Turkish Journal of Agriculture - Food Science and Technology, 9(4): 646-649, 2021
DOI: https://doi.org/10.24925/turjaf.v9i4.646-649.3715

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Antioxidant and Cytotoxic Potential of Local Endemic Plant *Pastinaca zozimoides* Fenzl

Cihan Düşgün[1,a,*], Teoman Kankılıç[1,b], Cemil İşlek[1,c], Dilara Fatma Balı[2,d], Özgür Kankılıç[1,e]

[1]*Faculty of Science Literature, Department of Biotechnology, Niğde Ömer Halisdemir University, 51240 Niğde, Turkey*
[2]*Faculty of Medicine, Medical Biology, Niğde Ömer Halisdemir University, 51240 Niğde, Turkey*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 02/07/2020
Accepted : 10/04/2021

Keywords:
Total phenolic
Antioxidant
Anticarcinogenic
Pastinaca zozimoides
Local endemic

ABSTRACT

P. zozimoides is local endemic in Nigde province, Turkey. There has been no previous examination of the chemical composition and bioactivity of that plant extract. In this study, we studied total phenolic content, antioxidant properties and cytotoxic effect of methanolic extracts of plant sample. According to the Folin-Ciocalteau method, the total phenolics of the extracts were determined spectrophometric. The antioxidant activity was conducted DPPH (1,1'-diphenyl-2-picrylhydrazyl) radical scavenging method and free radical reducing power assay. The cytotoxic effect was studied using MTT assay cell viability on cancer cell lines as well as on Caco-2 cell lines. The total phenolic content of *P*. zozimoides extract was found 28.79±0.68 µg GAE /mg. The free radical scavenger activities of the DPPH was 31.69%±1.61, 85.15%±0.13, 86.96%±0.085 for 1, 5 and 10 mg/mL, respectively. The free radical reducing power assay was 0.375±0.52, 1.587±0.71, 1.798±0.84 for 1, 5 and 10 mg/mL compared to ascorbic acid standard. On Caco-2 cell lines, the extract of plant showed no cytotoxic potential. Because of its phenolic constituents and its antioxidant capacity it can be considered a healthy nutrient.

[a] *cihandusgun@gmail.com* *https://orcid.org/0000-0003-2796-8356*
[c] *cemilislek@gmail.com* *https://orcid.org/0000-0002-6690-2846*
[e] *ozgurkankilic@gmail.com* *https://orcid.org/0000-0001-5565-0260*
[b] *teomankankilic@gmail.com* *https://orcid.org/0000-0002-9576-5887*
[d] *dilarafatmaakin@gmail.com* *https://orcid.org/0000-0002-0903-0017*

Introduction

Pastinaca is a genus that belongs to the Apiaceae family and have nine species in Turkey. Pastus means food in Latin. Parsnips are common name for the species of the genus. Since ancient times they have been used in human diet and as a traditional medicine. Five species are endemic: *Pastinaca armena* Fisch. & C.A.Mey., *Pastinaca gelendostensis* (Yıld. & B. Selvi) Hand., *Pastinaca trysia* Stapf & Wettst, *Pastinaca yildizii* Dirmenci and *Pastinaca zozimoides* Fenzl in Tukey,. *P. zozimoides* is endemic to southern parts of Turkey and distributed very small fields of Kızıltepe hill, Nigde, Turkey. It spreads in the alpine zone between 2500-3000 m. Structure of *P. zozimoides* is biennial or perennial plant. It grows in high mountain grasses. Characteristic structure is yellow flowers and elliptical fruits. It also has intense odour.

