. hortensis* türleri özellikle Trakya bölgesinde doğal olarak bulunmaktadır. Antik çağlardan beri baharat olarak kullanılan ve tıbbi özelliklerinden faydalanılan bu bitki, *Lamiaceae* familyasının tek yıllık üyesidir. *Lamiaceae* familyasından elde edilen doğal ürünlerde, diğerlerinin

Anahtar Kelimeler:
Biyoaktivite
Ekstraksiyon
Mikrodalga
S. hortensis L.
Ultrason

yanı sıra antioksidan özelliklerinden sorumlu olan polifenoller ve flavonoidler hakimdir. Araştırmada bitki örneklerinin farklı derişimlerdeki etanol (EtOH) ve üç farklı ekstraksiyon yöntemi ile ekstraktları elde edilmiştir. Bitki materyali (*Satureja hortensis L.)* Kırklareli ilinden temin edilmiştir. Geleneksel, ultrason ve mikrodalga olmak üzere üç farklı ekstraksiyon yöntemi ve üç farklı etanol çözücüsü (%40, %50 ve %60, hacim:hacim) ile fenolik madde ekstraksiyonu gerçekleştirilmiştir. Tüm örneklerin toplam fenolik madde miktarı ve toplam antioksidan aktivitesi (DPPH) karşılaştırılmıştır. Örnekler arasında geleneksel ekstraksiyon (TE), ultrasonik ekstraksiyon (15 dakika) (US-15) ve mikrodalga ekstraksiyon (2.5 dakika) (MW-2.5) ve etanol (%60, hacim:hacim) ile elde edilen ekstreler için en yüksek toplam fenolik madde miktarı (TPC) değeri belirlendi. Ultrasonik ekstraksiyon (30 dakika) (US-30) ve mikrodalga ekstraksiyon (5 dakika) (MW-5) ile elde edilen diğer örnekler için ise, etanol (50%, hacim:hacim) ile yapılan uygulama sonucunda en yüksek TPC değeri elde edildi. Diyabet için tedavi edici özelliğe sahip olduğu bilinen bazı Satureja türleri, aynı zamanda antioksidan özelliklere de sahiptir. Ancak, *S. hortensis L.* için herhangi bir araştırma bulunmamaktadır. Bu çalışma ile ilk kez ortaya koyulan özellikler ile, *S. hortensis L.* ekstrelerinin olası anti-hiperglisemik ve anti-obezite etkisi araştırılmıştır. Bu amaçla, tüm örnekler için amilaz ve lipaz enzim aktiviteleri belirlenmiştir. Böylece *S. hortensis L.* için elde edilen veriler gelecek araştırmalar ve endüstri uygulamaları için önem taşımaktadır.

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Introduction

Satureja hortensis L. is an annual herbaceous plant of *Lamiaceae Lindl. Family. S. hortensis L.*, is related to thyme and rosemary. It is also known by as "summer savory" and native to North Africa, Middle East, and Central Asia, Southern and Southeastern Europe (Bimbiraitė-Survilienė et al., 2021). The leaves, stems, and flowers of *S. hortensis* are frequently used in herbal tea and spice mixtures as the add aroma and flavour (Güllüce et al., 2003). Satureja species have different contents of many phenolic and volatile components such as carvacrol, γ-terpinene, thymol, p-cymene etc. (Pirbaloutia et al., 2014). As summer savory (*Satureja hortensis L.)* has anti-inflammatory, antioxidant, antifungal and antimicrobial properties because of containes volatile oils (carvacrol and thymol) (Ejaz et al., 2023). Besides it has also shown antispasmodic, antidiarrheal, antioxidant, sedative, and antimicrobial properties (Güllüce et al., 2003). It also possesses stimulating, carminative, antipyretic, and aphrodisiac properties (Mohammed et al., 2019).

