COMPARISON OF PROFILES, ANTIOXIDANT, ENZYME INHIBITORY AND CYTOTOXIC CAPACITIES OF PHENOLIC EXTRACTS OF FIVE Teucrium SPECIES FROM TURKIYE

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ABSTRACT

The study aims to investigate the phenolic compounds, antioxidant-enzyme inhibitory activities, and cytotoxic effects of the flavonoid subgroups and phenolic acid extracts of the *Teucrium chamaedrys* ssp *chamaedrys* (*TCC*), *T. chamaedrys* ssp *lydium* (*TCL*), *T. polium* (*TP*), endemic *T. alyssifolium* (*TA*), *T. kotschyanum* (*TK*) from Türkiye. Among the phenolics determined, phenolic acids were found to be in the highest concentration, particularly caffeic, p-coumaric, and t-cinnamic acids. In term of antioxidant activity, the best IC_{50} values of 1,1-diphenyl-2-picrylhydrazyl (23.21±0.78 µg/mL), hydroxyl (HO•) (1.01±0.01 µg/mL), nitric oxide scavenging (12.28±0.91 µg/mL) and metal chelating (20.10±0.66 µg/mL) were determined in *TP, TCL, TK*, and *TA*, respectively. Additionally, *TCL* has a 5.05-times higher quenching capacity of HO• compared to butylated-hydroxytoluene. The best acetylcholinesterase and tyrosinase inhibitions were detected in *TK* and *TCC*, respectively. While the cytotoxic properties of these extracts against HepG2, OE-33, HeLa, ACC-201, and MCF-7 cancer cells were detected, more extensive effect was observed against HepG2 in *TCC*, *TP, TA,* and *TCL*. According to the results, the richness of *Teucrium* species in phenolic compounds, the importance of their antioxidant-enzyme inhibitory properties and their cytotoxic effects make their potential as natural component sources in food and medical come to the fore.

Keywords: Antioxidant activity, cytotoxicity, enzyme inhibitory potential, extraction, phenolics, *Teucrium*.

1. INTRODUCTION

Plants are available resources of bioactive compounds and have been used as main resources in the areas of food, food additive, cosmetic, pharmacognostic, and medicine throughout history [1,2]. Phenolic compounds form common category of secondary plant metabolites with a broad variety of structures and actions. They are categorised like flavonoids, tannins, phenolic acids, lignans, and other compounds [3]. They show different biological possessions containing antioxidant activities, enzyme inhibitory, and cytotoxic properties [4-6]. Inhibition of acetylcholinesterase (AChE) via natural phenolic components is popular study due to its relation with Alzheimer's disorder [7]. On the other hand, the loss of melanin and obtaining depigmentation can possess important aesthetic and dermatological problems, while raised melanin production and accretion are related with different skin diseases, neurodegeneration led to Parkinson's disorder, and integument cancer [8]. One of the efficient procedures for inspecting skin pigmentation is prohibition of the tyrosinase enzyme, which catalyses the essential stages of melanogenesis. In addition, there is attention in detecting the potential of natural plant antioxidants due to the possible detrimental actions of synthetic antioxidants on human healthiness. Therefore, the phenolic compounds extraction with different structures and properties from plants utilising various procedures comes to the fore due to their various bioactivities and nutritional characteristics [3].

The species of the genus *Teucrium* are among the plants that attract attention in the world because they are overmuch for various secondary metabolites, especially phenolics, with very important bioactivities [9]. *Teucrium* is one of the genera of the Lamiaceae family contains over than 300 species spread in the Mediterranean zone, Africa, Europe, Asia, Australia, and America [10]. *Teucrium* species have been utilised to be medicinal plants for over than 2000 years, and generally in public medicine [11]. In addition, owing to the quantitative and qualitative conformation of their secondary biometabolites, *Teucrium* species display different bioactivities like antioxidant, anticancer, antidiabetic, antimicrobial and antiviral activity [4, 12]. There are 35 *Teucrium* species in Türkiye and 16 of these are endemic [13]. Based on traditional medicine, *Teucrium* species are utilised for curing many disorders, containing abdominal pain, flu, mouth ulcers, kidney infection, hearth disease, cancer, etc., due to have various secondary metabolites [11, 14]. When the phenolic components in extracts of *Teucrium* plants are examined, it is seen that they are especially plenty of phenylethanoid and phenylpropanoid, flavonoids, phenolic acids, and lignans [10]. The most studied species of *Teucrium* genus are *T. polium, T. chamaedrys,* and *T. montanum,* which are plenty of phenolics with important bioactivity [14-16]. The main flavonoids of *T. chamaedrys* are apigenin and luteolin, which are from the flavone subgroup [10]. Twenty extracts were prepared from the leave, flower and stem of from the leaves, flowers and stems of *T. montanum L. var. montanum, f. supinum (L.) Reichenb* from Central Serbia with water, methanol, acetone, ethyl acetate, petroleum ether [16]. While the total phenolic contents in the extracts altered betwixt 8.33 to 169.06 mg_{GA}/g, the contents of total flavonoids changed from 3.96 to 88.31mg_{RU}/g. The results of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging activity expressed as IC_{50} changed from 29.41 (for whole plant water-extract) to 2408.47 µg/mL. *T. flavum L., T. fruticans L.,* and *T. siculum* Rafin are *Teucrium* species commonly found in Italy, described by mono- and sesqui-terpene hydrocarbons, flavonoids, fatty esters and essential oils, and their total phenolic and total flavonoid amounts and antioxidant and their anti-bacterial effects were studied [17]. The findings found displayed that these extracts prepared by air-dried inflorescence with maceration in ethanol/water (80/20, v/v) possessed antimicrobial against the Gram (-) and (+) bacteria examined and also antioxidant effects. In the research studied by Özer et al. [18], phenolics and antioxidant activities of decoction as well as infusion samples of *T. polium* harvested from Turkey prepared with distilled water were reported, and the main compounds detected in the decoction sample were fumaric acid, luteolin-7-O-glycoside, luteolin-5-O-glycoside, and pelargonin, and were determined higher than those obtained in the infusion sample. It was stated that the antioxidant activities determined by DPPH•, β-carotene linoleic acid and CUPRAC in decoction sample have better than their infusion sample [18]. In Iran, *T. polium* L. is widely utilised in daily diet and for medicinal and nutritional properties, and phytochemical and bioactivity studies [19]. This plant aerial parts were extracted with solvents like petroleum ether, chloroform, methanol and water and methanol extract came to the fore in terms of major flavonoids such as rutin and apigenin and antioxidant activity. The best IC_{50} value for DPPH• scavenging was detected in the methanol extract as 20.1±1.7 ppm. The contents of total phenolic and total flavonoid and antioxidant properties of extracts that produced from aerial parts using twice maceration with dichloromethane, acetone, ethanol, hydro-alcohol (1:2, v/v) and water and essential oils of *T. polium* grown in Tunisia were evaluated and their levels were detected as 48.88±0.01 to 400.00±0.01 mg_{GAE}/g, and 2.75±0.01 to 38.85±0.04 mg_{QE}/g, respectively [20]. The best IC_{50} value for DPPH• quenching was recorded for acetone extract as 13±0.02 µg/mL. Additionally, ethanol extracts of *T. arduini*, *T. chamaedrys*, *T. montanum*, and *T. polium* from Croatia, containing rosmarinic and chlorogenic acids, luteolin and apigenin, have been reported to show inhibitory activity against AChE [12]. Also, Boghrati et al [21] found promising antioxidant and anti-tyrosinase activity in methanol extract of *T. polium* L. var. *gnaphalodes* from Iran. The anticancer effects of *Teucrium* species are related to high contents of various chemical secondary metabolites especially phenolics. Therefore, the effect of the obtained *Teucrium* extracts shows that it is not only an individual component but also a synergistic interaction [9].

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The methanol extract prepared by *T. polium* seeds from Saudi Arabia was found to possess the high concentration of phenolics, containing flavonoids, anthocyanins and saponins which were included in anti-cancer effect on MCF-7 (IC_{50}; 20.2 µg/mL) and HepG-2 (IC_{50}; 143.1 µg/mL) [22]. Stanković et al [23] reported that the best IC_{50} value of the methanol extract of *T. chamaedrys* aerial flowering parts from Serbia and Montenegro against HeLa cells determined as 146.47 µg/mL. To the best of our knowledge, there are no data for most of the examined *Teucrium* extracts containing the cytotoxicity against OE-33 cancer cell line [9] and AChE inhibitory properties, with the exception of *T. polium,* and *T. chamaedrys* [12]. Moreover, there is no comprehensive phytochemical and bioactivity studies especially on *T. alyssifolium* and *T. kotschyanum*.

The present study aims to research the bioactive compounds from the extracts of flavonoid subgroups such as flavanols, flavan-3-ols, flavan-3-ols with acid hydrolysis (AH), flavanones, flavones, and phenolic acid extracts of the leaves and flowers of the *Teucrium chamaedrys* ssp *chamaedrys (hairless), T. chamaedrys* ssp *lydium (hairy), T. polium,* endemic *T. alyssifolium, T. kotschyanum* from Türkiye in respect of their phenolic contents, antioxidant activity, enzyme inhibition and cytotoxic effects. Antioxidant activities like DPPH•, hydroxyl (HO•), and nitric oxide (NO•) radical scavenging; metal chelating, total reducing power, ferric reducing antioxidant power (FRAP) the total phenolic, flavonoid and, tannin contents were investigated in the phenolic extracts of these *Teucrium* species. Moreover, AChE and tyrosinase enzyme inhibitory properties of these extracts were also studied. Furthermore, the cytotoxicity effects of the *Teucrium* extracts against human cervical carcinoma (HeLa), oesophageal adenocarcinoma (OE-33), hepatocellular carcinoma (HepG2), breast adenocarcinoma (MCF-7), and gastric adenocarcinoma (ACC-201) cancer cells were investigated.

2. EXPERIMENTAL

2.1 Plant material

All samples of these *Teucrium* species were harvested from Türkiye at the time the flourishing duration in June 2018 and properties were presented in Table I. Taxonomic definitions of the harvested plant parts were performed in accord with Davis [24]. Voucher specimens were stored in the Dokuz Eylül University Herbarium of the Fauna and Flora Research and Application Centre (FAMER), Türkiye.