Phytochemicals in plants that have complex biochemical, physiological and pharmacological functions have been given focus in recent years. Plants that contain bioactive phytochemicals has potential to reduce the risk of diabetes, cancer and oxidative damage (John and Shahidi, 2019). Many aromatic plants contain chemical compounds which have antioxidant properties (Gonçalves et al., 2017). These effects are due to a range of phytochemicals involving alkaloids, flavonoids, carotenoids, lignans, terpenoids, basic phenolic compound and so forth (Aarland et al., 2017). Reactive oxygen species (ROS) have been concerned with occuring degenerative disorders. Several toxic cellular processes have been associated with free radical-induced oxidative stress, including oxidative protein structure and DNA damage, lipid oxidation of cell membrane, inactivation of enzymes and carcinogenic gene anomaly (Yeldu and Ishaq, 2017). Antioxidants suppress agents by passivating free radicals, which minimize oxidative damage to biological structures. These are compounds, which increase their shelf-life when added to lipids and food-containing lipids by retarding the lipid peroxidation process. They have also been commonly used to prevent food destruction and play an significant role in

the prevention of many diseases related aging in the way of life, being clearly tied to ROS formation and lipid peroxidation (Yeldu and Ishaq, 2017). ROS production is a natural and basic process throughout normal cell metabolism. An inequality in production of ROS and antioxidant defense systems results in oxidative stress. It has been identified as having a prominent function in the cause of many age-related and chronic disorders, cardiovascular and neurodegenerative diseases.

Taking enough antioxidants is important to prevent oxidative stress caused by radicals. Antioxidants ability of vegetables and plants has been reported to come from complete phenolics, anthocyanins and flavonoids (Earling et al., 2019; Li et al., 2017; Vicente and Boscaiu, 2018). Plants have many bioactive phytochemicals that has antioxidant, anti-inflammatory, and anticancer activities. Several reports have indicated, for example, that extracts from plants have beneficial cancer effects relative to chemotherapy or modern hormone therapies. (Eghbaliferiz and Iranshahi, 2016). Therefore, many plants were evaluated to determine for effective antioxidant and anticancer compounds, and to illustrate mechanisms for preventing apoptosis and cancer (Lopez de las Hazas et al., 2017).

P. zozimoides is located very small field. Very little is understood about this plant 's constituents and biological function. In this study, we aimed to determine the phenolic content of *P. zozimoides* Fenzl. It was also assesed its antioxidant and anticytotoxic activity by *in vitro*.

Material and Method

Plant Material

P. zozimoides samples were collected from plants growing spontaneously in their natural habitats. The material was authenticated by Dr. Ahmet SAVRAN (expert in botany at the department of Botanic, Nigde Ömer Halisdemir University, Department of Biology, Nigde, Turkey). Samples originating from the region of Kızıltepe hill in Nigde. It is endemic. The plants were harvested in the period between July and August 2019, corresponding to their full bloom period, and dried at room temperature (20–25°C) in the research unit of the Department of Biotechnology (Nigde, Turkey). The dried plant samples were ground using a mixer into fine powder and preserved, no more than two months, in dark glass containers until their fermentation.

Plant Extracts

P. zozimoides leaves were shade dried and powdered. Powder sample (5g) was subjected to maceration (using magnetic stirrer) with 70% ethanol solvent for 48 hours at room temperature, allowed to undergo solution completely, and then filtered using 0.22 µm Whatman filter paper. The respective filtrates were concentrated by rotary flash evaporator separately. Finally, the concentrated extracts were lyophilized and stored at +4°C until use.

Total Phenolic Content

Content of phenolics was calculated using folin-ciocalteu reaction methods. For standard curve, various gallic acid concentrations (10-50 µg/ml) were prepared. 0.1 ml of these different gallic acid concentrations were put test tubes and added 0.5 ml of Folin-Ciocalteu solution. After than, 20% anhydrous sodium carbonate solution was added to 1.5 ml, and water was used to make up to 10 ml of volume. The absorbance was measured at 760 nm, after 2 hour of incubation at 25°C. 0.1 ml extract solution (1 mg/ml concentration) is taken and treated as previously. Absorbance of all samples was determined and equivalents of gallic acid (GAE) were calculated by the normal curve analysis. Results of absorbance were described as µgGAE/mg.

