

***In vitro* anti-urease, antioxidant, anticholinesterase, cytotoxic and *in vivo* anti-inflammatory potential of *Satureja cuneifolia* Ten.**

Turgut TAŞKIN^{1*}, Murat DOĞAN², Muhammet E. CAM^{1,3,4},
Talip ŞAHİN¹, İsmail ŞENKARDEŞ¹

¹University of Marmara, Faculty of Pharmacy, 34668, Istanbul, Turkey; turguttaskin@marmara.edu.tr (*corresponding author);
muhammet.cam@marmara.edu.tr; talipsabin34@gmail.com; isenkardes@marmara.edu.tr

²University of Sivas Cumhuriyet, Faculty of Pharmacy, Sivas, Turkey; ecz.murat44@hotmail.com

³Center for Nanotechnology and Biomaterials Application and Research (NBUAM)

⁴University College London, Department of Mechanical Engineering, Torrington Place, London WC1E 7JE, UK

Abstract

Satureja cuneifolia Ten. (wild savoury) belongs to the Lamiaceae family and is used to produce essential oil and aromatic water. This plant is also used as a condiment and herbal tea due to its stimulating, tonic and carminative effects. The *in vitro* antioxidant, anti-urease, anticholinesterase and cytotoxic activities of the different extracts from the plant's aerial parts were examined. Besides, the *in vivo* anti-inflammatory activities of the fraction and direct methanol extracts were determined comparatively. In the current study, fraction methanol extract exhibited the strongest ABTS (52.34 mM trolox/mg extract) radical scavenging and ferric reduction (17.22 mM Fe²⁺/mg extract) activity. It was also found that the fraction methanol extract had stronger anti-urease (12.52%) and anticholinesterase (69.02%) activity than other extracts. The XTT results showed that fraction methanol extract had the most cytotoxic activity on MCF-7 cell lines (39.92%). According to the results of *in vivo* anti-inflammatory activity, it was found that both fraction and direct methanol extracts exhibited close and significant anti-inflammatory activity. The fact that methanol extracts have significant biological activity suggests that these may be used as a natural source in the future.

Keywords: anti-urease; anticholinesterase; anti-inflammatory; cytotoxic; *S. cuneifolia*

Introduction

Satureja L. (Lamiaceae) is a genus aromatic plant and has 200 species that grow wild in the Middle East, Mediterranean region to Europe, West Asia, North Africa, the Canary Islands and South America. These species have their flavours and are generally consumed as medicinal tea and spices. The leaves, flowers, and stems of *Satureja* genus are used, in traditional medicine to treat various ailments, such as cramps, muscle pains, nausea, indigestion, diarrhoea, and infectious diseases. According to literature survey, *Satureja* species have been found to have antioxidant, antimicrobial, cytotoxic, acetylcholinesterase inhibitory, antiviral, anti-inflammatory, herbicidal, hepatoprotective and antigenotoxic activities. *Satureja cuneifolia* Ten. (wild savoury)

belongs to the Lamiaceae family and is used to produce essential oil and aromatic water. Also, this plant is used as a condiment and herbal tea due to its stimulating, tonic and carminative effects. In studies in Turkey, plant's essential oil has been found to include the major carvacrol and thymol compounds (Bezi *et al.*, 2005; Oke *et al.*, 2009; Tepe and Cilkiz, 2015; García-Rellána *et al.*, 2016).

Alzheimer's is a neurodegenerative disease and is estimated to account for 50-60% of dementia cases in people over 65 years of age. Anticholinesterase drugs used for the treatment of dementia (for example, tacrin, donepezil, physostigmine, galantamine and heptilfizostigmin) are known to have various dangerous side effects such as hepatotoxicity, short biological effect time, low bioavailability, negative cholinergic side effects. Therefore, the number of studies on the cholinesterase activity of natural products has increased (Mogana *et al.*, 2014; Butterfield and Halliwell, 2019; Henstridge *et al.*, 2019; Long *et al.*, 2019).