Table I. *Teucrium* species investigated in this study.

Plant name and abbreviation	Sections	Harvested region from Türkiye	Voucher specimens
T. chamaedrys ssp *chamaedrys (hairless)- TCC*	Chamaedrys	Gündalan Plateu, Bozdağ/Izmir, 1453m; 37º35′14″N, 29º 07′31″E	FAMER 1450
T. chamaedrys ssp *lydium (hairy)- TCL*	Chamaedrys	Gömbe Plateu, Kaş/Antalya, 1754m; 37º35′14″N, 29º 07′31″E	FAMER 1410
T. polium-TP	Polium	Kurucuova, Sandras Mountain/Muğla, 1794m; 37º35′14″N, 29º 07′31″E	FAMER 0602
T. alyssifolium-TA (endemic)	Teucrium	Sandras Mountain/Muğla, 1707m; 37º35′14″N, 29º 07′31″E	FAMER 1121
T. kotschyanum-TK	Scorodonia	Nif Mountain/Izmir, 560m; 37º35′14″N, 29º 07′31″E	FAMER 1476

2.2. Preparation of *Teucrium* extracts

The leaves and flowers of *Teucrium* species were air dried in darkness. Equal amounts of flower and leaf mixtures were prepared and ground, and the *Teucrium* samples were weighed to 20 g and refluxed with n-hexane for 6 h in a Soxhlet tool for eliminating the oils. The n-hexane liquid phase was discarded for each sample and then the solid part was lyophilized. The samples were deposited at -20 °C before preparing the phenolic extracts.

2.2.1. Extractions of flavonoid subgroups including the flavanols, flavan-3-ols, flavan-3-ols AH, flavanones, and flavones

3 g dried *Teucrium* samples in the mixture of 150 mL of aqueous methanol (95%) and 45 mL of 25% HCl via a Soxhlet system for 1 h were extracted for the flavanols [25]. After filtration, sequential solid-liquid extraction with 60 mL of methanol was applied two times for 10 min. The mixed extracts were evaporated and then lyophilised.

3 g dried plant samples in 60 mL of methanol were extracted for the flavan-3-ols in an ultrasonic bath at 65 °C for 2 h and thereafter centrifuged [26]. The supernatants were separated and evaporated. Then the obtained extracts were lyophilised.

For the flavan-3-ol AH extraction, the pellet separated as a result of the flavan-3-ols extraction process described above was exposed to acid hydrolysis in 100 mL of HCl/methanol medium (4/1, v/v) at 100 °C for 2 h after sequential liquid-liquid extraction with 45 mL of diethyl ether and 45 mL of ethyl acetate [27]. The obtained extracts were combined, evaporated, and lyophilised.

3 g dried plant samples were extracted for the flavanones with 80% ethanol/water (5/1, v/v) at 85 °C for 2 h and centrifugated [28]. The liquid phases of the extracts were evaporated and lyophilised.

3 g dried *Teucrium* samples in 225 mL of diethyl ether medium for 20 min were extracted for the flavones by stirring [29]. After the blend was filtered, the solid part was extracted twice in 100 mL of diethyl ether for 10 min. The obtained liquid phases were mixed, evaporated in a rotary evaporator system, and lyophilised.

2.2.2. Extraction of phenolic acids

First, 7 g dried *Teucrium* samples were prepared by combining the liquid phases after two extraction procedures for 1h under room conditions in the presence of 80% methanol/water (5/1, v/v) [30]. Half of the evaporated and then lyophilised extract was solved in 14 mL of water at pH 2.0, and then liquid-liquid extraction with 14 mL of diethyl ether was repeated three times. The ether phase consisting of free phenolic acids (FPAs) was evaporated. The water part was set to pH 7.0 with 2 M NaOH and then evaporated. The obtained extract was redissolved in 14 mL of 2 M NaOH and subsequently mixed at 25 °C for 4 h. The resulting mixture was set to pH 2.0 with 6 M HCl and thereafter extracted with diethyl ether as defined above. The ether part in the extract contained base-hydrolysed phenolic acids (BHPAs). The water part was again combined with 14 mL of 6 M HCl at 95 °C for 20 min and extracted with diethyl ether. The resulting fraction was defined as acid-hydrolysed phenolic acids (AHPAs). At this stage, the other half of the solid part remaining from the methanol extraction process described above was hydrolysed in 65 mL of 2 M NaOH at 25 °C for 4 h. The liquid phase was separated and subsequently hydrolysed again in 6 M HCl at 95 °C for 1 h. These extracts are referred to in the following text as the phenolic acid: solid-base hydrolysis 1 ($PASB_1$) and phenolic acid: solid-acid hydrolysis 2 ($PASA_2$) fractions, respectively. Finally, the remaining half of the solid sample was subjected to acid hydrolysis and thereafter base hydrolysis under the same conditions. These extracts were referred to as the phenolic acid: solid-acid hydrolysis 1 ($PASA_1$) and phenolic acid: solid-base hydrolysis 2 ($PASB_2$) parts, respectively.

2.3. Analysis of phenolic compounds by RP-HPLC-DAD

Analyses of phenolic components in the *Teucrium* extracts were conducted utilising an HPLC-Agilent 1200 Series including an UV-DAD and fluorescence detectors. HPLC analysis was performed with a C18 reverse-phase column (150 mm × 4.6 mm, 5 µm particles). Dried all extracts and phenolic standards used (1 mg/mL) were solved in methanol (99.7%), filtered (0.45 µm), and analysed in triplicate with the HPLC system. Quantitative determination of phenolics was carried out considering the peak areas of calibration curves created according to the Rt values of the chromatograms of the standards. The standards used, the corresponding Rt values, and calibration curve equations (Supporting Information, Table S1) are presented as supplementary data with the chromatograms and phenolic amounts of the extracts (Supporting Information, Figs. S1-S40, Tables S2-S31). In the HPLC analysis, the injection volume was 20 µL, and the temperature of column was 30 °C.

Determination of flavanols was carried out by the method recommended by Olszewska [25]. A mobile solvent mixture containing (A) 0.5% orthophosphoric acid/water with (B) methanol was applied with the following gradient system at room temperature: 0-10 min, 40-60% B; 10-21 min, 60% B; 21-23 min, 60-40% B; 23-26 min, 40% B; 26-30 min, 40% B. The flow speed was 1 mL/min, and the procedure was conducted at 370 nm and 254 nm.

Determination of flavan-3-ols was achieved following the assay recommended by de Villiers et al [26]. The mobile phase including (A) 2% acetic acid/water with (B) 70/30 acetonitrile/water was applied with the following gradient system: 0-3 min, 5% B; 3-8 min, 5-15% B; 8-10 min, 15-20% B; 10-12 min, 20-25% B; 12-20 min, 25-40% B; 20-30 min, 40-80% B at a flow speed was 1.2 mL/min. The analysis was performed at 280 nm.

Determination of flavanones was conducted by the assay suggested by Pellati et al [28]. The mobile solvent mixture containing (A) 0.6% acetic acid/water with (B) methanol was applied with the gradient system: 0-5 min, 20-40% B; 5-8 min, 40% B; 8-12 min, 40-60% B; 12-25 min, 60% B; 25-30 min, 60-20% B at a flow speed was 0.4 mL/min. Flavanone analyses were achieved at 285 nm.

Determination of flavones was achieved following the assay recommended by Valentão et al [29]. The mobile solvent mixture including (A) water/formic acid (19/1, v/v) and (B) methanol was applied with the gradient system: 0-5 min, 50% B; 5-30 min, 80% B; 30 min, 80% B at a flow speed of 1 mL/min. Measurements were performed at 350 nm.

Determination of phenolic acids by HPLC was achieved to the assay suggested by Kim et al [30]. A mobile solvent mixture containing (A) acetonitrile with (B) 2% acetic acid/water was applied with the following gradient system at a flow speed of 1 mL/min: 0-30 min, 85-100% B; 30-55 min, 50-85% B; 55-60 min, 0-50% B; 60-65 min, 0-100% B. Derivatives of hydroxybenzoic and hydroxycinnamic acid were analysed at 280 and 320 nm, respectively.

2.4. Total phenolic, total flavonoid, and total tannin contents

Total phenolic contents were detected by utilising the Folin-Ciocalteu reagent and the obtained data were recorded in milligram of gallic acid equivalents per gram dry extract (mg_{GAE}/g_{DWE}) [31]. Shortly, the procedure is dependent on the potential of Folin-Ciocalteu reagent, that a phosphomolybdotungsten hetero acid structure, to act with phenols and produce dense blue. The analysis of the complex obtained was monitored at 765 nm.

Total flavonoid contents were detected by aluminium chloride method [32]. Methanolic extracts and a reaction medium with $AlCl_3.6H_2O$ (10%, w/v) and potassium acetate (1 M) were mixed. The medium was put at 25°C in the dark for 30 min. Thereafter, absorbance was monitored at 415 nm. The contents of each extract were recorded in milligrams of quercetin equivalents per gram of dry extract utilising quercetin to be the standard (mg_{QE}/g_{DWE}).

Total tannin contents were determined with vanillin-HCI assay [33]. A reaction mixture containing methanolic extracts, vanillin solution (4%, w/v), and concentrated HCl was prepared, mixed, and put at 25 °C in the darkness for 20 min. Thereafter, the analysis was monitored at 500 nm. The total contents of tannin in the *Teucrium* extracts were explained as mg tannic acid equivalent per g dry weight extract (mg_{TAE}/g_{DWE}).

2.5. Determination of antioxidant activities

DPPH˙ scavenging: Different concentrations of extracts obtained in methanol were mixed with 1 mM DPPH˙ solution and put in the darkness for 30 min under room conditions [34]. After incubation, the analyses of these extracts were monitored at 517 nm. Ascorbic acid (AA) and butylated hydroxytoluene (BHT) were utilised to be standards.

HO˙ scavenging: The mixture was prepared in a 1.0 mL containing $FeCl_3$ (100 µM), EDTA (104 µM), H_2O_2 (100 µM), AA (100 µM), deoxyribose (2.8 mM), phosphate buffer (20 mM, pH 7.4), and the *Teucrium* extracts at different concentrations [35]. The mixture was kept at 37 °C for 60 min, and afterwards 1 mL of thiobarbituric acid (1%, w/v) and 1 mL of trichloroacetic acid (TCA) (2%, w/v) were included. The reaction medium was kept in a boiling water condition for 15 min. Thereafter, analysis was monitored at 535 nm. BHT was utilised to be a standard.