DPPH Radical Scavenging Activity

Scavenging activity of free radical of plant extracts was measured using the procedure described by Brand-Williams et al. (1995), with some modifications. In a tube, 150 µL of 0.1 mM DPPH (2,2-diphenyl 1-picrylhydrylase) was mixed with 100 µL sample at various concentrations (1, 5, 10 mg/mL). After stirring for 30 minutes at room temperature in darkness, absorbances were measured against methanol at 517 nm. The percentage of DPPH scavenging activity was calculated using following formula;

$$\%DPPH=\frac{AB-AS}{AB}\times 100$$

where A_B is the absorbance of the control, and A_S the absorbance of the sample (Brand-Williams et al., 1995).

Reducing Power Activity

The reducing power activity was determined by the procedure described by Sujatha and Sekar (2019) only minimal improvements. 0.5 mL of extract of sample, 0.5 mL of phosphate buffer (0.2 M, pH: 6.6) and 0.5 mL of 1%potassium ferricyanide were added a test tube. The mixture was incubated for 20 min at 50°C. In order to end the reaction, 1.5 mL of 10% trichloroacetic acid solution was mixed after cooling. Added 0.5 mL of 0.1% ferric chloride and estimated absorbance in 700 nm. Increased absorbance of the mixture of reactions suggests increased power reduction.

Cell Culture

Caco-2 cell line (ATCC HTB-37) cells were kept at 37°C in an incubator at 5 percent CO_2/95 percent $_O2$ humidified atmosphere. Cells were developed in the updated Dulbecco Eagle medium (DMEM) containing 5 percent bovine fetal serum (FBS), 100 µg/ml streptomycin, 100 units/ml penicillin, and 2 mM L-glutamine.

Cell Viability

The MTT assay, which is based on the cleavage of a tetrazolium salt by mitochondrial dehydrogenases in viable cells, measured the cell viability (Hansen et al., 1989). ATCC HTB-37 cells were seeded at 1.2 x 105 cells/ml in a 96 well plate. Cells were treated with various concentrations of total extracts (4, 20, and 100 µg / ml) sixteen hours after plating and 1 hour later 1 mM of H2O2 was added to culture. The cells were incubated at 37°C for another 24 hours. The cells were incubated at 37°C with 20 µl of MTT stock solution (5 mg/ml) in a 200 µl medium over the last 4 hours. Samples were then taken with acidic isopropanol and an ELISA reader was used to measure absorbance (Bio-Rad, USA) at 570 nm. The relative

viability of the cells was determined by the quantity of MTT transferred to insoluble formazan salt. The formazan's optical density formed in the control cells was taken as viability at 100%. Data is average percentage of viable cells versus the controls concerned.

Results and Discussion

Total Phenolic Content

Phytochemical screening of extract shows there is presence of flavonoids, phenolics, steroids/ saponins etc. Since it was reported that the antioxidant activity of a plant extract often originates from phenolic compounds (Ghafoor et al., 2019; John and Shahidi, 2019; Lou et al., 2016). Total phenolic content in plant extract is showed in Table 1. Total phenolic content in methanolic extract of plant extract as estimated by Folin-ciocalteu Reagent method in the present study shows that plant extract show phenolic content in gallic acid equivalent (GAE). This is due to vary in nature of active ingredients in various samples. Total phenolic content was observed in *P. zozimoides* 28.79±0.68 µg GAE/mg. Phenolic content was reported to show antioxidant activity (Aryal et al., 2019). Another study of phenolic content conducted some plant ethanolic extracts showed that phenolic content worked in this study may be good antioxidant agent (Koley et al., 2016). A study showed that *Pastinaca* genus shows phenolic content (Jianu et al., 2020). Therefore, *P. zozimoides* may be consumed as a natural source for antioxidant phenols and be attributed health benefits.

