Determination of biological activity of *Tragopogon porrifolius* and *Polygonum cognatum* consumed intensively by people in Sivas

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**ABSTRACT**

**Objective:** This study was aimed to investigate the *in vitro* antioxidant, antimicrobial, cytotoxicity, and enzyme inhibition activities of *Tragopogon porrifolius* and *Polygonum cognatum* extracts which are naturally grown and consumed intensively by people in Sivas, Turkey.

**Methods:** Plant materials were extracted with aqueous ethanol by maceration method. The components of the extracts were determined using the Gas Chromatography Mass Spectrometry. Antimicrobial, cytotoxic and enzyme inhibition activities of the extracts were investigated by micro dilution, XTT assay and 96-micro-well plate methods, respectively. The antioxidant activity evaluated using the DPPH radical scavenging, thiobarbituric acid and reducing power methods. The total phenol and total flavonoid content was also examined.

**Results:** GC–MS analysis revealed the presence of 31 compounds in *P. cognatum* extract and 29 compounds in *T. porrifolius* extract. According to the results, *T. porrifolius* extract showed high level of antioxidant activity in comparison to *P. cognatum* extract. *T. porrifolius* exhibited higher α-glucosidase inhibitory activity, and both extract showed strong α-amylase inhibition activity compared to reference drug acarbose. *T. porrifolius* and *P. cognatum* ethanolic extracts exhibited antimicrobial activity in the concentration range of 0.039–2.5 mg/ml. Both extracts also exhibited significant anticancer effect on MDA-MB-231 breast cancer cells. The IC\(_{50}\) values of *T. porrifolius* and *P. cognatum* extracts in MDA-MB-231 cells were determined as 0.0625 mg/mL and 0.053 mg/mL, respectively.

**Conclusion:** Our findings demonstrated that *T. porrifolius* and *P. cognatum* ethanolic extracts have promising effect on antioxidant, antimicrobial and cytotoxic activity as well as enzyme inhibition activity, and hence further studies required to identify specific compounds responsible for these activities.

1. Introduction

*Tragopogon porrifolius* L. which belongs to the Asteraceae family is an annual or biennial plant. It has three subspecies which are *T. porrifolius* subsp. *australis*, *T. porrifolius* subsp. *cupani* and *T. porrifolius* subsp. *porrifolius* [1–3]. Various parts of the plant are consumed in Southern and Central Europe, North America and United Kingdom and also used to treat cancer in Lebanese folk medicine [4,5]. Phytochemical investigations on this plant revealed that it contains monounsaturated fatty acids, essential fatty acids, vitamins, and polyphenol components [6,7]. Moreover, the antioxidant activity of *T. porrifolius* has been shown because of its naturally occurring chemical components [8].

*Polygonum cognatum* Meissn. is a perennial plant belonging to the Polygonaceae family [9,10]. The *P. cognatum* young shoots and leaves are used for treatment of several diseases in central parts of Turkey [9,11]. The plant is known as “madmak” in Turkey, and it is commonly consumed as salad and dishes by local people in Sivas. *P. cognatum* was previously reported to contain phenolic compounds vitamin C and carotenoids [9,12,13]. It was also reported to possess antioxidant, antimicrobial, diuretic, and, antiadipic activity [9,14].

Reactive oxygen species (ROS) play important roles in various biological process majorly by oxidizing some biological molecules. Reactive oxygen species are generally scavenged by phenolic
compounds. Antioxidants are substances that decrease or prevent the oxidation of readily oxidizable materials [15]. In addition, the body’s cellular defense system is improved by antioxidant molecules against oxidative damage [16]. However, the protection offered by antioxidants is limited. When the formation rate of ROS exceeds the antioxidant capacity of biological systems, oxidative stress occurs. For this reason, the intake of antioxidants into the body as a food supplement plays an important role in prevention various diseases and conditions such as cancer, cardiovascular diseases and the aging process [17,18]. Additionally, many studies have revealed that oxidative stress and impaired iron regulation are associated with the Alzheimer’s disease [19,20].

Alzheimer’s disease (AD) is the most common form of neurodegenerative disease that mainly affects the elderly. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are two important enzymes necessary for the healthy functioning of the nervous system especially in AD. In numerous cholinergic pathways of the central and peripheral nervous systems, AChE and BuChE play important role in the termination of impulse transmission with the rapid hydrolysis of the neurotransmitter acetylcholine (Ach) and butyrylcholine (Bch). AChE is found in both nerves and red blood cells, while BuChE is found only in the liver cells. AChE and BuChE enzyme inactivation or excessive hydrolysis of Ach and Bch leads to the reduction of Ach and Bch level. Therefore, anti-cholinesterase inhibitors, inhibit the breakdown of Ach and Bch, which increase the level and duration of the neurotransmitter action, commonly used in the treatment of neurodegenerative disorders [21]. The common used synthetic anticholinesterase inhibitors are limited in use because of their adverse effects [22]. Thus, in recent years, researchers are searching potent anticholinesterase inhibitors with fewer side effects from natural sources.