The increasing interest in this plant is due to its chemical composition, which provides significant biological activity. Obesity, dyslipidemia, glycemic index imbalance, glucose intolerance, or hypertension are early signs of potential development of chronic diseases such as type 2 diabetes. Additionally, oxidative stress, an endogenous toxin, is a significant determinant of type 2 diabetes complications. Inhibiting these enzymes is an important

strategy for controlling blood sugar levels. α-amylase is responsible for the breakdown of long-chain carbohydrates. The inhibitory activity of extracts against α-Amylase functions to impede the breakdown of starch and oligosaccharides, thereby diminishing glucose absorption and subsequently mitigating the rise in postprandial glucose levels. Pancreatic lipase, identified as a pivotal enzyme in lipid absorption within the digestive system, catalyses the hydrolysis of triglycerides into glycerol and free fatty acids. Consequently, inhibiting pancreatic lipase activity is linked with the prevention of obesity-related disorders. Notably, phenolics, bioactive compounds abundant in plants, have been shown to inhibit both α-amylase and α-lipase enzymes. Hence, the simultaneous provision of antioxidants and α-amylase and lipase inhibitors through diet is a potential and feasible method for managing type 2 diabetes and obesity. Due to its natural source potential, we evaluated the obesity and antidiabetic potential of this plant traditionally used in herbal medicine in our study. In this study, all extracts exhibit inhibitory activity against amylase and lipase enzymes. Depending on the extraction method and solvent ratio, the percentage of extract inhibitor values ranges between 8.55% to 50.77% for amylase and 17.41% to 36.56% for lipase at a concentration of 0.4 mg mL^{-1}. In a previous study, the known *Moringa oleifera* extract's effects on diabetes and obesity reported lipase and amylase activities' 50% inhibition concentration values as 1.0877 mg mL^{-1} and 0.1802 mg mL^{-1}, respectively (Ogundipe et al., 2022).

The use of advanced extraction techniques such as organic solvent extraction, ultrasound-assisted extraction, and microwave-assisted extraction is crucial for investigating the impact of biologically active compounds. The chemical profile of these compounds can vary significantly depending on factors such as the Satureja species, geographical origin, and prevailing climatic conditions. This study primarily aims to investigate the quantity and effects of bioactive components in *Satureja hortensis L*. (summer savory) extracts. Therefore, the study will evaluate the properties of *S. hortensis L.* cultivated in Kırklareli using a series of in vitro analyses, including the determination of total phenolic compounds, radical scavenging activity through DPPH and ABTS assays, reducing power, and the inhibitory effects on lipase and α-amylase activities.

Material and Methods

Plant Material and Reagents

Satureja hortensis L. (summer savory) were gathered from the wild flora in Devecatagi, Kirklareli, located in the western region of Türkiye (41.7350° N, 27.2253° E), during the 2022 growing season. The plants were harvested before the blooming period in August at the

point when the volatile oil content reached its peak percentage in relation to the volatile compounds under investigation. Following this, the samples underwent drying in shaded conditions at an ambient temperature of approximately 30 °C and relative humidity of approximately 40-60 % for 48 hours. The dried material was subjected to grinding utilizing an analytical laboratory mill (A 11 basic Analytical Mill; IKA Werke, Staufen, Germany).

All standard chemicals used for LC-MS analysis were procured from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany). The reagents employed for assessing antioxidant activity, total phenolic content, and enzyme activities were sourced from Sigma Aldrich (St. Louis, MO, USA), Merck Co. (Darmstadt, Germany), and Fluka Chemical (USA), respectively.

Extraction Procedures

Extraction using organic solvents

Traditional extraction of phenolic compounds was performed according to the traditional extraction method (Şahin, 2019). 1 g of dried and powdered samples were weighed by precision scales (Weightlab WSA-224) at room temperature and transferred to a volumetric glass balloon with a volume of 100 mL. Three different concentrations of ethanol solution (40:60, 50:50 and 60:40, v:v, in water) were prepared as a solvent. It was completed to the volume line by adding ethanol solution to it. The balloon was capped and mixed several times by inverting the balloon to allow the solvent to contact the material. It was kept at room temperature (26 ^{o}C) and in the dark for 72 hours. At the end of 72 hours, the samples were filtered by paper filter and then PVDF membrane filter (0.45 μm) into amber-coloured flasks. Then the samples were stored frozen at -18^{o}C until further analysis. Extractions were performed in two replicates and three parallels.

