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AN IN VITRO ASSESSMENT OF THE BIOACTIVE, CYTOTOXIC, AND ANTIDIABETIC POTENTIAL OF DIFFERENT PARTS OF TUMBLEWEED (*GUNDELIA TOURNEFORTII* L.)

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Kutlu, G., Erol, K. F. (2025). Kenger (*Gundelia tournefortu* L.) bitkisinin farklı kısımlarının biyoaktif, sitotoksik ve antidiyabetik potansiyelinin in vitro değerlendirilmesi. *GIDA (2025) 50 (1) 28-41* doi: 10.15237/ gida.GD24096

ABSTRACT

This study aimed to investigate the physicochemical properties, bioactive compounds, cytotoxicity, and antidiabetic potential of the tumbleweed plant's roots (TR), stems & leaves (TSL), and flowers (TF). Results indicated TF as the richest part in bioactive compounds and antioxidant capacity, with the highest crude protein (13.91%), crude oil (15.50%), total phenolic content (214.64 mg GAE/g), total monomeric anthocyanin content (1132.96 mg/g), ABTS (25.48 mg TE/g), CUPRAC (203.92 mg TE/g), and FRAP (32.63 µmol Fe^{2+}E/g) activities. Mineral contents varied, with Mg, K, and Ca present in significant amounts across all parts. TF also showed the highest α-glucosidase (61.99%) and α-amylase (54.92%) inhibition, suggesting strong antidiabetic potential. Cytotoxicity was weak across samples, with IC_{50} values ranging from 1049.76 to 1641.08 µg/ mL against HEK-293 and Caco-2 cells. These findings highlight TF as the most bioactive and nutritionally rich part of the tumbleweed plant.

Keywords: *Gundelia tournefortii* L., bioactive properties, antidiabetic potential, antiproliferative characteristics, mineral content

KENGER (*GUNDELIA TOURNEFORTII* L.) BİTKİSİNİN FARKLI KISIMLARININ BİYOAKTİF, SİTOTOKSİK VE ANTİDİYABETİK POTANSİYELİNİN IN VITRO DEĞERLENDİRİLMESİ

ÖZ

Bu çalışma, kenger bitkisinin kökleri (TR), gövde ve yaprakları (TSL) ile çiçeklerinin (TF) fizikokimyasal özelliklerini, biyoaktif bileşiklerini, sitotoksisite ve antidiabetik potansiyelini incelemeyi amaçlamıştır. Sonuçlar, en yüksek ham protein (%13.91), ham yağ (%15.50), toplam fenolik içeriği (214.64 mg GAE/g), toplam monomerik antosiyanin içeriği (1132.96 mg/g), ABTS (25.48 mg TE/g), CUPRAC (203.92 mg TE/g) ve FRAP (32.63 µmol Fe^{2+}E/g) aktiviteleri açısından en zengin kısmının TF olduğunu göstermiştir. Magnezyum, potasyum ve kalsiyum gibi mineraller tüm

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bitki kısımlarında belirgin miktarda bulunmuştur. TF, aynı zamanda en yüksek α-glukozidaz (%61.99) ve α-amilaz (%54.92) inhibisyonu göstererek güçlü antidiabetik potansiyele işaret etmiştir. Tüm örneklerde sitotoksisite zayıf bulunmuş olup HEK-293 ve Caco-2 hücrelerine karşı IC_{50} değerleri 1049.76-1641.08 µg/mL arasında değişmiştir. Bu bulgular, kenger bitkisinin biyoaktif ve besin öğeleri açısından en zengin kısmının TF olduğunu göstermektedir.
Anahtar kelimeler: *Gundelia tournefortii L.*, biyoaktif özellikler, antidiyabetik potansiyel, antiproliferatif özellikler, mineral içeriği

INTRODUCTION

Plants are a vital natural resource for humanity, offering a wide range of phytochemicals and producing various secondary metabolites. The therapeutic properties of plants, specific to certain species or groups, align with the idea that the unique composition of secondary metabolites within each plant is taxonomically specific (Diab et al., 2021). Given that current food production will be insufficient to meet future demands, integrating crops from wild edible plants is essential for creating a sustainable food system. This strategy aims to enhance food security and nutrition through innovative and adaptive agricultural practices (Heywood, 2011). Edible flowers have long been associated with traditional cuisines and cultures worldwide. They are commonly incorporated into foods and beverages for their medicinal or pharmaceutical benefits (Yasar et al., 2022).

Tumbleweed (tumble thistle, *Gundelia tournefortii* L.), a member of the Asteraceae (Compositae) family, is native to various regions including Türkiye, Egypt, Iran, Israel, Jordan, Azerbaijan, Turkmenistan, and Syria. This spiny, thistle-like plant naturally grows in Türkiye's sandy and loamy soils, thriving in well-drained, moist conditions and unable to survive in shaded environments (Matthäus and Özcan, 2011; Mehrzadeh et al., 2024). Tumbleweed offers several edible parts, including its leaves, stems, roots, and immature flower buds. The tender, white parts of the young leaves are often utilized in soups. Moreover, the plant's seeds, stems, leaves, and flowers are used as food, underlining its role as a crucial dietary element and a notable medicinal herb in Eastern Anatolia (Coruh et al. 2007; Matthäus and Özcan, 2011; Mehrzadeh et al., 2024). The leaves and stems of tumbleweed are commonly used in soups and salads, while in the Middle East, the young flower buds are sold in local markets. The seeds are often used in pickling, and the fruits are preserved in vinegar or lemon juice with salt to serve as a garnish. Additionally, leaves, young stems, and the inflorescences are key ingredients in the traditional Palestinian dish known as "Akoob" (Tarhan et al., 2023). Traditionally, this genus has been used to treat various ailments, including kidney stones, bronchitis, infections, and stomach pain. Recent studies have shown that its extracts possess hypolipidemic, hepatoprotective, anti-inflammatory, antioxidant, and antimicrobial properties. Additionally, several phytochemicals such as sterols, terpenoids, fatty acids, along with essential minerals and vitamins, have been identified in the genus (Mehrzadeh et al., 2024).

