FABAD J. Pharm. Sci., 49, 3, 481-490, 2024
Doi: 10.55262/fabadeczacilik.1533909
RESEARCH ARTICLE

Assessment of the Antioxidant, Antiproliferative and Antityrosinase Potential of Unripe Fruit of *Prunus x domestica* L.

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Assessment of the Antioxidant, Antiproliferative and Antityrosinase Potential of Unripe Fruit of Prunus x domestica L.

SUMMARY

Prunus x domestica L. is a valuable plant that belongs to the Rosaceae family and is distributed worldwide. The aim of this study is to investigate the biological effect of the unripe green fruits of P. domestica, which have only been studied to a limited extent so far. The antioxidant capacity of the fruit extracts and subfractions was investigated by DPPH, TEAC and CUPRAC assays. The antiproliferative effect was investigated against the cell lines L929, CaCo-2 and PC-3. While the highest antiproliferative effect against cancer cell lines was found in the unripe fruit ethyl acetate subfraction, extracts and fractions showed no cytotoxic effect on the healthy cell line L929. The fruit ethyl acetate fraction showed a strong inhibition of the enzyme tyrosinase with an IC50: 51.83 µg/mL compared to standard compound kojic acid (IC50: 21 µg/mL). It was found that the methanol extract from unripe fruits as well as the ethyl acetate and aqueous subfractions exhibited strong antioxidant activity, showed concentration-dependent cytotoxic activity on PC-3 and CaCo-2 cells, but had no cytotoxic effect on healthy L929 cells. The high antioxidant capacity of the fruits, their selective cytotoxic effect on prostate and colon cancer cells and their strong tyrosinase inhibitory activity suggest that they could be a new, safe and cost-effective source for the pharmaceutical and cosmeceutical industries.

Key Words: *Prunus domestica L., unripe fruit, antioxidant, antiproliferative, tyrosinase inhibition.*

Prunus x domestica L. Olgunlaşmamış Meyvelerinin Antioksidan, Antiproliferatif ve Antitirozinaz Potansiyellerinin Değerlendirilmesi

ÖZ

Prunus x domestica L., Rosacea familyasına ait olan ve dünya çapında yaygın bulunan değerli bir bitkidir. Bu çalışmanın amacı; bugüne kadar sınırlı sayıda çalışılan P. domestica olgunlaşmamış yeşil meyvelerinin biyolojik etkisinin incelenmesidir. Meyve ekstraktlarının antioksidan kapasiteleri DPPH, TEAC, CUPRAC yöntemleriyle incelendi. Antiproliferatif aktivite L929, CaCo-2 ve PC-3 hücre hatlarına karşı araştırıldı. Kanser hücre hatlarına karşı en yüksek antiproliferatif etki meyve etilasetat alt fraksiyonunda bulunurken, tüm ekstreler L929 sağlıklı hücre hattında sitotoksik etki gösterdi. Meyve etilasetat fraksiyonu standart olarak kullanılan kojik asitle (IC50: 21 µg/mL) kıyaslandığında IC50: 51.83 µg/mL değeri ile güçlü tirozinaz enzim inhibisyonu gösterdi. Olgunlaşmamış meyve metanol ekstresi ile etilasetat ve sulu alt fraksiyonların güçlü antioksidan aktiviteye sahip olduğu, PC-3 ve CaCo-2 hücreleri üzerinde konsantrasyona bağlı olarak sitotoksik aktivite gösterdiği, ancak sağlıklı L929 hücreleri üzerinde sitotoksisite etkisi olmadığı görüldü. Meyvelerin yüksek antioksidan kapasitesi, prostat ve kolon kanser hücreleri üzerindeki seçici sitotoksik etkisi ve güçlü tirozinaz enzim inhibitör etkisi ile ilaç ve kozmesötik endüstrisi için yeni, toksik olmayan ve ucuz bir kaynak olabileceğini düşündürmektedir.

Anahtar Kelimeler: *Prunus domestica L., olgunlaşmamış meyve, antioksidan, antiproliferatif, tirozinaz inhibisyonu.*

Received: 15.08.2024
Revised: 22.09.2024
Accepted: 01.10.2024

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INTRODUCTION

Nature has always been the leading supplier of compounds, which are the leading raw materials for medicines, important for disease prevention and cure, and the inspiration for medical developments. In addition to drug discovery, organic synthesis, and biotechnological research, research into the development of natural compounds is one of the most important contributions to drug discovery and is becoming increasingly important. For example, chemotherapeutics derived from natural sources are becoming increasingly crucial as better therapeutic options for the cancer treatment. In addition, plants with high antioxidant activity also show promising results in the prevention and treatment of various diseases. Fruit, which are mainly consumed as food, are considered a potential source of cosmetic products thanks to their rich content of phenolic substances and their safe use (Kabir et al., 2021; Lu et al., 2021; Madhuri & Pandey, 2009; Maheshwari & Sharma, 2023; Sharafan et al., 2023).