Ultrasound assisted extraction procedure

Ultrasound assisted extraction of phenolic compounds was performed according to the method applied by Wang et al. (2008). An ultrasonic water bath (Advantage Lab AL04-12-230, 800W, 38 kHz, 12.75 L, Belgium) was used for extraction. The treatment conditions were determined according to the literature (Mohammed et al., 2019; Popovici et al., 2019; Şahin, 2019; Chambre et al., 2020; Shanaida, 2021). Three different ethanol solutions prepared at three different concentrations as solvent (40:60, 50:50 and 60:40, v:v, in water) and two different extraction times (15 min and 30 min) were determined as the treatments. 1 g of the ground and powdered samples were weighed into a 100 mL volumetric balloon and

ethanol solution was added up to the line. The closed and mixed balloon was placed in the ultrasonic bath. The Temperature of the ultrasonic bath was adjusted to 30 ^{o}C using the adjustment button on it and the extraction process was carried out at the specified times. At the end of the time, the samples were cooled to room temperature and filtered using paper filter and later 0.45 μm pore size PVDF membrane filter into amber coloured flasks. The filtrate was taken into sealed plastic sample containers and stored in a deep freezer at -18 ^{o}C. Extraction was repeated three times for each experiment.

Microwave assisted extraction procedure

Microwave extraction was carried out using a domestic microwave oven (Altus ALMD-17BY 20L, Arçelik, Istanbul, Türkiye) with a maximum power output of 850 W. Plant samples (1 g) were combined with identical solvents (100 mL) as those used in the standard procedure in a volumetric flask. Microwave irradiation followed the protocols outlined by Pan et al. (2003) and Şahin (2019) for varying durations (2.5 min and 5 min), ensuring avoidance of super-boiling. The samples were filtered using a paper filter and later 0.45 μm pore size PVDF membrane filter into amber coloured flasks, then the filtrate was stored in a deep freezer at -18 ^{o}C. Extraction was repeated three times for each experiment.

Determination of Analyses

Total phenolic compounds content

The total phenolic compounds content (TPC) evaluation was realized using Folin-Ciocalteu method as described in the literature (Popovici et al., 2019; Ergün, 2022). The samples were diluted to a predetermined concentration and left to incubation for 5 minutes at room temperature. Following the addition of 2 mL of sodium carbonate, the samples were further incubated for 60 minutes at room temperature, after which the absorbance was measured at 760 nm using a UV-VIS spectrophotometer (UV-2550 Shimadzu, Kyoto, Japan). The calibration curve (Figure 1) was obtained using gallic acid (GA) (Sigma-Aldrich, St Louis, MO, USA). The results were expressed in mg GAE g^{-1} dry material (DM).

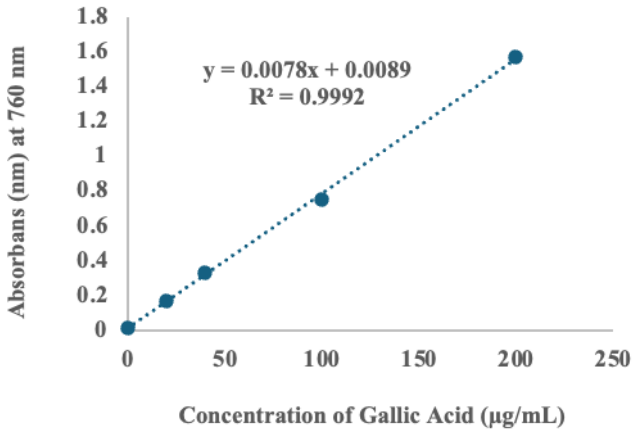

Figure 1. Calibration curve of gallic acid

Radical scavenging assay (DPPH assay)

The determination of the extract's free radical scavenging capacity (DPPH) was applied according to the method of Sanchez-Moreno et al. (1998). The extracts dissolved in methanol were prepared at various concentrations (50-1500 µg mL^{-1}). After incubating at room temperature, the absorbance of the samples at 517 nm was read against an ethanol blank. All experiments were performed in triplicate. Free radical inhibition was calculated as a percentage (I%) of DPPH :

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100 \quad (1)$$

IC50 (50% inhibition) values for the extracts were calculated.

