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Original Article

Organ-specific antioxidant capacities and cytotoxic effects of *Thermopsis turcica* extracts in breast cancer

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ABSTRACT

Background and Aims: *Thermopsis turcica* is an endemic species present in Türkiye and it is seen as a source of functional compounds such as antioxidant phenolics. Even though some biological activities of the aerial parts of *T. turcica* have been determined, knowledge regarding the organ-specific chemical composition and effects on human breast cancer is still scarce. Therefore, the present study aims to evaluate the antioxidant capacities, phenolic acid profiles, and potential biological activities of methanol extracts obtained from the leaf, flower, and stem tissues of *T. turcica*.

Methods: The antioxidant capacities of methanol extracts of *T. turcica* was tested with complementary methods (TAC, CUPRAC, FRAP, and DPPH). While the total phenol (TPC) and flavonoid contents (TFC) of the extracts were determined spectrophotometrically, their phenolic acid profiles were determined by high-performance liquid chromatography (HPLC). The cytotoxic effects of extracts on the human normal breast cell line (MCF-10A cells) and the breast tumor cell lines (MCF7, MDA-MB-231, and SKBR3) were also analyzed after 24 h treatment.

Results: The leaf extracts were found to have higher antioxidant capacity, which was associated with the presence of higher amounts of TPC and TFC. The HPLC analysis revealed the presence of quercetin, hesperidin, and rosmarinic acid as the main compounds in the leaf extracts, while a high amount of benzoic acid was found in the flower extract. Leaf and flower extracts also showed stronger cytotoxic activity against MCF-7 cells (IC_{50} values were 0.65 mg/mL and 0.55 mg/mL, respectively) as compared to stem extract (IC_{50} value was 1.10 mg/mL). Leaf extracts were the most active extract against SKBR3 cells with IC_{50} of 0.75 mg/mL. All extracts exhibited weak cytotoxic effects against MDA-MB-231 cells and IC_{50} values (1.53-1.75 mg/mL) were similar to the MCF-10A cells (IC_{50} values: 1.59-1.69 mg/mL).

Conclusion: In conclusion, extracts derived from *T. turcica* have the potential to serve as a valuable source of bioactive metabolites with antioxidant and antiproliferative properties.

Keywords: Antioxidant capacity, breast cancer, cytotoxic activity, phenolic content, *Thermopsis turcica*

INTRODUCTION

Plants produce a wide variety of substances, including biologically active compounds formed during secondary metabolism (Salmeron-Manzano, Garrido-Cardenas, & Manzano-Agugliaro, 2020). In addition to their ecological importance, these phytochemicals have important applications in industries such as pharmacology (Leicach & Chludil, 2014). Among secondary metabolites, phenolic compounds are taken into consideration because of their significant effects on plant metabolism. Their response to biotic and abiotic factors and signaling mechanisms are excellent examples (Lone et al., 2023). Investigations can show the characteristics of various plants and can lead to new perspectives for several industrial materials due to their antifungal, antimicrobial, antibacterial, antiviral, antitumor, and antioxidant properties (Manzoor, Yousuf, Pandith, & Ahmad, 2023). Phenolic compounds have potential pharmacological properties especially in the daily diet due to their radical scavenging activity (Elgadir, Chigurupati, & Mariod, 2023). Therefore, they have considerable economic attention (Elshafie, Camele, & Mohamed, 2023).

Thermopsis is a genus of the Fabaceae family spread over

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the temperate areas of North America and East Asia (Wojciechowski, 2003). The *Thermopsis* genus includes an important plant species with high medicinal value. For instance, it is known that *Thermopsis lanceolate* has many pharmacological effects such as antimicrobial and anticancer (Zhang et al., 2022). Recently, it has been shown that ethanol extracts of *Thermopsis rhombifolia* aerial parts showed the *in vitro* cytotoxicity and antiproliferative effect against colorectal adenocarcinoma (HT-29), malignant glioblastoma (M059K) and normal lung fibroblast (WI-38) cell lines. Furthermore, flavone luteolin isolated from *T. rhombifolia* has shown to have the potential to arrest the cell cycle by inhibiting protein kinase activity (Tuescher et al., 2020). *Thermopsis turcica* is a poisonous plant and is an endemic species spreading in a narrow area in southwestern Turkey (Tan, Vural, & Küçüködük, 1983). Previous studies demonstrated that various extracts of *T. turcica* have antimicrobial, antioxidant, and anticancer activities (Liman, Eren, Akyil, & Konuk, 2012; Bali et al., 2014; Yıldız et al., 2020). In a previous study, Bali et al. (2014) showed that ethanol and ethyl acetate extracts (20-100 µg/mL) from the aerial parts of *T. turcica* had substantial antiproliferative effects on promyelocytic leukemia cells while being relatively nontoxic to human gingival fibroblast cells. However, methanol extracts (0.5-2.5 mg/mL) of the flower and leaf tissues of *T. turcica* have been shown to have cytotoxic activity against HeLa cells lines (Yıldız et al., 2020).