Interest in research on *P. domestica* fruits (plums) has increased significantly since the 1990s due to the high content of phenolic substances in plums, including anthocyanin and flavonoids. Studies have shown that plums fruits have many health benefits, including improving bone health, boosting memory, antioxidants, anti-inflammatory and anti-cancer effects, and relieving constipation. These positive effects on health can be attributed to the antioxidant effect of plums, which is linked to their high phenol content (Ayub et al., 2023; Igwe & Charlton, 2016).

Many epidemiological studies show that a diet rich in vegetables and fruit can improve health and reduce the risk of major diseases. Fruit and vegetables are rich in vitamins, minerals and antioxidants, they are a source of fiber and have a low energy density, which makes them valuable for health (Angelino et al., 2019; Ayub et al., 2023). In this respect, the rich content of minerals and phytochemicals of *P. domestica* fruits appears to be a promising species identifying bioactive natural products. Fruit are an essential source of magnesium, calcium, and fiber in the daily diet. They contain various valuable phytochemicals such as abscisic acid, lignans anthocyanins and flavonoids, pectins and carotenoids, glycosides, and carbohydrates. Anthocyanins, flavonoids, and hydroxycinnamic acid, which are abundant in plums, are considered secondary metabolites responsible for the antioxidant effect (Ayub et al., 2023; Shukla, Shukla, & Singh, 2021; Usenik, 2021).

The studies carried out so far have shown that there are mainly studies on phytochemical and biological effects on ripe fruits, but very few studies have been found on unripe fruits. In this study, the antiproliferative effects of the methanol extract, ethyl acetate and aqueous fractions obtained from the unripe fruits of *P. domestica* were analyzed against L929, PC-3, CaCo-2 and the total antioxidant activity by DPPH, CUPRAC and TEAC methods. By studying the tyrosinase enzyme inhibition of the extracts, their effect on the healthy fibroblast cell line L929, and their antioxidant activity, their safe and effective potential cosmetic effects were investigated.

MATERIAL AND METHODS

Plant Material

The unripe green fruits of *Prunus x domestica* L. were collected in April 2022 at the Sıhhiye Campus of Hacettepe University. A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey [HUEF 22059]. The voucher specimen was identified by Prof Dr. A. Ahmet Basaran (Department of Pharmacognosy, Faculty of Pharmacy, Baskent University, Ankara, Turkey).

Preparation of extract and fractions from *Prunus x domestica* L.

Unripe fruits of *P. domestica* (20 g) were extracted three times with methanol (200 mL) at a temperature of not more than 40 °C. The three parts obtained were added to each other and evaporated with the

evaporator, yielding the main extract of methanol (0.95 g). The extract was then suspended in water and a liquid-liquid partitioning was performed with petroleum ether (PE) to separate lipophilic compounds, especially chlorophylls. The aqueous fraction was then extracted with 2x100 mL ethyl acetate (EtOAc), the solvents were evaporated, and the ethyl acetate (0.11 g) and aqueous subfractions (0.54 g) were recovered separately. These were stored at 4 °C to be used for biological effect studies.

Mushroom tyrosinase enzyme inhibition assay

Inhibition of tyrosinase enzyme assay was performed with some modifications to the method developed by Kim et al.(Güven et al., 2022; Güven, Saracoglu, Nagatsu, Yilmaz, & Basaran, 2023; Kim et al., 2017). In this method, in which L-tyrosine was used as a substrate, the absorbance of the dopachrome formed during the reaction between the substrate and the enzyme was measured spectrophotometrically at a wavelength of 475 nm and compared with the blank value. Kojic acid was applied as a standard compound (positive control).

DPPH radical scavenging effect

The DPPH radical scavenging effect was determined by spectroscopic evaluation of the color change from purple to yellow in the reaction mixture of methanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (Harput, Genc, & Saracoglu, 2012). All analyses were performed in triplicate. Gallic acid was used as a standard compound to compare the results, and the values obtained were expressed as gallic acid equivalents.

