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Extraction, characterization, and biological activities of anthocyanin pigment from *Prunus domestica* L. fruit peels

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ABSTRACT

The scientific community has developed an interest in anthocyanins due to their potential applications. This study aimed to extract anthocyanin from *Prunus domestica* and subsequently characterize it using UV-vis spectroscopy (UV-vis), Fourier transforms infrared spectroscopy (FTIR), and Gas chromatography-Mass spectrometer (GC-MS) techniques. Furthermore, this study aimed to explore the biomedical applications of these products, including their potential as antibacterial agents, antioxidants, and anticancer agents. Additionally, an *in vivo* examination of bodily functions was conducted through the assessment of parameters such as glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), urea, creatinine, and random blood sugar (RBS). The results of anthocyanins dye as antibacterial agent was (26.166±0.42, 23.766±0.42, 19.46±0.50, 15.5±0.42, 8.4±0.42, and 22.26±0.42 mm) for *Serratia marcescens*, *Bacillus cereus*, *Pseudomonas aerogenes*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Candida albicans* respectively. Its role as an antioxidant, as its effectiveness was highest at the highest concentration 86.66±0.017. Its role as an anticancer breast cancer (MCF-7) IC₅₀=34.92 µg/mL, and tests GOT, GPT, ALP, urea, creatinine and RBS also showed the role of anthocyanins dye in regulating various body functions when given at a concentration of orally administered 100 µg/Kg/day for different periods (1–4 weeks). Our research illustrated the beneficial effectiveness of *P. domestica* anthocyanins regarding their antibacterial, antifungal, antioxidant, and anticancer characteristics. Moreover, anthocyanins demonstrated the capacity to influence liver and kidney enzymes as well as maintain blood glucose levels consistently over the course of a day.

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1. Introduction

Plant extracts, rich in phenolic compounds and dietary fiber, can significantly improve human health and well-being by mitigating oxidative stress, reducing the risk of various metabolic-related diseases, and reducing susceptibility to diseases like diabetes, cancer, and cardiovascular issues. These compounds also reduce the risk of coronary heart disease, hypertension, stroke, obesity, and specific gastrointestinal disorders (Lin et al., 2016). Anthocyanins (such as malvidin, petunidin, pelargonidin, cyanidin, delphinidin, and peonidin) are water-soluble pigments that belong to the flavonoids, an important group of polyphenols, which are plant-derived secondary

metabolic products (Khoo et al., 2017). Anthocyanins are found in many fruits and vegetables, including plums, grapes, purple potatoes, cherries, berries, purple cabbage, red pepper, black carrots, beetroot, strawberries, eggplant peels, etc. (Liu et al., 2018). Regular consumption of anthocyanins has numerous health benefits, as they are capable of eliminating harmful free radicals in the human body due to their antioxidant effect (Gonçalves et al., 2021). Anthocyanin pigment lowers the risk of developing diabetes, rheumatoid arthritis, cardiovascular disease, and cancer (Diaconeasa et al., 2018). Certain anthocyanin and other flavonoids have been shown to possess antiviral, antibacterial, and antifungal properties (Saito and Harborne, 1992).

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Plum (*P. domestica*) is a seasonal fruit that is a significant source of calcium, potassium, magnesium, phosphorous, carbohydrates (glucose, fructose, sucrose), fiber, minerals, enzymes, and vitamins (vitamin C, vitamin A, vitamin K). The consumption of plums and their derived products confers considerable health advantages, particularly in preventing the onset of various diseases (Ayub et al., 2023). They are an essential source of compounds that promote human health. This fruit, abundant in nutrients, harbors primary and secondary metabolites that possess pharmacological attributes, including antioxidant and anti-atherosclerotic effects. Plums have the potential to exert a beneficial influence on both bone health and cardiovascular risk factors. Additionally, these properties can help reduce blood pressure and blood sugar levels (Turturica et al., 2016). *P. domestica* is recognized as one of the fruits that contain anthocyanins within its peels; it is abundant in bioactive constituents, encompassing phenolic acids, carotenoids, flavanols, and other aromatic compounds (Diaconeasa et al., 2018). Antibiotic overuse increases antibiotic resistance, making treatment more difficult and expensive and posing a significant public health risk due to the loss of ability to treat serious bacterial infections (Llor and Bjerrum, 2014). Antibiotics “fight” against bacteria by targeting specific structural or cellular components (Murathan et al., 2020). Still, bacteria can “defeat” antibiotics by mutations, rapid multiplication, natural selection, or the frequent use of alcohol and cleaning products, which are powerful pesticides for microbes (Llor and Bjerrum, 2014). The frequent use of cleaning agents can lead to the destruction of beneficial bacteria in the human body (skin, mouth, stomach), thus increasing the proportion of harmful bacteria causing various diseases (Mahmood et al., 2022). Therefore, it is necessary to continuously discover antibacterials to overcome the problem of resistant strains (Mohammed et al., 2020).