NO˙ scavenging: The reaction medium containing sodium nitroprusside (10 mM), phosphate-buffered saline (20 mM, pH 7.4), and the *Teucrium* extracts was kept for 150 min at 25 °C [36], and thereafter 0.5 mL was transferred from this mixture and 0.5 mL of Griess reagent (1/1, v/v of 2% (w/v) sulphanilamide and 0.2% (w/v) naphthyl ethylenediamine dihydrochloride) was added. This mixture was kept at 25 °C for 30 min. A_o was formed by including buffer to this medium in place of sample. The analysis of the *Teucrium* extracts was performed at 546 nm. AA was used as a positive standard.

Metal chelating capacity: The metal chelating capacity was detected by monitoring the production of the Fe^{2+}-ferrozine complex [37]. First, this medium including $FeCl_2$ (1 mM), *Teucrium* extracts, and acetate buffer (100 mM, pH 4.0) was kept under room condition for 30 min, and then ferrozine (5 mM) was added. The analysis of the last-coloured complex, declarative of Fe (II) ion chelation with ferrozine, was monitored at 562 nm. EDTA was utilised to be a standard.

IC_{50} values, indicating the concentration at which 50% inhibition has achieved, were detected for each of these radical quenching and metal chelating tests.

Total reducing power: The analysis medium was formed by $K_3Fe(CN)_6$ (1%, w/v), phosphate buffer (0.2 M, pH 6.6), and different concentrations of the sample [38]. After mixing, it was kept at 50 °C for 30 min. Then, TCA (10%, w/v) was added, and this medium was centrifuged. Samples from the upper phase were blended with $FeCl_3$ (0.1%, w/v). Thereafter, the analysis at 700 nm was monitored. A rise in absorbance values represents a rise in total reducing power. The total reducing powers of the *Teucrium* samples were recorded to be mg AA equivalent per g dry weight extract (mg_{AAE}/g_{DWE}).

FRAP assay: The assay is dependent on the decline of achromatic ferric-complex to blue colourful ferrous-complex by the reaction of electron-donating antioxidants at low pH. The reaction mixtures consisted of varying concentrations of *Teucrium* extracts and FRAP solution (25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM 2,4,6-Tris (2-pyridyl)-s-triazine, and 2.5 mL of 20 mM $FeCl_3$) [39]. Then, they were kept for 30 min at 37 °C in the darkness. The change of absorbance at 593 nm was monitored. The FRAP of each extract was recorded to be mg_{AAE}/g_{DWE} [40].

2.6. Determination of enzyme inhibitory potential

AChE inhibition was detected the assay suggested by Grochowski et al [41]. The analysis medium contained 50 µL of extract, 125 µL of 3 mM 5,5-dithio-bis(2-nitrobenzoic) acid (DTNB) and 25 µL of 15 mM acetylthiocholine iodide (ATCI) was prepared. Then, 25 µL of 0.28 U/mL AChE was transferred into the medium, and it was kept for 15 min. Afterward, it was monitored the absorbance at 405 nm. Galantamine was utilised to be a positive standard.

Tyrosinase inhibition was detected by the assay conducted by Uysal et al [42]. The reaction medium including 25 µL of extract, 40 µL of 200 U/mL enzyme, 100 µL of 40 mM phosphate buffer pH 6.8, and 40 µL of 10 mM L-Dopa was added to a microplate. The microplate was then kept at 25 ºC for 5 min and then the analysis was monitored at 492 nm. Kojic acid was utilised to be a standard.

IC_{50} values of *Teucrium* extracts for inhibitions of AChE and tyrosinase enzymes were recorded as µg/mL.

2.7. MTT cell proliferation analysis

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) test is dependent on the biotransformation of MTT to blue formazan crystals by living cells [43]. Cancer cells in suspensions (HeLa (ATCC® CCL-2), ACC-201 (DSMZ 23132/87), OE-33 (DSMZ ACC706), HepG2 (ATCC HB-8065), and MCF-7 (ATCC HTB-22)) were inoculated at 1×10^5 cells per well in 96-well plates and incubated in a humidified environment of 5% CO_2 in air at 37 °C. The cancer cells were kept with different extract concentrations ranging betwixt 20 and 150 ppm in 10% DMSO for 24 and 48 h. Afterwards, MTT (5 mg/mL) was put to each well and then the plates were put for 4 h at 37 °C. The formazan crystals that produced, a sign of alive and active cells, were subsequently solved in dimethyl sulfoxide (DMSO) and analysis was performed at 570 nm. The IC_{50} values of these *Teucrium* extracts were recorded as µg/mL.

2.8. Statistical analyses

All experiments were repeated in triplicate, and the obtained data are offered to be mean±standard deviation. Statistical significance was analysed utilising the ANOVA Student's t-test.

3. RESULTS AND DISCUSSION

Phenolic compounds, which are secondary biometabolites, are natural antioxidants that protect living systems against oxidative stress and prevent damage to intracellular biomolecules [44]. Phenolic compounds are important free radical scavengers owing to their hydrogen donating ability [45].

3.1. Phenolics extraction and profile

In this study, the extraction methods were performed for phenolic compounds like flavonoids containing flavanone, flavanol, flavan-3ol, flavone subgroups, and phenolic acids containing hydroxybenzoic and hydroxycinnamic acid substances. Phenolic components and their amounts, determined by RP-HPLC-DAD in extracts of flavonoid subgroup obtained from the *TCC, TCL, TP, TA, TK* plants examined in this study are shown in Table 2.

Table 2. Flavonoid compounds in the extracts of the *Teucrium* samples determined by RP-HPLC-DAD analysis. Results were expressed as mean±standard deviation of three independent replicates.

Subgroup Extracts of Flavonoids (λ_{nm})	*TCC*	*TCL*	*TP*	*TA*	*TK*
			mg/g_{DWE}[a]		
***Flavanone* (285nm)**					
Hesperidin	nd[b]	1.84±0.04	0.48±0.05	7.75±0.08	9.91±0.12
Naringenin	0.79±0.07	0.34±0.01	0.80±0.07	nd	1.14±0.12
Hesperetin	nd	0.87±0.03	0.45±0.04	0.52±0.04	0.32±0.03
***Flavonol* (254, 370nm)**					
Rutin	nd	nd	nd	nd	nd
Myricetin	3.46±0.07	0.97±0.03	nd	nd	nd
Quercetin	nd	0.40±0.01	nd	nd	nd
Kaempherol	1.17±0.04	0.36±0.01	1.17±0.04	2.41±0.05	0.39±0.01
Isorhamnetin	1.21±0.11	0.84±0.03	1.49±0.02	0.73±0.02	1.88±0.04
Isoquercitrin	nd	nd	nd	nd	nd
Quercitrin	0.63±0.02	1.48±0.04	nd	nd	2.45±0.06
***Flavan-3-ol* (280nm)**					
Catechin	nd	nd	nd	nd	nd
Epicatechin	nd	nd	0.94±0.03	2.79±0.06	1.32±0.04
Epigallocatechin	nd	nd	nd	nd	7.81±0.08
***Flavan-3-ol AH* (280nm)**					
Catechin	nd	nd	nd	nd	nd
Epicatechin	4.19±0.07	4.68±0.08	14.28±0.15	3.12±0.07	2.18±0.05
Epigallocatechin	nd	nd	34.65±1.22	nd	46.23±1.21
***Flavone* (350nm)**					
Apigenin	24.06±0.82	49.50±1.10	35.38±0.92	1.61±0.04	1.62±0.04
Luteolin	4.35±0.07	5.89±0.09	5.14±0.08	5.78±0.08	4.81±0.07
Eupatorin	1.71±0.04	1.83±0.04	21.48±0.85	37.15±0.95	0.59±0.01
Diosmin	nd	nd	nd	0.48±0.01	7.37±0.10
∑ Flavonoids	40.94	69	116.26	62.34	88.02

[a] mg/g_{DWE} mg/g dry weight extract; [b] nd: not detected

When the determined basic flavonoids were compared in terms of their total amounts for each *Teucrium* species examined, the ranking was as follows: *TP* > *TK* > *TCL* > *TA* > *TCC.* As seen in Table 2, the compounds determined in flavonoid extracts come to the forefront in terms of quantity and number of species, primarily flavone, flavan-3-ol AH and flavanone extracts. A total of 17 flavonoid components were detected in the examined *Teucrium* species and rutin, isoquercitrin, and catechin could not be identified. The highest flavonoid levels determined in the examined *Teucrium* flavonoid subgroups were apigenin in *TCC, TCL*, and *TP*-flavone extracts, while for *TA* it was eupatorin in the same extract. In addition, epigallocatechin was detected in the flavan-3-ol AH extract of *TK*. According to the results, the top three components of flavonoids in terms of quantity, apigenin, epigallocatechin and eupatorin were determined in *TCL*-flavone (49.50±1.10 mg/g_{DWE}), *TK*-flavan-3-ol AH (46.23±1.21 mg/g_{DWE}) and *TA*-flavone (37.15±0.95 mg/g_{DWE}) extracts, respectively.

The free phenolic acids in methanol extracts of the five *Teucrium* species examined, as also the hydroxybenzoic and hydroxycinnamic group compounds, and their amounts determined in the acid-base hydrolysed extracts of their liquid and solid phases are showed in Tables 3a and 3b.

Table 3a. Hydroxybenzoic acid components in the extracts of the *Teucrium* samples determined by RP-HPLC-DAD analysis. Results were expressed as mean±standard deviation of three independent replicates.