Recent research has increasingly focused on the physicochemical characteristics, phytochemical properties —including phenolic profile, antioxidant activity, and total phenolic content (TPC)—along with antidiabetic and cytotoxic effects, as well as the pharmacological effects of wild tumbleweed plant (Coruh et al. 2007; Haghi et al., 2011; Matthäus and Özcan, 2011; Asghari et al., 2015; Ereifej et al. 2015; Kadan et al., 2018; Saraç et al., 2019; Özaltun and Daştan 2019; Keskin et al., 2021; Tarhan et al., 2023). Although significant studies on these aspects are present in the literature, there is a lack of comprehensive studies examining different plant parts. Moreover, in vitro antidiabetic activity studies are very limited, and cytotoxicity studies on Caco-2 and HEK-293 cell lines have been conducted for the first time in this study. This research aims to fill this gap by evaluating three different parts of the tumbleweed plant: roots (TSL), stems and leaves (TSL), and flowers (TF). The objective of the study is to comprehensively address the physicochemical properties, bioactive compounds, antidiabetic potential, and cytotoxicity of these plant parts.

MATERIALS AND METHOD

Materials

Wild tumbleweed (*Gundelia tournefortii* L.) plants were gathered in June 2023 from the Central Anatolian Region of Türkiye, specifically from İnkışla, Gemerek, Sivas. The study utilized a variety of reagents and materials, including α-amylase, pepsin, and pancreatin, (all Sigma–Aldrich, St. Louis, MO, USA). Dulbecco's Modified Eagle Medium (DMEM) was sourced from Gibco Inc. (USA). Other chemicals included bile salts, Folin-Ciocalteu phenol reagent, sodium carbonate (Na_2CO_3), gallic acid, malvidin-3-glucoside, ABTS, Trolox, TPTZ, and potassium persulfate (all Sigma-Aldrich, Steinheim, Germany), as well as MTT (Serva GmbH, Germany) and 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB, Sigma–Aldrich). Additionally, metals such as copper (Cu), zinc (Zn), and iron (Fe) were sourced from Merck, and phosphorus (P) was obtained from Supelco. Ethanol were also provided by Merck. Ultrapure water was prepared using a Milli-Q purification system (Millipore, France).

Preparation of plants

The plants were thoroughly washed 2 times with running tap water followed by rinsing with distilled water to remove dust and other impurities. The manually harvested plants were then divided into three distinct parts: roots (TR), stems & leaves (TSL), and flowers (TF). The separated plant sections were air-dried at room temperature in ventilated place for 10 days, ensuring they were not exposed to direct sunlight. After drying, the samples were finely ground using a laboratory mill. The powdered samples were stored in beakers sealed with parafilm and kept in a refrigerator (Arçelik, Türkiye) at +4°C for future analyses. In order to increase the clarity and comprehensibility of the analyses carried out within the scope of the study, these analyses are visually summarised in a graphical abstract as presented in Figure 1.

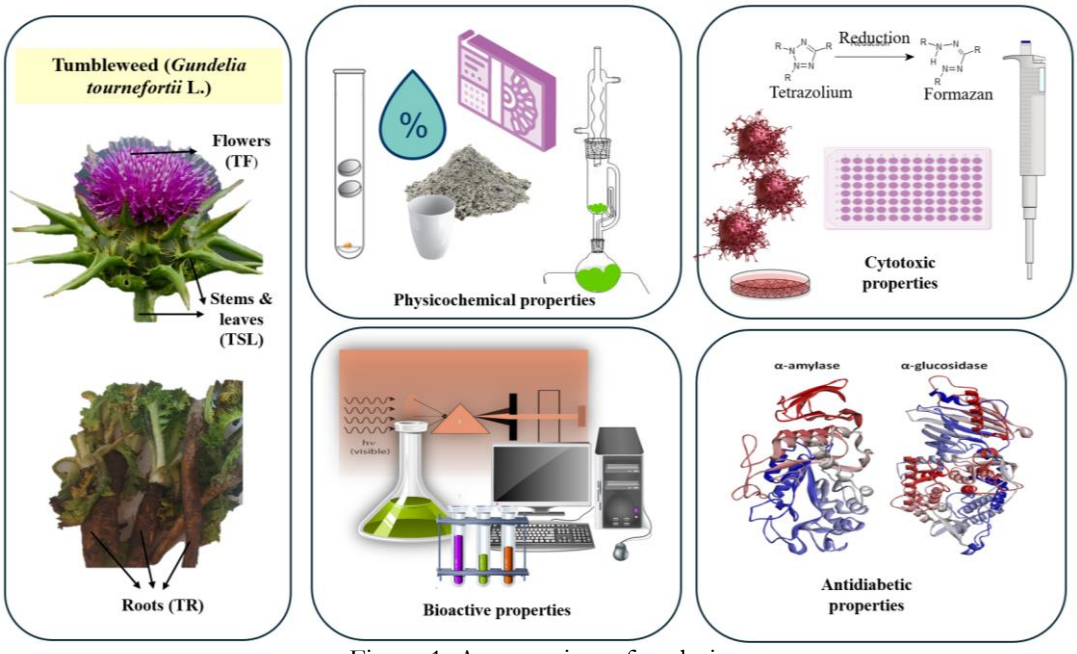

Figure 1. An overview of analysis.

Physicochemical analysis

Determination of moisture content

The moisture content of tumbleweed plant parts was measured using the oven-drying method. Approximately 3 g of the sample were weighed and placed in an oven (Memmert UF-110, Germany) at 105°C for 3 h. The percentage of moisture content was calculated based on the weight loss recorded after drying, as described by Kutlu et al. (2024a).

Determination of ash content

The ash content of tumbleweed plant parts was determined using an electric muffle furnace (WiseTherm-Daihan FH-03, Korea) with high-temperature capabilities. Porcelain crucibles were initially heated to 650°C to achieve a constant weight. Approximately 2 g of the sample were placed in these crucibles. The samples underwent a pre-ashing process with ethanol, followed by incineration at 650°C until all black residues were eliminated. The ash content percentage was then calculated based on the dry matter, as outlined by Kutlu (2015).