DPPH Radical Scavenging Activity

Free radicals are considered to be a major factor in biological destruction, and DPPH was used to determine natural antioxidant free radical-scavenging activity. DPPH, which is a radical itself with a violet color, transforms into a stable compound with a yellow color when reacting with an antioxidant and the degree of the reaction depends on the antioxidant's hydrogen donating ability. Table 2 displays the DPPH radical plant extract scavenging activity that is measured in percentage terms. *P. zozimoides* had activity against antioxidants. Less difference with plant sample was observed at lower concentration in DPPH scavenging activities. But as concentration increases the disparity in the study of scavenging events becomes more important.

Measurement of Reducing Power

The ability to reduce the oxidation potential of oxidants is determined by reducing the power method for determining antioxidant activity. The sample's reducing powers due to the capacity to donate hydrogen. Standard ascorbic acid was used in this assay. Increased reaction mixture absorbance indicates an increase in power reductions. In the present analysis for the determination of the reduction potential of different extracts as well as normal indicates, there is an increase in absorbance with respect to the change in concentration. In the present analysis for the determination of plant extract power reduction as well as normal indicates, there is an improvement in absorbance as regards concentration growth. Extract of *P. zozimoides* shows good antioxidant action (Table 3).

Table 1. Total Phenolic Content (µg GAE /mg of extract)

	P. zozimoides extract
Total phenolic content	28.79±0.68

Values expressed in mean ± S.D. (n=3) GAE, Gallic Acid Equivalent

Table 2. DPPH radical scavenging IC50 values (%)

Concentration	*P. zozimoides* extract
1 mg/mL	31.69±1.61
5 mg/mL	85.15±0.13
10 mg/mL	86.96±0.085

Table 3. Free radical reducing power assay

Concentration	*P. zozimoides* extract	Ascorbic acid
1 mg/mL	0.375±0.52	0.571±0.38
5 mg/mL	1.587±0.71	1.754±0.62
10 mg/mL	1.798±0.84	1.984±0.75

Values expressed in mean ± S.D. (n=3).

Cytotoxic Activity

P. zozimoides extract showed antioxidant properties. It is known that free radicals play an important role in cancer formation (Godwin et al., 2019; Pérez-Sánchez et al., 2017; Zhdanov et al., 2017). Therefore, it was thought that the plant extract would have anticancer properties. Concentrations of 1, 5 and 10 mg/mL of plant samples on cell lines were studied. As a result, plant extracts did not show any cytotoxic effects at any concentration.

Conclusion

Plants are important natural sources for biological activity. In this study, the biological activity of *Pastinaca zozimoides* has been assessed for the first time. In this study, *Pastinaca zozimoides* showed antioxidant activity. Incidentally this extract also showed the high phenolic content. This plant sample has not the anticarcinogenic effect on cell lines. The findings indicate that the plant samples showed a connection between the overall phenolic content and antioxidant activity. As a result, the methanolic extracts of this plant can be used as a source of antioxidants. However, it is not suitable to be used as an anti-carcinogenic agent. It is thought that these results will be lead to the subsequent works that will be done on *P. zozimoides.*

References

Aarland RC, Bañuelos-Hernández AE, Fragoso-Serrano M, Sierra-Palacios EdC, Díaz de León-Sánchez F, Pérez-Flores LJ, Rivera-Cabrera F, Mendoza-Espinoza JA. 2017. Studies on phytochemical, antioxidant, anti-inflammatory, hypoglycaemic and antiproliferative activities of *Echinacea purpurea* and *Echinacea angustifolia* extracts. Pharmaceutical Biology, 55(1): 649-56.

Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. 2019. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants, 8(4): 96-108.

Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology, 28(1): 25-30.

Earling M, Beadle T, Niemeyer ED. 2019. Açai Berry (*Euterpe oleracea*) dietary supplements: variations in anthocyanin and flavonoid concentrations, phenolic contents, and antioxidant properties. Plant Foods for Human Nutrition, 74(3): 421-29.