Diabetes mellitus (DM) which is characterized by elevation of blood glucose levels and severe complications in a chronic metabolic disease. It is characterized by reduction of insulin secretion in pancreatic beta cells or insulin resistance at the peripheral tissues. Currently, the synthetic hypoglycemic agents such as acarbose, voglibose used for DM therapy have been reported to have serious adverse effects like hypoglycemia, hepatotoxicity and gastrointestinal problems. Therefore, there is a need to develop new potential antidiabetic drugs with fewer adverse effects [23]. Various studies have shown that some plants have excellent hypoglycemic activity [24].

Tyrosinase, found widely in plant and mammalian cells, plays an important role in melanin biosynthesis. Tyrosinase catalysis the two-step reaction in the formation of melanin. In this context, melanin synthesis inhibitors are popular for cosmetic formulation due to its reducing hyperpigmentation in human skin. Besides, overproduction of melanin in food leads to browning, which is an unwanted situation in terms of the appearance of food [25]. Therefore, it is important to use tyrosinase enzyme inhibitors for dermatological skin whitening. Hence it is important to identify potential compounds with fewer side effects from natural sources.

The main goal of this study was to investigate the inhibition effects of *T. porrifolius* and *P. cognatum* ethanol extract on AChE, BChE, a-glucosidase, a-amylase, and tyrosinase. In addition, *in vitro* antioxidant, antimicrobial, and anticancer activities were also investigated. The GC–MS analysis was also conducted to determine the chemical compositions of the two plant extracts.

2. Material and method

This study was performed in the Faculty of Pharmacy laboratories, Sivas Cumhuriyet University, in 2018. *T. porrifolius* and *P. cognatum* plants were collected from the wild flora of Sivas and identified by Dr. Ayşın Aktopal. The herbarium specimen of *T. porrifolius* (NO: S125 AA) and *P. cognatum* (NO: 5120 AA) have been deposited at the Herbarium of Faculty of Science, Cumhuriyet University. The experiments were performed in randomized design with three replications. Leaf samples of the collected plants were milled with a grinder after dried up to constant weight in shade. In order to determine the biological activity of *T. porrifolius* and *P. cognatum*, the plant samples were extracted and were analyzed by different methods.

2.1. The chemical components

Dry plant leaves were ground and extracted with ethanol. The obtained extracts were analyzed by using the Gas Chromatography–Mass Spectrometry (GC–MS). The standards (BHT, BHA, Ascorbic acid, and gallic acid) and chemicals obtained from Sigma chemical Co, St Louis, USA.

2.2. Preparation of the extracts

Firstly, the dried plant leaves were grounded with a blender (Blue house). Then, 10 g of powder was soaked in 50 mL of ethanol for 24 h with intermittent shaking. After the extraction, it was filtrated with the help of No. 1 Whatman filter paper. The filtrate was concentrated to dryness under reduced pressure with a rotary evaporator at 40 °C and this procedure was repeated three times. Then, the filtrate was stored at −20 °C until experimental studies. The obtained extracts were analyzed using the GC–MS. [26].

2.3. Antioxidant assay

The antioxidant activity of the ethanol extracts was tested by DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity, ferric thiocyanate (FTC), thiobarbituric acid (TBA) methods, and reducing power methods.

2.3.1. DPPH radical scavenging activity

The free radical scavenging activity of ethanol extracts was performed using the previously reported method [27]. The 150 μL of ethanol extract was mixed with 50 μL of 1.0 × 10⁻³ M DPPH solution that was freshly prepared in methanol. Control experiment was performed using only methanol. It is incubated at room temperature for 30 min, then for the measurement of the reduction of the DPPH free radical, the absorbance was recorded at 517 nm with a spectrophotometer (Biotech, USA). Butylated hydroxytoluene (BHT) was used as positive control. Percent inhibition was calculated using the following equation;

\[
\text{% Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100
\]

2.3.2. ABTS radical scavenging activity

ABTS radical scavenging activity of the extracts was performed according to Re et al. method [28] with slight modification. ABTS stock solution was prepared by reacting 7 mM ABTS solution with 2.4 mM of potassium persulfate solution in equal volume for 16 h. Then, the working solution was obtained by diluting the stock ABTS solution with methanol to give an absorbance of 0.7 ± 0.02 units at 734 nm using a spectrophotometer. In each assay, the ABTS solution was prepared freshly. Fifty μL of extract was mixed with 150 μL ABTS working solution and were put for 10 min in darkness. All the analyses were performed in triplicate and the results expressed as mean ± standard deviation. Appropriate blanks (methanol) and positive control- BHT (Butylated hydroxytoluene) were run simultaneously. The absorbance was measured at 734 nm in a microplate reader (Epoch, USA).