Oxidative stress plays a critical role in the pathogenesis and aging processes of various diseases such as cardiovascular, neurodegenerative, inflammatory diseases, cancer and diabetes. In recent years, natural antioxidants from medicinal plants have been extensively researched to find compounds that can protect against a range of diseases related to oxidative stress and free radical damage (Fang *et al.*, 2002; Kindl *et al.*, 2015; Ayoub *et al.*, 2017; Ramos *et al.*, 2020).

The primary physiological role of urease is to supply the organisms with nitrogen in the form of ammonia for their growth. The urease activity of *Helicobacter pylori* plays an important role in the pathogenesis of the stomach and peptic ulcer. Therefore, there has been a rapid increase in the number of studies on urease inhibitors in recent years (Hanif *et al.*, 2012; Mimica-Dukic *et al.*, 2018; Holleczech *et al.*, 2019).

As far as we know, no data has been published about the *in vitro* anti-urease, cytotoxic and *in vivo* anti-inflammatory effects of *S. cuneifolia*, and there is a lack of information about the anticholinesterase activity of this species. The study aims to reveal the *in vitro* (anti-urease, anticholinesterase, antioxidant, cytotoxic), *in vivo* (anti-inflammatory) biological effects of different extracts from *S. cuneifolia*.

Materials and Methods

Identification of plant material

Satureja cuneifolia Ten. was collected from the province of Balıkesir in Turkey during the flowering period and taxonomically identified by Dr. Ismail Senkardes. The voucher specimens, representative samples of the plant material, were archived in the Marmara University, Pharmacy Faculty herbarium and documented with the herbarium number of MARE: 18815.

Preparation of Satureja cuneifolia extracts

The aerial parts of *S. cuneifolia* were dried at 25 °C in the shade. Dried parts of the plant (50 g) were extracted with organic solvents such as petroleum ether (SFPE), chloroform (SFC) and methanol (SFM) using the maceration method respectively until colourless. In addition, 20 grams of sample were extracted with direct methanol (SDM) solvent using the maceration method until the colourless. The different extracts from the plant were concentrated by a rotary vacuum evaporator.

DPPH radical scavenging assay

240 µL DPPH solution (0.1 mM) was added to 10 µL sample of the extracts. The prepared mixture was stirred for 1 min and placed at 25 °C for 30 min. The mixture absorbance was determined against the reference at 517 nm. The control sample was carried out under the same conditions using 10 µL of methanol instead of experimental and standard materials and the control sample was daily measured. The investigation was performed three times. The data gained from the investigation was given as IC₅₀=mg/mL (Taskin, 2018).

ABTS⁺ scavenging assay

50 µL of extracts, 50 µL of ABTS⁺ working solution and 150 µL distilled water were added on the prepared extracts. The mixture absorbance was determined against the reference at 734 nm for 6 min. The control sample was prepared under the same conditions with the use of 50 µL distilled water instead of experimental and standard materials. The control sample was daily measured. ABTS radical scavenging determination was applied to trolox solutions prepared at different concentrations (0.2-1 mM). The results of this study were given as mM trolox/mg extract (Re *et al.*, 1999).

FRAP assay

The method of Benzie and Strain (1996) was applied to the extracts to estimate the ferric reducing ability. The FRAP reagent was incubated for 30 minutes at 37 °C. Then, 190 µL FRAP reagent was mixed with 10 µL extract and the mixture absorbance was determined at 593 nm after 4 min. FRAP values of the extracts were given as mM Fe2+/mg extract (Benzie *et al.*, 1996).

Anti-urease activity assay

Stock solutions were prepared from different extracts obtained from the plant and these solutions were diluted to prepare working solutions. A working solution (100 µL) was taken and urease (500 µL) was added to it. The mixture was incubated at 37 °C for 30 min. Then, urea (1100) µL was added on this mixture and incubated at 37 °C for 30 min. R1 (1% phenol, 0.005% sodium nitroprusside) and R2 (0.5% NaOH, 0.1% sodium hypochlorite) reagents were added to the mixture, respectively. After the incubation period at 37 °C for 2 h, the absorbance of samples was measured at 635 nm (Taskin, 2018).