Oxidation processes in the body lead to damage to cell membranes, lipids, cellular proteins, and DNA. The metabolism of oxygen produces unstable molecules known as free radicals, which take electrons from other molecular structures, consequently inflicting damage upon DNA and other cells. The body manages free radicals and necessitates their presence for optimal functionality. Nonetheless, the damage arising from the protracted accumulation of free radicals may become irreversible, culminating in certain diseases, including cardiovascular and hepatic conditions, atherosclerosis, vascular disorders, arthritis, Alzheimer’s disease, and certain malignancies

attributed to DNA damage in cells (Dadgostar, 2019). Some factors accelerate the accumulation of free radicals, such as smoking, excessive alcohol consumption, sunlight, environmental pollution, and oxidation. Oxidative stress has been documented to correlate with the development of numerous metabolic, chronic disorders, or cancers (Finkel and Holbrook, 2000; Reuter et al., 2010). Given that antioxidants possess the capacity to eliminate free radicals within cells and hinder or diminish the harm stemming from oxidation, the ingestion of flavonoid-rich foods proves advantageous. Owing to their elevated antioxidant levels, these foods have the potential to avert various metabolic disorders or instances of cancer (Pham-Huy et al., 2008). Breast cancer ranks as the most prevalent form of cancer subsequent to skin cancer, exhibiting a higher incidence in females compared to males (Dawidowicz et al., 2012). BRCA1, BRCA2, and PALB-2 are classified as tumor suppressor genes, and alterations in these genes are linked to an augmented risk of developing breast and ovarian cancer (Shareef et al., 2016). Phytochemicals show great potential in fighting breast cancer due to their promising anticancer effects, bolstering the immune system, preventing carcinogenesis and metastatic spread, shielding cells from malignant transformation, and boosting the efficacy of traditional anticancer treatments (Mazurakova et al., 2022). The use of plant extracts can be safer and may decrease the side effects of chemotherapy used for breast cancer patients (Jawad et al., 2021). The plant extracts encompass compounds that have been evidenced to possess antioxidant and anti-inflammatory properties and may also exhibit the capacity to inhibit the proliferation and dissemination of cancer cells (Llor and Bjerrum, 2014; Mahmood et al., 2022). Their use is safe in terms of use and environmentally friendly, and they are abundant in nature and can be obtained through daily meals. They do not contain toxic compounds or chemical compounds harmful to human health.

The main objective of this study is to explore the extraction of anthocyanins pigment from *P. domestica* fruits. The research involves a comprehensive analysis of the properties of anthocyanins pigment, including UV-vis, FTIR, and GC-MS. In addition, the study addresses the biomedical applications of these extracted compounds, focusing on their potential antibacterial, antioxidant, and anticancer activities. The main aims of the in vivo experiments with mice are to clarify the impact of anthocyanins on physiological functions by evaluating key hematological parameters such as GOT, GPT, ALP, urea, creatinine,

and RBS. This in-depth examination seeks to unravel the role of anthocyanins in modulating essential physiological processes within the body.

2. Materials and methods

2.1. Chemicals and solvents

The solvents used (Ethanol 75% and dimethyl sulfoxide (DMSO)), trypsin/EDTA; Fetal bovine serum, the source of the Roswell Park Memorial Institute 1640 medium (RPMI), DPPH and ascorbic acid were purchased from Sigma Co. (St. Louis, MO, USA). Antibiotic disks were obtained from Liofilchem, Roseto degli Abruzzi, Italy. Muller–Hinton agar was obtained from Mast, Liverpool, England.