2.3.3. Determination of total phenolic (TPC)

For the measuring of the total phenolic content in the extracts, the spectrophotometric Folin–Ciocalteu (F-C) method was used [29]. One
hundred μL F-C reagent freshly diluted 1/10 with distilled water was added the 20 μL of extract diluted appropriately in DMSO. After five minutes, 80 μL 7.5 % Na2CO3 solution was added the solution, and mixture was incubated for 30 min at 25 °C. Absorbance was measured at 650 nm in a microplate reader (Epoch, USA). All the analyses were performed in triplicate and the results were given as mean ± standard deviation. Appropriate blanks (DMSO) and standard (gallic acid in DMSO) were run simultaneously, after which the total phenolic content (TPC) was calculated as milligrams gallic acid equivalents per gram of dry extract.

2.3.4. Estimation of total flavonoids (TFC)
For the determination of total flavonoid concentration, the aluminum chloride colorimetric method was used according to Molan et al. [30]. Catechine was used as a reference standard. In summary, 25 μL of test sample solution with concentration of 1 mg/mL, 100 μL of distilled water and 7 μL of 5 % NaNO3 were mixed together in 96-well plates. Then, 7 μL of 10 % (w/v) AlCl3 was added and the mixture was incubated for 15 min at room temperature. Five min later, 50 μL of 1 M NaOH and 60 μL of distilled water were added to each wells. After that, the absorbance was measured at 490 nm. All experiments were carried out in triplicates. The total flavonoids content was given as mg of catechene equivalent per g of dry weight of extract.

2.3.5. Iron chelating activity
For the determination of iron chelating activity of the extracts according to their interaction with the formation of ferrozine-Fe2+ complex, a previously described procedures were used [31]. Each extract was dissolved in DMSO to prepare stock solution. For the preparation of working FRAP reagent, the 0.3 M acetate buffer (pH 3.6), a solution of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM FeCl3 solution was mixed in the ratio of 10:1:1 at the time of use. The mixture of the 30 μL of sample solution and 270 μL of FRAP working solution were added in 96-well plates and warmed at 37 °C for 4 min. The absorbance was measured at 593 nm. All experiments were performed in triplicate. By using different concentrations of FeSO4 solution, standard calibration curve was drawn. All solutions were prepared freshly and the results were expressed as FRAP value.

2.3.6. Ferric reducing antioxidant power (FRAP) assay
FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method. The FRAP assay was performed as previously described [32,33]. Each extract was dissolved in DMSO to prepare stock solution. For the preparation of working FRAP reagent, the 0.3 M acetate buffer (pH 3.6), a solution of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM FeCl3 solution was mixed in the ratio of 10:1:1 at the time of use. The mixture of the 30 μL of sample solution and 270 μL of FRAP working solution were added in 96-well plates and warmed at 37 °C for 4 min. The absorbance was measured at 593 nm. All experiments were performed in triplicate. By using different concentrations of FeSO4 solution, standard calibration curve was drawn. All solutions were prepared freshly and the results were expressed as FRAP value.

2.4. Enzyme inhibition activity
2.4.1. Acetylcholinesterase (AChE)/ Butryrylcholinesterase (BChE) inhibition assay
For determining of the AChE inhibitory activity of the extracts, a 96-well plate colorimetric method was used according to Ellman et al. [34]. The experiments were performed with three replications. Galanthamine and buffer were utilized as positive control and blank, respectively. Briefly, 140 μL of 0.1 mM phosphate buffer (pH 6.8), 20 μL of extracts prepared in buffer from 50 mg/mL stock sample solution in DMSO, 20 μL AChE from Electric eel (5 × 10−3 M)/BChE from equine serum (5 × 10−3 M) were added in each well of microtiter plate. After 10 min of incubation, 10 μL of 3 mM DTNB was added. After that, by the adding of 10 μL of 0.71 μM Acetylcholine iodide (AChI)/0.2 mM Butryrylcholine iodide (BChI), the reaction was initiated and the absorbance was measured at 412 nm with microplate reader (Epoch, USA).