ABTS scavenging activity

The ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity of the extracts and fractions was evaluated spectrophotometrically by the decolorization assay, with absorbance measured at 734 nm. Trolox was used as the standard compound, and the total antioxidant activity was calculated as equivalent to Trolox and expressed as mg Trolox/g extract (Re et al., 1999).

Copper Reducing Antioxidant Capacity (CUPRAC)

The antioxidant capacity that reduces copper ions was determined by the method developed by Özyürek et al., and the experimental results were calculated by measuring the absorbance values at 450 nm (Ozyurek, Bektasoglu, Guclu, & Apak, 2009). Standard curve was prepared with different Trolox concentrations. The unit of total antioxidant activity was expressed as mg Trolox/g extract.

Antiproliferative activity

The effect of different extracts of *P. domestica* fruit, such as methanol, ethyl acetate, and water on cell viability was examined using Cell Viability Detection Kit-8 (CVDK8, Ecotech Biotechnology, Erzurum, Türkiye) following the manufacturer's protocol (Barlak et al., 2021). Cell viability was measured every 24 hours for two days. The L929 (CRL-2148, ATCC), PC-3 (CRL-1435, ATCC) and CaCo-2 (HTB-37, ATCC) cells obtained from the American Type Culture Collection (ATCC). *P. domestica* fruit extracts were prepared at different concentrations (800-400-200-100-50 µg/mL) with serum medium. PC-3, CaCo-2, and L929 cells were treated with these extracts for 24 and 48 hours. PC-3, CaCo-2 cells were seeded in 96-well plate wells with 4.5 $x10^3$ cells per well, while L929 cells were seeded in 96-well plate wells with 4.2$x10^3$ cells per well. They were incubated for 24 and 48 hours. At the end of the incubation period, the medium in the wells was removed and the medium was added to each well as a 10% CVDK-8 solution. The cells were incubated again in the incubator for 3 hours. The viability of the cells was determined by measuring the optical density at 450 nm using a Benchmark Plus microplate spectrophotometer (Biorad, Segrate-Milano, Italy). The change in cell viability was calculated by comparing the absorbance values of the control groups. Experiments were performed with at least three replicates.

Statistical analysis

The data were analyzed using the Statistical Package for Social Science (SPSS) program. The Kolmogorov-Smirnov test was used to determine

whether the data were normally distributed. Since the data showed normal distribution, the results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied to the results, and the differences were considered significant at $p < 0.001$. Two-way analyses of variance (ANOVA) were used to evaluate both factors. The results were assessed at 95% confidence interval and $p<0.001$ was considered statistically significant.

RESULTS AND DISCUSSION

Evaluation of the antiproliferative potential of the extract and fractions

The main methanol extract of *P. domestica* fruits, and the remaining EtOAc and H_2O subfractions were prepared separately. To evaluate the cytotoxicity of the obtained extracts and fractions their effects on cell proliferation of two cancer cell lines PC-3 (human prostate carcinoma), CaCo-2 (human colorectal adenocarcinoma) and normal L929 (murine fibroblast) cells were determined using the CVDK method.

The results showed a dose-dependent cytotoxicity of the EtOAc fraction against the cell lines PC-3 and CaCo-2 in concentration ranges of 50-800 µg/mL (Figures 1 and 2). The ethyl acetate fraction inhibited PC-3 cells by 41.36 % at the highest concentration (Figure 1.). The cytotoxic effect was found to be limited against all cell lines in the aqueous fractions. The main fruit extract and fractions showed negligible antiproliferative activity in the L929 cell line (Figure 3). Cell viability was 93.84%, even at the highest concentration of the ethyl acetate fraction (800 µg/mL) Miljic et al. tested the cytotoxic effects of fruit wines obtained from three plum varieties common in Serbia on Hep2c, RD and L2OB cancer cell lines. A significant decrease in cell viability was observed with an $IC_{50} < 50$ µg/mL value against all three cell lines (Miljić et al., 2016).