Phenolic acids (mg/g_{DWE})[a]						
Hydroxybenzoic acid components						
Phenolic Acid Extracts (λ=280nm, 320nm)	**Gallic**	**Proto-catechuic**	**4-hydroxy-benzoic**	**Vanillic**	**Syringic**	**Benzoic**
Free Phenolic Acids (FPA)						
TCC	0.35±0.04	nd[b]	nd	0.37±0.02	nd	nd
TCL	nd	nd	nd	nd	nd	nd
TP	nd	nd	0.48±0.04	nd	0.47±0.02	nd
TA	nd	nd	nd	0.72±0.03	nd	nd
TK	nd	nd	0.46±0.05	2.50±0.13	nd	nd
Base Hydrolysed Phenolic Acids (BHPA)						
TCC	0.29±0.01	nd	2.74±0.06	10.89±0.12	3.52±0.06	9.89±0.12
TCL	0.32±0.02	7.72±0.08	nd	13.36±0.15	3.04±0.07	7.69±0.08
TP	nd	nd	nd	1.32±0.04	25.62±0.82	13.24±0.15
TA	0.49±0.05	19.52±0.37	5.77±0.08	3.84±0.07	22.81±0.74	69.15±1.17
TK	nd	nd	nd	21.90±1.26	9.41±0.10	nd
Acid Hydrolysed Phenolic Acids (AHPA)						
TCC	25.70±0.85	nd	nd	12.46±0.15	nd	3.41±0.08
TCL	20.01±0.72	9.43±0.82	nd	5.44±0.02	0.31±0.01	1.96±0.04
TP	0.49±0.02	0.96±0.09	nd	4.57±0.07	3.40±0.07	2.97±0.06
TA	nd	13.17±1.08	0.52±0.03	5.37±0.15	4.04±0.06	nd
TK	10.80±0.11	4.22±0.12	0.86±0.03	24.67±0.80	1.03±0.04	3.58±0.07
Phenolic Acids of Solid Hydrolysis						
$PASB_1$						
TCC	nd	nd	6.36±0.09	15.46±0.25	nd	5.74±0.08
TCL	nd	nd	8.10±0.10	19.71±0.66	2.40±0.05	50.09±1.12
TP	nd	nd	nd	3.47±0.14	26.07±0.85	nd
TA	nd	nd	2.60±0.05	4.51±0.07	2.62±0.06	21.09±0.70
TK	nd	13.53±1.07	4.35±0.07	6.74±0.09	3.67±0.06	15.82±0.33
$PASA_2$						
TCC	2.29±0.05	2.17±0.05	1.97±0.06	15.26±0.23	2.37±0.06	nd
TCL	2.14±0.05	4.86±0.07	6.33±0.09	nd	nd	nd
TP	nd	2.43±0.05	7.05±0.09	16.94±0.31	49.27±1.13	2.00±0.07
TA	nd	1.27±0.04	4.91±0.08	2.71±0.06	3.42±0.06	nd
TK	nd	3.71±0.13	4.33±0.07	13.78±0.15	3.70±0.07	nd
$PASA_1$						
TCC	nd	2.61±0.05	2.90±0.06	8.76±0.11	2.33±0.05	nd
TCL	0.45±0.05	2.93±0.06	2.98±0.19	8.39±0.10	2.20±0.16	nd
TP	nd	2.99±0.06	3.33±0.07	5.97±0.43	23.58±1.37	nd
TA	1.50±0.04	1.89±0.04	1.43±0.04	2.29±0.05	2.56±0.18	nd
TK	nd	nd	1.48±0.04	3.39±0.07	0.81±0.05	nd
$PASB_2$						
TCC	nd	nd	nd	6.35±0.09	nd	nd
TCL	nd	nd	nd	8.27±0.10	0.67±0.03	nd
TP	nd	0.53±0.02	0.63±0.03	0.83±0.04	4.08±0.07	nd
TA	nd	nd	2.28±0.05	3.28±0.06	1.68±0.04	nd
TK	nd	nd	0.95±0.04	2.13±0.05	0.52±0.02	nd

[a] mg/g_{DWE}; mg/g dry weight extract; [b] nd; not detected

Table 3b. Hydroxycinnamic acid components in the extracts of the *Teucrium* samples determined by RP-HPLC-DAD analysis. Results were expressed as mean±standard deviation of three independent replicates.

Phenolic acids (mg/g_{DWE})[a]							
Hydroxycinnamic acid components							
Phenolic Acid Extracts (λ=280nm, 320nm)	**Caffeic**	**p-Coumaric**	**o-Coumaric**	**Ferulic**	**Sinapic**	**Rosmarinic**	**t-Cinnamic**
Free Phenolic Acids (FPA)							
TCC	0.31±0.04	0.29±0.01	0.56±0.06	0.15±0.005	nd[b]	nd	15.24±0.72
TCL	0.21±0.02	0.44±0.05	nd	0.20±0.03	nd	0.77±0.03	3.96±0.45
TP	0.28±0.03	1.26±0.04	nd	0.20±0.01	nd	0.55±0.02	80.79±1.32
TA	nd	0.22±0.01	0.22±0.03	nd	nd	nd	6.96±0.71
TK	nd	nd	0.27±0.03	nd	nd	0.58±0.06	15.24±0.25
Base Hydrolysed Phenolic Acids (BHPA)							
TCC	154.81±7.19	16.56±1.27	5.16±0.61	59.83±2.92	nd	4.26±0.07	33.67±1.35
TCL	85.87±1.43	13.70±0.15	2.22±0.16	56.42±1.22	nd	1.90±0.04	13.69±1.23
TP	1.91±0.05	11.89±0.13	nd	5.55±0.08	nd	0.72±0.03	nd
TA	23.60±0.77	37.38±1.05	6.12±0.07	55.65±1.20	8.98±0.10	13.43±0.15	43.02±1.14
TK	6.10±0.07	85.71±1.41	0.38±0.04	nd	1.46±0.12	6.48±0.08	51.90±3.08
Acid Hydrolysed Phenolic Acids (AHPA)							
TCC	nd	nd	0.96±0.07	1.14±0.12	nd	17.48±1.12	69.12±3.74
TCL	3.81±0.25	12.23±0.24	0.21±0.02	0.89±0.02	nd	17.48±0.36	18.48±1.05
TP	nd	nd	0.64±0.05	nd	0.56±0.06	2.08±0.05	4.45±0.11
TA	nd	0.31±0.02	nd	2.98±0.13	5.87±0.08	1.11±0.03	13.10±0.14
TK	3.68±0.07	18.49±0.32	0.87±0.09	2.05±0.05	nd	6.28±0.08	6.26±0.55
Phenolic Acids of Solid Hydrolysis							
$PASB_1$							
TCC	0.84±0.07	22.31±1.08	0.36±0.03	13.08±0.20	nd	0.54±0.02	6.61±0.35
TCL	3.41±0.27	31.54±1.38	1.21±0.10	50.38±1.15	nd	4.01±0.07	11.84±0.85
TP	5.76±0.24	45.96±2.37	1.43±0.09	35.67±1.02	1.07±0.04	nd	12.64±0.83
TA	1.09±0.08	15.32±1.03	nd	18.17±0.38	0.50±0.03	3.96±0.17	7.05±0.09
TK	1.90±0.11	126.65±5.17	nd	45.44±1.11	nd	0.30±0.01	2.79±0.09
$PASA_2$							
TCC	1.50±0.12	nd	0.67±0.03	0.46±0.04	nd	0.47±0.02	41.19±1.15
TCL	1.90±0.04	1.48±0.05	2.73±0.06	0.42±0.02	nd	2.97±0.06	2.72±0.15
TP	15.17±0.27	4.46±0.07	1.04±0.11	5.05±0.08	0.30±0.03	3.78±0.06	4.17±0.21
TA	nd	0.58±0.02	nd	nd	nd	0.85±0.04	3.24±0.21
TK	0.58±0.02	1.18±0.07	0.29±0.03	0.51±0.02	0.24±0.02	1.13±0.09	1.18±0.07
$PASA_1$							
TCC	11.37±1.05	nd	nd	0.91±0.08	nd	4.47±0.08	13.68±0.16
TCL	1.21±0.10	0.22±0.03	3.72±0.07	nd	0.36±0.04	1.45±0.04	12.50±0.50
TP	nd	0.22±0.02	3.16±0.06	nd	0.87±0.04	nd	38.18±0.72
TA	nd	nd	0.34±0.04	nd	nd	1.29±0.11	13.81±0.15
TK	0.34±0.03	nd	1.21±0.04	nd	nd	nd	13.20±0.16
$PASB_2$							
TCC	nd	nd	nd	1.60±0.13	nd	0.54±0.02	2.95±0.06
TCL	1.59±0.11	nd	0.50±0.02	1.67±0.04	0.93±0.04	nd	4.70±0.07
TP	1.71±0.15	0.24±0.03	nd	0.18±0.005	nd	nd	nd
TA	0.20±0.02	3.40±0.21	nd	2.03±0.05	nd	0.55±0.02	12.82±0.37
TK	0.29±0.03	1.31±0.12	nd	0.29±0.02	nd	nd	nd

[a] mg/g_{DWE} mg/g dry weight extract; [b] nd: not detected

As presented in Tables 3a and 3b, hydroxybenzoic and hydroxycinnamic acid groups of phenolic acids were commonly detected in AHPA and BHPA extracts, respectively. The highest amounts of phenolic acids as hydroxybenzoic acid derivatives determined in the five *Teucrium* species examined were gallic and vanillic in *TCC*- and *TK*-AHPA, benzoic in *TCL*-$PASB_1$ and *TA*-BHPA, and syringic acids in *TP*-$PASA_2$ extracts ($p<0.05$). When this situation was evaluated for the hydroxycinnamic group in Table 3b, caffeic in *TCC*- and *TCL*-BHPA, t-cinnamic in *TP*-FPA, ferulic in *TA*-BHPA, and p-coumaric acids in *TK*-$PASB_1$ extracts were detected ($p<0.05$). No significant variation was obtained betwixt the amounts of ferulic acid in *TCC*-, *TCL*- and *TA*-BHPA extracts and also betwixt *TCL*- and *TK*-$PASB_1$ ($p>0.05$).

When examined in terms of the type of phenolic acids, the top three components with regard to quantity were determined from hydroxycinnamic acid derivatives as caffeic (154.81±7.19 mg/g_{DWE}), p-coumaric (126.65±5.17 mg/g_{DWE}), and t-cinnamic (80.79±1.32 mg/g_{DWE}) acids ($p<0.05$).