Anticholinesterase activity assay

Inhibition activities of acetylcholinesterase (AChE) were measured using a microplate reader. Acetylcholinesterase as an enzyme source derived from electric fish, acetyl thiocolin iodide was used as a substrate. Yellow-colored 5,5-dithiobis- (2-nitrobenzoic acid) (DTNB) was used for the measurement of activity. As a control, ethanol and galantamine, the alkaloid type drug isolated from the *Galanthus* plant, were used as controls.

AChE % inhibition test: AChE (20 µL) and different concentrations of extracts (20 µL) were added to phosphate buffer solution (pH 8.2 0.1 M, 40 µL). This mixture was incubated at 25 °C for 10 min. After incubation, DTNB (100 µL) and AcI (20 µL) as substrate were added to the mixture. The same procedure was applied to the galantamine used as standard. 5-thio-2-nitrobenzoic acid was spectrophotometrically measured at 412 nm. Anticholinesterase activity of the extracts was calculated using the following equation as% inhibition relative to control (Ellman *et al.*, 1961).

$$\%I = (A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100$$

Total phenolic contents assay

The total phenolic contents of the four different extracts from the plant were determined using the FCR method. Briefly, 0.1 mL extract (5 mg/mL-0.5 mg/mL) was taken in the tube and 4.5 mL of water was added. Then 0.1 mL of Folin-Ciocalteu reagent (diluted 1/3 with distilled water) and 0.3 mL of 2% sodium carbonate solution were added to the mixture. The mixture was allowed to stand at room temperature for 2 hours, and then absorbance was measured at 760 nm against the reference. The total phenolic contents in the extracts were given as mg gallic acid equivalents/mg extract (Ozsoy *et al.*, 2008).

In-vitro cytotoxicity assay

This study was performed by modifying the study of Wolf *et al.* (2009). Cytotoxicity of *Satureja cuneifolia* extracts was measured by the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide) cell viability assay, using MCF-7 and L-929 cell lines. Cell lines were cultured in low glucose DMEM containing 10% FBS, 1 % L-glutamine, 100 IU/mL penicillin and 10 mg/mL streptomycin in 25 cm² polystyrene flasks. The cells were kept at 37 °C within 5% CO₂ humidified atmosphere and were passaged when they had reached 85-90% confluence. Cells were seeded at 1 x 10⁴ cells/well in 96-well plates with 100 µL DMEM including 10% FBS and incubated overnight. The extracts of *S. cuneifolia*, containing petroleum ether, chloroform, and methanol were dissolved in dimethyl sulfoxide (DMSO). These extracts with concentration 1 mg/ml were suspended with DMEM medium and extracts were put in the 96-well plates. Moreover, the same amount of DMSO was put in the positive control group. The cells were incubated for 24 h. Then, the medium was removed and wells were washed with 200 µL phosphate-buffered saline (PBS). Following these periods, for determination of living cells, 200 µL DMEM and 50 µL XTT labelling solution were added to each well and the plates were incubated for 4 h. The absorbance values of XTT-formazan were measured using microplate (ELISA) reader at 450 nm against the control, as untreated cells. All experiments were performed three times and cell proliferation was expressed compared to control (100 % of viability).

In vivo anti-inflammatory activity

Carrageenan-induced paw edema was applied in female Sprague-Dawley rats (200-300 g) by sub-plantar injection of 0.1 ml of 1% (w/v) carrageenan in saline in the right hind paw. There are 4 groups, treated orally with saline (10 ml/kg), SFM (fraction methanol extract) (200 mg/kg), SDM (direct methanol extract) (200 mg/kg) and indomethacin (5 mg/kg) 1 h before the administration of carrageenan. The volume of the edema development and its duration was determined for 4 hours using a plethysmometer. The study was approved by Marmara University, Animal Experiments Local Ethics Committee (MÜHDEK-58.2017.mar) (Vazquez *et al.*, 2015; Taskin *et al.*, 2019).

