Emirates Journal of Food and Agriculture. 2023. 35(4): 271-279
doi: 10.9755/ejfa.2023.v35.i4.3049
http://www.ejfa.me/

RESEARCH ARTICLE

Phenolic screening and anticancer potential of various wild savory extracts

Ela Nur Şîmşek Sezer*

Department of Biology, Faculty of Science, Selçuk University, Konya, Turkey

ABSTRACT

Cancer is one of the leading causes of death in our age. In addition to the treatments used in cancer, plants are frequently used in complementary or alternative approaches. Plants of the genus *Satureja* are used worldwide for various purposes, primarily as complementary therapies. For this purpose, in this study, the antioxidant potential and phenolic content of the *Satureja cuneifolia* plant, which is frequently consumed as a spice and tea among the public, as well as its cytotoxic and apoptosis-inducing effects on cancer cells were tried to be revealed. The cytotoxic effect of the extracts prepared with three different solvents (methanol, ethanol and water) was determined by the MTT test in colorectal cancer (DLD1) and promyelocytic leukaemia cell lines (HL60), and then the expression levels of five apoptotic gene regions (*apaf-1, bax, bcl2, card4, casp3* and *tp53*) were evaluated with Real Time PCR. The antioxidant potential was determined via DPPH test and HPLC was used to screen for phenolic substances. As a result, it was determined that the extracts have cytotoxic effects and have a variable but positive effect on pro-apoptotic gene expressions. When the antioxidant potential was evaluated, it was determined that the methanolic extract had more radical scavenging effect ($IC_{50:}$ 105.02 ± 0.034 µg/ml). In conclusion, the cytotoxic and apoptosis-inducing potentials of the extracts obtained from the *S. cuneifolia* plant were revealed the first time upon DLD1 and HL60 cell lines in this study, and this study is a pioneer for future studies in cancer therapy.

Keywords: Apoptosis; qRT-PCR; Spice; Turkey; Wild savory

INTRODUCTION

Phytotherapy has become a very important strategy for many diseases, especially cancer, in recent years, thanks to the advantages it provides in the treatment and prevention of diseases (Pan et al., 2010; Song et al., 2018). In general, the use of plants in the treatment of cancer has a long history, and for this reason, plants have been the main source of effective traditional and complementary therapies in cancer treatment (Sewell and Rafieian-Kopaei, 2014). About 20% of known plants have been used in pharmaceutical studies, affecting the health system in positive ways, such as in the treatment of cancer and harmful diseases (Naczk and Shaidi, 2006). The absence of effective therapeutic strategies for cancer control yet is the most important factor causing high mortality rates (Siegel et al., 2016; Gezici and Şekeroğlu, 2019). A large number of plant species used in cancer treatment have been reported since ancient times, and today, there is an increasing interest in studies evaluating the effects of plants and phytochemicals obtained from plants. More than half of the anticancer drugs used today are derived from natural sources such as sea creatures, plants and microorganisms (Cragg and Newman, 2005).

The genus *Satureja* grows in Western Asia and the Eastern Mediterranean region, spreading in vast geographies such as Anatolia, Turkmenistan, Caucasus, Iraq and Iran. In Turkey, the genus represented by 15 species (Güner et al., 2012). *Satureja* taxa are medicinal plants that are notable for the existence of secondary metabolites such as flavonoids, terpenoids and tannins. Due to these crucial compounds, there are many studies carried out to determine its essential oil content, chemical content, ethno medical effects as well as pharmacological activities and antimicrobial activities (Lamaison et al., 1991; Mueller-Riebau et al., 1995). Moreover, they have long been known for their healing properties and have been used as traditional folk remedies to treat various illnesses such as nausea, indigestion, diarrhoea, cramps, muscle aches, and infectious diseases (Bezić et al., 2009).

More recently, interest in herbs and spices has increased not only for their spice and aroma properties but also for their

***Corresponding author:**
Ela Nur Şimşek Sezer, Department of Biology, Faculty of Science, Selçuk University, Konya, Turkey. **E-mail:** elasimsek@selcuk.edu.tr

Received: 03 July 2022; **Accepted:** 08 March 2023

biological potential (Albano and Miguel, 2011). *Satureja cuneifolia* is a perennial shrub and sprouts every spring with new branches full of leaves. *S. cuneifolia* Ten. is known as "Dağ Kekiği" in Turkey and commonly used as a spice (Kan et al., 2006). It is used to produce essential oils and aromatic water, in the mountainous regions in Turkey's Aegean and Mediterranean regions is a well-known and aromatic plant widely used. The essential oil of this plant is applied to flavouring spices, soups, flavours, canned meats, spicy table sauce and sausages. The composition and antimicrobial effects of oils, is depending on the local conditions and growth stages of plant species (Bezić et al., 1999; Milos et al., 2001). As far as we know, there is broad literature information on various biological activities of *S. cuneifolia* (Milos et al., 2001; Taslimi et al., 2020; Taskin et al., 2020; Eminagaoglu et al., 2007; Tepe and Cilkiz, 2016; Oke et al., 2009; Bezić et al., 2005). Moreover, although there are many studies on the content of this plant, the bioactive properties and chemical composition of the plant can vary significantly from one region to another, depending on the changing habitat and environmental conditions (Ganamé et al., 2021; Le et al., 2020). According to the present literature, there are reported many studies that reveal the volatile components related to the species and as a result, there is too much data about that subject. As a different approach to gain new information, therefore, it is aimed to work on mostly phenolic compounds in this study. *S. cuneifolia* has been previously reported to exhibit *in vitro* anti-Alzheimer, antidiabetic, antimicrobial, antioxidant, antiurease anticholinesterase and cytotoxic potential (Taslimi et al., 2020; Taskin et al., 2020; Eminagaoglu et al., 2007; Tepe and Cilkiz, 2016), but there is no study on its cytotoxic and apoptosis-inducing effects on colorectal cancer and leukaemia cells. The main objective of this study is to reveal the phytotherapeutic potential of the extracts prepared using different solvents from *S. cuneifolia*, which is frequently used as a tea and spice. This study aims to show the phenolic contents, antioxidant capacities and cytotoxic and apoptosis-inducing effects of extracts obtained from the plant. This article is the first effort to understand the cytotoxic and apoptosis-inducing potential of different *S. cuneifolia* extracts.