Determination of oil content using the soxhlet method

To measure the oil content in tumbleweed plant parts, approximately 4 g of the sample were weighed and wrapped in filter paper. The filter papers were placed into a cartridge, which was packed with cotton and inserted into an extractor. Hexane was used as the solvent with heating set to achieve a slow boil, and the reflux condenser was activated. The extraction continued for 8 h with continuous hexane flow. After extraction, the hexane was evaporated using a rotary evaporator (BUCHI-Vacuum Controller V-850, Switzerland). Any residual hexane was removed by placing the samples in an oven at 105°C (Memmert UF-110, Germany) until a constant weight was achieved in a desiccator. The final weight of the flask was recorded, and the percentage of oil content was calculated (Kutlu, 2015).

Determination of protein content

Approximately 3 g of the plant samples were weighed and placed into a Kjeldahl flask. Subsequently, 25 mL of concentrated H_2SO_4 and one catalyst tablet (containing a mixture of potassium sulfate (K_2SO4), sodium sulfate (Na_2SO_4), titanium dioxide (TiO_2), and copper sulfate ($CuSO_4$) were added. The sample was subjected to a digestion process at 200-250°C for 2 h and then at 350-400°C for 8 h. During the digestion, the color of the samples changed from black to a light, transparent color. After allowing the samples to cool, 5 mL of 40% NaOH and 3 mL of 3% boric acid were added, and the distillation process was initiated (Behr Distillation Unit-S5, Germany). After 4 min of distillation, titration was performed using 0.1 N HCl, and the volume of HCl consumed was recorded in mL. The percentage of crude protein was then calculated as follows (Kutlu, 2015):

$$Nitrogen\ (\%) = \frac{((V_1 - V_0) * N * 0.014) * 100}{m} \quad (1)$$

V_1 is the volume of HCl consumed during titration (mL); V_0 is the volume of HCl consumed in the blank titration (mL); N is the normality of the HCl solution used in titration (0.1 N), 0.014 is the milliequivalent weight of nitrogen, m is the mass of the food sample taken (g).

Mineral content

The concentrations of essential (Mg, P, Ca, Na, and K) and trace elements (Zn, Fe, and Cu) in the tumbleweed plant parts were analysed using an atomic absorption spectrophotometer (Perkin Elmer-Analyst 800, Norwalk, CT) following the method outlined by Erol et al. (2024); Erol and Kutlu (2024). 1.0 g of the sample was treated with 1.5 mL of 30% hydrogen peroxide and 6.5 mL of concentrated 69% nitric acid, then subjected to microwave digestion (MD). The MD was conducted under conditions of 220 PSI and 180 °C, with a gradual increase in temperature over 15-25 min, followed by a stabilization period of 10-15 min. After cooling to room temperature, the solution was diluted to 50 mL with high-purity water. Following the digestion, essential and trace elements were determined using an atomic absorption spectrophotometer, with calibration standards prepared from 1000 μg mL^{-1} stock solutions of the tested elements in deionized water.

Extraction of dried tumbleweed plants

The extraction of dried tumbleweed plant parts was performed following the method described by Sasidharan et al. (2021), with minor modifications. The dried and ground parts of the tumbleweed plant were combined with absolute ethanol at a 1:10 (w:v) ratio and left to stand at room temperature for 5 days. Following this, the mixtures were centrifuged at 5000 rpm for 5 min at 25°C to separate the solid residues. The resulting supernatant was concentrated using a

rotary evaporator (Heidolph Instruments GmbH and Co.KG, Schwabach, Germany). The concentrated extract was then frozen at -18ºC for 12 h before being lyophilized (Christ β 1,8-LSC plus, Martin Christ GmbH, Osterode am Harz, Germany) to obtain a dry extract. The lyophilized extract was further pulverized into a fine powder using a porcelain mortar. The prepared extracts were stored in the dark at 4ºC in stoppered flasks until further use

Estimation of total polyphenol content (TPC)
The TPC of different parts of tumbleweed plants was determined using the Folin–Ciocalteu (Sigma-Aldrich) method, following the procedure described by Demirkan et al. (2024); Erol and Kutlu (2024). In brief, 0.5 mL of the sample was placed into a 10 mL test tube, and then 0.5 mL of 2 N Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and 0.5 mL of 7.5% sodium carbonate solution (Sigma Aldrich Co., Singapore) were added. The mixture was shaken vigorously and left to incubate in the dark at room temperature for 30 min. After the incubation period, the absorbance was measured at 760 nm using a SHIMADZU (UV-1800) UV-VIS-NIR spectrophotometer (Japan). A standard curve was prepared with gallic acid, and the TPC results were reported as milligrams of gallic acid equivalents per gram of dried extract (mg GAE g^{-1} dried extract), demonstrating a linear range of 2.5 to 80 μg/mL ($R^2 = 0.999$).

Total monomeric anthocyanin content (TAC)
The pH differential method employed for the determination of monomeric anthocyanins was adapted from the protocol described by Ağçam and Akyıldız (2015). This method is based on the principle that, at pH 1, the colored oxonium form of monomeric anthocyanins predominates, while at pH 4.5, the colorless hemiketal form is more prevalent. Accordingly, the difference in absorbance values measured at pH 1 and pH 4.5 is directly proportional to the anthocyanin concentration. To adjust the pH values to the desired levels, 0.025 M potassium chloride (KCl, adjusted to pH 1 with HCl) and 0.4 M sodium acetate ($CH_3COONa \cdot 3H_2O$, adjusted to pH 4.5 with HCl) buffer solutions were utilized. The volumes of the potassium chloride (pH 1.0) and sodium acetate buffer solutions (pH 4.5) were determined based on the optimal dilution ratio. This dilution ratio was applied to each solution, and readings were taken after allowing 15 min for equilibrium to be established. For the analysis, 0.5 mL of extract was taken into two glass tubes. To the first tube, 7.5 mL of the 0.025 M potassium chloride solution was added, while 7.5 mL of the 0.4 M sodium acetate solution was added to the second tube. Absorbance readings were taken at 700 nm wavelength absorbance (λ_{max}) to determine turbidity. Following the 15-min incubation period, absorbance measurements were performed using a Perkin Elmer Lambda 25 UV/VIS spectrophotometer (Massachusetts, USA, 2005) in 1 cm thick disposable cuvettes. To identify the wavelenghth absorbance of the tumbleweed plant parts anthocyanins, the absorbance measurements were conducted between 470 and 570 nm. The maximum absorbance was determined to be at 518 nm, and calculations were based on the absorbance values at this wavelength. The amount of monomeric anthocyanins in tumbleweed plant parts was calculated based on the malvidin-3-glucoside using the equation provided below. The results are expressed as a concentration corresponding to 1 g of extract (mg/g). The concentration of monomeric anthocyanins was estimated using the following formula:

$$Monomeric\ anthocyanin\ content\ \left(\frac{mg}{g}\right) = \frac{A*MW*SF)}{(\varepsilon*L)} * 100 \quad (2)$$