2.2 Plant material, sample preparation, and extraction of anthocyanin

Fruits of *P. domestica* were procured from a local market situated in Baghdad, Iraq. The fruits underwent washing with tap water and were subsequently placed in a freezer to facilitate the peeling process. The frozen fruits were dipped in warm water for 1–2 min, and the peels were easily removed from the fruit and kept in the freezer until use. Anthocyanins

were extracted from *P. domestica* peels using the Soxhlet device (Mohammed et al., 2020); every 50 g of fresh peels were extracted with 300 mL of 75% ethanol at 40°C for 7 h. The extract was separated by ethanol condensation, and the remaining amount of the extract was added to a glass Petri dish and placed in an air-dry oven at 50°C in order to evaporate the remaining ethanol. The final extract was kept until use (Figure 1).

2.3. Characterization of *P. domestica* peels extract

UV visible, FTAR, and GC-MS analyses were performed to characterize anthocyanin, verify the presence of a dye, identify the functional group, and determine the biological activity of anthocyanin. *P. domestica* peels extract was optically analyzed using UV-vis (UV-1900, Shimadzu, Japan) at the Department of Medical Engineering, University of Technology, within the spectral range of 200–800 nm. FTIR Thermo Fisher Scientific, USA, was used with a spectral range of 500–4000 nm. GC/MS was analyzed by Agilent (7820A) USA GC Mass Spectrometer Analytical Column Agilent HP-5ms Ultra Inert (30 m length × 250 µm inner diameter × 0.25 µm film thickness). FTIR was done in Iraq's Ministry of Science and Technology.