Aksoy, Kolay, Ağılönü, Aslan, & Kargıoğlu (2013) reported that methanol and acetone extracts of the aerial parts of *T. turcica* have high phenolic content and accordingly high antioxidant capacity. Similarly, total *T. turcica* extracts prepared with different solvents were found to have antioxidant and cytotoxic effects (Bali et al., 2014). To our knowledge, no organ-specific antioxidant and biological activities have been reported in *T. turcica* extracts. In this study, therefore, it was aimed to determine total phenolic and flavonoid contents, total antioxidant activity, free radical scavenging activity, and phenolic acid profiles in methanol extracts of the leaf, flower, and stem tissues of *T. turcica*. Furthermore, the organ-specific cytotoxic effects of *T. turcica* extracts on human breast cancer cell lines were evaluated.

MATERIALS AND METHODS

Plant collection and preparation of extracts

The aerial parts of *Thermopsis turcica* were collected at undisturbed areas near Lake Eber, Afyonkarahisar, Türkiye. The plant specimen was identified by co-author Dr. Mustafa Yıldız. The aerial parts were separated into leaf, flower, and stem tissues and dried under laboratory conditions (in shade at room temperature). It has been suggested that methanol is the effective solvent for extracting phenolic compounds from plants (Cheynier, 2012). Therefore, dried tissues (3 g) were finely powdered and incubated overnight with 30 mL methanol at +4°C. After filtration with filter paper, extracts were vacuum-dried with a rotary evaporator at 50°C. For the determination of phenolic contents and antioxidant capacities, a portion of dry extracts (10 mg/mL) was dissolved in methanol. Another portion of extracts (10 mg/mL) was dissolved in 0.1% dimethyl sulfoxide (DMSO) to determine cytotoxic effects on breast cancer cell lines.

Determination of total phenolic and flavonoid contents

The total phenolic content (TPC) in the extracts (1 mg/mL) was determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965). The TPC was determined by the gallic acid (GA) standard (1, 0.5, 0.25, 0.125, and 0.0625 mg/mL) curve and presented as gallic acid equivalents (µg GAE/mg extract). The total flavonoid content (TFC) in the extracts (1 mg/mL) was evaluated by the aluminum chloride colorimetric method of Deng & van Verkel (1998). The TFC was determined by the quercetin (Q) standard (10, 20, 30, 40, and 50 µg/mL) curve and expressed as quercetin equivalents per mg of extracts (µg QE/mg extract).

Determination of antioxidant capacity

The antioxidant capacities of *T. turcica* extracts were determined via four *in vitro* methods (TAC, CUPRAC, FRAP, and DPPH assays). The total antioxidant capacities (TAC) of the extracts (1 mg/mL) were determined through the phosphomolybdenum assay (Prieto, Pineda, & Aguilar, 1999). The antioxidant capacities were expressed as µg ascorbic acid (AA) equivalents per mg of extract (µg AAE/mg extract). The cupric ion-reducing antioxidant capacities (CUPRAC) of the extracts (1 mg/mL) were determined according to the total antioxidant capacity measurement method based on the Cu^{2+} reducing capacity (Apak et al., 2007). The CUPRAC results were expressed as trolox (TR) equivalents per mg of extracts (mM TRE/mg extract). The ferric-reducing ability potential (FRAP) of the extracts (0.5 mg/mL) was determined according to the method based on the reduction of $[Fe\ (III)\ (TPTZ)_2]^{3+}$ to $[Fe\ (II)\ (TPTZ)_2]^{2+}$ (Tuberoso et al., 2010). The FRAP results were expressed as trolox equivalents per mg of extracts (mM TRE/mg extract). The free radical scavenging activities of the extracts (0.1-2 mg/mL) were determined according to the DPPH (2,2-diphenyl-1-picrylhydrazil) method (Espín, Soler-Rivas, & Wichers, 2000). Ascorbic acid was used as a positive control, and DPPH scavenging capacity was calculated using the equation:

$$\text{Inhibition of DPPH radical (\%)} = [(Abs_{Methanol} - Abs_{Extract})/Abs_{Methanol}] \times 100$$