A represents the absorbance difference measured at pH 1 and 4.5, calculated as:

$$A = ((A_{\lambda max} - A_{700})_{pH1} - (A_{\lambda max} - A_{700})_{pH4.5}) \quad (3)$$

MW is the molecular weight of the anthocyanin of interest (655.6 g/mol); SF is the dilution factor; ε is the molar absorptivity coefficient (26,900 L/(mol·cm)); L represents the path length of the absorbance cuvette (1 cm).

Determination of reducing power of antioxidants by CUPRAC and FRAP method

The cupric ion reducing antioxidant capacity (CUPRAC) of different parts of tumbleweed plants was evaluated according to the method described by Yasar et al. (2022). The assay involved mixing a sample solution (1 g) with a premixed reaction mixture composed of $CuCl_2$ (1 mL, 0.01 mM), neocuproine (1 mL, 7.5 mM) in 100% ethanol, NH_4Ac buffer (1 mL, 1 M, pH 7.0) in distilled water, and 1 mL of distilled water. If the sample possesses antioxidant properties, it reduces Cu (II) to Cu (I), with neocuproine forming a yellow chromophore complex with Cu (I). The mixture was incubated at room temperature for 1 h in the dark within a sealed test tube. After incubation, the absorbance was measured at 450 nm against a reagent blank, and the CUPRAC activity was expressed as milligrams of trolox equivalents per gram of extract (mg TE/g dry extract). The standard curve exhibited a linear range of 25 to 400 μM, with an R^2 value of 0.993.

The ferric reducing antioxidant power (FRAP) assay was conducted according to the method provided by Kutlu (2024). A sample solution (20 μL) was added to a FRAP reagent mixture (2 mL), which included acetate buffer (300 mM, pH 3.6), TPTZ (10 mM) dissolved in 40 mM HCl, and ferric chloride (20 mM) in a 10:1:1 volumetric ratio. After 30 min of incubation at room temperature, the absorbance was recorded at 593 nm. FRAP activity was expressed as micromoles of Fe^{2+} equivalents per gram of dry weight (mmol Fe^{2+}E/g). The standard curve demonstrated a range of 0.008 to 0.5 mg/mL, achieving an R^2 value of 0.999.

Determination of scavenging activity by ABTS method

The ABTS assay of various parts of tumbleweed plants was performed as described by Erol et al. (2023). In summary, a mixture of x mL of the sample solution and (4 − x) mL ethanol was prepared, with 4 mL of ethanol serving as the reagent blank. A 1 mL portion of a 1:10 diluted ABTS radical cation solution, which had been prepared by combining a 7-mM ABTS solution with a 2.45-mM potassium persulfate solution and allowing it to react in the dark at room temperature for 12–16 h, was added to the mixture at 15-sec intervals and mixed thoroughly. Absorbance readings were taken at 734 nm after a 6-min incubation, using ethanol as the reference. The decrease in absorbance (A_0) observed in the presence of antioxidants was proportional to their concentration. The antioxidant capacity of the samples, determined using a Trolox calibration curve, was expressed as milligrams of Trolox equivalents per gram of dry extract (mg TE/g dry extract). Standar curve consisted of a range of 0.008 to 0.5 mg/mL, achieving an R^2 value of 0.999.

Phenolic profile

Phenolic compounds in various parts of tumbleweed plants were identified using high-performance liquid chromatography (HPLC) coupled with a diode array detector (HPLC-DAD, Shimadzu Corp., Kyoto, Japan). Prior to analysis, the extracts were filtered through a 0.45-μm membrane filter, and 1 mL of the filtrate was injected into the HPLC system (equipped with a CMB-20A communications bus module, SPDM20A DAD detector, DGU-20A5R degasser, SIL-20A HT autosampler, LC-20AD pump, and CTO-10ASVP column oven, all from Shimadzu Corp., Kyoto, Japan). The separation process was performed at 40°C on a reversed-phase Intersil® ODS C-18 column (250 mm × 4.6 mm, 5 μm particle size, GL Sciences, Tokyo, Japan). The mobile phases consisted of solvent A (distilled water containing 0.1% acetic acid, v/v) and solvent B (acetonitrile with 0.1% acetic acid, v/v). Gradient elution was programmed as follows: 10% B from 0–2 min, 10%–30% B from 2–27 min, 30%–90% B from 27–50 min, and 90%–100% B from 51–60 min, returning to the initial conditions at 63 min. The flow rate was maintained at 1 mL/min, and chromatograms were monitored at wavelengths between 254 and 356 nm. Identification and quantification of phenolic compounds were achieved by comparing retention times and standard curves, with the results expressed as μg/g for different parts of the tumbleweed plants (Kayacan et al., 2020).

Assessment of antidiabetic activities

To evaluate in vitro antidiabetic activity, α-amylase and α-glucosidase inhibition assays were conducted as per the method described by Kutlu (2024). For the α-glucosidase assay, extracts were combined with the enzyme and para-nitrophenyl-α-D-glucopyranoside (P-NPG), and the absorbance was recorded at 405 nm. In the α-amylase assay, the enzyme was pre-incubated with the samples, followed by the addition of starch and DNSA reagent; absorbance was measured at 405 nm after heating.

Cytotoxic activity

For assessing in vitro cytotoxicity of various parts of tumbleweed plants, Caco-2 and HEK-293 cell lines were cultured in DMEM supplemented with FBS, penicillin-streptomycin, and L-glutamine. The MTT assay was performed by plating the cells in 96-well plates and exposing them to different concentrations of tumbleweed plant extracts. After incubation with MTT and DMSO, absorbance was measured to determine cell viability, which was inferred from the reduction of MTT to formazan crystals (Erol and Kutlu, 2024).