2.4.2. Alpha-glucosidase inhibition activity
The alpha-glucosidase inhibition was assessed using the Kumar et al. method [35]. Acarbose and phosphate buffer were utilized as positive control and negative control in place of sample, respectively. Each concentration was carried out in triplicate. Twenty-five μL of sample solution diluted with buffer was mixed with 25 μL of a-glucosidase (0.5 U/mL), incubate for about 10 min at 25 °C. After the addition of 25 μL of 0.5 mM para-nitrophenyl-α-D-glucopyranoside (P-NPG) substrate to each well as substrate, the mixture was incubated for 30 min at 37 °C. Then, 100 μL of 0.2 M sodium carbonate was added for terminating the reaction and the absorbance was read at 405 nm.

2.4.3. Alpha-amylase inhibition activity
The alpha-amylase inhibition was evaluated according to Kumar et al. method [36]. Acarbose and phosphate buffer (0.02 M PBS, pH 6.9) were utilized as positive control and negative control in place of sample, respectively. Each evaluation of the samples was carried out in triplicate with different concentrations. The reaction mixture containing 50 μL of sample solution diluted with buffer, 25 μL of α-amylase (0.5 mg/mL) incubated for about 10 min at 25 °C. After the addition of freshly prepared 50 μL of 0.5 % starch solution (w/v) to each well as substrate, the mixture was incubated for 10 min at 25 °C. After incubation period, 100 μL of 1 % 3,5-dinitrosalicylic acid (DNS) color reagent was added as color reagent and heated in water bath for 10 min. The absorbance was read at 540 nm.

2.4.4. Tyrosinase enzyme inhibition activity
Tyrosinase enzyme inhibition was determined according to Jeong et al. method [37]. In 96 well plate, 20 μL of sample solution diluted with buffer, 100 μL of phosphate buffer were mixed with 20 μL of tyrosinase (250 U/mL) in each well and incubate for about 10 min at 25 °C. After the addition of 20 μL of 3 mM L-tyrosine as substrate, the mixture was incubated for 30 min at 25 °C. After incubation period, the absorbance was read at 492 nm. Kojic acid and phosphate buffer (100 mM PBS, pH = 6.8) were used as positive control and negative control in place of sample, respectively. Each sample was carried out in triplicate with different concentrations.

2.5. Antimicrobial activity
The Minimum Inhibitory Concentration (MIC) values against bacteria and fungi for the ethanol extracts was determined according to the microdilution method [38]. In this study, Enterococcus faecalis (ATCC 29212), Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 29213), Pseudomonas aeruginosa (ATCC 27853) and Candida albicans (ATCC 10231) strains were used. The ethanol extracts were dissolved with 8 % DMSO. 10 μL sample was added in the first line of microtiter plate which was diluted with 90 μL broth. Next, 50 μL sample was added in second line of microtiter plate and serially diluted two-fold with broth. The concentration of plant extract in the wells ranged from 5.00 to 0.02 mg/mL. Final inoculum size was 5 × 10^5 CFU/mL at bacteria and 0.5–2.5 × 10^5 CFU/mL at Candida every well. Bacteria and Candida culture were diluted with Mueller Hinton Broth (Accumin® AM1072) and Sabouraud Dextrose Broth (Himedia ME033), respectively. Fifty μL of bacteria and fungi suspension were added on prepared samples. Samples which added bacteria were incubated at 37 °C and samples which added Candida were incubated at 35 °C for 16 – 24 h. Then, to indicate microbial growth, 50 μL (2 mg/mL) 2, 3, 5-Triphenyltetrazolium chloride (TTC) (Merck, Germany) was added to each well. The microtiter plates were further incubated at 37 °C for 2 h. After incubation, the reduction in density of formazan’s red color was accepted the MIC value. The experiment was performed twice and the standard deviation was zero.
2.6. Cell culture

Human breast cancer cell line MDA-MB-231 (HTB-26) and mouse subcutaneous connective tissue cell line I.929 (CRL-6364) were obtained from ATCC. Dulbecco’s modified Eagle’s medium, fetal bovine serum and phosphate buffer saline were supplied from PAA Ltd. (France). Trypsin-EDTA was purchased from Biological Industries Ltd. (Haemek, Israel). l-glutamine–penicillin–streptomycin solution was obtained from Sigma-Aldrich. XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) reagent was purchased from Roche Diagnostic. Both cell lines were maintained in DMEM containing 10 % FBS, 1 % L-glutamine, 100 IU/mL penicillin, and 10 mg/mL streptomycin and cultured in a humidified atmosphere with 5 % CO₂ at 37 °C. The cells were used for the experiments when they reached 85–90 % confluence.