MATERIALS AND METHODS

Plant material and preparation of plant extracts

Plant specimen was collected from its natural habitat and A sample material was stored in the herbarium of Selçuk University. The locality information of the plant specimen is Afyon, Emirdağ, around Karakuyu village,1000 m, 38°05'22"N, 30°12'58"E and collected in 2018. The plant specimen was collected and identified by Dr Tuna UYSAL. The collection number of the plant specimen is TU-3750. After the collection, the aerial parts of the sample were dried in a sun-free environment and made ready for analysis. The sample was pulverized with herb grinder under sterile conditions and extracted with absolute methanol and ethanol for 6-8 hours using a Soxhlet apparatus (Raaman, 2006). The aqueous extract was prepared via maceration method. Obtained total extracts were evaporated at 40 ° C. Extracts were coded as, methanolic extract SCUmet; ethanolic extract SCUet and the aqueous extract was SCUaq, respectively.

HPLC analyses

For the determination of phenolic compounds in the content of the extract, 100mg of each extract sample was dissolved in methanol (% 100) and the final volume of the solution was adjusted to 10 ml. After the mixture was filtered through a 0.22μM sterile filter, HPLC analyses were performed at Selçuk University Research and Development Center. Analyses were carried out via Shimadzu LC-20AD device equipped with UV/VIS (SPD-20A) detector and with using the INERTSIL ODS-3V (5μm; 4.6 x 250 mm) column. The method used by Wen et al., 2005 in the analyses was used with some minor modifications (Wen et al., 2005). In our study, 6 phenolic substances were scanned and quantified. The used standards are; phenolic acids (protocatechuic, caffeic, syringic and trans-p-coumaric), flavonoids (rutin trihydrate and naringenin). HPLC analyses were performed with at least three parallel tests and mean values were taken into consideration.

Antioxidant assay

DPPH analyses to reveal the antioxidant capacity were performed by following the Chu method with minor amendments (Chu et al., 2000). Absorption of the extracts and standards were measured at 490 nm using an Elisa reader.

Cytotoxicity assay

Satureja extracts prepared at different concentrations (0.09-0.75 mg/ml) were applied on the cells and incubated in two time periods (24-48 hours). Cell lines DLD1 (colorectal cancer) and HL60 (leukemia) used in cytotoxicity determination were kindly provided by Dr Ali Uğur URAL and Dr Zerrin CANTURK. Cells were routinely cultured in RPMI 1640 medium containing 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin and grown under conditions of 37 °C and 5 % CO_2. Cell proliferation experiments were performed via MTT (Sigma-Aldrich GmbH, Sternheim, Germany) assay. The optical density of the plates was measured at 570 nm. Each experiment was performed in triplicate with at least three wells in the plate. Vinblastine was used as a positive control. IC50 concentrations were calculated from dose-response curves.

PBMC Isolation and cytotoxicity assay

Peripheral blood mononuclear cells (PMBCs) were isolated from fresh human blood with Ficoll reagent. The isolated cells cultured with DMEM medium and were grown under conditions at 5 % CO_2 and 37 ° C for 24h. Then the extracts applied for 24-48h. Cell proliferation experiments were performed via XTT (XTT Cell Proliferation Kit, Biological Industries) assay. The optical density of the plates was measured at 450-500 nm. Each experiment was performed in three replicates, at least two wells in the plate.

RNA isolation and RT-PCR

Total RNA was isolated with the AxyPrep Multisource Total RNA Miniprep Kit according to the manufacturer's recommendations, and the concentration and quality of the RNA samples were estimated with Nanodrop 2000 (Wilmington, DE, USA). Total RNA (0.5-1 µg) was converted to cDNA via the First strand cDNA synthesis kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). Gene expression experiments were performed following the previously reported procedure (Şimşek Sezer & Uysal, 2021). The data were analysed comparatively with the CT method and the fold change was calculated with the formula $2^{-}\Delta\Delta^{CT}$ (Livak et al., 2013).

Statistical analysis

All of the results were presented as mean values. Untreated cells were used as control both MTT assay and RT-PCR. The statistical analysis was conducted using one-way analysis of variance (ANOVA), Dunnett and Games Howell tests were used as post hoc tests. Statistical analysis was performed using SPSS version 22 for Windows (SPSS Inc., Chicago, IL, USA) and p less than 0.05 was selected as the level of significance. Pearson correlation analysis was performed to determine the relationship between antioxidant effect and cytotoxic effect.

RESULTS

HPLC analyses

In the present study, 6 phenolic substances in the extract composition of *S. cuneifolia*; protocatechuic, caffeic, trans-p-coumaric and syringic acid, rutin trihydrate and naringenin were screened by HPLC and their amounts were determined. When the results were evaluated, different phenolic substances were determined in different amounts depending on the solvent (Fig. 1). Rutin trihydrate was common for methanol and ethanol extract, while naringenin was detected only in the ethanolic extract. On the other hand, four phenolic; protocatechuic, caffeic, trans-p coumaric and syringic acid were detected only in aqueous extract. The HPLC chromatogram of the *S. cuneifolia* extracts is shown in Fig. 1, together with analytical standards. The phenolic components and amounts were given in Table 1.