Statistical analysis

Each sample was analyzed in triplicate to ensure accuracy. Results are presented as means ± standard deviations. Statistical analysis was performed using JMP 6.0 software (SAS Institute, Inc., Cary, USA). Prior to conducting ANOVA, the normality of the data distribution was assessed using the Shapiro-Wilk W test, with a p-value of ≥ 0.05 indicating normal distribution. Tukey's honestly significant difference (HSD) test was used for post-hoc comparisons. Statistical significance was assessed using one-way ANOVA with a significance level using set at $P < 0.05$ (Erol and Kutlu, 2024).

RESULT AND DISCUSSION

Proximate profile

Table 1 provides a summary of the proximate composition, including moisture, protein, total fat, and ash content in different parts of tumbleweed. Moisture content is one of the most commonly measured properties in food materials (Alam et al., 2020). The moisture content was recorded as 9.32% for TR, 13.35% for TSL, and 9.92% for TF. In terms of crude protein, the values for TR, TSL, and TF were 4.05%, 9.12%, and 13.91%, respectively. The crude ash content was 8.89% for TR, 5.85% for TSL, and 8.88% for TF. Additionally, the crude oil content varied, with TR showing the lowest value at 6.16%, followed by 8.95% for TSL, and the highest oil content of 15.50% in TF. These findings align with previous studies, such as Matthäus and Özcan (2011), who reported 8.6% moisture, 16.2% crude oil, 12.6% protein, and 8.7% ash in the ripe flower buds of tumbleweed. Similarly, Ereifej et al. (2015) observed 94.2% dry matter, 14.6% protein, 1.6% fat, and 18.7% ash in the stems and leaves of *G. tournefortii* L. collected from Jordan. These comparisons highlight the variation in physicochemical properties across different parts of the tumbleweed plant, emphasizing its nutritional and functional potential.

Table 1. Some physicochemical properties of tumbleweed parts.

The name of samples	Moisture (%)	Crude protein (%)	Crude ash (%)	Crude oil (%)
TR	9.32±0.73^{b}	4.05±0.38^{c}	8.89±0.40^{a}	6.16±0.50^{c}
TSL	13.35±1.30^{a}	9.12±0.76^{b}	5.85±0.56^{b}	8.95±0.49^{b}
TF	9.92±0.45^{b}	13.91±0.93^{a}	8.88±0.62^{a}	15.50±0.93^{a}

TR: The roots of tumbleweed; TSL: The stems & leaves of tumbleweed; TF: The flowers of tumbleweed

Minerals are essential micronutrients that support bone health, enzyme activity, nerve function, and oxygen transport, making adequate intake crucial for overall health and preventing deficiencies (Kutlu et al., 2024b). The mineral profile (Table 2) of the tumbleweed plant showed significant variation depending on the part analyzed ($P < 0.05$). For example, the mineral composition of the TR section, ranked from highest to lowest, included Mg (1047 mg/100 g), K (464 mg/100 g),

Ca (340.33 mg/100 g), P (22.33 mg/100 g), Na (12.51 mg/100 g), Fe (3.34 mg/100 g), Zn (2.18 mg/100 g), and Cu (1.27 mg/100 g). In contrast, the TSL section showed the highest mineral concentration for Mg (898.33 mg/100 g), followed by Ca (394 mg/100 g), K (353.67 mg/100 g), P (45.00 mg/100 g), Na (17.65 mg/100 g), Fe (4.81 mg/100 g), Zn (3.49 mg/100 g), and Cu (3.27 mg/100 g). Similarly, the TF section exhibited a mineral content trend similar to the TR section, with Mg (1164.67 mg/100 g) as the dominant mineral, followed by K (528.33 mg/100 g), Ca (376.67 mg/100 g), P (47.67 mg/100 g), Na (22.30 mg/100 g), Fe (7.57 mg/100 g), Zn (5.50 mg/100 g), and Cu (5.04 mg/100 g). According to the recommended dietary allowance (Anonymous, 2017), 100 g of various parts of the tumbleweed plant can fulfill 21.8–55% of the daily Zn requirement, 127–344% of the Cu requirement, 23.86–54.07% of the Fe requirement, 42.54–49.25% of the Ca requirement, 239.56–310.58% of the Mg requirement, 17.68–26.41% of the K requirement, and 3.19–6.81% of the P requirement. These findings align with previous studies, such as Matthäus and Özcan (2011), who reported P (1,834.02 mg/kg), K (412.08 mg/kg), Ca (316.27 mg/kg), Mg (266.56 mg/kg), and Na (32.44 mg/kg) as the major minerals in the flower buds of tumbleweed. Additionally, Ereifej et al. (2015) identified Ca (31.4 mg/g), Na (15.5 mg/g), K (19.7 mg/g), Cu (0.14 mg/g), Fe (1.7 mg/g), Mg (23.9 mg/g), Mn (0.12 mg/g), Zn (0.4 mg/g), and P (4.6 mg/g) in the stems and leaves of *G. tournefortii* L.. Previously, Rana et al. (2019) reported that the variations in mineral content may be attributed to factors such as soil quality, environmental and climatic conditions, and species. This comparison highlights the notable differences in mineral composition across various plant parts and studies, underscoring the diverse mineral content of tumbleweed based on plant section and geographical location.

Table 2. The mineral content (mg/100 g dw) of tumbleweed parts.

Element type	RDA (mg/ day)	Unit	The name of tumbleweed parts		
			TR	TSL	TF
Zinc (Zn)	10	mg/100 g	2.18±0.32^{c}	3.49±0.48^{b}	5.50±0.65^{a}
Copper (Cu)	1	mg/100 g	1.27±0.20^{c}	3.27±0.35^{b}	5.04±0.64^{a}
Iron (Fe)	14	mg/100 g	3.34±0.19^{c}	4.81±0.21^{b}	7.57±0.40^{a}
Calcium (Ca)	800	mg/100 g	340.33±11.59^{b}	394.00±13.45^{a}	376.67±10.69^{a}
Magnesium (Mg)	375	mg/100 g	1047.00±49.79^{b}	898.33±18.58^{c}	1164.67±31.47^{a}
Sodium (Na)	*	mg/100 g	12.51±1.66^{c}	17.65±1.13^{b}	22.30±1.08^{a}
Potassium (K)	2000	mg/100 g	464.00±10.58^{b}	353.67±11.06^{c}	528.33±17.50^{a}
Phosphorus (P)	700	mg/100 g	22.33±0.58^{a}	45.00±2.00^{b}	47.67±2.08^{a}

TR: The roots of tumbleweed; TSL: The stems & leaves of tumbleweed; TF: The flowers of tumbleweed; RDA: Recommended daily allowance.
Statistical differences within the same row are denoted by lowercase letters ($^{a\text{-}c}$) ($P < 0.05$).