In a previous work, p-coumaric acid, luteolin, isoquercitrin, rutin and quercitrin from ethanolic extract of *T. chamaedrys* from Romania were determined as 25.68±0.33, 20.42±0.47, 524.8±2.75, 85.42±0.9, and 18.52±0.49 µg/g plant material, respectively [46]. Additionally, quercetin, ferulic and caftaric acids could not be identified in this extract. It can be said that rutin could not be detected in these *Teucrium* extracts, but quercetin and ferulic acid were determined. The amounts of gallic, caffeic, ferulic, luteolin, apigenin and hydrated quercetin in the methanolic extract of *T. chamaedrys* from Greece were determined by RP-HPLC as 0.6±0.02, 0.7±0.03, 1.1±0.01, 1.2±0.02, 0.9±0.02 and 0.2±0.01 mg/100 g dry sample, respectively [47].

In research performed by Bilušić et al [48], quercetin, kaempferol and isorhamnetin were determined at levels lower than 0.2 mg/kg in *T. chamaedrys* from Croatia that underwent acid hydrolysis after infusion. In general, it can be said that higher levels of phenolic compounds were determined than those obtained in these studies.

In another previous research, the aerial parts of *T. barbeyanum* from Libya were extracted in 70% aqueous methanol after defatting procedure [4]. As a result, eleven phenolics were determined as tetramethoxyflavone, salvigenin, 5-hydroxy-6,7,3',4'-tetramethoxyflavone, chrysoplenetin, cirsilineol, cirsimaritin, cirsiliol, apigenin, luteolin, methyl caffeate and 4-hydroxybenzoic acid. In their study, Djordjevic et al [49] determined six flavonoids (catechin, apigenin, rutin, myricetin, quercetin, luteolin) and five phenolic acid (chlorogenic, p-coumaric, gallic, vanillic, caffeic acids) compounds in the methanolic extract of *T. polium* harvested from Serbia. In the study performed by Sadeghi et al [10], the most common phenolic acids found in *Teucrium* species were shown to be ferulic, sinapic, rosmarinic, caffeic, p-coumaric, 4-hydroxybenzoic and syringic acids, which also support the results of this study.

Plants constitute the primary source of phenolics, and even in the same plant species, the content distribution of phenolic components can be affected by climate-geographic conditions, ripening-harvesting time and shape of the plant, and agro-environmental factors. Additionally, polarity and solvent affect the amounts of phenolic components derivatised from plants [50]. The differentials in the extraction capability of solvents may be the result of the phenolics alterations in the *Teucrium* extracts.

3.2. The contents of total phenolic, flavonoid and tannin

The contents of total phenolic, flavonoid and tannin in a plant extract are an indicator of the antioxidant capacity of that extract [44]. As seen in Table 4, there is no significant variation betwixt *TCL* and *TK* and also *TA* and *TP* in total phenolic contents ($p>0.05$). The highest value was determined as 479.66±4.28 mg_{GAE}/g_{DWE} for *TCC*. Total flavonoid contents are in the order *TCC*> *TCL*> *TK*> *TP*> *TA* and the highest value found for *TCC* was 123.60±1.92 mg_{QE}/g_{DWE}.

While the total tannin contents of the examined *Teucrium* species were highest in *TA* as 346.43±4.65 mg_{TAE}/g_{DWE}, there were no significant variations betwixt the values of *TCC, TP*, and *TK* ($p>0.05$). The lowest value was determined for *TCL* as 212.52±3.95 mg_{TAE}/g_{DWE}. The findings are in consistent with those of Vlase et al. [46] and Bilušić et al [48] who studied that *T. chamaedrys* contained the highest contents of total phenolics to be 243.65 mg_{GAE}/g plant material and 2061±42 mg_{GAE}/L, respectively.

Table 4. Total phenolic, total flavonoid and total tannin contents of *Teucrium* species. Results were expressed as mean±standard deviation of three independent replicates.

Teucrium species	Total Phenolic	Total Flavonoid	Total Tannin
	mg_{GAE}/g_{DWE} [a]	mg_{QE}/g_{DWE} [b]	mg_{TAE}/g_{DWE} [c]
TCC	479.66±4.28	123.60±1.92	260.71±4.12
TCL	296.04±4.15	107.69±1.81	212.52±3.95
TP	218.95±3.95	79.89±1.75	276.79±4.10
TA	231.80±4.11	65.04±1.52	346.43±4.65
TK	288.54±4.13	71.68±1.60	266.07±4.03

[a] mg_{GAE}/g_{DWE}: mg gallic acid equivalent/g dry weight extract; [b] mg_{QE}/g_{DWE}: mg quercetin equivalent/g dry weight extract; [c] mg_{TAE}/g_{DWE}: mg tannic acid equivalent/g dry weight extract.

Total contents of phenolic of the investigated *Teucrium* species were detected to be higher than those obtained from methanol extracts prepared with *Teucrium* species harvested from Serbia and Montenegro by Stanković et al [16]. When the contents of total phenolics and flavonoids were compared, the *TCC* was higher than the extracts obtained from South Serbian *T. chamaedrys* leaves (208.17±0.18 mg_{GAE}/g water extract and 110.13±0.41 mg_{RU}/g ethyl acetate extract, respectively) [15] and methanol extract obtained from Iranian *T. hyrcanicum* aerial parts (69.36 mg_{GAE}/g and 68.95 mg_{QE}/g extract, respectively) [51]. It was determined that the total phenolic contents of the water extracts from the leaves, flowers and stem parts of *T. alyssifolium* collected from Sandıras Mountain-Türkiye were varied between 13.99-41.54 mg_{GAE}/g extract, while the total flavonoid content was detected between 16.82-49.52 mg_{RU}/g extract [52]. In a previous study, the highest total phenolic amount (296±12.72 mg/g) was reached in the n-butanol extract of *T. montanum* collected from Serbia [53]. In a study which the polyphenol amount of acetone, water, ethanol and methanol extracts of *T. trifidum* collected from South Africa was examined, the highest and lowest total tannin contents were obtained in the aqueous and acetone extracts as 99.395±1.490 mg_{CE}/g and 77.339±1.068 mg_{CE}/g, while the total flavonoid amount was found to be the opposite as 3.398±0.241 mg_{QE}/g and 53.253±0.638 mg_{QE}/g, respectively [50]. The existence of polyphenolics depends on the plant species, genetic properties, geographical region, variations in growth, the soil type, the period and season of collect, the extraction process and solvent, drying, and storage [46]. The fact that higher results were found in the present study suggests that the investigated *Teucrium* plants may be regarded a possible resource of polyphenols.

3.3. Antioxidant activities

The balance between oxidative stress and antioxidant capacity in living cells is of great importance for a healthy life [54]. The antioxidant capacity of plants, constitutes one of the most effective bioactive properties in their applications in food, cosmetic, and medicine.

DPPH• scavenging: DPPH• is a durable nitrogen radical and receives an electron or hydrogen radical to convert into a more durable diamagnetic molecule. The free radical quenching capability of the extracts of the *Teucrium* species examined in this research was determined by DPPH• (Tables 5a and 5b).

Table 5a. Antioxidant activity results of the *Teucrium* flavonoid extracts. Results were expressed as mean±standard deviation of three independent replicates.

Phenolic Extracts	DPPH·	HO· scavenging	NO·	Metal chelating	Total reducing power	FRAP
	IC_{50} (µg/mL)				$µg_{AAE}/g_{DWE}$[a]	
Flavanone						
TCC	29.59±1.10	3.70±0.07	30.84±1.10	364.01±4.97	1018.04±15.15	594.50±6.73
TCL	31.57±1.03	2.10±0.02	55.13±1.17	410.23±5.15	719.91±9.21	396.84±5.06
TP	31.72±1.12	3.55±0.06	59.23±1.20	159.56±2.51	624.96±8.10	239.46±4.33
TA	39.06±1.25	5.20±0.08	33.95±1.12	932.49±12.80	479.21±5.93	227.03±4.25
TK	29.53±1.23	3.50±0.06	34.30±1.15	1343.02±20.17	834.75±11.56	457.08±5.92
Flavonol						
TCC	34.78±1.24	3.61±0.07	74.35±1.30	2059.80±25.10	631.60±8.15	346.76±4.88
TCL	44.98±1.30	3.90±0.07	69.52±1.25	31.81±1.15	649.25±8.52	227.03±4.30
TP	54.54±1.18	11.20±0.12	86.10±.30	1162.19±16.34	463.71±5.94	110.32±1.98
TA	45.13±1.26	9.01±0.10	61.21±1.20	1228.05±18.87	649.24±8.47	228.16±4.37
TK	44.05±1.27	1.20±0.01	76.55±1.27	1008.91±15.18	501.31±6.25	329.07±4.92
Flavan-3-ol						
TCC	29.58±1.23	4.51±0.08	32.34±1.18	1100.92±16.03	790.61±10.37	417.55±5.21
TCL	31.39±1.12	4.30±0.08	30.36±1.15	1498.60±22.19	644.81±8.58	296.69±4.80
TP	39.25±1.25	3.70±0.06	72.82±1.25	459.01±5.47	397.50±5.01	140.08±2.03
TA	36.06±1.08	3.41±0.05	62.26±1.23	745.00±9.81	501.31±6.23	177.33±2.66
TK	28.94±1.21	4.50±0.08	13.45±0.15	145.01±2.29	715.50±9.73	298.57±4.91
Flavan-3-ol AH						
TCC	56.33±1.27	3.50±0.07	39.41±1.17	928.60±12.52	488.04±6.03	246.24±4.09
TCL	49.72±1.30	3.01±0.03	48.16±1.21	2133.50±28.71	492.45±6.10	207.08±3.88
TP	51.89±1.25	5.02±0.07	67.33±1.25	5415.01±47.23	494.66±6.12	284.64±4.75
TA	49.73±1.22	2.85±0.05	61.76±1.20	5412.00±47.01	461.51±6.05	231.93±3.97
TK	54.02±1.35	2.95±0.05	61.15±1.21	764.98±9.93	278.20±4.80	300.45±4.81
Flavone						
TCC	69.17±1.17	3.32±0.07	72.14±1.27	47.74±1.20	452.71±5.46	143.82±1.47
TCL	49.57±1.11	2.80±0.05	90.63±1.35	184.98±2.67	415.20±5.13	212.73±3.90
TP	49.78±1.15	2.70±0.05	149.39±2.27	150.01±2.30	379.81±4.90	46.94±1.17
TA	50.35±1.24	4.20±0.08	124.51±1.93	146.45±2.27	536.60±6.68	214.98±4.12
TK	56.08±1.26	3.61±0.07	178.70±2.64	123.75±1.95	452.71±5.42	80.20±1.30
Positive controls						
BHT*	26.80±1.40	5.1±0.2	-	-	-	-
AA**	18.72±0.17	-	36.76±1.03	-	-	-
EDTA**	-	-	-	5.5±0.3	-	-

[a] $µg_{AAE}/g_{DWE}$: mg Ascorbic Acid Equivalent/g Dry Weight Extract;
[*] Butylated hydroxytoluene;
[**] Ascorbic acid;
[***] Ethylenediaminetetraacetic acid

Table 5b. Antioxidant activity results of the *Teucrium* phenolic acid extracts. Results were expressed as mean±standard deviation of three independent replicates.