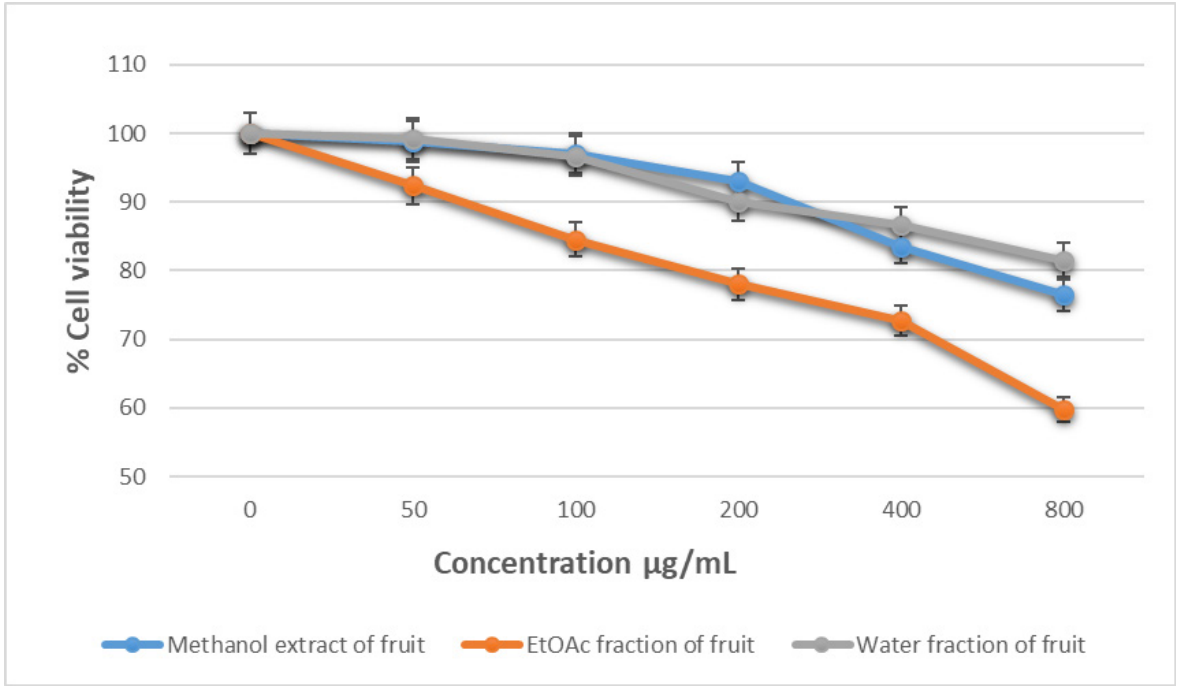

Figure 1. Antiproliferative activities of the *P. domestica* unripe fruit extract and fractions against PC-3 cell line

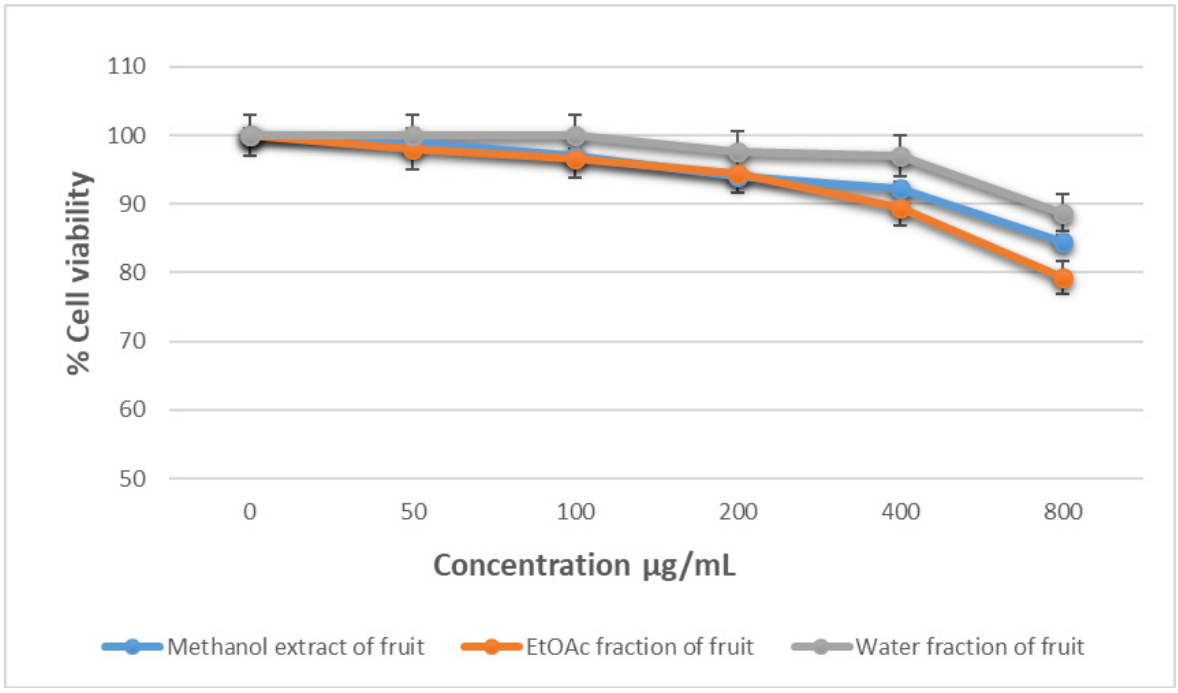

Figure 2. Antiproliferative activities of the *P. domestica* unripe fruit extract and fractions against CaCo-2 cell line