Bioactive properties

Phenolic compounds, a diverse group of secondary metabolites in plants, are known for their biological activities, such as anti-inflammatory, anticancer, antidiabetic, and anti-atherosclerotic effects, primarily due to their antioxidant properties. Antioxidants are essential in neutralizing free radicals, which contribute to tissue damage. Recently, wild plants have attracted significant interest as natural sources of antioxidants, offering a safer alternative to synthetic options, which may carry health risks (Rana et al., 2019; Alam et al., 2020; Erol and Kutlu, 2024).

The TPC, TAC, and antioxidant activity values (ABTS, CUPRAC, and FRAP) of the tumbleweed plant are presented in Table 3. The TPC of different parts of the tumbleweed plant was quantified, revealing a range from 62.71 to 214.64 mg GAE g^{-1} of dried extract. Moreover, the TAC levels of the tumbleweed plant were determined to be between 705.01 and 1132.96 mg g^{-1} in dry weight. The highest TPC and TAC contents were observed in the TF section, while the lowest values were found in the TR section. Moreover the antioxidant activity measurements followed a similar trend to the TPC and TAC values. Specifically, the ABTS activity of the samples ranged from 13.14 to 25.48 mg TE g^{-1} extract, CUPRAC activity was measured between 88.03 and 203.92 mg TE g^{-1} extract, and FRAP activity values were recorded between 15.87 and 32.63 μmol Fe^{2+} E g^{-1}. These findings indicate that the TF section exhibits the highest antioxidant potential, correlating with its elevated phenolic and total monomeric anthocyanin content, whereas the TR section shows the lowest antioxidant capacity. Such variations in antioxidant activity and bioactive compound content highlight the potential of different parts of the tumbleweed plant as sources of natural antioxidants, depending on their phenolic and anthocyanin concentrations.

Table 3. Some bioactive and antidiabetic properties of tumbleweed parts.

The name of sample	Bioactive properties					Antidiabetic properties	
			Antioxidant activities				
	TPC (mg GAE g^{-1} of dried extract)	TAC (mg g^{-1} in dry weight)	ABTS activity (mg TE g^{-1} of dry extract)	CUPRAC activity (mg TE g^{-1} of dry extract)	FRAP activity (μmol Fe^{+2} E g^{-1} of dry extract)	α-Glucosidase from *Saccharomyces cerevisiae* (%)	α-Amylase from porcine pancreas (%)
TR	62.71±3.20^{c}	705.01±9.73^{c}	13.14±1.58^{c}	88.03±5.91^{c}	15.87±1.41^{c}	38.46±1.16^{c}	29.01±1.08^{c}
TSL	108.13±6.68^{b}	866.64±11.74^{b}	17.78±1.66^{b}	130.09±4.30^{b}	24.81±2.04^{b}	50.97±0.39^{b}	52.80±0.64^{b}
TF	214.64±11.40^{a}	1132.96±28.24^{a}	25.48±2.12^{a}	203.92±6.93^{a}	32.63±3.05^{a}	61.99±0.66^{a}	53.67±0.46^{ab}
Acorbose	-	-	-	-	-	52.01±0.68^{b}	54.92±0.26^{a}

TR: The roots of tumbleweed; TSL: The stems & leaves of tumbleweed; TF: The flowers of tumbleweed; GAE: Gallic acid equivalent; TE: Trolox equivalen

Statistical differences within the same column are denoted by lowercase letters ($^{a\text{-}c}$) ($P < 0.05$).

Similarly, Coruh et al. (2007) found that the TPC was 64.4 μg/mg in the aerial parts and 105.1 μg/mg in the seeds of *G. tournefortii* L. Moreover, Ereifej et al. (2015) observed that the stems and leaves of *G. tournefortii* L. contained 375.5 mg GAE/100 g of TPC and 100.1 mg/100 g of anthocyanin concentration. Furthermore, according to the findings of Dalar et al. (2019), the ethanol extract of *Gundelia rosea* seed demonstrated a significant TPC, measuring 55.3 mg GAE per g of extract. Additionally, *G. tournefortii* L. exhibited strong reducing power, with FRAP and CUPRAC values of 1683 μmol Fe^{2+} and 214.1 mg TE per gram of extract, respectively, as well as notable radical scavenging activity, indicated by an ABTS value of 141.2 mg TE per gram of extract. Variations in the TPC among different plant parts are expected and can be attributed to the presence of a diverse range of phenolic compounds and their various derivatives in each part. Moreover, Karakas et al (2017) noted that the antioxidant properties of phenolic compounds are likely linked to their strong hydrogen-donating capacity, making them highly effective in neutralizing free radicals and preventing oxidative damage.

Phenolic profile

An analysis was conducted to identify and quantify the most prevalent polyphenols across different parts of tumbleweed, as shown in Table 4. The results revealed significant variability in both the profile and concentration of polyphenolic compounds among the studied parts. Notably, gallic acid, protocatechuic acid, p-hydroxybenzoic acid, caffeic acid, syringic acid, kaempferol, rutin, ferulic acid, o-coumaric acid, myricetin, and quercetin were detected in all parts of the plant, whereas chrysin was absent only in the TR. The concentration of phenolic

compounds ranged from 0.93 to 119.98 μg/g, with the highest levels of gallic acid (119.98 μg/g), protocatechuic acid (19.68 μg/g), syringic acid (5.62 μg/g), kaempferol (4.60 μg/g), rutin (80.05 μg/g), chrysin (1.65 μg/g), ferulic acid (13.53 μg/g), o-coumaric acid (8.60 μg/g), myricetin (32.01 μg/g), and quercetin (5.45 μg/g) recorded in the TF. Conversely, the highest concentration of p-hydroxybenzoic acid (11.85 μg/g) was found in the TSL, and caffeic acid was most abundant in the TR. This comprehensive profiling of polyphenolic compounds highlights the diverse phenolic content across the various plant parts, emphasizing their potential bioactive properties.