Statistical analysis

All the experiments were done in triplicates. The results of the antioxidant, anticholinesterase and anti-urease experiments were demonstrated as mean ± SD. All the data were analysed by the Graphpad Prism 5 program. Statistical differences between the study groups were analysed using two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The data obtained from the anti-inflammatory activity were analysed by PASW Statistics. The significance of the difference between means was determined by Mann Whitney U test and p-values less than 0.05 were considered statistically significant.

Results and Discussion*Antioxidant activity of extracts*

The antioxidant activities of plant's different extracts were shown in Table 1. The fraction chloroform (IC₅₀: 0.03 mg/mL) and direct methanol (IC₅₀: 0.07 mg/mL) extracts exhibited stronger DPPH free radical scavenging activity than other extracts. It was also found that fraction petroleum ether extract had the lowest DPPH radical scavenging activity. In this study, the free radical scavenging activities of the plant's different extracts were found to be lower compared to the standards ascorbic acid (IC₅₀: 0.006 mg/mL) and BHT (IC₅₀: 0.009 mg/mL) used. In the ABTS experiment, it was determined that all the extracts from the plant showed close ABTS radical scavenging activity. When the TEAC values of all extracts were compared, it was found that the fraction methanol (52.34 mM trolox/mg extract) extract had the highest TEAC value and also showed activity close to the BHA compound (52.63 mM trolox/mg) as standard. In the FRAP experiment, direct

methanol (17.08 mM Fe²⁺/mg extract), fraction chloroform (17.15 mM Fe²⁺/mg extract) and fraction methanol (17.22 mM Fe²⁺/mg extract) extracts were found to have close ferric reduction activity and were also found to have stronger ferric reduction activity than the BHT (14.12 mM Fe²⁺/mg) and BHA (16.91 mM Fe²⁺/mg).

Phenolic compounds contained in plant extracts were determined by FCR method and according to the data obtained, it was determined that direct methanol (0.071 mg GAE/mg extract) and fraction methanol (0.074 mg GAE/mg extract) extracts contained close phenolic contents. In this study, when the relationship between phenolic contents amount and antioxidant activities were evaluated, a linear relationship was found between the amount of phenolic contents contained in the fraction methanol extract and ABTS and FRAP activities.

Table 1. The antioxidant activity and total phenolic contents of the plant's different extracts

Samples	DPPH (IC ₅₀ : mg/mL)	ABTS (mM trolox/mg extract)	FRAP assay (mM Fe ²⁺ /mg extract)	Total phenolic (mgGAE/mg extract)
SFPE	0.27±0.01 ^a	51.08±0.016 ^a	9.31±0.04 ^a	0.043±0.001 ^a
SFC	0.03±0.004 ^b	52.14±0.008 ^b	17.15±0.007 ^b	0.062±0.009 ^b
SFM	0.07±0.0007 ^c	52.34±0.016 ^{c,b}	17.22±0.009 ^{c,b}	0.074±0.002 ^c
SDM	0.04±0.006 ^d	51.37±0.016 ^d	17.08±0.011 ^d	0.071±0.005 ^d
Ascorbic acid	0.006±0.01 ^e			
BHT	0.009±0.001 ^f		14.12±0.07 ^e	
BHA		52.63±0.009 ^e	16.91±0.02 ^f	

Values are mean of triplicate determination (n = 3) ± standard deviation; Means with different superscripts (^{a-e}) are significantly different, *p*<0.05; SFPE: fraction petroleum ether extract, SFC: fraction chloroform extract, SFM: fraction methanol extract, SDM: direct methanol extract, BHT: Butylated hydroxytoluene, BHA: Butylated hydroxyanisole, GAE: gallic acid equivalent

Urease and acetylcholinesterase inhibitory activity

The results of anti-urease and anticholinesterase activity of different extracts are shown in Table 2. The fraction methanol (12.52%) and direct methanol (9.65%) extracts exhibited stronger anti-urease activity than other extracts. When the enzyme inhibitor activities of the extracts and standard compound were compared, it was determined that all extracts showed a lower percentage of enzyme inhibition than thiourea (70.05%) compound. In anticholinesterase assay, the acetylcholinesterase enzyme inhibition activities of the different extracts at a concentration of 500 µg/ mL were examined and it was determined that the fraction methanol (69.02%) and direct methanol (48.96%) extracts showed the highest enzyme inhibition. According to this study, all the extracts had lower anticholinesterase activity than galantamine (94.52%).