Eghbaliferiz S, Iranshahi M. 2016. Prooxidant activity of polyphenols, flavonoids, anthocyanins and carotenoids: updated review of mechanisms and catalyzing metals. Phytotherapy Research, 30(9): 1379-91.

Ghafoor K, Ahmed IAM, Doğu S, Uslu N, Fadimu GJ, Al Juhaimi F, Babiker EE, Özcan MM. 2019. The effect of heating temperature on total phenolic content, antioxidant activity, and phenolic compounds of plum and mahaleb fruits. International Journal of Food Engineering, 15: 11-12.

Godwin MA, Mahithashri K, Shiney OJ, Bhagat M, Praseetha PK. 2019. Metal Incorporated g-C3N4 Nanosheets as Potential Cytotoxic Agents for Promoting Free Radical Scavenging in Cancer Cell Lines. Journal of Nanoscience and Nanotechnology, 19(9): 5448-55.

Gonçalves S, Moreira E, Grosso C, Andrade PB, Valentão P, Romano A. 2017. Phenolic profile, antioxidant activity and enzyme inhibitory activities of extracts from aromatic plants used in Mediterranean diet. Journal of Food Science and Technology, 54(1): 219-27.

Hansen MB, Nielsen SE, Berg K. 1989. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. Journal of Immunological Methods, 119(2): 203-10.

Jianu C, Goleț I, Stoin D, Cocan I, Lukinich-Gruia AT. 2020. Antioxidant activity of *Pastinaca sativa* L. ssp. *sylvestris* [Mill.] Rouy and Camus Essential Oil. Molecules, 25(4): 869-80.

John JA, Shahidi F. 2019. Phenolic content, antioxidant and anti-inflammatory activities of seeds and leaves of date palm (*Phoenix dactylifera* L.). Journal of Food Bioactives, 5: 120–30.

Koley TK, Kaur C, Nagal S, Walia S, Jaggi S. 2016. Antioxidant activity and phenolic content in genotypes of Indian jujube (*Zizyphus mauritiana* Lamk.). Arabian Journal of Chemistry, 9: 1044-52.

Li D, Li B, Ma Y, Sun X, Lin Y, Meng X. 2017. Polyphenols, anthocyanins, and flavonoids contents and the antioxidant capacity of various cultivars of highbush and half-high blueberries. Journal of Food Composition and Analysis, 62: 84-93.

Lopez de las Hazas M-C, Mosele JI, Macià A, Ludwig IA, Motilva M-J. 2017. Exploring the colonic metabolism of grape and strawberry anthocyanins and their in vitro apoptotic effects in HT-29 colon cancer cells. Journal of Agricultural and Food Chemistry, 65(31): 6477-87.

Lou S-N, Lai Y-C, Hsu Y-S, Ho C-T. 2016. Phenolic content, antioxidant activity and effective compounds of kumquat extracted by different solvents. Food Chemistry, 197: 1-6.

Pérez-Sánchez A, Sánchez-Marzo N, Herranz-López M, Barrajón-Catalán E, Micol V. 2017. Rosemary (*Rosmarinus officinalis* L) extract increases ROS and modulates Nrf2 pathway in human colon cancer cell lines. Free Radical Biology and Medicine, 108: 79.

Sujatha S, Sekar T. 2019. Free-radical scavenging activity leaf extract of *Lisea laevigata* gamble. International Journal of Pharmacy and Pharmaceutical Sciences, 11: 96-103.

Vicente O, Boscaiu M. 2018. Flavonoids: Antioxidant compounds for plant defence and for a healthy human diet. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 46(1): 14-21.

Yeldu MH, Ishaq S. 2017. Changes in lipid peroxidation, free radical scavengers and tumour necrosis factor-alpha in serum of wistar rats with induced thyroid dysfunction. Annual Research & Review in Biology: 1-14.

Zhdanov AV, Andreev DE, Baranov PV, Papkovsky DB. 2017. Free radical production and detoxification in complex IV deficient cancer cells. Free Radical Biology and Medicine, 108: 104.