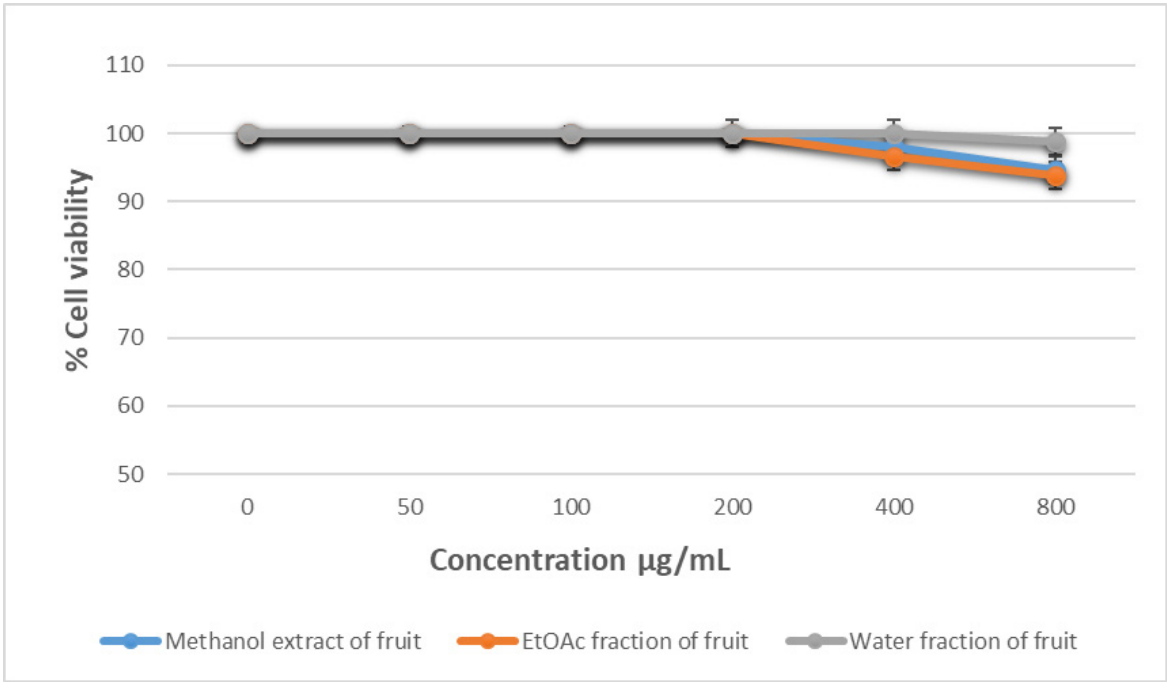

Figure 3. Antiproliferative activities of *P. domestica* unripe fruit extract and fractions against L929 cell line

Antioxidant capacity of the extract and the fractions

Antioxidant activity was determined using CUPRAC, TEAC and DPPH assays. While the EtOac fraction proved to most effective in all methods, the aqueous fractions showed weak antioxidant activity (Table 1). Gallic acid was used as the standard compound to determine DPPH radical scavenging activity and the results were expressed as equivalent to gallic acid. For the TEAC and CUPRAC methods, the results are expressed as Trolox equivalent.

Table 1. Antioxidant capacity of unripe fruit of *P. domestica* extract and fractions

	DPPH[a]	TEAC[b]	CUPRAC[b]
Methanol extract of fruit	101.65 ±1.98	179.16 ±2.68	212.06 ±2.85
EtOAc fraction of fruit	120.64 ±2.07	180.45 ±2.56	251.43 ±1.84
Water fraction of fruit	64.36 ±1.08	78.44 ±1.36	107.18 ±1.24

Data are presented as mean ± SD, n=3 experiments, ($p < 0.001$)
a: mg gallic acid/g extract
b: mg trolox/g extract

In this study, the ethyl acetate fractions of the unripe fruits of *P. domestica* were the most effective fraction according to the antioxidant capacity studies tested. The fruit water fraction showed the least activity. When comparing the antioxidant capacity of the extracts and fractions, the ethyl acetate fraction showed the highest effect for all methods, which was 251.43 and 180.45 mg Trolox/g extract for the CUPRAC and TEAC methods, respectively.