Phenolic Extracts	DPPH·	HO· scavenging	NO·	Metal chelating	Total reducing power	FRAP
	IC_{50} (µg/mL)				$µg_{AAE}/g_{DWE}$ [a]	
Free Phenolic Acid (FPA)						
TCC	36.63±1.07	4.50±0.08	50.33±1.20	1592.32±23.15	379.81±4.93	87.73±1.32
TCL	49.27±1.18	3.41±0.07	85.14±1.33	75.14±1.25	359.91±5.01	56.65±1.22
TP	40.32±1.11	3.42±0.07	75.29±1.25	95.09±1.33	287.10±4.11	63.25±1.25
TA	40.12±1.10	3.30±0.06	38.87±1.08	20.10±0.81	417.41±5.17	105.42±1.52
TK	40.21±1.10	4.01±0.07	69.86±1.23	130.01±2.17	419.61±5.15	80.20±1.27
Base-Hydrolysed Phenolic Acid (BHPA)						
TCC	28.31±0.95	3.77±0.07	30.60±1.03	843.33±11.75	850.20±11.81	628.01±8.15
TCL	36.55±1.05	3.75±0.08	11.97±0.12	959.26±12.85	571.95±6.56	636.29±8.29
TP	49.04±1.25	3.71±0.06	64.47±1.17	105.02±1.85	351.10±4.77	88.855±1.40
TA	39.52±1.25	3.90±0.05	33.58±1.15	487.26±5.91	587.41±6.69	648.34±8.24
TK	38.14±1.14	3.95±0.05	12.28±0.18	244.12±4.27	578.61±6.55	289.53±4.83
Acid-Hydrolysed Phenolic Acid (AHPA)						
TCC	31.07±1.03	4.50±0.07	34.70±1.17	5235.01±45.22	821.51±11.47	296.31±4.97
TCL	28.50±1.10	4.35±0.08	23.35±0.92	260.34±4.60	920.87±12.73	76.51±1.30
TP	41.36±1.20	4.30±0.07	76.84±1.33	5582.01±45.46	510.10±6.12	176.21±2.71
TA	38.09±1.13	3.87±0.05	64.14±1.30	202.11±3.93	662.51±8.51	1087.35±15.21
TK	40.89±1.25	4.15±0.06	62.66±1.20	131.82±2.27	541.04±6.23	166.42±2.64
Phenolic Acids of Solid Hydrolysis						
$PASB_1$						
TCC	77.51±1.30	4.50±0.07	77.39±1.33	50.01±1.24	333.45±4.93	112.95±2.09
TCL	63.14±1.17	1.01±0.01	36.59±1.20	63.74±1.28	317.99±4.87	143.07±2.15
TP	67.30±1.22	1.30±0.01	40.88±1.21	85.32±1.35	461.52±5.24	165.66±2.68
TA	67.25±1.15	5.11±0.05	68.94±1.25	74.35±1.30	428.42±5.01	136.67 ±2.30
TK	64.55±1.28	8.11±0.10	40.39±1.11	78.85±1.32	357.75±4.75	120.08±2.12
$PASA_2$						
TCC	41.50±1.18	3.81±0.07	43.63±1.17	131.51±2.23	468.22±5.90	189.38±2.75
TCL	35.64±1.11	6.20±0.09	29.48±1.12	159.75±2.45	638.21±8.18	273.34±4.65
TP	23.21±0.30	2.11±0.02	62.90±1.28	1486.25±22.16	713.32±9.65	299.32±4.59
TA	58.24±1.27	2.55±0.02	32.16±1.15	219.41±4.23	408.50±5.17	180.35±2.71
TK	49.41±1.13	4.01±0.03	7.60±1.30	5231.02±45.59	472.61±5.95	173.95±2.70
$PASA_1$						
TCC	37.21±1.17	6.40±0.09	63.01±1.20	216.99±3.98	545.45±6.25	283.89±4.77
TCL	33.31±1.05	3.21±0.07	34.38±1.15	365.42±4.73	682.40±8.83	275.60±4.86
TP	32.38±1.12	6.50±0.08	34.96±1.18	5230.01±45.23	587.41±6.41	198.04±2.77
TA	35.78±1.15	6.01±0.07	65.01±1.25	373.12±4.82	565.31±6.35	241.17±4.13
TK	36.82±1.09	1.41±0.01	72.12±1.30	186.78±2.74	569.70±6.47	196.16±2.58
$PASB_2$						
TCC	83.46±1.42	11.10±0.12	76.55±1.33	69.46±1.25	278.20±4.80	43.05±1.15
TCL	77.06±1.32	6.70±0.09	70.83±1.30	78.12±1.32	326.81±4.87	104.29±1.80
TP	65.81±1.30	13.12±0.75	80.68±1.35	340.07±4.77	384.25±4.90	105.04±1.75
TA	69.06±1.33	11.11±0.42	40.05±1.11	59.19±1.13	415.16±5.12	121.24±1.95
TK	84.93±1.40	3.81±0.05	67.62±1.25	79.19±1.35	302.52±4.85	109.94±1.83
Positive controls						
BHT*	26.80±1.40	5.1±0.2	-	-	-	-
AA**	18.72±0.17	-	36.76±1.03	-	-	-
EDTA**	-	-	-	5.5±0.3	-	-

[a] mg_{AAE} /g_{DWE}: mg Ascorbic Acid Equivalent/g Dry Weight Extract;
[*] Butylated hydroxytoluene;
[**] Ascorbic acid;
[***] Ethylenediaminetetraacetic acid

The best IC_{50} values for DPPH• quenching of these extracts were detected in *TP*-$PASA_2$, *TCC* -BHPA, *TCL*-AHPA and *TK*-flavan-3-ol as 23.21±0.30, 28.31±0.95, 28.50±1.10 and 28.94±1.21 µg/mL, respectively. Additionally, the best IC_{50} value of *TA* in quenching this radical was determined as 36.06+1.08 µg/mL in flavan-3-ol extract, which was not significantly variation from flavanone ($p>0.05$). The IC_{50} scavenging values of DPPH• for BHT and AA used as positive controls were 26.80±1.40 and 18.72±0.17 µg/mL, respectively. *Teucrium* extracts with the best values were found to have similar values with BHT, which is synthetic and widely used. In recent studies, the negative effects of BHT, which is used as a preservative additive in foods, on human health have led researchers to investigate the food preservative properties of natural plant-derived antioxidants [55]. Therefore, according to the results obtained, it can be said that these extracts have scavenging potential. In the *TP*-$PASA_2$ extract, where the best scavenging value was obtained, syringic, vanillic, caffeic acids were in the foreground. These phenolic compounds are antioxidant components with high radical quenching [56]. In the research of Sharififar et al [19], the best IC_{50} value in DPPH• quenching was detected as 20.1 ppm in *T. polium* methanolic extract. For DPPH• scavenging, IC_{50} values in methanol extracts of *T. polium* collected from Morocco [57] and *T. hyrcanicum* harvested from Iran [51] were determined as 0.41±0.03 mg/mL and 44.32±5.5 µg/mL, respectively. While the best IC_{50} value was 24.51±1.32 µg/mL with the root methanol extract of *T. chamaedrys* from Southern Serbia, this value was 29.46±0.99 µg/mL in the whole plant methanol extract [15]. The antioxidant activity of aqueous extracts from the leaves, flowers and stem parts of *T. alyssifolium* collected from Sandıras Mountain-Turkey was examined by *in vitro* DPPH• quenching and it was shown to have IC_{50} values ranging between 13.52-132.55 µg/mL [52]. The best IC_{50} value for this radical was obtained in the infusion of *T. chamaedrys* as 1.90±0.02 mg/mL [48].

The maximum percentage of DPPH• inhibition was detected as 83% in the presence of 200 ppm of methanol extract of the flowering stage aerial parts of *T. polium* harvested from Iran [58]. In research performed by Ersoy et al [59], the best DPPH• scavenging IC_{50} values in the extracts of the aerial parts of *T. polium* from the Elazığ Province, and *T. parviflorum* from the Batman Province, Turkey prepared by maceration in ethanol were 67.39±1.33 and 133.49±1.64 µg/mL, respectively. Also, it was showed that these extracts containing naringenin, luteolin, epigallocatechin gallate and hesperidin were effective in the scavenging of DPPH• [59]. It was declared that the IC_{50} value of the *T. chamaedrys* ethanolic extract obtained from Romania was 26.70±0.96 ppm in DPPH• scavenging and that the chlorogenic, p-coumaric, gentisic acids, luteolin, isoquercitrin, rutin and quercitrin present in the extract were effective in this activity [46]. In another study, after the extraction of *T. chamaedrys* L. from Serbia with water, methanol, ethyl acetate, acetone and petroleum ether in the Soxhlet apparatus, the IC_{50} values of this radical quenching were determined as 31.79±0.45, 29.46±0.80, 269.41±0.93, 35.73±1.2 and 341.08±0.85 µg/mL, respectively [15]. These observed differences in this radical scavenging could be ascribed to the extraction type used and plants from different geographic conditions. In addition, to the outcomes of this research, it can be said that *Teucrium* extracts have this radical scavenging activity owing to their hydrogen donation or electron transfer abilities of their phenolic compounds. It can be stated that generally better results were obtained for the quenching of this radical than the values in the literature [48, 51, 57, 59].