Analysis of phenolic compounds via HPLC

Quantitative analysis of phenolic components was carried out using a chromatographic system (Agilent 1200) coupled with an UV-diode array detector (DAD) and a reversed-phase column Supelco LC18 (250 × 4.6 mm^2, 5 µm). The leaf, flower, and stem extracts (10 mg/mL) of *T. turcica* were prepared in HPLC-grade methanol. After centrifugation at 10,000 × g for 10 min, the resulting supernatants were filtered using 0.45 µm filters. The injection volume was 20 µL and the flow rate was 0.8 mL min^{-1}. UV region at 278 nm was used for peak detection. The mobile phase consisted of acetic acid (2%) and methanol. The quantifications were calculated by comparing the peak surface areas with phenolic compounds standards of 3-hydroxy benzoic acid, benzoic acid, caffeic acid, catechin hydrate, chlorogenic acid, epicatechin, gallic acid, hesperidin, p-coumaric acid, quercetin, rosmarinic acid, sinapic acid, syringic acid, t-cinnamic acid, and t-ferulic acid (Caponio, Alloggio, & Gomes, 1999). The method was evaluated according to Koc et al. (2020). The correlations of standard curves of each phenolic substance are given in Table 2. The phenolic compounds were identified by comparing their retention time and UV spectra with those obtained from standard solutions. Quantification of phenolic components was performed by normalization method based upon the area percent reports obtained by HPLC-DAD.

Cell culture and viability assay

The human normal breast cell line (MCF-10A cells) and the breast tumor cell lines (MCF7, MDA-MB-231, and SKBR3) were obtained from Medicinal Genetics Department, Afyonkarahisar Health Sciences University. The human normal breast cell line (MCF-10A cells) was cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) supplemented with 5% horse serum, 20 ng/mL epidermal growth factor, 0.5 µg/mL hydrocortisone, 100 ng/mL cholera toxin, 10 µg/mL insulin, and 1% penicillin-streptomycin. The breast tumor cell lines (MCF7, MDA-MB-231, and SKBR3) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids, and 1% penicillin-streptomycin. Cells were grown in a humidified incubator set at 37°C with 5% CO_2. The viability of cells was assessed using the WST-1 assay (Roche Diagnostics, Switzerland). Briefly, cells were seeded in a 96-well plate at a density of $1x10^4$ cells per well. After 24 hours of incubation at 37°C, the cells were treated with varying concentrations (0 – 4 mg/mL) of leaf, flower, and stem extracts of *T. turcica* or 0.1% DMSO for 24 hours. Following this treatment period, 10 µL of WST-1 reagent was added to each well and further incubated for 4 hours. Optical absorbance was measured using a Multiscan GO microplate reader (Thermo Scientific, USA) at a wavelength of 450 nm. The IC_{50} values were calculated from the linear regression of the dose-log response curves.

Statistical analysis

All statistical analyses were performed using SPSS software (version 22.0, SPSS, USA). For the comparisons of means, one-way ANOVA followed by post hoc test (Tukey's test) was employed. Values are expressed as the mean ± standard error.

RESULTS

Alterations in total phenolic and flavonoid contents

TPC and TFC of the different tissue extracts of *T. turcica* are presented in Table 1. The highest concentrations for TPC and TFC were found for leaf extract (145.8 ± 5.9 µg GAE/mg extract, and 76.6 ± 1.3 µg QE/mg extract, respectively), followed by flower extract (87.2 ± 3.6 µg GAE/mg extract, and 53.7 ± 4.2 µg QE/mg extract, respectively). The lowest values were determined in stem extract (TPC; 70.8 ± 4.9 µg GAE/mg extract, TFC; 32.2 ± 2.4 µg QE/mg extract).

Table 1. Total phenolic content (TPC), total flavonoid content (TFC), and *in vitro* antioxidant capacities (TAC, CUPRAC, and FRAP) of the different tissue extracts of *T. turcica*.