2.6.1. Cell proliferation assay

Antiproliferative activity of T. porrifolius and P. cognatum extracts was evaluated using the XTT cell proliferation assay against the MDA-MB-231 and I.929 cells. Firstly, the cancer and healthy cells were seeded at a density of 5 × 10³ cells per well in 96-well culture plates and were allowed to attach overnight before treatment. Then, these cells were treated with various concentrations (0.0625 – 1 mg/mL) of T. porrifolius and P. cognatum extracts for 24 h. DMEM (contain 0.5 % DMSO) was used as negative control. After treatments, wells were washed with PBS. Then, in order to determine living cells, 100 μL DMEM without phenol red and 50 μL XTT mixture were added to each well and the plates were incubated for 4 h. The absorbance of XTT-formazan was read with micro plate reader (Epoch) at 450 nm.

2.7. Statistical analysis

The statistical significance for the bioactivity assays was performed using an ANOVA test with post hoc Dunnett’s by Graphpad Prism 7.0 (Graphpad). The results obtained from the experiments were expressed as the mean ± standard deviation (SD).

3. Results

3.1. Chemical composition of the extracts

The chemical composition of ethanol extracts prepared from Tragopogon porrifolius and Polygonum cognatum was analyzed using the GC-MS. GC-MS analysis results showed that the ethanol extracts contain different chemical components (Table 1). When comparing the most abundant compounds in the extracts, T. porrifolius ethanolic extract was found to contain 4H-Pyran-4-one, 2,3-dihydropyrido-6-methyl-, (5,6-dihydro-2-phenyl-4-pyridone), (acrylic acid dodecyl ester), (Hexadecanoic acid (CAS)), (Hexadecanoic acid, ethyl ester (CAS)), (Phytol), (Linoleic acid ethyl ester), (9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-), (Ethyl linoleate), (9-Octadecanamide, (Z)-(CAS)), (5-Eicosene, (E)-) (respectively 3.98 %, 3.14 %, 4.54 %, 7.20 %, 11.61 %, 14.31 %, 5.51 %, 17.03 %, 7.01 %, 3.04 %, 3.93 %), while P. cognatum ethanolic extract was increased with the increase of extract concentration, which is similar with the standard compound BHT. Moreover, T. porrifolius extract exhibited higher antioxidant activity than P. cognatum extract. The P. cognatum extract also showed relatively low levels of DPPH scavenging activity, however in ABTS radical scavenging assay, there is no significant difference between the two extracts (Fig. 1b). Both extracts scavenged the DPPH and ABTS radical in concentration-dependent manner. As shown in Fig. 2, the total phenol content of T. porrifolius extract.

Table 1 Chemical compositions of ethanolic extracts of T. porrifolius and P. cognatum.