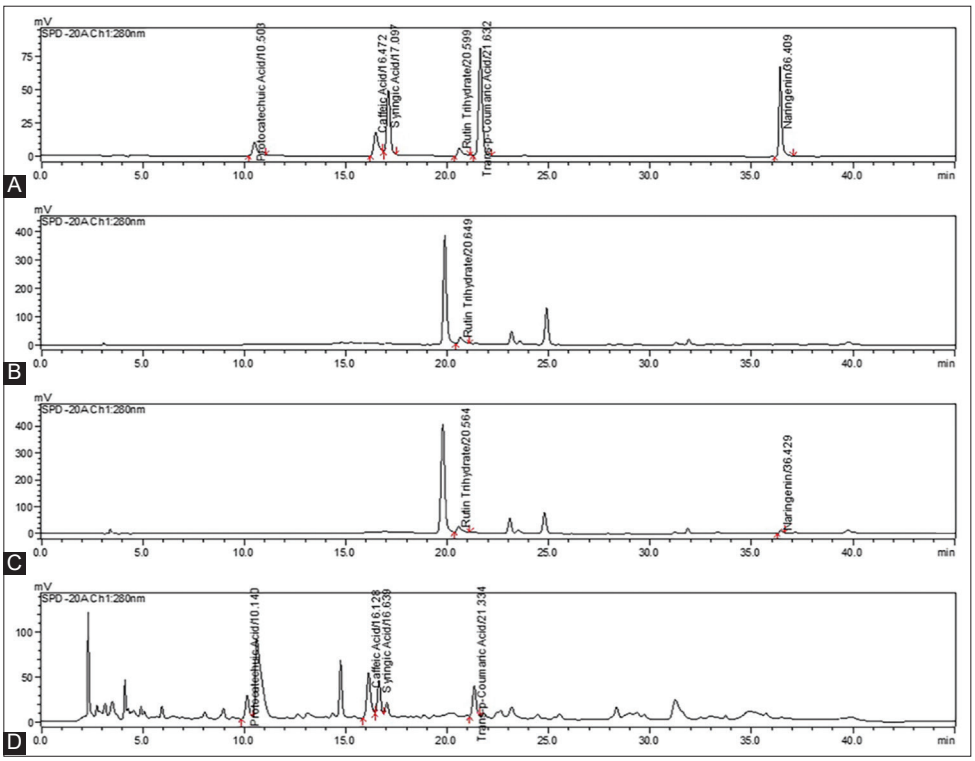

Fig 1. HPLC chromatograms of studied samples (A: Analytical standards B: SCUmet; C: SCUet; D: SCUaq).

Antioxidative potential of extracts

The antioxidant potentials of plants and plant-derived compounds can be defined as the level of prevention or inhibition of the oxidation of various biomolecules in the environment (Gülçin, 2012). Antioxidant, or free radical scavenging, activities of the *S. cuneifolia* extracts were determined using DPPH assay. DPPH radical scavenging activity of extracts was compared with ascorbic acid. The results are displayed in Table 2. When we evaluated the antioxidant activity results in general, it was found that the extracts had antioxidant potential but they have a lower antioxidant effect than ascorbic acid, and SCU-MET extract has the most antioxidant effect. When we list the antioxidant capacities of the extracts, it is SCU-MET>SCU-ET>SCU-AQ. Although there are various studies in the literature revealing the antioxidant properties of *S. cuneifolia*, the plant localities, and extraction methods used in these studies are different and the essential oil of the plant was generally studied. The antioxidant capacity of *S. cuneifolia* essential oil and methanolic extracts were evaluated and it was reported that methanol extracts of *S. cuneifolia* exhibited greater antioxidant activity (Taslimi et al., 2020; Eminagaoglu et al., 2007; Oke et al., 2009). Our results consistent with previous studies.

Cytotoxic effect of extracts

In order to reveal the cytotoxic effect of *S. cuneifolia* extracts on cancer cells, two cancer cell lines (DLD1 and HL60) were used. MTT assay was used for cytotoxicity assignment. In order to determine the cytotoxic effects, they were applied over 4 different doses (0.09-0.75 mg/ml) on cell lines and two different time intervals (24 and 48 h). The MTT assay results were given as a % viability graph in proportion to the control group. It was determined that all extracts showed a cytotoxic effect on DLD1 cells in a dose and time-dependent manner. (Fig. 2). When we evaluate the cytotoxic effects of the extracts on the HL60 cell line in general we can say that the extracts have merely a dose-dependent cytotoxic effect (Fig. 3). The IC_{50} values of the extracts were given in Table 2. According to the MTT results, the DLD1 cell line was more sensitive than the HL60 cell line to all extracts treated Therefore, DLD1 cells were selected for further study. On the other hand, no cytotoxic effect was observed on PBMC's (Fig. 3). Especially this finding is very important because of showing the selective effects of the *S. cuneifolia* extracts.

Table 1: Comparative phenolic content of *S. cuneifolia* extracts.

Phenolic compounds	SCU MET	SCU ET	SCU AQ
		(mg/L)	
Protocatechuic Acid	-	-	11.60±0.43
Caffeic Acid	-	-	13.13±0.04
Syringic Acid	-	-	5.93±0.07
Rutin Trihydrate	218.63±9.43	219.10±5.40	-
Trans-p-Coumaric Acid	-	-	3.55±0.03
Naringenin	-	11.90±0.35	-

Effects of extracts on apoptotic gene expressions

In addition to the fact that a plant extract performs cell death, it is desirable to have this cell death by apoptotic pathways. In order to reveal the potential anticancer mechanism at the molecular level of *S. cuneifolia* extracts qRT-PCR analysis was used to detect the gene expression levels of *apaf-1, bax, bcl2, card4, casp3* and *tp53* genes in DLD1 cells. In gene expression determination studies, β-actin was used as an internal control and relative expression graphs were created. Real-Time PCR results were evaluated in general, it is seen that the extracts have variable effects on different apoptosis-related gene regions. When the expression levels of apoptotic gene regions were evaluated, it was found that methanolic and ethanolic extracts caused an increase in *apaf-1, bax/bcl2*, *card4*, *casp3* and *tp53* gene expressions that trigger apoptosis. On the other hand, there is a different situation in aqueous extract than others, it caused an increase in apaf-1, bax and card4 expressions, while it did not cause a significant difference in other gene regions. The relative expression graphs of studied gene regions were given in Fig. 4.

DISCUSSION

Today, chemotherapeutic drugs are used in a wide range in the treatment of many diseases such as cancer, and new treatments continue in the field due to their side effects and damage to the immune system. Plants are a well-known potential source of many bioactive compounds,

Table 2: IC_{50} values of DPPH and MTT assays of *S. cuneifolia* extracts.