Table 2. The enzyme inhibitory activity of different parts from *S. cuneifolia*

Samples	Urease inhibition (%) (12.5 µg/mL)	Acetylcholinesterase inhibition (%) (500 µg/mL)
SFPE	6.20±0.77 ^a	15.24±1.08 ^a
SFC	3.98±0.05 ^b	35.46±2.04 ^b
SFM	12.52±0.66 ^c	69.02±0.39 ^c
SDM	9.65±0.45 ^d	48.96±0.49 ^d
Thiourea	70.05±0.75 ^e	
Galantamine		94.52±0.14 ^e

Values are mean of triplicate determination (n = 3) ± standard deviation; Means with different superscripts (^{a-e}) are significantly different, *p*<0.05; SFPE: fraction petroleum ether extract, SFC: fraction chloroform extract, SFM: fraction methanol extract, SDM: direct methanol extract

Cytotoxicity result of extract

The cytotoxic activities of *S. cuneifolia* extracts on MCF-7 and L 929 cell lines were examined and the rate of cell death is presented in Figure 1. The results showed that methanol extracts of the plant had the most cytotoxic activity on both MCF-7 and L-929 cell lines. Cell death rates of extracts on MCF-7 cell line were $28.29 \pm 1.34\%$ (petroleum ether), $32.73 \pm 1.67\%$ (chloroform), $39.92 \pm 1.82\%$ (methanol). Moreover, cell death rates of extracts on L-929 cell line were $22.95 \pm 2.31\%$ (petroleum ether), $27.07 \pm 2.40\%$ (chloroform), $27.61 \pm 1.44\%$ (methanol) respectively. Cell viability below 70% is considered to be a cytotoxic effect (Wolf *et al.*, 2000). The XTT results showed that the extracts of *S. cuneifolia* were not cytotoxic on the L-929 cell line. However, the extracts of the plant were cytotoxic effect on the MCF-7 cell line. The extracts of plant were cytotoxic on MCF-7 cell line, but were not cytotoxic on L-929 cell line is a promising and crucial result.

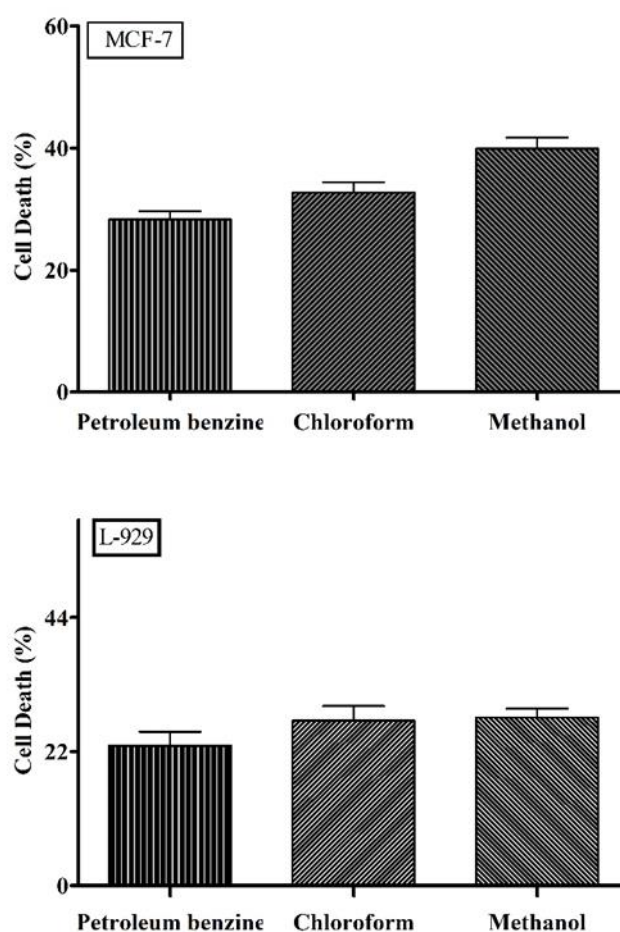


Figure 1. Cell death rate of MCF-7 and L-929 after the treatment of extracts of *S. cuneifolia*. The cell proliferation of extracts was expressed compared to control (100 % of viability)