HO• scavenging: Among the oxygen radicals, HO• is the radical that reacts fastest with intracellular biomolecules and causes cell damage [54]. Therefore, it is important for a healthy life to control the intracellular level of this radical very well and keep it at a low level. As seen from Tables 5a and 5b, the best IC_{50} values for HO• quenching were detected to be 1.01±0.01, 1.20±0.01, 1.30±0.01, and 1.41±0.01 µg/mL in *TCL*-$PASB_1$, *TK*-flavanol, *TP*-$PASB_1$ and *TK*-$PASA_1$ extracts, respectively. These values were found as 3.32±0.07 and 2.55±0.06 µg/mL for *TCC*-flavanol and *TA*-$PASA_2$, respectively. For this radical quenching, IC_{50} value of BHT utilised as a positive standard was 5.10±0.2 µg/mL, and *Teucrium* extract, from which the best value was obtained, have a 5.05 times higher quenching capacity of this radical compared to BHT. It is noteworthy that all tested *Teucrium* samples had IC_{50} values for HO• scavenging generally lower than 4.50±0.12 µg/mL as shown in Tables 5a and 5b. Ferulic, benzoic, p-coumaric, vanillic, and t-cinnamic acids were determined in *TCL*-$PASB_1$ extract, where the best IC_{50} value was obtained. The value for the HO• quenching of the n-butanol extract of the *T. montanum* was 80 ppm and found to contain gallic, protocatechuic, gentisic, vanillic, syringic, chlorogenic, caffeic, coumaric, ferulic, 3,5-dimethoxy-4-hydroxy-cinnamic acids, and rutin [53].

In previous research, the best IC_{50} value for this radical quenching was detected to be 3.05±0.0129 ppm with ethyl acetate/water extract of *T. sandrasicum* leaves collected from the Muğla Province of Turkey [6]. Considering results, it can be concluded that the investigated *Teucrium* species are a more active than BHT for HO• quenching.

NO• scavenging: Nitric oxide has a physiological role as a new signalling molecule in many cellular events like the regulation of cell growth and metabolism, immunity and apoptosis, and high concentrations may be associated with diseases. For this reason, studies on the examination of natural herbal source extracts in NO• quenching are remarkable and come to the fore [60,61]. Among the extracts of examined *Teucrium* species, the best IC_{50} values for NO• quenching were detected as 7.60±1.30 µg/mL and 11.97±0.12 µg/mL for *TK*-$PASA_2$ and *TCL*-BHPA, respectively. Additionally, it was determined any significant difference for *TCC*-flavanone, -flavan-3 ol, and -BHPA extracts ($p>0.005$). Also, there was no significant variation betwixt the NO• scavenging values of *TP*-$PASA_1$ extract as 34.96±1.18 µg/mL and *TA*-$PASA_1$ as 32.16±1.15 µg/mL ($p>0.005$). For the positive control, AA, this value was 36.76±1.03 µg/mL, and it can be said that the NO• scavenging levels of these *Teucrium* extracts are 2.7-4.8 times higher than AA. In the presence of 200 ppm of aerial part methanol extract at the flowering stage of *T. polium* collected from Iran, the maximum NO• scavenging was found as 52% [58]. The NO• quenching IC_{50} values of acetone, water, ethanol and methanol extracts of *T. trifidum* shrubs collected from South Africa were detected to be 0.290, 0.292, 0.150, and 0.304 mg/mL, respectively [50]. In *TK*-$PASA_2$, where the highest this radical quenching activity was observed, vanillic, 4-hydroxybenzoic, protocatechuic, syringic, and p-coumaric acids were found at the forefront. The characteristic of phenolic compounds in this extract may be due to the electron donating nature of the –OH group at position 4 of each of them. These phenolic compounds, with antioxidant activities, compete with oxygen for the reaction with NO molecules and thus a reduction in the formation of free radicals can occur owing to the transformation into their reductive products and therefore prevent the damage of cell [56].

Metal chelating: In metabolism, some metal ions like iron and copper injury the structure and actions of proteins, DNA and lipids, and also cause the production of toxic chelate complexes with proteins due to their capability to generate active radicals [62]. As seen in Tables 5a and 5b, the best IC_{50} values in metal chelating were detected as 20.10±0.81, 31.81±1.15, 47.74±1.20, and 50.01±1.24 µg/mL in the *TA*-FPA, *TCL*-flavanol, *TCC*-flavone, and -$PASB_1$ extracts, respectively. It was reported that maximum Fe^{2+} chelation was found as 32% in the presence of 200 ppm of methanol extract of *T. polium* harvested from Iran [58]. It has been stated that iron ion chelating activity is related to phytochemical compounds such as phenolics and flavonoids obtained in the extract.

Total reducing power and FRAP: As a result of the antioxidant capacity of declining components in extracts of plant and the transfer of hydrogen atoms or electrons to free radicals, these radicals are neutralized and their harmful effects are prevented [62]. As seen in Tables 5a and 5b, the best total reducing power results obtained from *Teucrium* extracts were found in *TCC*-flavanone, *TCL*-AHPA and *TCC*-BHPA extracts (1018.04±15.15, 920.87±12.73, and 850.20±11.81 $\mu g_{AAE}/g_{DWE}$, respectively) while FRAP values were determined in *TA*-AHPA, *TA*-BHPA, *TCL*- and *TCC*-BHPA extracts (1087.35±15.21, 648.34±8.24, 636.29±8.29, and 628.01±8.15 $\mu g_{AAE}/g_{DWE}$, respectively). Extractions of *T. polium* aerial parts collected from Algeria were carried out in methanol, ethanol, ethyl acetate, chloroform, hexane and water on the Soxthlet apparatus, and their Fe (III) reduction potential was compared based on the absorbance values at 700 nm and it was stated that it increased as their concentration increased. The best reducing power, absorbance value at 100 ppm methanol extract was determined as 0.900 [63]. It was expressed that the best FRAP level was found in the butanol extract of *T. barbeyanum* from Libya as 4350.9±4.9 µmol Trolox equivalent/g [4] and in the infusion of *T. chamaedrys* as 6952±20 mg/L [48].

The diverse extracts showed important free radical quenching activity therefore *Teucrium* species examined can probably be utilised to be a natural resource of antioxidant compounds. This may be owing to the high phenolic content of the extract, and this could have supported to the electron transfer/hydrogen donation [64]. The existence of various phenolics with antioxidant capacity in the extracts may have supported to the synergistic effect [9].

The radical quenching capacities of the extracts of flavonoid and phenolic acid of the *Teucrium* species examined in this study are remarkable and especially important for their usability as additives in the food industry. The results are promising, especially in terms of the possibility of creating an alternative to food preservatives such as BHT, which have been extensively used in recently and due to studies showing findings regarding their harm to health [55].

3.4. Enzyme inhibition potentials

Overproduced free radicals can often lead to oxidative stress that may result in health problem and disorders like cancer and neurodegenerative diseases [62]. Additionally, synthetic enzyme inhibitors display secondary acts determined in the disease of the gastrointestinal system and hepatoxicity [9]. A series of studies has been performed to describe and identify the enzyme inhibitory attitude of native plant resources and to determine the relation betwixt their inhibitory functions and structural characteristics [65].

AChE inhibition: When AChE inhibition by these *Teucrium* extracts was investigated, the only Flavone extracts of the *Teucrium* species excluding *TA* showed inhibition on the AChE varying levels. The other *Teucrium* extracts exhibited weaker anti-AChE activity than that of Flavone extracts. When the IC_{50} values of the AChE inhibition were compared, the ranking was as follow; *TK* < *TCC* < *TP* < *TCL* as 137.58±3.07; 144.37±10.07; 648.25±9.05; 4851.96±10.25 µg/mL, respectively. Considering the best IC_{50} (137.58±3.07 µg/mL), *TK*-flavone extract containing diosmin, luteolin, apigenin, eupatorin showed higher AChE inhibition compared to the rest. Since flavones showed higher anti-AChE activities than the other phenolics, it was concluded that the existence of the C2-C3 double bond is important for this enzyme inhibitory ability. Also, these obtained results corroborated previous results of various medicinal plants expressed to show AChE inhibitory activity, like *T. arduini*, *T. chamaedrys*, *T. polium*, and *T. montanum* ethanol extracts containing 1 mg/mL [12]. Golfakhrabadi et al [66] reported that the IC_{50} value for the methanol extract of *T. hyrcanicum* from Iran on the AChE inhibition was 2.12 mg/mL.

Tyrosinase inhibition: When the anti-tyrosinase activity results of phenolic extracts obtained from five *Teucrium* species are examined, it can be said that phenolic acid extracts are more effective (Table 6). When the IC_{50} values of the anti-tyrosinase activity were compared, the ranking was as follow; *TCL*-$PASB_1$ < *TK*-$PASB_1$ < *TP*-$PASA_1$ as 251.65±4.28; 257.60±3.32; 284.13±5.23 µg/mL, respectively. According to the findings the principal compounds of the examined *Teucrium* extracts were ferulic, benzoic, p-coumaric, t-cinnamic, and vanillic acids. Korkmaz et al. [67] reported that the tyrosinase inhibitory activity may base on the hydroxyl items of the phenolics, like chlorogenic acid, benzoic acid, protocatechuic acid, gallic acid, ellagic acid, kojic acid, resveratrol, and various catechins that may produce a hydrogen bond to a centre in the enzyme, superior to lower enzymatic activity.

Table 6. Tyrosinase enzyme inhibition results of the *Teucrium* phenolic acid extracts. Results were expressed as mean±standard deviation of three independent replicates.