ABTS radical scavenging capacity assay

ABTS•+ (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity of extracts was determined spectrophotometrically based on the method of Askin and Atik (2016). A standard curve was obtained using Trolox analysis, and the results were expressed as Trolox equivalent antioxidant capacity (TEAC) in mM Trolox mL^{-1}.

Reducing power assay

The reducing power of the extracts (RP) was determined according to Oyaizu (1986). The mixture absorbance was measured at 700 nm against the blank (ethanol). The inhibitory concentration (IC50), defined as the extract concentration producing 0.5 absorbance units at λ700nm, was used to describe the reducing capacity (Oliveira et al., 2009).

Enzymatic Inhibitions

The percentage of α-amylase (EC 3.2.1.1) and lipase inhibition was calculated with the given formula:

$$\%\text{Inhibition} = [(A_{blank} - A_{sample})/A_{blank}] \times 100 \quad (2)$$

where A_{blank} is the absorbance of the control (blank, without extract),
and A_{sample} is the absorbance in the presence of the extracts.
The plant concentration with different levels were evaluated. The best concentration had the highest inhibitory was determined for 400 μg dried plant extract mL^{-1}.

α-Amylase inhibition assay

The α-amylase inhibitory activity of the extracts was assessed following the protocol outlined by Uysal et al. (2017). Enzyme activities were measured in the presence of the extracts at a concentration of 400 μg DW mL^{-1}, and absorbance readings were taken at 630 nm. Blanks were prepared for each sample by substituting buffer for enzyme.

Lipase inhibition assay

The lipase inhibition assay followed the procedure outlined by De Camargo et al. (2016), utilizing p-NPB as the substrate. Blanks were prepared for each sample by substituting enzyme with buffer. Enzyme activities were measured in the presence of the extracts at a concentration of 400 μg DW mL^{-1}, with absorbance readings taken at 412 nm.

Statistical Analysis

Every experiment was conducted in triplicate, and standard deviations were computed accordingly. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Duncan test, employing the statistical software JMP 19.0 (SAS Institute Inc., Cary, NC). Significance was determined at the level of $P<0.05$.

Results and Discussions

The extraction techniques represent the initial crucial phase in acquiring bioactive compounds from botanical sources. Typically, the extraction of phenolic compounds from plant matrices is accomplished through either traditional or innovative methods employing various solvents due to their intricate nature and interactions with other bioactive compounds within the plant matrices (Chew et al., 2011; Alara et al., 2020).

Long time-consuming is the most important disadvantage of the traditional solvent extraction method. Microwave and ultrasound extractions are alternative extraction methods to solve time-consuming (Proestos and Komaitis, 2008; Ince et al., 2013). It is important to evaluate different methods for the desired product to achieve optimal efficiency, quality, and cost in extraction (Alara et al., 2020).

Table 1. Total phenolic contents (TPC) and total flavonoid contents (TF) of *Satureja hortensis L.* extracts obtained using different extraction methods with different solution ratios.

Extraction Method	Extraction Time	TPC (µg GA mg DW^{-1})	TF (µg mg DW^{-1})
Traditional (%40 EtOH)	72h	235.15 ±2.18^{f}	14.32±1.25^{cd}
Traditional (%50 EtOH)	72h	255.88±5.00^{e}	15.70±1.26^{bc}
Traditional (%60 EtOH)	72h	270.15±5.10^{d}	19.63±1.36^{a}
US (%40 EtOH)	15 min	93.38±0.20^{l}	08.11±0.90^{e}
US (%50 EtOH)	15 min	131.71±0.21^{j}	08.61±0.92^{e}
US (%60 EtOH)	15 min	144.73±0.94^{i}	08.53±1.08^{e}
US (%40 EtOH)	30 min	149.10±1.77^{i}	08.40±0.62^{e}
US (%50 EtOH)	30 min	173.06±0.52^{h}	09.12±1.02^{e}
US (%60 EtOH)	30 min	149.10±3.43^{i}	09.18±1.21^{e}
MW (%40 EtOH)	2.5 min	120.04±8.96^{k}	08.78±1.03^{e}
MW (%50 EtOH)	2.5 min	213.27±5.10^{g}	09.94±1.16^{e}
MW (%60 EtOH)	2.5 min	271.81±2.60^{d}	10.23±1.30^{e}
MW (%40 EtOH)	5 min	310.77±2.60^{c}	12.59±1.48^{d}
MW (%50 EtOH)	5 min	447.33±5.83^{a}	16.97±1.34^{b}
MW (%60 EtOH)	5 min	390.88±8.12^{b}	17.32±1.36^{b}