The antioxidant capacity and tyrosinase enzyme inhibition results were found to be parallel. The ethyl acetate fraction of the fruits showed the highest tyrosinase of the enzyme tyrosinase with IC_{50}: 51.83 µg/mL, and the antioxidant effect was also the highest for all tested methods.

In a study, the antioxidant effects of methanol extracts of flowers of *P. domestica* were analyzed by ABTS (65 µmol TE g^{-1}), DPPH (47.50 µmol TE g^{-1}) and CUPRAC (1.04 mmol TE g^{-1}) (Dundar, Sahin, Parlak, & Saricaoglu, 2023). The DPPH radical scavenging activity of plum wines produced from two varieties of *P. domestica,* Crvena ranka and Požegača, was investigated, and the results were determined to be 0.94 and 1.33 mg Trolox L^{-1}, respectively (Ljekocevic et al., 2019).

There are numerous studies in the literature comparing the antioxidant capacity of ripe and unripe fruit (Benmohamed et al., 2023; Hwang, Kim, & Shin, 2020; Yang et al., 2024). In a study comparing the antioxidant effect of ripe and unripe fruits of *P. persica*, it was found that the antioxidant capacity of the unripe fruits was higher (Giovanoudis et al., 2023). In another study the change in antioxidant capacity and chemical content of *Prunus persica* fruits during the ripening period was investigated. Both ascorbic acid and polyphenol contents and antioxidant activities decreased significantly during the ripening period (Liu, Cao, & Jiang, 2015).

Mushroom tyrosinase enzyme inhibition

The antityrosinase potential of the main methanol extract of the fruit, ethyl acetate, and aqueous fractions was investigated using L-tyrosine as a substrate. The IC_{50} values of the extract, the fractions, and the kojic acid (positive control) are shown in Table 2. It was found that all extracts and fractions showed very potent inhibition compared to the standard compound. The ethyl acetate subfraction showed higher antityrosinase effects than the others with IC_{50}: 51.83 µg/mL value.

Table 2. Mushroom tyrosinase enyme inhibition of unripe fruit of *P. domestica* extract and fractions (IC_{50} µg/mL)

Methanol extract	EtOAc fraction	Water fraction	Kojic acid
102.034 ±1.28	51.83±0.82	121.06±1.08	21±0.28

Data are presented as mean ± SD, n=3 experiments, ($p < 0.001$).

A study by Wahyuningsih et al. evaluated the effect of 2% plum extract application on preventing the expression of tyrosinase enzyme and the increase in the amount of melanin in the skin of male guinea pigs exposed to UV-B light. According to the results, it was found that the expression of the enzyme tyrosinase in the skin of guinea pigs decreased and the increase in the amount of melanin was prevented (Wahyuningsih, Pangkahila, & Winarti, 2023).

In another study, the effect of plum leaf extracts on the enzyme tyrosinase was investigated. The extracts showed weak tyrosinase inhibition (from 0.0 to 11.6%) compared to licorice extract (91.6%) and kojic acid (78.9%), which were used as standards. The highest activity was found in EtOAc extracts (Stierlin, Azoulay, Massi, Fernandez, & Michel, 2018).

Mocan et al. showed that the tyrosinase enzyme inhibition of leaf samples from different cultivars of *P. domestica* between 23.07 mg KAE/g extract for cultivar Ialomita and up to 8.90 mg KAE/g extract for the cultivar Minerva (Mocan et al., 2018).

CONCLUSION

P. domestica is a globally widespread species that has always been consumed as food and is characterised by its fruits with a high nutrient content. In this study the antiproliferative, antioxidant, and antityrosinase effects of extracts and fractions of different polarities of the unripe fruits of *P. domestica* were investigated. The extracts, which showed no significant cytotoxic effect on healthy L929 fibroblast cells even at high concentrations, exhibited a cytotoxic effect on PC-3 (human prostate carcinoma) and CaCo-2 (human colorectal adenocarcinoma) cancer cells, depending on the concentration. Unripe fruits of *P. domestica* are a natural source that has no toxic effect on normal cells, has a selective cytotoxic effect on cancer cells, has a strong antioxidant effect in DPPH, TEAC, and CUPRAC methods, and has a strong antityrosinase effect compared with kojic acid. Therefore, it can be a safe and effective source for developing new natural products for the cosmetics and pharmaceutical industries. So far, studies have mainly focused on ripe fruits, but there are few studies on unripe fruits. Therefore, unripe fruits can be considered as a source of bioactive natural compounds and have economic value.