Parameters	Plant tissues		
	Leaf	Flower	Stem
TPC (µg GAE/mg extract)	145.8 ± 5.90	87.2 ± 3.63 [a]	70.8 ± 4.88 [a]
TFC (µg QE/mg extract)	76.6 ± 1.26	53.7 ± 4.24 [a]	32.2 ± 2.36 [a,c]
TAC (µg AAE/mg extract)	110.3 ± 2.10	87.2 ± 3.53 [a]	94.7 ± 2.82 [b]
CUPRAC (mM TRE/mg extract)	1.13 ± 0.06	0.62 ± 0.03 [a]	0.48 ± 0.06 [a]
FRAP (mM TRE/mg extract)	1.26 ± 0.04	0.58 ± 0.03 [a]	0.39 ± 0.04 [a,d]

a P<0.001 vs Leaf group, b P<0.01 vs Leaf group, c P<0.001 vs Flower group, d P<0.01 vs Flower group.

Alterations in antioxidant capacity

Antioxidant capacities of the leaf, flower, and stem extracts from *T. turcica* evaluated using four complementary assays are given in Table 1. All tissue extracts exerted a total antioxidant capacity, the most active being leaf extract (110.3 ± 2.1 µg AAE/mg extract), followed by stem extract (94.7 ± 2.8 µg AAE/mg extract) and flower extract (87.2 ± 3.5 µg AAE/mg extract). The highest antioxidant capacity was detected for leaf extract, both in CUPRAC and FRAP assays (1.13 ± 0.06 mM TRE/mg extract and 1.26 ± 0.04 mM TRE/mg extract, respectively), followed by flower extract (0.62 ± 0.03 mM TRE/mg extract and 0.58 ± 0.03 mM TRE/mg extract, respectively). The stem extract displayed the lowest antioxidant capacity in CUPRAC and FRAP assays (Table 1). All tested *T. turcica* extracts showed the potential to reduce DPPH (Figure 1). Results

showed that tissue extract differentially affected the antioxidant capacity. The best reducer of DPPH was leaf extract after the positive control ascorbic acid.

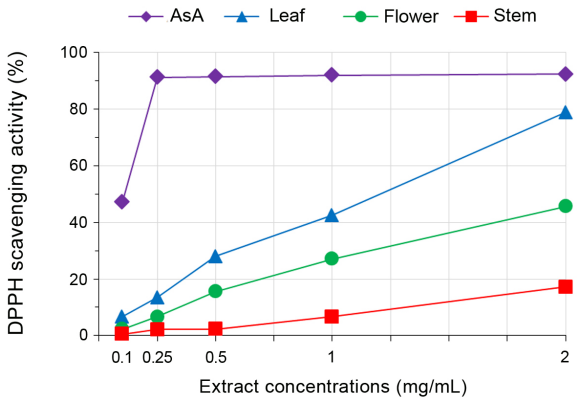

Figure 1. DPPH radical-scavenging activity of the leaf, flower, and stem extracts of *T. turcica.*

Phenolic acid composition of *T. turcica* extracts

Sixteen phenolic acids were analyzed by reverse-phase HPLC. The HPLC chromatograms obtained from the leaf, flower, and stem extracts showed similar phenolic profiles (Figure 2). In order of retention time, the phenolic compounds are given in Table 2. Among them, 3-hydroxybenzoic acid was detected in the leaf and stem samples, while it was not detected in the flower samples. Catechin hydrate and caffeic acid were determined only in the leaves, whereas sinnapic acid was determined only in the flowers. Moreover, syringic acid was not detected in all tissues. Among the sixteen phenolic compounds, the most abundant phenolic acids were quercetin (58.11 ± 0.48 µg/g DW), hesperidin (29.12 ± 1.29 µg/g DW), and rosmarinic acid (11.77 ± 2.34 µg/g DW) in the leaf tissues. Additionally, hesperidin, quercetin, rosmarinic acid, t-cinnamic acid, and gallic acid were found in the leaves more than in stem and flower samples. Moreover, benzoic acid (46.24 ± 3.86 µg/g DW) was found as the main compound in the flower extract of *T. turcica* (Table 2).