In vivo evaluation of the anti-inflammatory activity

The injection of carrageenan showed a significant increase in the volume of paw, reaching its maximum of 4 h post-injection (Taskin *et al.*, 2019). Indomethacin (5 mg/kg) indicated maximum anti-inflammatory effect 4 hours after carrageenan injection by 56,8% (Table 3, $p < 0.001$). SDM demonstrated a more prominent and intensive anti-inflammatory effect at first hour with 65.9% of inhibitive capacity in the altered edema size. However, SDM lost its anti-inflammatory effect rapidly in 4 hours. On the other hand, SFM protected its anti-inflammatory effect in 4 hours ($\%27.1$, $p < 0.01$) compared to the control group (Figure 2).

Table 3. Effects of SFM and SDM on carrageenan-induced paw edema in rats

Treatment	0 h	1h	2h	4h
Control	1.17 ± 0.05	1.61 ± 0.07	1.83 ± 0.10	2.35 ± 0.14
SFM	1.10 ± 0.04	1.29 ± 0.02 *	1.52 ± 0.05*	1.96 ± 0.05 **,++
SDM	1.15 ± 0.03	1.30 ± 0.05 *	1.56 ± 0.07 ⁺	2.21 ± 0.07 ***
indomethacin	1.05 ± 0.04	1.21 ± 0.06 **	1.27 ± 0.06 ***	1.56 ± 0.08 ***

Values are expressed as Mean ± SEM (Standard error mean); Values are calculated using two-way ANOVA followed by Bonferroni posttests; * p < 0.05, ** p < 0.01, *** p < 0.001 vs. control; ⁺ p < 0.05, ⁺⁺ p < 0.01, ⁺⁺⁺ p < 0.001 vs. indomethacin.

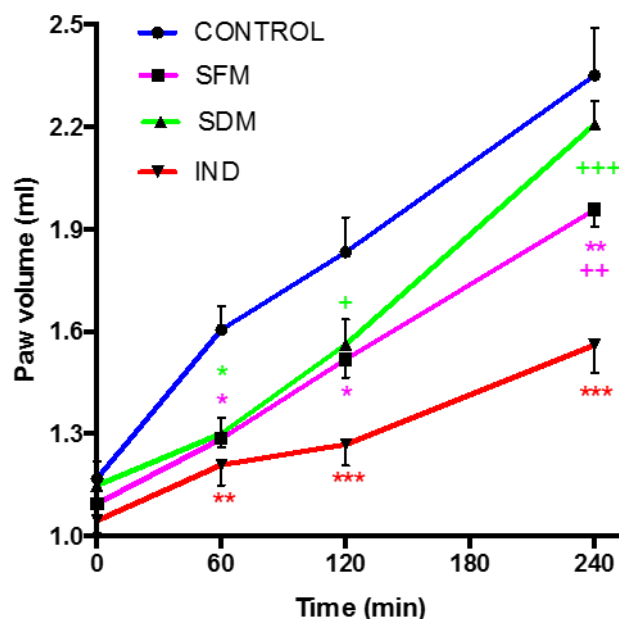


Figure 2. Values are expressed in volume of paw before carrageenan injection and mean ± SEM (Standard error mean) from 6 animals in the treatment group, * p < 0.05, ** p < 0.01, *** p < 0.001 vs. control; ⁺ p < 0.05, ⁺⁺ p < 0.01, ⁺⁺⁺ p < 0.001 vs. indomethacin

The chemical composition of the essential oil from the plant's aerial parts was analysed by the GC-MS method, and the antioxidant activities of the essential oil and methanol extracts which is prepared using the Soxhlet method were examined. It was found that carvacrol and *p*-cymene compounds were found to be major in essential oil. It has been determined that methanol extract has stronger DPPH radical scavenging (IC₅₀ 26.0 µg/mL) and β-carotene bleaching (95.2%) activity than essential oil (IC₅₀ 65.1 µg/mL, 84.5%) (Oke *et al.*, 2009).