AUTHOR CONTRIBUTION STATEMENT

Concept: Z.B.G., A.A.B..; Design: Z.B.G., A.A.B.., Control: Z.B.G., A.A.B., Sources: Z.B.G., N.Y., A.A.B., Materials: Z.B.G., A.A.B.., Data Collection and/or Processing: Z.B.G., N.Y.; Analysis and/or Interpretation: Z.B.G., N.Y.; Literature Review: Z.B.G., N.Y., Manuscript Writing: Z.B.G.; Critical Review: Z.B.G., N.Y., A.A.B.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Angelino, D., Godos, J., Ghelfi, F., Tieri, M., Titta, L., Lafranconi, A., . . . Sciacca, S. (2019). Fruit and vegetable consumption and health outcomes: An umbrella review of observational studies. *International journal of food sciences and nutrition, 70*(6), 652-667.

Ayub, H., Nadeem, M., Mohsin, M., Ambreen, S., Khan, F. a., Oranab, S., . . . Zarlasht, M. (2023). A comprehensive review on the availability of bioactive compounds, phytochemicals, and antioxidant potential of plum (*Prunus domestica*). *International Journal of Food Properties, 26*(1), 2388-2406.

Barlak, N., Capik, O., Kilic, A., Sanli, F., Aytatli, A., Yazici, A., . . . Karatas, O. F. (2021). MicroRNA-145 transcriptionally regulates Semaphorin 3A expression in prostate cancer cells. *Cell Biology International, 45*(5), 1082-1090.

Benmohamed, M., Guenane, H., Messaoudi, M., Zahnit, W., Egbuna, C., Sharifi-Rad, M., . . . Boubekeur, S. (2023). Mineral profile, antioxidant, anti-inflammatory, antibacterial, anti-urease and anti-α-amylase activities of the unripe fruit extracts of pistacia atlantica. *Molecules, 28*(1), 349.

Dundar, A. N., Sahin, O. I., Parlak, M. E., & Saricaoglu, F. T. (2023). Drying kinetics and change in bioactive compounds of edible flowers: *Prunus domestica*. *Journal of Food Process Engineering, 46*(10), e14405.

Giovanoudis, I., Athanasiadis, V., Chatzimitakos, T., Kalompatsios, D., Mantiniotou, M., Bozinou, E., . . . Lalas, S. I. (2023). Antioxidant Capacity in Two Different Cultivars of Ripe and Unripe Peaches Utilizing the Cloud-Point Extraction Method. *AgriEngineering, 5*(4), 2139-2154.

Güven, Z. B., Dogan, Z., Saracoglu, I., Picot, L., Nagatsu, A., & Basaran, A. A. (2022). Food plant with antioxidant, tyrosinase inhibitory and antimelanoma activity: Prunus mahaleb L. *Food Bioscience, 48*, 101804.

Güven, Z. B., Saracoglu, I., Nagatsu, A., Yilmaz, M. A., & Basaran, A. A. (2023). Anti-tyrosinase and antimelanogenic effect of cinnamic acid derivatives from Prunus mahaleb L.: Phenolic composition, isolation, identification and inhibitory activity. *Journal of Ethnopharmacology, 310*, 116378.

Harput, U. S., Genc, Y., & Saracoglu, I. (2012). Cytotoxic and antioxidative activities of Plantago lagopus L. and characterization of its bioactive compounds. *Food Chem. Toxicol., 50*(5), 1554-1559. doi:10.1016/j.fct.2012.01.019

Hwang, H., Kim, Y.-J., & Shin, Y. (2020). Assessment of physicochemical quality, antioxidant content and activity, and inhibition of cholinesterase between unripe and ripe blueberry fruit. *Foods, 9*(6), 690.

Igwe, E. O., & Charlton, K. E. (2016). A systematic review on the health effects of plums (*Prunus domestica* and Prunus salicina). *Phytotherapy Research, 30*(5), 701-731.