Cell Line	DPPH	MTT			
		DLD1		HL60	
Extracts	IC_{50} (μg/ml)	24H IC_{50} (mg/ml)	48H IC_{50} (mg/ml)	24H IC_{50} (mg/ml)	48H IC_{50} (mg/ml)
SCU MET	105.02±0.034	0.276	0.239	0.252	0.242
SCU ET	132.4±0.438	0.280	0.254	0.311	0.267
SCU AQ	160±1.824	0.306	0.249	0.33	0.34
Ascorbic acid (DPPH)/Vinblastine (MTT)	21.6±0.5	27 μM	9.75 μM	49 μM	2.4 μM

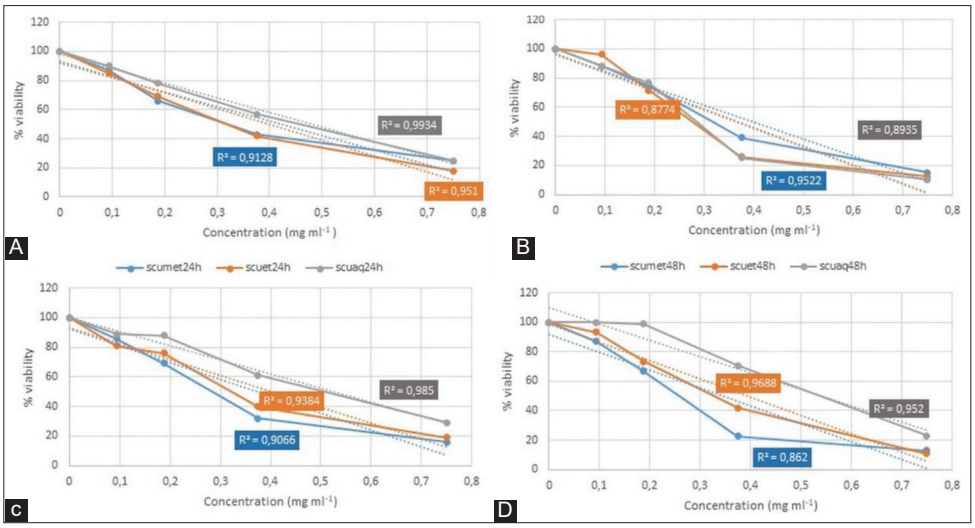

Fig 2. Dose response curves of MTT assay graphs of *S. cuneifolia* extracts for two-time intervals (A and B: DLD1 cell line, C and D: HL60 cell line; control: untreated cells; SCUmet: methanolic extract, SCUet: ethanolic extract and SCUaq: aqueous extract).

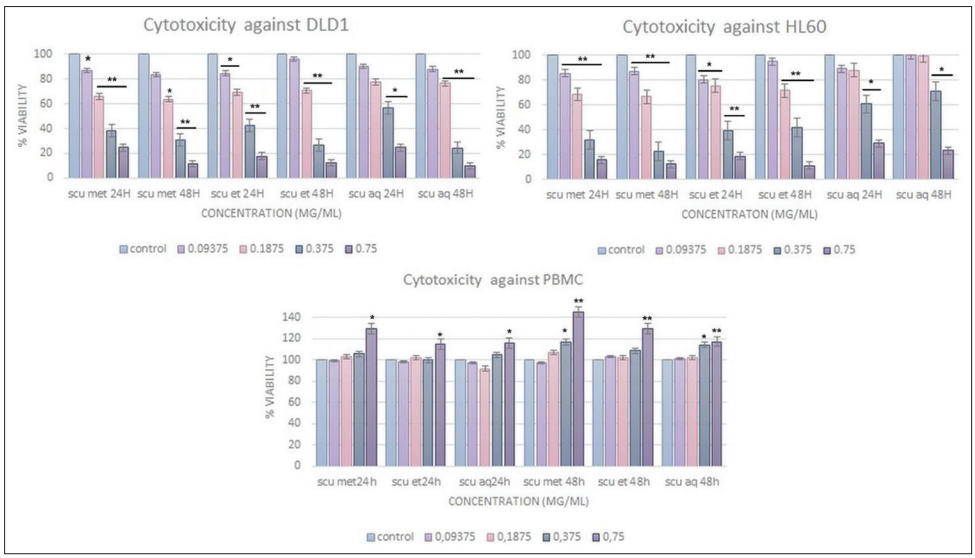

Fig 3. MTT assay graphs of *S. cuneifolia* extracts for two-time intervals (control: untreated cells; SCUmet: methanolic extract, SCUet: ethanolic extract and SCUaq: aqueous extract).

and plant-derived compounds are preferred over others (Atanasov et al., 2021). Recently, it is very popular to reveal the various biological properties of plants consumed as food and the many components derived from them. The present study was conducted to reveal the potential anticancer activity of different extracts of *S. cuneifolia*, a well-known and aromatic plant. The cytotoxic effect of *S. cuneifolia* extracts against liver (HepG2) and breast cancer (MCF-7) has been reported in previous studies (Taskin et al., 2020; Yücel, 2018). As previous studies were limited to two cancer cell lines, the efficacy of the plant extract against other cancer cell lines is still lacking and further study of more cell lines is required. Therefore, this study is the first report on the anticancer potential of different extracts of *S. cuneifolia* on DLD1 and HL60 cell lines.

According to the literature survey, the antioxidant and free radical scavenging potential of *S. cuneifolia* is well known. Dorman, et al. (Dorman et al., 2004) reported the antioxidant properties of aqueous extracts from some Lamiaceae species grown in Turkey, including *S. cuneifolia*. Particularly *S. cuneifolia* extract, has been reported to be having usable *in vitro* antioxidant properties. In another study, the *in vitro* antioxidative properties of the essential oils and methanol extracts of *S. cuneifolia* were investigated and reported that they exerted antioxidant activities and especially methanolic extract showed greater activity in the DPPH system ($IC_{50:}$ 68.0 ± 1.76 μg/ml) (Eminağaoğlu et al., 2007). Oke et al.,(2009), also determined DPPH radical scavenging activity of the methanolic extract (IC_{50} = 26.0 ± 1.2 μg/ml) and the essential oil