In our study, when IC₅₀ values of direct methanol (IC₅₀ 40 µg/mL) extract from aerial parts of plant were compared with the study above, the DPPH radical scavenging activity obtained in this study was found to be lower than the DPPH radical scavenging activity above (IC₅₀ 26.0 µg/mL). In other studies, β-cubebene, camphor, camphene, spathulenol, and β-caryophyllene were found as major compounds in essential oil from aerial parts of plant. Also, it has been found that essential oils showed strong antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Saccharomyces cerevisiae*, *Amaranthus hybridus* and *Conyza canadensis* (Bezi *et al.*, 2005; García-Rellána *et al.*, 2016). In the last study, the antioxidant, anti-Alzheimer, antidiabetic activities of methanol and water extracts from the aerial parts of the plant was examined and the phenolic contents was analysed by LC-MS/MS (Taslimi *et al.*, 2020). The methanol extract was found to show stronger anti-Alzheimer (AChE: IC₅₀ 63.69 µg/mL; BChE: IC₅₀ 23.17 µg/mL) and antidiabetic (α-

glycosidase: IC₅₀ 10.66 µg/mL) activity than water extract. It has also been determined that this extract has stronger DPPH (IC₅₀ 26.03 µg/mL), FRAP (λ 700 1.344), CUPRAC (λ 450 1.203) and ABTS (IC₅₀ 14.98 µg/mL) radical scavenging activity than water extract. It was determined that rutin, kaempferol-3-*O*-rutinoside and fumaric acid compounds were the most common in the plant.

In our study, it was determined that direct methanol extract (IC₅₀ 40 µg/mL) showed lower DPPH radical scavenging activity than the above study (IC₅₀ 26.03 µg/mL) as well as in parallel with this study, it was found that it showed significant activity in ABTS, FRAP and CUPRAC methods. Also, in this study, the activity of both fraction and direct methanol extracts on cholinesterase enzyme was examined, and it was found that the fraction methanol extract (69.02%) showed stronger inhibition than direct methanol extract (48.96%).

The anti-inflammatory and analgesic activities of essential oil, hydroalcoholic and polyphenolic extracts of the seeds of the *Satureja hortensis* were examined with acetic acid and formalin (analgesic methods) and carrageenan-induced rat paw edema (anti-inflammatory) tests. In this study, it was found that these extracts decreased acetic acid- induced abdominal twitches and paw edema (Hajhashemi *et al.*, 2012; Jafari *et al.*, 2016). Antinociceptive and anti-inflammatory activity of hydroalcoholic extract of *S. khuzestanica* was evaluated using formalin and carrageenan-induced rat paw edema test. In this study, the hydroalcoholic extract of plant [150 mg/kg; intraperitoneally (i.p.)] has been found to have as effective anti-inflammatory activity as indomethacin (4 mg/kg; i.p.). Besides, the hydroalcoholic extract was found to show significant antinociceptive activity compared to morphine (Amanlou *et al.*, 2005). In our study, it was found that methanol extracts of the plant in parallel with the above literature data showed significant anti-inflammatory activity compared to the control.