Kabir, M. T., Rahman, M. H., Akter, R., Behl, T., Kaushik, D., Mittal, V., . . . Abdel-Daim, M. M. (2021). Potential Role of Curcumin and Its Nanoformulations to Treat Various Types of Cancers. *Biomolecules, 11*(3), 392. Retrieved from https://www.mdpi.com/2218-273X/11/3/392

Kim, J. H., Yoon, J.-Y., Yang, S. Y., Choi, S.-K., Kwon, S. J., Cho, I. S., . . . Choi, G. S. (2017). Tyrosinase inhibitory components from Aloe vera and their antiviral activity. *J. Enzyme Inhib. Med. Chem., 32*(1), 78-83. doi:10.1080/14756366.2016.1235568

Liu, H., Cao, J., & Jiang, W. (2015). Evaluation of physiochemical and antioxidant activity changes during fruit on-tree ripening for the potential values of unripe peaches. *Scientia Horticulturae, 193*, 32-39.

Ljekocevic, M., Jadranin, M., Stankovic, J., Popovic, B., Nikicevic, N., Petrovic, A., & Tesevic, V. (2019). Phenolic composition and DPPH radical scavenging activity of plum wine produced from three plum cultivars. *J. Serb. Chem. Soc., 84*(2), 141-151. doi:10.2298/jsc180710096l

Lu, W., Shi, Y., Wang, R., Su, D., Tang, M., Liu, Y., & Li, Z. (2021). Antioxidant activity and healthy benefits of natural pigments in fruits: A review. *International journal of molecular sciences, 22*(9), 4945.

Madhuri, S., & Pandey, G. (2009). Some anticancer medicinal plants of foreign origin. *Curr. Sci., 96*(6), 779-783.

Maheshwari, N., & Sharma, M. C. (2023). Anticancer properties of some selected plant phenolic compounds: future leads for therapeutic development. *Journal of Herbal Medicine*, 100801.

Miljić, U., Puškaš, V., Velićanski, A., Mašković, P., Cvetković, D., & Vujić, J. (2016). Chemical composition and in vitro antimicrobial and cytotoxic activities of plum (*Prunus domestica* L.) wine. *Journal of the Institute of Brewing, 122*(2), 342-349.

Mocan, A., Diuzheva, A., Carradori, S., Andruch, V., Massafra, C., Moldovan, C., . . . Zara, S. (2018). Development of novel techniques to extract phenolic compounds from Romanian cultivars of *Prunus domestica* L. and their biological properties. *Food and chemical toxicology, 119*, 189-198.

Ozyurek, M., Bektasoglu, B., Guclu, K., & Apak, R. (2009). Measurement of xanthine oxidase inhibition activity of phenolics and flavonoids with a modified cupric reducing antioxidant capacity (CUPRAC) method. *Anal. Chim. Acta, 636*(1), 42-50. doi:10.1016/j.aca.2009.01.037

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med., 26*(9/10), 1231-1237. doi:10.1016/S0891-5849(98)00315-3

Sharafan, M., Malinowska, M. A., Ekiert, H., Kwaśniak, B., Sikora, E., & Szopa, A. (2023). Vitis vinifera (Vine Grape) as a Valuable Cosmetic Raw Material. *Pharmaceutics, 15*(5), 1372.

Shukla, R. K., Shukla, A., & Singh, R. (2021). Evaluation of nutritive value, phytochemical screening, total phenolic content and in-vitro antioxidant activity of the seed of *Prunus domestica* L. *Plant Science Today, 8*(4), 830–835-830–835.

Stierlin, E., Azoulay, S., Massi, L., Fernandez, X., & Michel, T. (2018). Cosmetic potentials of *Prunus domestica* L. leaves. *J. Sci. Food Agric., 98*(2), 726-736. doi:10.1002/jsfa.8520

Usenik, V. (2021). The influence of the production system on the composition of phytochemicals in *Prunus domestica* L. fruit. *Journal of Food Composition and Analysis, 95*, 103701.

Wahyuningsih, M. D., Pangkahila, W., & Winarti, N. W. (2023). The Administration of 2% Plum (*Prunus domestica* L.) Extract Cream Inhibited the Increase of Tyrosinase Enzyme Expression and the Amount of Skin Melanin in Male Guinea Pigs (Cavia porcellus) Skin Exposed to UV B Light. *European Journal of Biomedical Research, 2*(3), 12-16.

Yang, Q.-N., Deng, W., Wu, D.-T., Li, J., Liu, H.-Y., Yan, H.-L., . . . Huang, J.-W. (2024). Characterization, Antioxidant Capacity, and Anti-Inflammatory Activity of Polyphenol-Enriched Extracts Obtained from Unripe, Mature, and Overripe Fruits of Red-Fleshed Kiwifruit Cultivars. *Foods, 13*(18), 2860.