| Chemical Components | RT (%) | P. cognatum (mg/mL) | T. porrifolius (mg/mL) |
|---------------------|--------|---------------------|-----------------------|
| Ethanol, 2,2’-iminobis- (CAS) | 4.603 | 0.17 | —— |
| Pyrazine, methyl- (CAS) | 6.057 | 0.09 | —— |
| 1,3-Cyclopentandione | 9.026 | 1.67 | —— |
| 2,4-Dimethyl-2-oxazoline-4-methanol | 12.225 | 0.54 | —— |
| Benzenecacetaledhyde | 13.386 | 4.15 | —— |
| 2,5-Dimethyl-4-hydroxy-3(2H)-furaneone | 14.193 | 2.22 | —— |
| Ethanone, 1-cyclohexyl- (CAS) | 15.744 | 3.41 | —— |
| Ethanamine, N-ethyl-N-nitroso- | 16.522 | 3.95 | —— |
| 4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl- | 16.871 | 15.00 | —— |
| 1,4,3-Dihydrone-alpha- d-glucopyranoside | 18.576 | 4.03 | —— |
| 2,3-dihydro-benzofuran | 18.891 | 0.90 | 3.15 |
| Isorosbide | 21.512 | 6.30 | —— |
| 2-Methoxy-4-vinylphenol | 22.473 | 5.17 | —— |
| Phenol, 2,6-dimethoxy- | 24.127 | 1.29 | 0.27 |
| Benzenamine, N,N-diethyl- | 25.694 | 1.74 | —— |
| Cycloedcane | 28.195 | 1.44 | —— |
| Cyclopropane, nonyl- | 28.206 | 0.81 | —— |
| 1,4-Benzenedimedian, N,N-dimethyl- | 28.784 | 1.14 | —— |
| Phenol, 2,4-bis(1,1-dimethylyl)- | 29.299 | 0.71 | 0.42 |
| 1-hydroxy-2-ethoxybenzene | 30.495 | 0.03 | —— |
| 2,6-Dimethyl-3-(methoxymethyl)-p-benzonquinone | 30.667 | 0.54 | —— |
| 1,2,3-Tetrahydro-cyclopenta[b]indolone | 31.033 | 0.73 | —— |
| Ethyl, alpha.-d-glucopyranoside | 32.332 | 0.51 | —— |
| 6H-Purin-6-one, 2, (dimethylamino)-1,7-dihydro- (CAS) | 32.566 | 2.70 | —— |
| Maltoxazine | 32.572 | 0.32 | —— |
| 5,6-dihydro-2-phenyl-4-pyridone | 33.230 | 3.14 | —— |
| acrylic acid dodecyl ester | 33.436 | 4.54 | —— |
| Tetradeconoic acid | 34.838 | 1.18 | —— |
| (-)-Loliolide | 35.113 | 2.80 | 1.50 |
| 2-(t-butyl)-1,3-benzothiazole | 35.782 | 0.70 | —— |
| Neoptadiene | 36.188 | 0.24 | —— |
| 2-Pentadecanone, 6,10,14-trimethyl- | 36.314 | 0.89 | —— |
| Hexadeconoic acid (CAS) | 38.471 | 3.03 | 7.20 |
| Ethyl 9-hexadecenoate | 38.729 | 1.01 | —— |
| Hexadecanoic acid, ethyl ester (CAS) | 38.849 | 1.16 | 1.29 |
| 2-Tetracene, (E)- | 39.170 | 2.79 | 0.93 |
| Phytol | 40.737 | 1.03 | 14.31 |
| 9,12,15-Octadecatrienatoic acid, methyl ester, (Z,Z,Z)- | 41.098 | 1.97 | —— |
| Ethyl linoleate | 41.264 | 7.66 | 2.00 |
| Ethyl linoleate | 41.407 | 1.02 | —— |
| Linoleic acid ethyl ester | 41.447 | 5.51 | —— |
| 9,12,15-Octadecatrienatoic acid, ethyl ester, (Z,Z,Z)- | 41.579 | 1.03 | 17.03 |
| Octadecanoic acid, ethyl ester (CAS) | 41.876 | 0.49 | —— |
| Stigmasn-5-en-3-ol, (3.beta.,24S)- (CAS) | 42.471 | 1.03 | 0.83 |
| 9-Octadecanamide, (Z)- (CAS) | 44.256 | 0.75 | —— |
| 9-Octadecanamide, (Z)- (CAS) | 44.302 | 3.04 | —— |
| 3-Keto-isovitio | 45.887 | 1.35 | —— |
| 5-Eicosene, (E)- | 45.933 | 3.93 | —— |
| Heptfluorobutanoic acid, heptadecyl ester | 48.983 | 0.66 | —— |
| Stigmasan-3,5-diene | 55.134 | 0.87 | —— |

Phytol.

3.2. In vitro antioxidant activity

The DPPH radical scavenging activity of T. porrifolius and P. cognatum extracts were shown in Fig. 1a. T. porrifolius and P. cognatum extracts antioxidant activity was increased with the increase of extract’s concentration, which is similar with the standard compound BHT. Moreover, T. porrifolius extract exhibited higher antioxidant activity than P. cognatum extract. The P. cognatum extract also showed relatively low levels of DPPH scavenging activity, however in ABTS radical scavenging assay, there is no significant difference between the two extracts (Fig. 1b). Both extracts scavenged the DPPH and ABTS radical in concentration-dependent manner.

As shown in Fig. 2, the total phenol content of T. porrifolius extract.
(74.71 ± 5.59 mg GAE/g) was about two-fold higher than *P. cognatum* extract (31.59 ± 2.02 mg GAE/g). Total flavonoid contents of the two plant extracts were found to be almost the same, *T. porrifolius* 12.33 ± 0.23 mg and in *P. cognatum* 12.36 ± 0.435 mg, catechin equivalent per gram of extract on the dry weight basis.

Both extracts exhibited iron chelating activity in a concentration-dependent manner. Furthermore, *T. porrifolius* ethanolic extract showed relatively higher level of iron chelating activity than *P. cognatum* ethanolic extract. In addition, at the concentration lower than 250 μg/mL, the *T. porrifolius* ethanolic extract demonstrated relatively higher level of metal chelating activity than *P. cognatum* ethanolic extract (Fig. 3a). Ferric reducing assay showed that the FRAP value is higher for *T. porrifolius* extract than *P. cognatum* extract (Fig. 3b).

### 3.3. Enzyme inhibition activity

*T. porrifolius* and *P. cognatum* ethanolic extracts were evaluated for their inhibitory effects against AchE and BchE, which are AD-related enzymes, and α-amylase and α-glucosidase inhibition activity related with DM (Table 2).

AchE and BchE inhibitory activities of *T. porrifolius* and *P. cognatum* leaf extracts were evaluated and the results were given in Table 2. The two extracts exhibited nearly the same inhibitory activity (51.36 ± 3.55 %, 56.68 ± 2.38 % for *T. porrifolius* extract vs 50.61 ± 6.05 %, 50.45 ± 0.98 % for *P. cognatum* extract respectively), however they showed less activity compared to standard drug galanthamine (93.87 ± 0.56 %, 89.89 ± 0.01 %).