Note: Means with different letters differ significantly at P= 0.05 (a-l: in the same column). All data is three replicates of mean ± SD

Based on the statistical analysis, it is evident that a 5-minute extraction duration sufficed for the complete release of phenolic compounds. This indicates that all accessible phenolics efficiently permeated into the solvent during microwave extraction, resulting in their rapid extraction within a short timeframe. Microwaves engage with polar molecules in the extraction medium, thereby increasing the internal pressure exerted on solid materials. This interaction leads to enhanced extraction efficiency. As a result, microwave-assisted extraction enhances extraction effectiveness (Ince et al., 2013). The decrease in extraction duration is

due to microwaves' heating mechanism, which raises the internal pressure of solid materials. This increase in pressure aids in extraction, enabling phenolic compounds to be extracted in shorter periods compared to traditional methods (Bayramoglu et al., 2008). The shortened extraction time under microwave conditions likely reduced the degradation of phenolic compounds compared to traditional extraction techniques.

In this study, ethanol and water were selected as the extraction solvents due to their safer handling characteristics when compared to alternative organic solvents such as methanol and acetone. Furthermore, their compatibility with human consumption played a significant role in their selection. As described in Table 1, ethanol concentration showed a significant effect ($P<0.05$) on TPC, except MW-5 samples and US-30 samples.

In ultrasound extraction, the duration of extraction significantly influenced the TPC of the extracts. Extraction periods of 15 and 30 minutes exhibited statistically significant differences regarding TPC (Table 1). Based on statistical analysis, the optimal conditions for ultrasound extraction of *Satureja hortensis L.* were determined to be 50% ethanol extraction for 30 minutes. This suggests that hydrogen bonds formed between solvents and phenolic compounds contribute to the enhanced extractability of phenolic acids. Similar trends in the relationship between total phenolic content (TPC) and extraction time have been observed in previous studies. Additionally, according to the literature, the lower TPC observed in extracts obtained at higher ultrasound power levels may be attributed to the degradation of certain phenolic compounds (Farahmandfar et al., 2020; Askin, 2021).

The increased solvent concentration has led to a higher yield of phenolic extract in MW extraction for 2.5 min. There was no significant difference between the TPCs obtained by microwave and traditional extractions by 60% ethyl alcohol ($P>0.05$). Nevertheless, the total phenolic content (TPC) of extracts obtained for 5 minutes exceeded that of the extract obtained at 2.5 minutes. When comparing the TPC of a microwave-extracted sample obtained over 5 minutes to that of conventionally extracted ones, the microwave extraction also yielded a higher TPC within a shorter duration. As mentioned, the TPC content of samples treated with microwave extraction for 5 minutes and ultrasound extraction for 30 minutes increased with the use of 50% ethanol. Additionally, ethanol concentration did not cause significant differences in TF and antioxidant properties ($p<0.05$). For MW-5 and US-30 extraction the samples, TPC values were increased as the ethanol concentration was increased up to 50%. The TPC values were significantly ($P<0.05$) decreased with 60% ethanol concentration for the same samples.

TPC experimental results are consistent with previous studies reporting different solvent concentration from plant samples (Nawaz et al., 2006; Turkmen et al., 2006; Kim et al., 2007; Yang and Zhang, 2008; Chew et al., 2011). However, the best ethanol concentration determined for TF is completely different from TPC. The TF values were found to be influenced only by concentration for the traditional method and MW-5 ($P<0.05$), while concentration did not significantly affect the TF value in other sample groups ($P>0.05$). Galvan d'Alessandro et al. (2012) similarly attributed this to the fact that ethanol increases mass transfer and, accordingly, more phenolic substances are extracted. While soluble phenolic substances are generally found in cell vacuoles, the presence of flavonoids, lignin, and insoluble polyphenols bound to proteins and polysaccharides in the cell membrane has shown that water and low concentration of ethanol can reach the cells, but high concentrations of ethanol cause protein denaturation and prevent phenolic substance extraction. In the other literatures, 50% ethanol concentration was defined as the best value for TF extraction from plants (Yagcioglu, 2015; Xie et al., 2016). It has been determined that ultrasound-assisted extraction causes more degradation of flavonoids than other methods. Yağcıoğlu (2015), who reached a similar conclusion, stated that myricetin, quercetin, camperol, and rhamnetin flavonoids are the most degraded flavonoids, depending on the amount of hydroxyl groups they contain.