Phenolic Extracts IC_{50} (µg/mL)	***Teucrium* species**				
	TCC	***TCL***	***TP***	***TA***	***TK***
Flavanone	ni[a]	ni	3834.91±12.51	ni	ni
Flavan-3-ol	1028.25±16.03	2258.33±22.19	ni	693.68±9.81	ni
Flavone	10697.37±21.20	451.33±9.67	ni	ni	ni
FPA	ni	715.67±11.25	419.65±8.33	527.01±6.81	ni
$PASB_1$	672.27±8.24	251.65±4.28	627.31±7.35	414.51±5.30	257.60±3.32
$PASA_2$	ni	639.15±4.73	700.06±15.23	ni	12703.12±7.74
$PASA_1$	ni	433.68±4.73	284.13±5.23	ni	1458.73±2.74
$PASB_2$	322.96±6.25	ni	ni	933.77±5.13	ni
***Positive control* IC_{50} (µg/mL)**					
Kojic acid, 94.70±1.38					

[a]ni: not inhibited

Previous researches of the inhibitory activity of *Teucrium* species extracts on the AChE and tyrosinase enzymes indicate that these extracts have a strong/moderate capability to inhibit these enzymes [9, 59]. According to the findings, flavone subgroup from flavonoid extracts is prominent in AChE inhibition, while $PASB_1$ and $PASA_1$ from phenolic acid extracts are prominent in anti-tyrosinase activity. Considering the results, it can be said that *Teucrium* phenolic compounds are effective in the inhibition of these enzymes and also antioxidant activities.

3.5. Cytotoxic effects of *Teucrium* species

Phenolic compounds have important roles in controlling cell death by inhibiting the proliferation and metastasis of cancer cell lines by regulating intracellular signalling mechanisms with single or multiple synergetic effects [44]. Therefore, the diversity and quantity of the nutritional profile of plants is important to maintain a healthy life and suppressing some diseases. In this research, the cytotoxic activities of the prepared *Teucrium* phenolic extracts against HeLa, ACC-201, OE-33, HepG2 and MCF-7 cells were investigated according to the MTT test in the range of 20-150 ppm (Figure 1 and Figure 2**)**. Data with IC_{50} values of 125 µg/mL and below, defined as 50% inhibition of MTT results compared to controls, were considered.

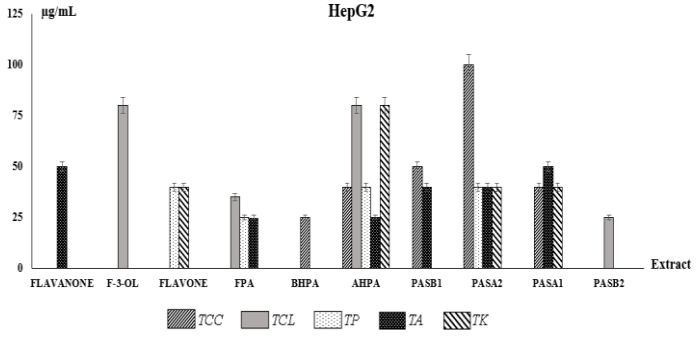

Figure 1. The IC_{50} values of *Teucrium* plant extracts against the HepG2 cancer cell line after 48 hours incubation. Results were expressed as mean±standard deviation of three independent replicates.

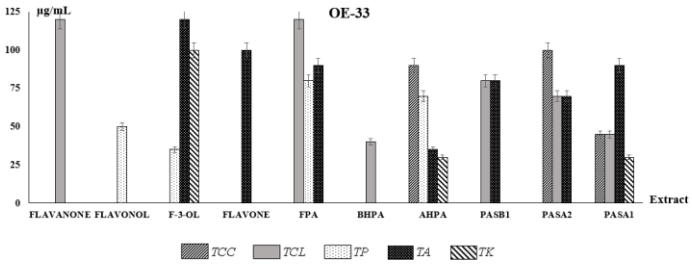

Figure 2. The IC_{50} values of *Teucrium* plant extracts against the OE-33 cancer cell line after 24 hours incubation. Results were expressed as mean±standard deviation of three independent replicates.

The best IC_{50} value against HeLa cancer cell line was determined as 65.13±2.47 µg/mL for the 24 h of incubation in *TK*-FPA extract. In this extract, where cytotoxic effects are observed, t-cinnamic, vanillic, rosmarinic and o-coumaric acids come to the fore. In the present study, more widespread cytotoxic effects were observed in these *Teucrium* extracts prepared against HepG2 cancer cells after 48 h of incubation. The best IC_{50} value of 25 µg/mL against the HepG2 was determined in *TCC*-BHPA, *TP*-FPA, *TA*-FPA, *TA*-AHPA, and *TCL*-$PASB_2$ extracts with no significant diverse between them ($p>0.05$). According to the findings, caffeic, ferulic, t-cinnamic, p-coumaric, vanillic acids in *TCC*-BHPA; t-cinnamic, p-coumaric, rosmarinic, 4-hydroxybenzoic, syringic acids in *TP*-FPA; t-cinnamic, vanillic, o-, p-coumaric acids in *TA*-FPA; procatechuic, t-cinnamic, vanillic, sinapic, syringic, ferulic acids in *TA*-AHPA; vanillic, t-cinnamic, sinapic, ferulic, gallic, syringic acids in *TCL*-$PASB_2$ stand out. The best IC_{50} values against OE-33 were detected after 24 h of incubation as 30 µg/mL in *TK*-AHPA and -$PASA_1$ extracts, with no significant variation betwixt them ($p>0.05$). To the data, t-cinnamic, vanillic, 4-hydroxybenzoic, and o-coumaric acids were found in *TK*-$PASA_1$, and vanillic, p-coumaric, gallic, t-cinnamic, rosmarinic, and caffeic acids were determined in *TK*-AHPA. To the US National Cancer Institute (USNCI), an IC_{50} value of plant extracts against a cancer cell line <30 ppm generally means that the extract has cytotoxic activity i*n vitro* [68]. According to MTT results, the best IC_{50} values determined from these *Teucrium* extracts can be interpreted as values with acceptable cytotoxic activity, especially against HepG2 and OE-33 cancer cells, according to the USNCI. In the investigated *Teucrium* extracts, limited cytotoxic effects with IC_{50} values >100 ppm were detected against ACC-201 cancer cell line. In addition, the best IC_{50} value of 57.31±3.28 µg/mL was obtained in *TP*-FPA extract against MCF-7 cancer cell line, and t-cinnamic, p-coumaric, rosmarinic, 4-hydroxybenzoic, and syringic acids are at the forefront in this extract. According to these findings, it can be said that phenolic acid compounds stand out compared to other phenolic compounds in the observed effect. Thus, phenolic acids i.e. benzoic acid and cinnamic acid derivatives with higher hydroxyl substitutions could be considered as potentially effective in inhibiting these cancer cell proliferation. These extracts contain diverse phenolics with various chemical structures, so they may generate different effects in comparison with each component. Their cytotoxic capacities are not only the finding of activities of each compound, but also of their interactions, since they react in a synergistic, additive or antagonistic effect [44].

In the research of Bilušić et al (2024), the best antiproliferative activities of 1 mg/mL of aqueous infusion of *T. chamaedrys* from Croatia, against MD-MBA-231, T24, and A549 cancer cells were detected to be 39.53, 32.52, and 40.82%, respectively. In previous research, the IC_{50} value of extract of the *T. polium* against hepatocellular cancer was determined as 90 ppm [68]. The best IC_{50} values of *T. chamaedrys* and *T. montanum* methanolic extracts collected from Bulgaria against MDBK cancer cells were determined as 1.92 and 2.49 mg/mL, respectively, and it was evaluated that secondary metabolites were responsible for this effect [70]. Moreover, the best IC_{50} values against A431 and MCF-7 cancer cells were found as 235.4 and 326.6 µg/mL, respectively, with the ethyl acetate extract of *T. hyrcanicum* L aerial parts collected from Iran [51]. In a work conducted by Tarhan et al [6] the IC_{50} values of the methanol/water extract of *T. sandrasicum* flowers collected from Turkey against HeLa and MCF-7 cells were determined as 20 ppm. To the best of our knowledge, this is the first study on the cytotoxic activity of these *Teucrium* extracts against OE-33 cancer cells. According to the results of this study, the abundance of *Teucrium* species in terms of phenolic compounds, the importance of their antioxidant properties and especially their activities against HepG2 and OE-33 cells highlight their potential to be natural component sources.

CONCLUSIONS

The focus of the present research was to comparative analysis of the phenolic profiles and bio-potentials (antioxidant, enzyme inhibitory and cytotoxicity) of phenolic extracts from five *Teucrium* species from Türkiye. *Teucrium* species distributed worldwide are traditional medicinal plants that are used for various disorders. In the present study, the samples of the flavonoid subgroups, and phenolic acids and their liquid and solid phase acid-base hydrolysis extracts were prepared by using different extraction methods. Among the phenolic compounds in the investigated *Teucrium* species detected by RP-HPLC-DAD analysis, phenolic acids come to the fore in terms of quantity. The top three components of phenolic compounds were detected to be caffeic, p-coumaric, and t-cinnamic acids in *TCC, TK* and *TP*, respectively, without any significant difference between them ($p>0.05$). The antioxidant activities of these extracts of *Teucrium* species contained important quenching of $DPPH^{\bullet}$, $HO^{\bullet}$, and $NO^{\bullet}$, along with total reducing power and metal chelating capacities. Among the studied antioxidant parameters, the *TCL* extract is 5.05 times more effective in scavenging $HO^{\bullet}$ than BHT, which is extensively utilised in the food industry, while the extract of *TCC* is 1.15 times more effective in quenching $DPPH^{\bullet}$. In addition, the *TK* extract is 4.84 times more effective in $NO^{\bullet}$ scavenging compared to AA. The obtained results are important in terms of usability of these *Teucrium* species as additives in the food industry. Additionally, the moderate AChE and tyrosinase inhibitory potentials were displayed by the *Teucrium* extracts. Furthermore, the best cytotoxic effects of these *Teucrium* extracts as indicated by IC_{50} values of 25.03±1.24 and 30.07±1.50 µg/mL were detected against the HepG2 and OE-33 cancer cell lines. To the best of our knowledge, there are no previous literature on the comparative data of the cytotoxic activities of five *Teucrium* species against OE-33 cancer cells. According to the results, the studied *Teucrium* species can be considered good natural resources of bioactive phenolic compounds with antioxidant, cytotoxic and enzyme inhibitory potential, making these plants effective alternatives to be used in the food, cosmetic and medical areas.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research.

ACKNOWLEDGEMENTS

This study was supported by the Scientific Research Projects Unit of Dokuz Eylül University with the project numbered 2020.KB.FEN.005.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supplementary data section at the end of this manuscript.

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