The cytotoxic activity of *S. intermedia* essential oil was evaluated by MTT method on human cancerous cells (esophageal squamous cell carcinoma and human bladder carcinoma cell lines). The results revealed that essential oil showed considerable activity ranging from 39 µg/mL to 1,000 µg/mL (IC₅₀ 156 µg/mL) (Sadeghi *et al.*, 2013). The cytotoxic activities of *S. sabendica* essential oil against MCF-7, Vero, SW480 and JET 3 cell lines was examined by MTT method and it was observed that the essential oil showed strong cytotoxic activity on MCF-7 (IC₅₀ 15.6 µg/mL), Vero (IC₅₀ 15.6 µg/mL), SW480 (IC₅₀ 125 µg/mL), and JET 3 (IC₅₀ 250 µg/mL) cell lines (Yousefzadi *et al.*, 2012). The cytotoxic activity of the methanol extract of *S. kitaibelii* was examined and it was found that methanol extract exhibited strong cytotoxic activity against Fem-X Human Malignant Melanoma cells (IC₅₀ 39.66 µg/mL) and moderate activity against MDA-MB-361 ((IC₅₀ 361 µg/mL) and HeLa ((IC₅₀ 173 µg/mL) cell lines. *Satureja odora* and *S. parvifolia* dichloromethane extracts were tested for toxicity on brine shrimp, and these extracts were a promising activity was observed (LC₅₀ value <200 µg/mL). As seen in the studies presented here, it has been determined that the extracts and essential oils of the *Satureja* species show cytotoxic activity. Besides, phytochemical compounds (rosmarinic acid, 5,7,3',5'-tetrahydroxy flavanone, 5,4'-dihydroxy-3'-methoxyflavanone-7-(6''-*O*-α-L-rhamnopyranosyl)-β-D glucopyranoside) of plants are known to have important cytotoxic activities (Tepe and Cilkiz, 2015). In our study, it was observed that the plant's methanol extract showed promising cytotoxic activity in cancer cell line (MCF-7) similar to the above data.

Inhibitory activities of essential oil or extracts (water, ethanol) of *S. khuzestanica*, *S. montana*, *S. parvifolia*, and *S. thymbra* were evaluated for their AChE and BChE. According to these study, essential oil or extracts of plants had a promising enzyme inhibition activity. According to literature reviews, *S. hortensis*, *S. khuzestanica* and *S. montana*, in general, exhibited strong antioxidant activity due to their oxygenated monoterpenes (especially, carvacrol and thymol contents) and polar phytochemicals (Tepe and Cilkiz, 2015). In our study, as in previous studies, it was determined that the plant's methanol extracts has strong antioxidant and anticholinesterase activity.

Satureja species are known to contain essential oils, phenolic acids (gallic acid, vanillic acid, caffeic acid, chlorogenic acid), flavonoids (luteolin, apigenin, cirsilineol, acacetin), and these compounds have various

biological activities (antioxidant, anticholinesterase, antispasmodic, anti-inflammatory etc.) (Tepe and Cilkiz, 2015). In the previous study by Taslimi *et al.*, phenolic acid (gallic acid, fumaric acid, ellagic acid) and flavonoid compounds (apigenin, luteolin, rutin) in the methanol extract of *Satureja cuneifolia* were analysed by LC-MS/MS technical (Taslimi *et al.*, 2020). In this study, *Satureja cuneifolia* is thought to exhibit strong biological activity due to the phenolic acid and flavonoid compounds in the methanol extracts.

As far as we know, data on the *in vitro* anti-urease, cytotoxic and *in vivo* anti-inflammatory effects of *S. cuneifolia* have not been published. In this study, unlike other literature information, the anti-urease, cytotoxic and anti-inflammatory effects of different extracts of the plant were investigated for the first time.

Conclusions

In the current study, it was found that the fraction methanol extract showed stronger ABTS and FRAP activity than other extracts as well as it contained a higher amount of phenolic contents. A linear relationship was determined between the amount of phenolic contents contained in this extract and antioxidant activity. The activity results of the extracts on the enzymes (urease, acetylcholinesterase) showed that the fraction methanol extract had the strongest anti-urease and anticholinesterase activity. XTT results showed that the fraction methanol extract had the most cytotoxic activity on MCF-7 cell lines, but it was a promising result that it was not cytotoxic on the L-929 cell line. It was determined that both extracts from the plant's aerial parts showed significant *in vivo* anti-inflammatory activity. The fact that methanol extracts have significant biological activity suggests that these may be used as a natural source in the future. Therefore, it is thought that more comprehensive studies are needed for these extracts.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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