The cytotoxic effects of *T. turcica* extracts on breast cancer cell lines

The cytotoxic effects of the different tissue extracts of *T. turcica* on the cell lines are shown in Figure 3. We observed that *T. turcica* extracts induced a significant decrease in the viability of MCF7, MDA-MB-231, and SKBR3 cells with increasing extract concentration. Determination of IC_{50} values for different tissue extracts of *T. turcica* on the cell lines exhibited various inhibitory patterns (Table 3). The leaf extracts of *T. turcica* manifested IC_{50} values of 1.63 ± 0.01 mg/mL, 0.65 ± 0.19 mg/mL, 1.62 ± 0.03 mg/mL, and 0.75 ± 0.18 mg/mL for MCF-10A, MCF7, MDA-MB-231, and SKBR3 cells, respectively. Of note, MCF7 ($P < 0.05$) and SKBR3 cells ($P < 0.05$) showed significantly lower IC_{50} values compared to those of MCF-10A cells. For the flower extracts, the IC_{50} values were found to be 1.59 ± 0.01 mg/mL for MCF-10A, 0.55 ± 0.02 mg/mL for MCF7, 1.53 ± 0.02 mg/mL for MDA-MB-231, and 1.11 ± 0.08 mg/mL for SKBR3 cells. Notably, the IC_{50} values were significantly lower in both MCF7 ($P < 0.001$) and SKBR3 ($P < 0.05$) cells when compared to MCF-10A cells. Similarly, *T. turcica* stem extracts showed IC_{50} values of 1.69 ± 0.04 mg/mL, 1.10 ± 0.58 mg/mL, 1.75 ± 0.06 mg/mL, and 1.30 ± 0.04 mg/mL for MCF-10A, MCF7, MDA-MB-231, and SKBR3 cells, respectively. The IC_{50} value of SKBR3 cells ($P < 0.05$) was significantly lower compared to that of MCF-10A cells (Table 3).

DISCUSSION

TPC is a crucial factor in determining the overall antioxidant capacity and is commonly employed to assess the antioxidant attributes of plant-based materials (Lamuela-Raventós, 2018). Given the diverse array of phenolic compounds and antioxidant constituents present in plants, each varying in structure, size, and polarity, the choice of extraction solvents can significantly impact the outcomes of such analyses (Xu et al., 2017). Our results showed significant differences in TPC and TFC of the different tissue extracts from *T. turcica*. The highest TPC and TFC of the extracts were obtained from the leaf extracts. In a previous study, Bali et al. (2014) evaluated the TPC of ethyl acetate, ethanol, and methanol extracts of the total aerial parts of *T. turcica* plants. Authors determined the highest TPC value in ethyl acetate followed by methanol extracts and the results ranged from 162.5 ± 1.2 to 44.9 ± 0.90 µg gallic acid/mg of dry extract. However, the highest TPC values were obtained when acetone was used as a solvent (Aksoy et al., 2013). Methanol extracts in plants have been found to contain high TPC (Molole, Gure & Abdissa, 2022), indicating better solubility of these compounds in polar solvents. Overall, the higher phenolic substance content in leaves is a well-documented phenomenon supported by scientific evidence. Understanding the role of phenolic compounds in leaves can provide valuable insights into plant defense mechanisms and potential health benefits. Further research in this area is warranted to explore the full potential of phenolic compounds in leaves.

It is known that there is a significant correlation between antioxidant capacity and phenolic substance content of medicinal plants (Cai, Luo, Sun, & Corke, 2004). *T. turcica* has been suggested as a natural source of antioxidants due to the phytochemicals of the aerial parts of the plant (Aksoy et al., 2013). Previous studies have shown that ethanol and water extracts of *T. turcica* had antioxidant effects (Çelik & Küçükkurt, 2016). Ethyl acetate, methanol, and ethanol extracts were also mentioned to be effective antioxidants due to the quantity of their