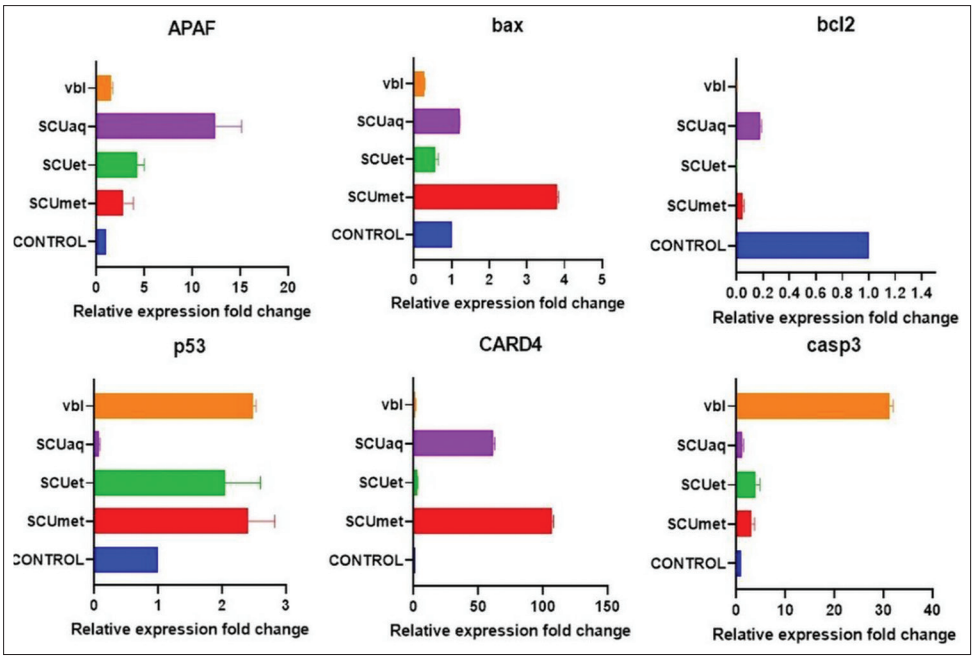

Fig 4. Relative expression fold graphs of studied gene regions (control: untreated cells; SCUmet: methanolic extract, SCUet: ethanolic extract and SCUaq: aqueous extract, vbl: vinblastine).

(IC_{50} = 65.1 ± 2.2 µg/ml) of *S. cuneifolia*. In another study, the composition and antioxidant capacities of volatile components of *S. montana* and *S. cuneifolia* essential oils were investigated in Croatia, and according to the DPPH results, these two species were reported to have very high reducing power ($IC_{50:}$ 7.04±0.55- 28.90±1.14 mg/ml), which may be interesting for pharmaceutical and food engineering studies (Ćavar et al., 2008). Taskin et al.,(2020), investigated various biological effects of *S. cuneifolia* fractions, including antioxidant effect, and reported that chloroform fraction and methanol fraction showed high antioxidant effect ($IC_{50:}$ 0.03- 0.07 mg/ml), but petroleum ether fraction had low efficacy in the DPPH test. In a recent study on the antioxidant capacity of the plant, the antioxidant capacity of the methanolic and water extracts were investigated by various tests and it was reported that the methanolic extract had a stronger radical scavenging effect than the water extract ($IC_{50:}$ 26.03- 30.63 µg/ml) (Taslimi et al., 2020). In this study, we found that the methanolic extract had the highest antioxidant effect. Although IC_{50} values differ, our results are consistent with previous reports. There are many studies on the antioxidant potential and the chemical composition of the plant, but it should not be ignored that locality, environmental conditions and especially preferred extraction methods can change the chemical composition and radical scavenging effect of the extract or essential oil (Ganamé et al., 2021; Le et al., 2020).

Considering the use of a plant for medicinal purposes, it is very important to reveal its various biological properties and content. In particular, the antioxidant and cytotoxic effect is thought to be closely related to the phenolic content of plants. There are many factors that play a critical role in successfully isolating phenolic compounds from plant materials. Among these, the solvent selection is the most important (Bhebhe et al., 2016). In the present study, 6 phenolic substances in the extract composition of *S. cuneifolia*; protocatechuic, caffeic, trans-p-coumaric and syringic acid, rutin trihydrate and naringenin were screened by HPLC and their amounts were determined. Protocatechuic Acid (PCA), one of the phenolic substances identified in this study, is a simple phenolic acid and is recommended as a potential chemotherapeutic agent with its antioxidative properties and pro-apoptotic effects (Lin et al., 2011; Semaming et al., 2015; Abotaleb et al., 2020; Quinn et al., 2017; Gani et al., 2019). Another compound, caffeic acid, has anti-inflammatory and antiproliferative properties and has been reported to have antioxidant properties in normal cells and pro-oxidant properties in cancer cells (Kanimozhi and Prasad, 2015; Pelinson et al., 2019). Many *in vitro* and *in vivo* studies have documented the beneficial role of syringic acids in various cancers and non-communicable diseases (Boutayeb and Boutayeb, 2005; Kumar and Pandey, 2013; Kowalczyk et al., 2019; Gheena and Ezhilarasan, 2019). On the other hand, many beneficial effects of routine, especially anti-inflammatory and antioxidant effects, in the content of screened extracts have been described (Chen et al., 2000; Jung et al., 2007). In addition, the routine has been previously described to cause in vitro cytotoxic effects on cancer cell lines, including human colon cancer cells (ben Sghaier et al., 2016; Guon and Chung, 2016; Kuntz et al., 1999; Menon et al., 1995).