Following the principle of "like dissolves like," solvents will solely extract compounds that share similar polarity with them (Spigno et al., 2007; Zhang, 2007; Yang and Zhang, 2008; Alara et al., 2020). Briefly, the phenolic compounds extracted from S. hortensis would have the same polarity as the extraction solvent. Besides, another factor affected by solvent concentration is the dielectric constant. Therefore, in the literature, it is stated that by adding a solvent with lower polarity such as ethanol into a polar solvent such as water to reduce the dielectric constant, medium polarity phenolic substances can be effectively extracted. For example, by 60% ethanol extraction, while polar components such as glycosides can be effectively extracted at higher concentrations. It is stated that good results cannot be obtained due to the decrease in resolution (Alara et al., 2020).

The antioxidant activities of the samples were assessed through three different methods; radical scavenging DPPH (1,1-diphenyl-2-picrylhydrazyl) free assay, $ABTS^{\bullet+}$ (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity, and the reducing power of the extracts. Ethanol concentration notably impacted the reducing power (RP) value for all samples ($P<0.05$). The in vitro antioxidant activities were evaluated using DPPH as a free radical, measuring the extracts' capacity to donate H or e- (Chambre et al., 2020). The results

indicated inhibitions ranging from 32% to 57% for the samples analysed, signifying significant antioxidant activity. Ethanol concentration exhibited a discernible influence on the antioxidant capacities of the extracts. While higher inhibition was observed for samples extracted via microwave (MW) for 5 minutes, no significant difference was found between extracts obtained through ultrasound (US) and traditional methods. Notably, ethanol concentration induced significant changes in DPPH and $ABTS^{\bullet+}$ values, particularly evident in traditional extraction. However, prolonged extraction times in MW and US extractions did not yield significant changes. Increasing the ethanol concentration in US-15 extracted samples resulted in a significant ($P<0.05$) decrease in $ABTS^{\bullet+}$ but had no significant ($P<0.05$) effect on DPPH, potentially due to the extraction of phenolic compounds with differing molecular weights (Paixão et al., 2007; Choi et al., 2014). Earlier research indicates that the DPPH assay demonstrates a higher reactivity towards low molecular weight phenolic compounds, suggesting that 60% ethanol may be more effective in extracting low molecular weight phenolic compounds with potent antioxidant properties from plants when compared to 40% ethanol.

Table 2. Antioxidant activity values in different methods of *Satureja hortensis L.* extracts obtained using different extraction methods with different solution ratios.