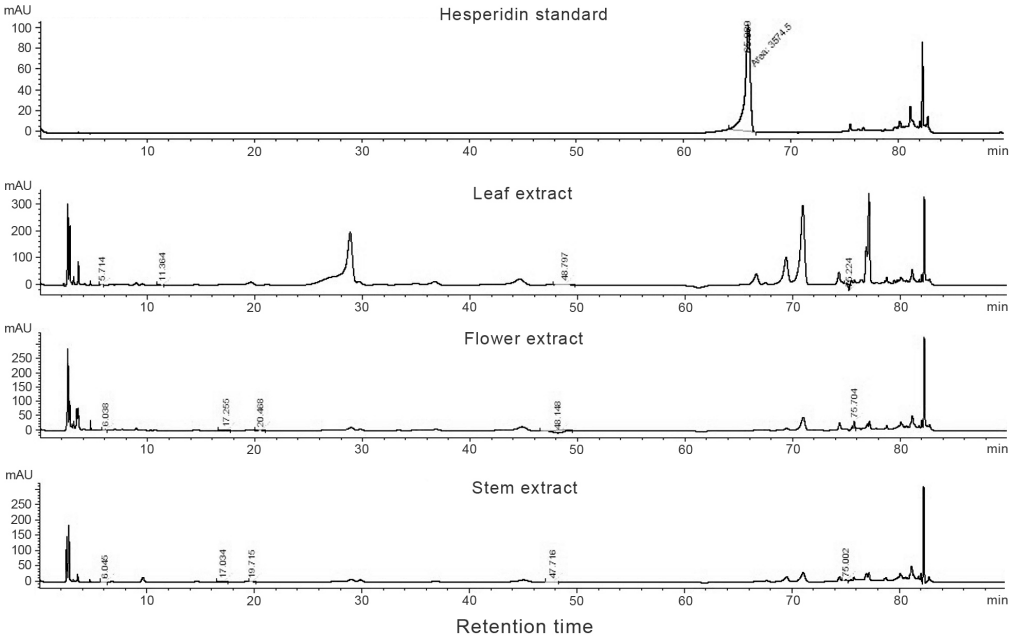

Figure 2. Representative HPLC chromatograms of the hesperidin standard and phenolic acids in the methanolic extracts of *T. turcica* tissues.

Table 2. Quantitative changes in phenolic compounds in different tissue extracts of *T. turcica.*

Phenolic compounds	Correlation (r^2)	RT (min)	Leaf (µg/g DW)	Flower (µg/g DW)	Stem (µg/g DW)
Gallic acid	0.99966	5.912	1.21 ± 0.17	0.92 ± 0.16	0.59 ± 0.12 [c]
Catechin hydrate	0.99906	11.499	3.00 ± 0.41	*ND*	*ND*
Chlorogenic acid	0.99970	16.239	*ND*	1.03 ± 0.02	*UC*
4-Hydroxy benzoic acid	0.99994	17.647	*UC*	0.89 ± 0.08	*ND*
Epicatechin	0.99879	20.169	*ND*	5.26 ± 0.30 [c]	7.64 ± 1.23 [b]
Caffeic acid	0.99892	21.476	1.09 ± 0.01	*ND*	*ND*
3-Hydroxy benzoic acid	0.99928	22.545	1.71 ± 0.45	*ND*	1.16 ± 0.04
Syringic acid	0.99839	22.628	*ND*	*ND*	*ND*
p-Coumaric acid	0.99982	33.597	0.57 ± 0.02	1.69 ± 0.12 [a]	0.62 ± 0.13 [d]
t-Ferrulic acid	0.99993	37.202	0.36 ± 0.09	0.28 ± 0.11	0.06 ± 0.02
Sinnapic acid	0.99925	38.264	*ND*	0.38 ± 0.05	*ND*
Benzoic acid	0.99986	47.629	21.07 ± 2.84	46.25 ± 3.86 [b]	*UC*
Hesperidin	0.99705	65.989	29.12 ± 1.29	4.79 ± 0.25 [a]	3.59 ± 0.13 [a]
Rosmarinic acid	0.99907	70.655	11.78 ± 2.34	3.21 ± 0.16 [b]	2.70 ± 0.07 [b]
t-Cinnamic acid	0.99998	75.207	2.21 ± 0.45	1.07 ± 0.01 [c]	0.46 ± 0.04 [b]
Quercetin	0.99962	76.313	58.11 ± 0.48	8.66 ± 0.66 [a]	14.10 ± 0.38 [a,e]

[a] P<0.001 vs Leaf group, [b] P<0.01 vs Leaf group, [c] P<0.05 vs Leaf group, [d] P<0.001 vs Flower group, [e] P<0.01 vs Flower group. *ND* = Not determined. *UC* = Uncalculated.