*T. porrifolius* leaf extract showed nearly same α-glucosidase inhibitory activity (55.48 %) and *P. cognatum* exhibited lower inhibitory activity (41.05 %) when compared to standard drug acarbose (57.56 %). Both *T. porrifolius* and *P. cognatum* leaf extracts have showed higher α-amylase inhibitory activity (80.8 % and 86.6 %, respectively) while acarbose exhibited 58.4 % α-amylase inhibitory activity. Tyrosinase inhibitory activity of *T. porrifolius* and *P. cognatum* leaf extracts were also evaluated. *P. cognatum* extract showed moderate inhibitory activity (18.28 %), while *T. porrifolius* extract showed higher inhibitory activity (24.23 %) than *P. cognatum*, which is comparable to reference drug kojic acid (29.46 %).

### 3.4. Antimicrobial activity

Antimicrobial activities against five bacteria and *C. albicans* of *T. porrifolius* and *P. cognatum* leaves were evaluated and the results were given in Table 2. The two extracts exhibited nearly the same inhibitory activity (51.36 ± 3.55 %, 56.68 ± 2.38 % for *T. porrifolius* extract vs 50.61 ± 6.05 %, 50.45 ± 0.98 % for *P. cognatum* extract respectively), however they showed less activity compared to standard drug galanthamine (93.87 ± 0.56 %, 89.89 ± 0.01 %).

*T. porrifolius* leaf extract showed nearly same α-glucosidase inhibitory activity (55.48 %) and *P. cognatum* exhibited lower inhibitory activity (41.05 %) when compared to standard drug acarbose (57.56 %). Both *T. porrifolius* and *P. cognatum* leaf extracts have showed higher α-amylase inhibitory activity (80.8 % and 86.6 %, respectively) while acarbose exhibited 58.4 % α-amylase inhibitory activity. Tyrosinase inhibitory activity of *T. porrifolius* and *P. cognatum* leaf extracts were also evaluated. *P. cognatum* extract showed moderate inhibitory activity (18.28 %), while *T. porrifolius* extract showed higher inhibitory activity (24.23 %) than *P. cognatum*, which is comparable to reference drug kojic acid (29.46 %).

Fig. 1. Radical scavenging activity of ethanolic extracts of *T. porrifolius* and *P. cognatum* leaves; DPPH radical scavenging activity; b) ABTS radical scavenging activity.

Fig. 2. Total phenol and total flavonoid contents in ethanolic extracts of *T. porrifolius* and *P. cognatum* leaves.

Fig. 3. Iron chelating activity (a) and ferric reducing power as FeSO₄ equivalent (b) of ethanolic extracts of *T. porrifolius* and *P. cognatum* leaves.
According to the XTT assay extracts were evaluated on MDA-MB-231 and L929 cell lines with the 3.5. Cytotoxicity

Furthermore, the cell proliferation results clearly showed that T. porrifolius and P. cognatum ethanol extracts were observed in the concentration range of 0.039–2.5 mg/mL (Table 3). Among the tested microorganisms, S. aureus was found to be more sensitive to T. porrifolius and P. cognatum extracts than others (MIC value was 0.039 mg/mL) and it is followed by P. aeruginosa and E. coli. Comparative antimicrobial activity assay of the two extracts showed variable activity in different microorganisms. T. porrifolius extract was found to be more active on S. aureus, while P. cognatum extract was found to be more active on E. coli (MIC value was 0.625 mg/mL) and P. aeruginosa (MIC value was 0.312 mg/mL). However, both the extracts showed relatively the same antimicrobial effect on the other tested microorganisms.

3.5. Cytotoxicity

In vitro cytotoxicity activity of T. porrifolius and P. cognatum ethanol extracts were evaluated on MDA-MB-231 and 1929 cell lines with the XTT assay and the results are given in Fig. 4. According to the XTT assay results, both plant extracts showed significant anticancer effect on breast cancer cell in a concentration-dependent manner (p < 0.05). Furthermore, the cell proliferation results clearly showed that T. porrifolius extract has stronger anticancer effect than the P. cognatum extract on MDA-MB-231 cells for all concentrations. The IC50 values of T. porrifolius and P. cognatum extracts in MDA-MB-231 cell lines were calculated as 0.0625 mg/mL and 0.053 mg/mL, respectively. However, neither of the extracts showed any significant cytotoxicity on L929 cell line at the concentration range (1-0.0625 mg/mL).