Extraction Method	Extraction Time	DPPH IC50 (µg DW mL^{-1})	$ABTS^{\bullet+}$ IC50 (µg DW mL^{-1})	RP IC50 (µg DW mL^{-1})
Traditional (40% EtOH)	72h	57.32 ± 3.65^{a}	27.50 ± 1.17^{ab}	423.11 ± 11.44^{b}
Traditional (50% EtOH)	72h	53.31 ± 2.34^{abc}	26.10 ± 1.20^{b}	270.27 ± 6.34^{f}
Traditional (60% EtOH)	72h	49.69 ± 2.13^{cd}	19.23 ± 0.91^{c}	122.54 ± 3.90^{j}
US (40% EtOH)	15 min.	55.45 ± 1.99^{bc}	30.11 ± 1.91^{a}	496.76 ± 13.89^{a}
US (50% EtOH)	15 min.	51.78 ± 2.77^{bcd}	28.27 ± 1.76^{d}	389.77 ± 13.62^{c}
US (60% EtOH)	15 min.	51.33 ± 2.76^{bcd}	26.78 ± 1.68^{b}	322.45 ± 13.56^{e}
US (40% EtOH)	30 min.	55.77 ± 3.23^{bc}	28.44 ± 2.02^{ab}	487.22 ± 14.09^{a}
US (50% EtOH)	30 min.	50.65 ± 2.90^{bcd}	27.55 ± 1.17^{ab}	367.55 ± 12.94^{d}
US (60% EtOH)	30 min.	50.58 ± 2.29^{cd}	26.03 ± 2.30^{b}	245.11 ± 11.46^{g}
MW (40% EtOH)	2.5 min.	47.72 ± 2.08^{d}	27.22 ± 0.89^{b}	367.29 ± 12.42^{d}
MW (50% EtOH)	2.5 min.	34.23 ± 1.90^{e}	10.33 ± 1.20^{d}	197.89 ± 10.69^{h}
MW (60% EtOH)	2.5 min.	34.29 ± 2.51^{e}	09.27 ± 0.69^{d}	119.55 ± 11.97^{j}
MW (40% EtOH)	5 min.	35.65 ± 2.20^{e}	11.14 ± 1.14^{d}	273.45 ± 11.76^{f}
MW (50% EtOH)	5 min.	33.93 ± 1.25^{e}	09.11 ± 0.40^{d}	168.45 ± 9.94^{i}
MW (60% EtOH)	5 min.	32.10 ± 1.37^{e}	08.55 ± 1.04^{d}	59.78 ± 5.64^{k}

Note: Means with different letters in the same column differ significantly at P= 0.05. All data is three replicates of mean ± SD

The results obtained from statistical evaluation between total phenolic content and antioxidant activity. Strong correlations were observed between TPC and IC50 values of DPPH, ABTS•+, and RP, as well as between TPC and total flavonoid content (Table 3). The phenolic hydroxyl group in *Satureja hortensis L.* serves as an effective hydrogen donor, enabling reaction with reactive oxygen species and preventing the generation of newer radical species. Despite potential variations in the impact of *S. hortensis L.* extract on cell survival, its antioxidant activity, linked to the polyphenols within the extract, was demonstrated. Existing literature supports a direct relationship between the content of polyphenolic compounds and the antioxidant activity observed in most plant extracts. These associations suggest that the robust antioxidant capacity of extracts is closely tied to the presence of both phenolic and flavonoid content (Fathi et al., 2013; Choi et al., 2014; Akyuz, 2019; Bimbiraitė-Survilienė et al., 2021).

Comparation ultrasonic method and traditional extraction, it was noted that processing time was reduced without significantly altering the total phenolic content of the extracts. On the other hand, microwave extraction is not only minimize the processing time but also notably increase the total phenolic content compared to traditional extraction methods.

Table 3. Correlation coefficients between colour characteristics for different treatment groups

	TPC	**TF**	**ABTS**	**DPPH**	**RP IC50**
TPC	1.000				
TF	0.800**	1.000			
ABTS	-0.810**	-0.480	1.000		
DPPH	-0.710**	-0.300	0.960**	1.000	
RP IC50	-0.730**	-0.630**	0.820**	0.770**	1.000

*: $p<0.05$; **: $p<0.01$

Obesity, dyslipidemia, glycemic index imbalance, glucose intolerance, or hypertension are early indicators of potential chronic diseases like type 2 diabetes. Moreover, oxidative stress, considered an endogenous toxin, is believed to play a pivotal role in type 2 diabetes complications (Knekt et al., 2002). Inhibiting these enzymes represents a crucial strategy for regulating blood sugar levels. α-Amylase is responsible for breaking down long-chain carbohydrates. The inhibitory activity of α-amylase by the extracts delays the hydrolysis of starch and oligosaccharides, thereby reducing glucose absorption and preventing the subsequent rise in postprandial glucose levels (Gao et al., 2000). It's well-established that pancreatic lipase is the primary enzyme involved in lipid absorption in the digestive tract,

breaking down triglycerides into glycerol and free fatty acids. Consequently, inhibiting pancreatic lipase is linked with preventing obesity-related diseases (Amoutzopoulos, 2013; Kim et al., 2020).