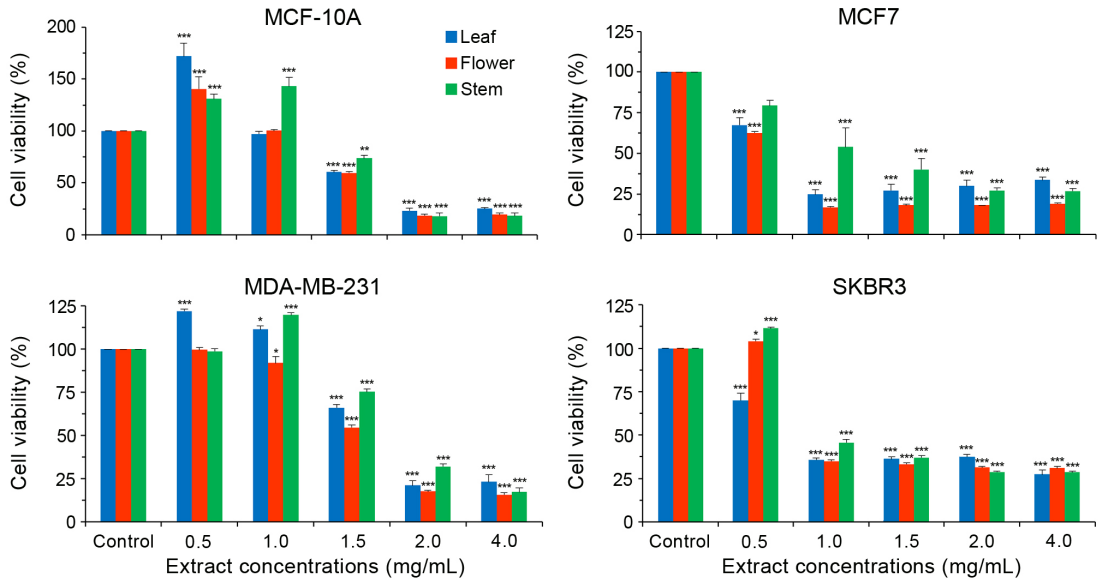

Figure 3. Cytotoxic effects of the leaf, flower, and stem extracts of *T. turcica* on the normal and breast tumor cell lines. Data is presented as mean ± SE. ***P < 0.001, **P < 0.01, and *P < 0.05 compared with the control group.

Table 3. IC_{50} values of the different tissue extracts of *T. turcica* for the normal and breast tumor cell lines.

Cell lines	Leaf	Flower	Stem
	IC_{50} values (mg/mL)		
MCF-10A	1.63 ± 0.01	1.59 ± 0.01	1.69 ± 0.04
MCF7	0.65 ± 0.19 [a]	0.55 ± 0.02 [b]	1.10 ± 0.58
MDA-MB-231	1.62 ± 0.03	1.53 ± 0.02	1.75 ± 0.06
SKBR3	0.75 ± 0.18 [a]	1.11 ± 0.08 [a]	1.30 ± 0.04 [a]

[a] P<0.05 vs MCF-10A group, [b] P<0.001 vs MCF-10A group.

total phenolic compounds (Bali et al., 2014). In our study, the results showed that leaf extracts exhibited antioxidant capacity more than flower and stem extracts. Indeed, TPC and TFC were highly correlated with the antioxidant capacity measured by TAC, CUPRAC, FRAP, and DPPH assays. This result suggested that there is a relationship between antioxidant capacity and the content of phenolic acids or flavonoid compounds for all extracts. Sinan et al. (2023) suggested the high antiradical and antioxidant activity of methanol extracts could be attributed to their high total phenolic and flavonoid contents. Kumar and Goel (2019) reported that substituents on the aromatic ring in phenolic acids impact the stabilization of the structure, thus influencing the radical-quenching ability. In fact, the antioxidant activity of the extracts may also be associated with other compounds with a specific antioxidant potential (Huang, Ou, & Prior, 2005).

Plant phenolics such as simple phenols, phenolic acids, and flavonoids are a special class of secondary metabolites. In addition to their important functions in plant metabolism, phenolic acids are the precursors of many bioactive compounds beneficial for human health (Kumar & Goel, 2019). There are no studies in the literature on the phenolic acid profiles of *T. turcica* extracts. In the present study, therefore, phenolic acid profiles of the leaf, flower, and stem extracts of *T. turcica* were analyzed qualitatively and quantitatively. Our findings revealed that there are organ-specific differences in the phenolic acid profiles of extracts. Among the analyzed sixteen phenolic compounds, hesperidin, quercetin, and rosmarinic acid were found as the main compounds in leaf extracts, while benzoic acid content was remarkable in the flower extracts of *T. turcica*. The health benefits of phenol compounds are linked to their function in preventing various ailments associated with the destructive impact of free radicals and ROS (Valko et al., 2007). Hesperidin, a flavonoid that falls under the flavanone group, has been demonstrated to have significant antioxidant, anti-inflammatory, and neuroprotective effects in various models of central nervous system disorders (Muhammad et al., 2019). Furthermore, hesperidin's anticancer potential has been described through different mechanisms of action (Pandey & Khan, 2021). Quercetin, another flavonoid, possesses potent antioxidant properties that allow it to scavenge free radicals, decrease oxidative stress, and safeguard against cellular damage. Quercetin's anti-inflammatory properties involve the inhibition of inflammatory cytokines and enzymes, making it a potential therapeutic agent for various inflammatory conditions (Aghababaei & Hadidi, 2023). Rosmarinic acid, which possesses antioxidant and anti-inflammatory properties, has been observed to have positive effects on cancer disease (Ijaz et al., 2023).