Since the phenolic substances in the extracts have both antioxidant and cytotoxic effects, it can be thought that the antioxidant capacity and cytotoxicity observed as a result of the extract application come from the phenolic substances. According to the Pearson correlation analysis, a significant ($p<0,05$) positive correlation was found between the DPPH inhibitory capacities and cytotoxic effects of the extracts (methanol and ethanol) (Correlation coefficient DLD1- DPPH; Scumet 24-48h: 0.914 and 0.902; Scuet 24-48h: 0.893 and 0.930; HL60 -DPPH; Scumet 24-48h: 0.934 and 0.942; Scuet 24-48h: 0.902 and 0.907 respectively). On the other hand, the antioxidant and cytotoxic activity of Scuaq had a positive relationship (correlation coefficient DLD1- DPPH; 24-48h 0,761 and 0,857; HL60 -DPPH; 24-48h: 0,791 and 0,675 respectively), but that was not significant ($p>0,05$). Apoptosis, a programmed cell death that does not affect surrounding cells in terms of its mode of action, is considered a good target for anticancer therapy (Pfeffer and Singh, 2018). We determined by qRT-PCR that *S. cuneifolia* extracts showed apoptosis-triggering effects on pro-apoptotic and anti-apoptotic gene expressions in DLD1 cells. In the present study, apaf-1, tp53, card4 and Casp-3 genes were found to be upregulated in cancer cells after exposure to these extracts; these proteins play an important role in tumour suppression, cell cycle arrest and application phase of cell apoptosis (Delbridge et al., 2012; Kao et al., 2015; Kobayashi et al., 2000; Mattson and Bazan, 2012; Umetani et al., 2004; Carneiro and El-Deiry, 2020; Yu et al., 2019). On the other hand, the increase in the interrelated expressions of card4 and apaf-1 in the apoptotic pathway with the administration of aqueous extract may indicate that the aqueous extract causes apoptosis, but the pathway may be different. Representative CARD-containing proteins in apoptosis signalling are associated with apoptotic protease activating factor-1 (apaf-1) and receptor-interactive serine/threonine-protein kinase (RIP) (Rodriguez and Lazebnik, 1999; Shakeri et al., 2017). Based on the data we have obtained; we can clearly say that more studies are needed to fully elucidate the apoptotic pathways followed by the extracts. Our results show that treatment of cancer cells with *S. cuneifolia* extracts leads to changes in the expression pattern of apoptosis-related genes. Although apoptosis-inducing effects of *S. cuneifolia* have not been previously reported, we have found reports of similar effects from extracts from other species of the genus *Satureja*. Asadipour, et al. (Asadipour et al., 2020) reported the anti-leukemic effects of dichloromethane and hexane extracts of *S. bachtiarica* due to induction of apoptosis on K562 and Jurkat cells. It was previously reported that *S. subspicata* and *S. horvatii* induce apoptosis in human lymphocyte cell culture in mice by regulating pro-apoptotic and anti-apoptotic gene expressions in vitro and in vivo (Čakar et al., 2018). In addition, in another study, it was reported that *S. khuzestanica* extract induced apoptosis in human breast cancer cells (MCF-7)(Esmaeili-Mahani et al., 2018).

CONCLUSIONS

This study once again showed that herbal treatment methods have an increasing potential and importance in the fight against cancer. It is of particular importance that this effect originates from a plant that is frequently consumed as a spice in meals. Such studies, which try to determine active ingredients from extracts or drugs obtained from wild plant sources and to reveal their pharmaceutical potential, are very important for more specific studies to be conducted in the future. This study once again revealed the potential effects of *Satureja* species. The use of extracts and/or active substances obtained from *Satureja* species in the treatment of cancer may be promising. While some of the results obtained in this study are reported for the first time, other findings are in agreement with previous studies. And this supports the need for further investigation of the molecular action pathways of the extracts. Among the studies planned to be done in the future is the discovery of the active ingredient(s) that may be found in the extracts. In the next step, it will be determined whether there is a single substance responsible for the effect or if there is more than one substance with a synergistic effect. In addition, it will be tried to determine at the molecular level which pathways are triggered in apoptotic cell death by using different markers.

ACKNOWLEDGEMENT

This study was financially supported by Selçuk University Scientific Research Projects Coordinatory (Project No: 18401144).

Authors' contribution

Simsek Sezer, EN designed the study, carried out the experiments and wrote the manuscript.

REFERENCES

Abotaleb, M., Liskova, A., Kubatka, P. and Büsselberg, D. 2020. Therapeutic potential of plant phenolic acids in the treatment of cancer. Biomolecules. 10: 221.

Albano, S. M. and M. G. Miguel. 2011. Biological activities of extracts of plants grown in Portugal. Ind. Crops Prod. 33: 338-343.

Asadipour, M., S. Malek-Hosseini and Z. Amirghofran. 2020. Anti-leukemic activity of *Satureja bachtiarica* occurs by apoptosis in human cells. Biotech. Histochem. 95: 506-513.

Atanasov, A. G., S. B. Zotchev, V. M. Dirsch and C. T. Supuran. 2021. Natural products in drug discovery: Advances and opportunities. Nat. Rev. Drug Discov. 20: 200-216.

ben Sghaier, M., A. Pagano, M. Mousslim, Y. Ammari, H. Kovacic and J. Luis. 2016. Rutin inhibits proliferation, attenuates superoxide production and decreases adhesion and migration of human cancerous cells. Biomed. Pharmacother. 84: 1972-1978.

Bezić, N., I. Šamanić, V. Dunkić, V. Besendorfer and J. Puizina. 2009. Essential oil composition and internal transcribed spacer (ITS) sequence variability of four South-Croatian *Satureja* species (*Lamiaceae*). Molecules. 14: 925-938.

Bezić, N., M. Skočibušić and V. Dunkić. 1999. Antimicrobial effect of *Satureja cuneifolia* Ten. Essential oil. Acta Bot. Croat. 58: 99-104.

Bezić, N., Skočibušić, M. and Dunkić, V. 2005. Phytochemical composition and antimicrobial activity of *Satureja montana* L. and *Satureja cuneifolia* Ten. Essential oils. Acta Bot. Croat. 64: 313-322.

Bhebhe, M., T. N. Füller, B. Chipurura and M. Muchuweti. 2016. Effect of solvent type on total phenolic content and free radical scavenging activity of black tea and herbal infusions. Food Anal. Methods. 9: 1060-1067.

Boutayeb, A. and S. Boutayeb. 2005. The burden of non-communicable diseases in developing countries. Int. J. Equity Health. 4: 1-8.

Čakar, J., N. K. Lojo, A. Haverić, M. Hadžić, L. Lasić, S. C. Zeljković, S. Haverić and K. Bajrović. 2018. *Satureja subspicata* and *S. horvatii* Extracts induce overexpression of the BCl-2 family of anti-apoptotic genes and reduce micronuclei frequency in mice. Nat. Prod. Commun. 13: 1934578X1801300617.