4. Discussion

Upon we evaluated the solubility of plant compounds in ethanol, two plants showed approximately the same solubility. As seen in the Table 1, the three main compounds identified in the P. cognatum extract are 9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z,Z), Phytol and Hexadecanoic acid, ethyl ester (CAS) respectively, and the three main compounds identified in the T. porrifolius extract are 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Isosorbide and 2-Methoxy-4-vinylphenol, respectively. Biological activity of the extracts may be due to these main compounds.

The exploration of phytotherapeutic agents for the treatment of oxidative stress has been increased in recent years. The medicinal plants can be coping with harmful effects of reactive species inside the body due to their antioxidant effects [39]. Generally, the antioxidant activity of T. porrifolius and P. cognatum extracts have been examined using different antioxidant assays. The antioxidant activity of T. porrifolius extract was found to be higher than P. cognatum extract. This may be related to the higher concentration of flavonoid and phenolic compounds in T. porrifolius than P. cognatum extract. To our knowledge, this is the first report on evaluation of antioxidant activities of T. porrifolius and P. cognatum extracts.

Acetylcholinesterase and butyrylcholinesterase, which are thought to be effective in the progression of age-related diseases, are extremely important enzymes for the brain. Therefore, cholinesterase inhibitors (ChEI) may play an important role in balancing cognitive functions in age-related diseases [40]. When AChE and BChE inhibitory activities of T. porrifolius and P. cognatum plants were evaluated, it was determined that both plants showed low levels of activity.

Inhibition of two important enzymes α-glucosidase and α-amylase for the digestive system reduces the digestion of carbohydrates. This prevents the increase in postprandial blood glucose levels. Thus, controlling postprandial blood glucose without insulin is particularly important for the treatment of type 2 diabetes [41]. So, upon T. porrifolius and P. cognatum plants were evaluated for enzyme activity values of α-glucosidas and α-amylase, which are diabetes mellitus-related enzymes, T. porrifolius showed more α-glucosidase enzyme inhibiting activity than P. cognatum, although both plants were highly α-amylase inhibitory activity.

Tyrosinase inhibitors used widely in skin whitening formulations and food industry due to it involve in melanin biosynthesis. However, tyrosinase inhibitors such as Kojic acid and arbutin reported to have some side effects [42]. In addition, T. porrifolius showed more tyrosinase inhibition activity than the P. cognatum. Previous study reported that flavonoids with –OH groups at A and B rings, presence of double bonds are highly important for tyrosinase inhibitors resulting in Cu2+ chelate formation [43–45].
Medicinal and aromatic plants, which have been used extensively since ancient times, are of great interest due to their antioxidant and antimicrobial effects. In particular, the resistance of pathogens to available drugs makes these plants even more important in terms of their antimicrobial properties [46,47]. When both plants were evaluated for their antibacterial properties, both of them were effective for S. aureus and only P. Cognatum was effective on P. aeruginosa. However, the extracts of these plants have been found to be effective on a limited number of microorganisms as a natural antimicrobial agent.

It is extremely important that both plant extracts have significant anticancer effects on breast cancer. Furthermore, the fact that T. porrifolius extract on MDA-MB-231 cells has stronger anticancer effect than P. cognatum extract can be attributed to different main components of T. porrifolius according to cell proliferation results. Similar anticancer activities were reported for the T. porrifolius extract in previous studies. Al-Rimawi et al., showed the strong anticancer activity of T. porrifolius ethanolic extract on aggressive human osteosarcoma cells, HOS and KHOS [48]. In a different study, Tenkerian et al., reported that T. porrifolius ethanolic extract has strong anticancer effects on breast and colon [16]. Similar results were reported for ethanolic extract of T. porrifolius in our study.

5. Conclusion
In this study, firstly GC–MS analysis of ethanolic extracts of two plants, P. cognatum and T. porrifolius, widely consumed in Sivas province of Turkey, was investigated. In addition, the in vitro antioxidant, antimicrobial, enzyme inhibition, and antiproliferative activities of these extracts was evaluated by different assays. The ethanolic extracts of P. cognatum and T. porrifolius was assessed to contain significant amounts of phenols and flavonoids. The two plants showed extract significant antioxidant, enzyme inhibition, and antiproliferative properties, and moderate antimicrobial activity. Hence, further studies are required to identify which specific compound/s is responsible for these activities.

CRediT authorship contribution statement
Nuraniyе Erugуur: Conceptualization, Methodology, Writing - original draft, Software, Validation, Writing - review & editing. Esra Ucar: Data curation, Writing - original draft, Writing - review & editing. Mehmet Atas: Supervision, Writing - review & editing. Merve Ergul: Visualization, Investigation, Writing - review & editing. Mustafa Ergul: Visualization, Investigation, Writing - review & editing. Fazli Sozen: Data curation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data
Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2019.12.002.
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