Table 4. Phenolic profile of various tumbleweed parts.

Phenolic compounds	TR (μg/g)	TSL (μg/g)	TF(μg/g)
Gallic acid	60.71±0.29^{c}	96.41±0.84^{b}	119.98±0.61^{a}
Protocatechuic acid	9.40±0.15^{c}	14.32±0.32^{b}	19.68±0.38^{a}
p-Hydroxybenzoic acid	7.56±0.41^{c}	11.85±0.40^{b}	9.96±0.53^{a}
Caffeic acid	14.72±0.30^{a}	10.97±0.23^{c}	13.29±0.13^{b}
Syringic acid	2.40±0.13^{c}	3.30±0.12^{b}	5.62±0.25^{a}
Kaempferol	1.57±0.24^{c}	2.99±0.23^{b}	4.60±0.34^{a}
Rutin	48.05±0.20^{c}	52.95±0.18^{b}	80.05±0.51^{a}
Chrysin	NPD	0.88±0.16^{b}	1.65±0.08^{a}
Ferulic acid	7.34±0.07^{c}	9.87±0.36^{b}	13.53±0.19^{a}
o-Coumaric acid	4.54±0.26^{c}	6.49±0.29^{b}	8.60±0.22^{a}
Myricetin	16.80±0.22^{c}	24.78±0.22^{b}	32.01±0.59^{a}
Quercetin	0.93±0.09^{c}	1.46±0.14^{b}	5.45±0.25^{a}

NPD: No peak is detected; TR: The roots of tumbleweed; TSL: The stems & leaves of tumbleweed; TF: The flowers of tumbleweed

Statistical differences within the same row are denoted by lowercase letters ($^{a\text{-}c}$) ($P < 0.05$).

These findings align with earlier research on the phenolic composition of *G. tournefortii* L. For instance, Haghi et al. (2011) conducted a detailed analysis of the phenolic profile of tumbleweed, revealing the presence of several key compounds, including chlorogenic acid, cryptochlorogenic acid, caffeic acid, and neochlorogenic acid. Similarly, Asgari et al. (2015) identified chlorogenic and caffeic acids as the predominant phenolics in *G. tournefortii* L. In a more recent study, Tarhan et al. (2023) reported the presence of fumaric acid, gallic acid, protocatechuic acid, gentisic acid, and chlorogenic acid in the root, stem, leaf, and flower of tumbleweed, further expanding the understanding of the plant's diverse phenolic composition. These findings highlight the rich array of bioactive compounds in *G. tournefortii* L, which could be valuable for future research into its antioxidant and therapeutic potential in various applications.

Antidiabetic activity

Alpha-amylase plays a crucial role in the breakdown of polysaccharides and is a key enzyme in the digestive process, primarily found in saliva and pancreatic juice. Inhibiting this enzyme is a potential strategy to reduce postprandial blood glucose levels. In contrast, α-glucosidase, an enzyme located in the mucosal brush border of the small intestine, is responsible for breaking down complex carbohydrates into absorbable sugars. Its inhibition provides an effective method to slow glucose absorption and regulate postprandial blood sugar, potentially helping to manage diabetes (Mechchate et al., 2021; Erol et al., 2023; Erol and Kutlu, 2024; Kutlu, 2024).

The inhibitory activities of different parts of the tumbleweed plant were assessed at a concentration of 2 mg/mL against α-glucosidase and α-amylase (Table 3). The α-glucosidase inhibition percentages ranged from 38.46% to 61.99%, while α-amylase inhibition varied between 29.01% and 54.92%. Acarbose, a commonly used α-glucosidase inhibitor in the treatment of type 2 diabetes mellitus (Kutlu, 2024), served as a reference. Among the extracts,

the TF section demonstrated the inhibitory activity against α-glucosidase (61.99%) from *Saccharomyces cerevisiae,* although none of the extracts showed inhibitory activity against α-amylase at the level of acarbose (54.92%) from porcine pancreas. The findings suggest that tumbleweed parts (TR, TSL, and TF) do not significantly inhibit alpha-amylase, indicating that they may not reduce glucose absorption through this pathway. However, it is noteworthy that the α-amylase inhibitory activity of the TF extract was found to be very close to that of acarbose. These results suggest that the TF part of the tumbleweed plant may possess significant potential as a natural inhibitor of α-glucosidase and α-amylase, which could be beneficial for managing hyperglycemia in type 2 diabetes. These findings align with previous research on *Gundelia tournefortii* L. In a study conducted by Keskin et al. (2021), the alpha-amylase and alpha-glucosidase enzyme inhibition capacities of ethanol and water extracts from the stalks and roots of *Gundelia tournefortii* L. were determined, revealing that the IC_{50} value of the acarbose standard was lower than those of the extracts. This suggests that the antidiabetic activity of *G. tournefortii* L. is relatively limited. Moreover, Kadan et al. (2018) found that *G. tournefortii* L. methanol extract enhances GLUT4 translocation in muscle cells, suggesting its potential antidiabetic effect through improved glucose metabolism. These studies, together with existing findings on tumbleweed, highlight the potential of plant-based extracts as natural agents in diabetes management.