Carneiro, B. A. and W. S. El-Deiry. 2020. Targeting apoptosis in cancer therapy. Nat. Rev. Clin. Oncol. 17: 395-417.

Ćavar, S., M. Maksimović, M. E. Šolić, A. Jerković-Mujkić and R. Bešta. 2008. Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. Food Chem. 111: 648-653.

Chen, S., J. Gong, F. Liu and U. Mohammed. 2000. Naturally occurring polyphenolic antioxidants modulate IgE-mediated mast cell activation. Immunology. 100(4): 471-480.

Chu, Y. H., C. L. Chang and H. F. Hsu. 2000. Flavonoid content of several vegetables and their antioxidant activity. J. Sci. Food Agric. 80: 561-566.

Cragg, G. M. and D. J. Newman. 2005. Plants as a source of anti-cancer agents. J. Ethnopharmacol. 100: 72-79.

Delbridge, A. R. D., L. J. Valente and A. Strasser. 2012. The role of the apoptotic machinery in tumor suppression. Cold Spring Harb Perspect Biol. 4: a008789.

Dorman, H. D., O. Bachmayer, M. Kosar and R. Hiltunen. 2004. Antioxidant properties of aqueous extracts from selected *Lamiaceae* species grown in Turkey. J. Agric. Food Chem. 52: 762-770.

Eminagaoglu, O., B. Tepe, O. Yumrutas, H. A. Akpulat, D. Daferera, M. Polissiou and A. Sokmen. 2007. The *in vitro* antioxidative properties of the essential oils and methanol extracts of *Satureja spicigera* (K. Koch.) Boiss. and *Satureja cuneifolia* ten. Food Chem. 100: 339-343.

Esmaeili-Mahani, S., M. R. Samandari-Bahraseman and M. M. Yaghoobi. 2018. *In-vitro* anti-proliferative and pro-apoptotic properties of *Sutureja khuzestanica* on human breast cancer cell line (MCF-7) and its synergic effects with anticancer drug vincristine. Iran. J. Pharm. Res. 17: 343.

Ganamé, H. T., Y. Karanga, I. Tapsoba, M. Dicato, M. F. Diederich, C. Cerella and R. W. Sawadogo. 2021. Phytochemical screening and antioxidant and cytotoxic effects of *Acacia macrostachya*. Plants (Basel). 10: 1353.

Gani, S. A., S. A. Muhammad, A. U. Kura, F. Barahuie, M. Z. Hussein and S. Fakurazi. 2019. Effect of protocatechuic acid-layered double hydroxide nanoparticles on diethylnitrosamine/phenobarbital-induced hepatocellular carcinoma in mice. PloS One. 14: e0217009.

Gezici, S. and N. Şekeroğlu. 2019. Current perspectives in the application of medicinal plants against cancer: Novel therapeutic agents. Anticancer Agents Med. Chem. 19: 101-111.

Gheena, S. and D. Ezhilarasan. 2019. Syringic acid triggers reactive oxygen species-mediated cytotoxicity in HepG2 cells. Hum. Exp. Toxicol. 38: 694-702.

Gülçin, I. 2012. Antioxidant activity of food constituents: An overview. Arch. Toxicol. 86: 345-391.

Güner, A., S. Aslan, T. Ekim, M. Vural and M. Babaç. 2012. Türkiye Bitkileri Listesi. Damarlı Bitkiler, Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, Oregon, p. 262.

Guon, T. E. and H. S. Chung. 2016. Hyperoside and rutin of Nelumbo nucifera induce mitochondrial apoptosis through a caspase-dependent mechanism in HT-29 human colon cancer cells. Oncol. Lett. 11: 2463-2470.

Jung, C. H., J. Y. Lee, C. H. Cho and C. J. Kim. 2007. Anti-asthmatic action of quercetin and rutin in conscious guinea-pigs challenged with aerosolized ovalbumin. Arch. Pharm. Res. 30: 1599-1607.

Kan, Y., U. S. Uçan, M. Kartal, M. L. Altun, S. Aslan, E. Sayar and T. Ceyhan. 2006. GC-MS analysis and antibacterial activity of cultivated *Satureja cuneifolia* Ten. Essential oil. Turk. J. Chem. 30: 253-259.

Kanimozhi, G. and N. Prasad. 2015. Anticancer effect of Caffeic acid on human cervical cancer cells. In: Coffee in Health and Disease Prevention. Elsevier, Netherlands, pp. 655-661.

Kao, W. P., C. Y. Yang, T. W. Su, Y. T. Wang, Y. C. Lo and S. C. Lin. 2015. The versatile roles of CARDs in regulating apoptosis, inflammation, and NF-κB signaling. Apoptosis. 20: 174-195.

Kobayashi, T., H. Sawa, J. Morikawa, W. Zhang and H. Shiku. 2000. Bax induction activates apoptotic cascade via mitochondrial cytochrome c release and Bax overexpression enhances apoptosis induced by chemotherapeutic agents in DLD-1 colon cancer cells. Jpn. J. Cancer Res. 91: 1264-1268.

Kowalczyk, T., P. Sitarek, E. Skała, M. Toma, M. Wielanek, D. Pytel, J. Wieczfińska, J. Szemraj and T. Śliwiński. 2019. Induction of apoptosis by *in vitro* and *in vivo* plant extracts derived from *Menyanthes trifoliata* L. in human cancer cells. Cytotechnology. 71: 165-180.

Kumar, S. and A. K. Pandey. 2013. Chemistry and biological activities of flavonoids: An overview. ScientificWorldJournal. 2013: 162750.

Kuntz, S., U. Wenzel and H. Daniel. 1999. Comparative analysis of the effects of flavonoids on proliferation, cytotoxicity, and apoptosis in human colon cancer cell lines. Eur. J. Nutr. 38: 133-142.

Lamaison, J., C. Petitjean-Freytet and A. Carnat. 1991. Medicinal *Lamiaceae* with antioxidant properties, a potential source of rosmarinic acid. Pharm. Acta Helv. 66: 185-188.