Breast cancer is one of the most marked common malignant tumors among women (Wang et al., 2022). The use of plant-

derived products in cancer treatment has gained great importance in recent years. Plant phenolics exert a great potency for the prevention and treatment of oxidative stress-related disorders such as cancer (Abotaleb, Liskova, Kubatka, & Büsselberg, 2020). Among the flavonoid components, quercetin is suggested to overcome tumor cells via modulation of proliferation and apoptosis. Previous research has demonstrated that quercetin modulates several signal pathways to inhibit the progression of breast cancer (Ranganathan, Halagowder, & Sivasithambaram, 2015; Liu, Lee, & Ahn, 2019). Hesperidin is a flavonoid that possesses various biological activities, suggesting therapeutic potential in the treatment of cancer (Madureira et al., 2023). Recently, Önder et al. (2023) reported that hesperidin exerts cytotoxic effects by inhibiting cellular proliferation and inducing apoptosis in MCF-7 and MDA-MB-231 breast cancer cell lines. Benzoic acid and its derivatives, which are included in a class of simple phenolic acids, have been reported to have biological activities such as inhibiting the growth of breast cancer cells (Lin, Chen, Chou, & Wang, 2011). In the present study, exposure of the human breast cancer cell lines (MCF7, MDA-MB-231, and SKBR3) to the *T. turcica* extracts caused a decrease in cell proliferation depending on the concentration and the type of each extract. The IC_{50} value (0.65 mg/mL and 0.55 mg/mL, respectively) of leaf and flower extracts in MCF-7 cells was found to be lower than the value of normal MCF-10A cells. Similar results were also determined for SKBR3 cell lines. However, IC_{50} values for MDA-MB-231 cells were similar to control cells for all extracts. There are very few studies providing data on the anticancer potential of *Thermopsis* species. For instance, ethanol extracts (50 and 500 µg/mL) of *T. rhombifolia* leaves were found to exert cytotoxic activity on human colon cancer (HT-29) and brain tumor cell lines (SHSY5Y). Twenty-four hours exposure of HT-29 and SHSY5Y cells to the extracts resulted in a decrease in cell viability with IC_{50} values of 220 and 183 µg/mL, respectively (Kernéis et al., 2015). Furthermore, ethanol extracts (0.1 - 1.000 µg/mL) of *T. rhombifolia* aerial parts also demonstrated anticancer activity on HT-29 (IC_{50}: 130 µg/mL), M059K malignant glioblastoma (IC_{50}: 90 µg/mL), and WI-38 normal lung fibroblast (IC_{50}: 240 µg/mL) cell lines after 96 hours exposure (Tuescher et al., 2020). However, luteolin extracted from *T. rhombifolia* has been shown to inhibit cyclin dependent kinase and arrested cells in the G1 phase of the cell cycle (Tuescher et al., 2020). The predominant compounds of *T. turcica* extracts such as quercetin, hesperidin, and benzoic acid may be recognized as inhibitors of breast cancer cell proliferation.

CONCLUSION

In summary, the current study presented a comparative analysis of the antioxidant capacity, phenolic acid profile, and biological activities of the different tissue extracts of *T. turcica*. High levels of TPC and TFC were highly correlated with the antioxidant capacity measured by TAC, CUPRAC, FRAP, and DPPH assays. The leaf extracts exerted the highest antioxidant activity for all assays. HPLC analyses showed high amounts of quercetin and hesperidin in leaf extract, while benzoic acid was found as the predominant compound in flower extract. These phytochemicals may be responsible for the cytotoxic effects of *T. turcica* on human breast cancer. However, there is a need to test the individual and synergistic effects of these phytochemicals.

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