In vitro antiproliferative activity

The anticancer potential of these ethanolic extracts was assessed using the MTT assay, which relies on the reduction of MTT to formazan crystals by mitochondrial dehydrogenases in living cells, with the activity being dose-dependent (Diab et al., 2021). The cytotoxicity of the extracts was first evaluated against HEK-293 cells, an in vitro model, using various concentrations (1–1000 µg/mL). The results showed that increasing concentrations of the plant extracts reduced the viability of HEK-293 cells in a dose-dependent manner, with cytotoxicity expressed as IC_{50} values (Table 5). Specifically, the cell viability for TR decreased from 100% to 75.33%, for the TSL from 100% to 67.67%, and for the TF from 100% to 59.00% over the concentration range. The lower the IC_{50} value, the more potent the extract is at inhibiting cell growth and inducing cytotoxicity. Among the extracts, TF exhibited the highest cytotoxic activity against HEK-293 cells, with the lowest IC_{50} value of 1049.76 µg/mL (Table 5), followed by TSL (1384.49 µg/mL) and TR (1641.08 µg/mL). In addition to HEK-293 cells, the cytotoxicity of the extracts was also assessed against Caco-2 colon cancer cell lines. Some parts of tumbleweed demonstrated potential anticancer activity against colon adenocarcinoma compared to the other parts, as indicated by their low IC_{50} values in the MTT assay. However, the various tumbleweed parts did not exhibit significant toxicity against Caco-2 cells, with IC_{50} values ranging from 820.62 to 889.98 µg/mL. The cell viability of Caco-2 cells decreased from 100% to 50.00% with TR, from 100% to 47.67% with TSL, and from 100% to 52.67% with TF in the tested concentration range of 1–1000 µg/mL. These findings suggest that while all parts of tumbleweed exhibit some degree of cytotoxic activity, the flowering part (TF) is the most potent against HEK-293 cells, while TSL shows the strongest effect on Caco-2 cells. This variability in cytotoxic response may be attributed to the different bioactive compounds present in the various plant parts.

These findings align with previous studies evaluating the cytotoxic and antiproliferative effects of tumbleweed extracts. In this regard, Kadan et al. (2018) also evaluated the toxicity of aerial parts of tumbleweed extracts and confirmed that both methanol and hexane extracts were safe for L6-GLUT4myc (rat skeletal muscle) cells at concentrations up to 250 µg/mL, as determined by MTT and LDH leakage assays. Furthermore, Özaltun and Daştan (2019) observed a reduction in the viability of MCF-7 (breast cancer) cancer cells and HUVEC (human endothelial) cells, which was dependent on both the dosage and exposure time. Additionally, Saraç et al. (2019) found that the aqueous extract of tumbleweed seeds showed moderate antiproliferative activity in cell lines such as MCF-7 (human breast

adenocarcinoma), MKN-45 (human gastric cancer), and PC3 (human prostate cancer), and but weak cytotoxicity in HUVEC (human umbilical vein endothelial) and L929 (mouse fibroblast) cells.

Table 5. Cell viability percentages and half maximal inhibitory concentration (IC_{50}) of different concentrations of tumbleweed parts on HEK-293 and Caco-2 cells.

Name of cell line	Sample name	Control	1 (μg mL^{-1})	50 (μg mL^{-1})	100 (μg mL^{-1})
HEK-293 (%)	TR	100.00±0.00^{Aa}	100.00±0.00^{Aa}	90.00±2.65^{Ab}	83.33±0.58^{Ac}
	TSL	100.00±0.00^{Aa}	100.00±0.00^{Aa}	91.67±1.53^{Ab}	81.00±1.00^{Bc}
	TF	100.00±0.00^{Aa}	100.00±0.00^{Aa}	86.00±1.00^{Bb}	74.67±1.53^{Cc}
Caco-2 (%)	TR	100.00±0.00^{Aa}	100.00±0.00^{Aa}	91.00±1.00^{Ab}	79.00±1.00^{Ac}
	TSL	100.00±0.00^{Aa}	100.00±0.00^{Aa}	92.33±1.53^{Ab}	80.67±1.53^{Ac}
	TF	100.00±0.00^{Aa}	100.00±0.00^{Aa}	91.67±1.53^{Ab}	74.00±1.00^{Bc}

Name of cell line	Sample name	250 (μg mL^{-1})	500 (μg mL^{-1})	1000 (μg mL^{-1})	IC_{50} (μg mL^{-1})
HEK-293 (%)	TR	79.33±1.53^{Ad}	75.33±0.58^{Ae}	71.33±1.15^{Af}	1641.08
	TSL	74.67±0.58^{Bd}	71.00±1.00^{Be}	67.67±0.58^{Bf}	1384.49
	TF	68.67±1.53^{Cd}	63.67±0.58^{Ce}	59.00±1.15^{Cf}	1049.76
Caco-2 (%)	TR	70.67±2.08^{Ad}	56.33±2.08^{Be}	50.00±1.00^{Bf}	837.65
	TSL	67.67±2.08^{ABd}	59.67±2.08^{ABe}	47.67±1.53^{Bf}	820.62
	TF	65.00±1.00^{Bd}	61.00±1.00^{Ae}	52.67±1.15^{Af}	889.98

TR: The roots of tumbleweed; TSL: The stems & leaves of tumbleweed; TF: The flowers of tumbleweed
Differences in cell viability findings among the extract concentrations of various tumbleweed parts are indicated by distinct capital letters within the same column, with significance set at $P < 0.05$. Variations within the same row for different tumbleweed parts are marked by different lowercase letters ($P < 0.05$).

CONCLUSION

This study evaluated the physicochemical properties, bioactive compounds, cytotoxicity, and antidiabetic potential of the roots (TSL), stems & leaves (TSL), and flowers (TF) of the tumbleweed plant. The findings indicate the promising potential of TF in regulating blood glucose levels, particularly through α-glucosidase inhibition. Cytotoxicity assays revealed that the inhibition of Caco-2 and HEK-293 cells was concentration-dependent, with higher extract concentrations from different plant parts (TR, TSL, and TF) exhibiting stronger cytotoxic effects. A positive correlation was also observed between the total phenolic content of various tumbleweed parts and their total monomeric anthocyanin content, ABTS, CUPRAC, and FRAP activities. This indicates that phenolic compounds in the roots, stems, leaves, and flowers play a significant role in the plant's antioxidant potential. Given their robust antioxidant properties, these parts hold substantial promise as natural antioxidants for potential application in the food and nutrition industries. Tumbleweed's ability to neutralize free radicals and prevent oxidative stress positions it as a viable natural alternative to synthetic antioxidants, responding to the increasing demand for plant-based, safer ingredients in food preservation and health-promoting products. Further investigation into its bioactive properties could pave the way for the commercial use of tumbleweed in functional foods and nutraceuticals.

CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Gozde KUTLU: Investigation, writing – original draft, review&editing, visualisation; Kubra Feyza EROL: Methodology, validation, investigation.

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