Le, N. T., M. G. Donadu, D. V. Ho, T. Q. Doan, A. T. Le, R. Ain, U. Donatella, G. Sanna, M. Mauro and U. Marianna. 2020. Biological activities of essential oil extracted from leaves of *Atalantia sessiflora* Guillauminin Vietnam. J. Infect. Dev. Ctries. 14:1054-1064.

Lin, H. H., J. H. Chen, F. P. Chou and C. J. Wang. 2011. Protocatechuic acid inhibits cancer cell metastasis involving the down-regulation of Ras/Akt/NF-κB pathway and MMP-2 production by targeting RhoB activation. Br. J. Pharmacol. 162: 237-254.

Livak, K. J., Q. F. Wills, A. J. Tipping, K. Datta, R. Mittal, A. J. Goldson,

D. W. Sexton and C. C. Holmes. 2013. Methods for qPCR gene expression profiling applied to 1440 lymphoblastoid single cells. Methods. 59: 71-79.

Mattson, M. P. and N. G. Bazan. 2012. Apoptosis and necrosis. In: Basic Neurochemistry. Elsevier, Netherlands, pp. 663-676.

Menon, L. G., R. Kuttan and G. Kuttan. 1995. Inhibition of lung metastasis in mice induced by B16F10 melanoma cells by polyphenolic compounds. Cancer Lett. 95: 221-225.

Milos, M., A. Radonic, N. Bezic and V. Dunkic. 2001. Localities and seasonal variations in the chemical composition of essential oils of *Satureja montana* L. and *S. cuneifolia* Ten. Flavour Fragr. J. 16: 157-160.

Mueller-Riebau, F., B. Berger and O. Yegen. 1995. Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. J. Agric. Food Chem. 43: 2262-2266.

Naczk, M. and F. Shahidi. 2006. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. J. Pharm. Biomed. Anal. 41: 1523-1542.

Oke, F., B, Aslım, S. Ozturk and S. Altundag. 2009. Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. Food Chem. 112: 874-879.

Pan, L., H. Chai and A. D. Kinghorn. 2010. The continuing search for antitumor agents from higher plants. Phytochem. Lett. 3: 1-8.

Pelinson, L. P., C. E. Assmann, T. V. Palma, I. B. M. da Cruz, M. M. Pillat, A. Mânica, N. Stefanello, G. C. C. Weis, A. de Oliveira Alves and C. M. de Andrade. 2019. Antiproliferative and apoptotic effects of caffeic acid on SK-Mel-28 human melanoma cancer cells. Mol. Biol. Rep. 46: 2085-2092.

Pfeffer, C. M. and A. T. Singh. 2018. Apoptosis: A target for anticancer therapy. Int. J. Mol. Sci. 19: 448.

Quinn, L., S. G. Gray, S. Meaney, S. Finn, O. Kenny and M. Hayes. 2017. Sinapinic and protocatechuic acids found in rapeseed: Isolation, characterisation and potential benefits for human health as functional food ingredients. Ir. J. Agric. Food Res. 56: 104-119.

Raaman, N. 2006. Phytochemical Techniques. New India Publishing, India.

Rodriguez, J. and Y. Lazebnik. 1999. Caspase-9 and APAF-1 form an active holoenzyme. Genes Dev. 13: 3179-3184.

Semaming, Y., P. Pannengpetch, S. C. Chattipakorn and N. Chattipakorn. 2015. Pharmacological properties of protocatechuic acid and its potential roles as complementary medicine. Evid. Based Complement Alternat. Med. 2015: 593902.

Sewell, R. D. and M. Rafieian-Kopaei. 2014. The history and ups and downs of herbal medicines usage. J. Herbmed Pharmacol. 3: 1-3.

Shakeri, R., A. Kheirollahi and J. Davoodi. 2017. Apaf-1: Regulation and function in cell death. Biochimie. 135: 111-125.

Siegel, R. L., K. D. Miller and A. Jemal. 2016. Cancer statistics, 2016. CA Cancer J. Clin. 66: 7-30.

Şimşek Sezer, E. N. and T. Uysal. 2021. Phytochemical analysis, antioxidant and anticancer potential of *Sideritis niveotomentosa*: Endemic Wild Species of Turkey. Molecules. 26: 2420.

Song, M., B. Vogelstein, E. L. Giovannucci, W. C. Willett and C. Tomasetti. 2018. Cancer prevention: Molecular and epidemiologic consensus. Science. 361: 1317-1318.

Taskin, T., M. Dogan, T. Sahin and I. Senkardeş. 2020. *In vitro* anti-urease, antioxidant, anticholinesterase, cytotoxic and *in vivo* anti-inflammatory potential of *Satureja cuneifolia* Ten. Not. Sci. Biol. 12: 222-232.

Taslimi, P., E. Köksal, A. C. Gören, E. Bursal, A. Aras, O. Kılıç, S. Alwasel and I. Gülçin. 2020. Anti-Alzheimer, antidiabetic and antioxidant potential of *Satureja cuneifolia* and analysis of its phenolic contents by LC-MS/MS. Arab. J. Chem. 13: 4528-4537.

Tepe, B. and M. Cilkiz. 2016. A pharmacological and phytochemical overview on *Satureja*. Pharm. Biol. 54: 375-412.

Umetani, N., A. Fujimoto, H. Takeuchi, M. Shinozaki, A. J. Bilchik and D. S. Hoon. 2004. Allelic imbalance of APAF-1 locus at 12q23 is related to progression of colorectal carcinoma. Oncogene. 23: 8292-8300.

Wen, D., C. Li, H. Di, Y. Liao and H. Liu. 2005. A universal HPLC method for the determination of phenolic acids in compound herbal medicines. J. Agric. Food Chem. 53: 6624-6629.

Yu, Z., Q. Li, Y. An, X. Chen, Z. Liu, Z. Li, J. Gao, L. H. H. Aung and P. Li. 2019. Role of apoptosis repressor with caspase recruitment domain (ARC) in cancer. Oncol. Lett. 18: 5691-5698.

Yücel, D. 2018. Cytotoxic effects of *Satureja cuneifolia* extract in liver cancer cell line (HepG2). Biol. Divers. Conserv. 11: 42-46.