Phenols, a bioactive compound abundant in plants, have been shown to inhibit both α-amylase and α-lipase. Thus, the simultaneous provision of antioxidants and inhibitors for α-amylase and lipase through dietary sources presents a promising and practical approach for managing type 2 diabetes and obesity (Ogundipe et al., 2022).

Table 4. Inhibition of amylase and lipase enzyme activities of of *Satureja hortensis L.* extracts obtained using different extraction methods with different solution ratios.

Extraction Method	Extraction Time	Amylase Activity (%)	Lipase Activity (%)
Traditional (40% EtOH)	72h	16.40±0.50^{ef}	17.41±1.14^{l}
Traditional (50% EtOH)	72h	18.73±1.05^{de}	21.37±0.18f^{g}
Traditional (60% EtOH)	72h	19.93±1.18^{d}	22.45±0.80^{ef}
US (40% EtOH)	15 min.	15.53±1.18^{f}	18.69±1.43^{hi}
US (50% EtOH)	15 min.	18.04±2.05^{de}	22.02±0.85^{ef}
US (60% EtOH)	15 min.	41.44±1.71^{b}	23.65±1.08^{de}
US (40% EtOH)	30 min.	24.11±1.49^{c}	19.98±0.65^{gh}
US (50% EtOH)	30 min.	24.10±0.00^{c}	24.78±1.12^{cd}
US (60% EtOH)	30 min.	49.51±2.42^{a}	25.69±1.11^{c}
MW (40% EtOH)	2.5 min.	08.68±0.89^{g}	23.87±1.12^{cde}
MW (50% EtOH)	2.5 min.	09.57±1.31^{g}	32.01±1.27^{b}
MW (60% EtOH)	2.5 min.	08.35±0.87^{g}	31.95±1.32^{b}
MW (40% EtOH)	5 min.	16.53±0.81^{ef}	25.78±1.05^{c}
MW (50% EtOH)	5 min.	18.04±1.19^{de}	35.09±1.29^{a}
MW (60% EtOH)	5 min.	24.18±1.27^{c}	35.56±1.31^{a}

Note: Means with different letters in the same column differ significantly at P= 0.05. All data is three replicates of mean ± SD

In the present study, it was assessed the obesity and antidiabetic potential of *S. hortensis L.*, a plant renowned in traditional medicine for its natural healing properties. All extracts exhibited inhibitory activity against amylase and lipase enzymes. The percentage inhibitory values of the extracts varied from 8.55% to 50.77% for amylase and 17.41% to 36.56% for lipase, depending on the extraction method and ethanol concentration, at a concentration of 400 μg mL^{-1}. Notably, these values align with findings from previous studies on Moringa oleifera extract, a plant recognized for its effects on diabetes and obesity, where the 50% inhibitory concentration values for lipase and amylase activities were reported as 1.0877 mg mL^{-1} and 0.1802 mg mL^{-1}, respectively (Lakka et al., 2020; Ogundipe et al., 2022).

Conclusion

In this study, the extraction methods of *Satureja hortensis L*. using microwave and ultrasound were compared. Microwave-assisted extraction resulted in a notable decrease in processing time and a significant enhancement in the total phenolic content compared to traditional extraction methods. Moreover, significant disparities in antioxidant activity were evident between microwave-assisted and traditional extracts. Conversely, while ultrasonic extraction reduced processing time compared to traditional methods, it did not yield higher total phenolic content in the extracts. Overall, microwave-assisted extraction exhibited superior advantages in terms of time efficiency and total phenolic content compared to other extraction techniques.

This study introduces the novel and innovative potential of microwave-assisted extraction, demonstrating its superior efficiency in reducing processing time and enhancing total phenolic content and antioxidant activity compared to traditional methods. Future research could focus on optimizing microwave-assisted extraction parameters for various plant species and exploring its combination with other advanced techniques to further improve extraction efficiency and bioactive compound stability.

Disclosure Statement

No conflicts of interest were reported by the authors.

Authors' Contributions

The authors of this manuscript would like to acknowledge the following contributions: The conceptualization and methodology were carried out by Buket Askin. The extraction procedures and analyses were Sercan Ozbek Yazıcı. Data evaluation was carried out by Buket Askin.

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