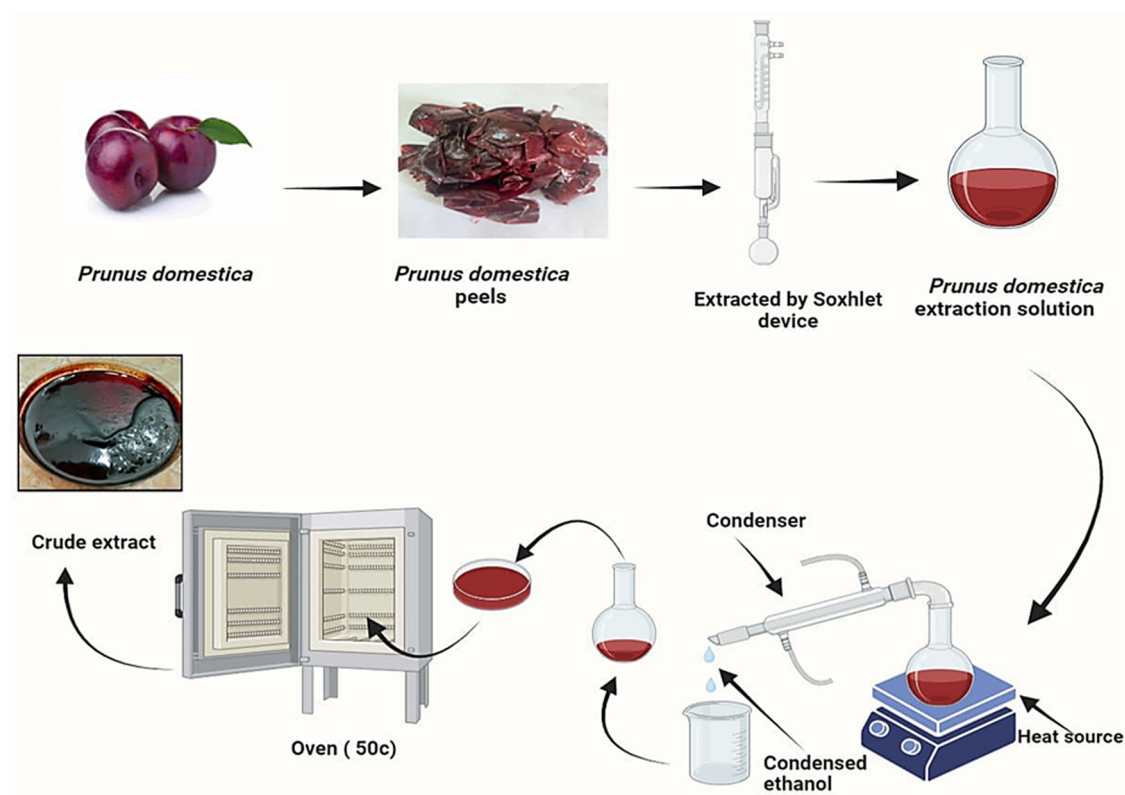


Figure 1. Schematic diagram for anthocyanin extraction from *P. domestica* peels using soxhlet device.

GC-MS was analyzed by Agelint (7820A) USA GC Mass Spectrometer Analytical Column Agelint HP-5ms Ultra Inert (30m length $\times$ 250 μ m inner diameter $\times$ 0.25 μ m film thickness). The GC-MS analysis using a 2 μ L from *P. domestica* dilute with distilled water for the phytochemical screening. The Elite-1 column, composed of 100% dimethylpolysiloxane and fused capillary silica (30m $\times$ 0.25 mm diameter, 1 mm thick), has been made available for the GC-MS system. Helium served as the carrier gas with a flowrate of 1.0ml/min and a split ratio of 1:10; the temperature range is 110° for 2min; it then climbs 5 min to 200°C and is kept for 9min; finally, it rises 5 min to 280°C and is maintained for 9min. The injector and detector were kept at a constant temperature of 280°C. Mass spectra covering the spectral range of 45–450m/z were recorded at 70eV. The mass detectors employed in the research based on the modification of mass spectra and chromatograms included Turbo-Mass Gold-Perkin Elmer and Turbo-Mass 5.2 (Altameme et al., 2022). The majority of components were categorized using the mass spectrum and NIST library. GC-MS was done in Iraq's Ministry of Science and Technology.

2.4. Biological activities of *P. domestica* peel extract

2.4.1 Microorganisms

The microorganisms used in antimicrobial assays were against *S. aureus*, *Serratia marcescens*, *P. mirabilis*, *Pseudomonas aeruginosa*, *B. cereus* and *C. albicans*. These cultures were obtained from Laboratory of Biotechnology Division, Department of Applied Sciences, University of Technology, Iraq.

2.4.2. Antimicrobial activities

The antibacterial activity of *P. domestica* peel extract was evaluated *S. aureus*, *Serratia marcescens*, *P. mirabilis*, and *Pseudomonas aeruginosa*. In contrast, the anti-fungal activity was evaluated against *C. albicans* by agar well diffusion method. Each inoculum was adjusted to 0.5 McFarland standard and applied *B. cereus* on the surface of Muller-Hinton Agar, then sterilized cork borer, and 6mm wells were made in each plate. Then, the wells were filled with different concentrations (1.25, 2.5, 5) μ g/mL of extract. Subsequently, the plates were incubated for 24h at 37°C; the inhibition area was measured as the previously described method (Hasoon et al., 2024; Mohanaparameswari et al., 2023; Yosif et al., 2024).

2.4.3 Antibiotic susceptibility test

To conduct the antibiotic susceptibility test, we utilized Muller-Hinton. Ten distinct antibiotic discs, specifically Amikacin, Rifampicin, Lincomycin, Cefotaxime, Amoxicillin, Tetracycline, Streptomycin, Nystatin, Ketoconazole, and Fluconazole, were employed. The experimental procedure was conducted utilizing the Kirby-Bauer (disc diffusion) methodology in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (2022). The effect of antagonism of *P. domestica* peels extract with some antibiotic. After extracting anthocyanins from *P. domestica* peels at a concentration of 5 μ g/mL, the antibiotic discs were immersed in the peels extract for (5–10 min) to be saturated. Well- diffusion method was used to test the antibacterial activity of *P. domestica* peels extract with antibiotics (Abdelmawgoud et al., 2021).

2.4.4. Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl [DPPH] radical scavenging assay was performed using the stated methodology (Dawidowicz et al., 2012). The peel extract was prepared by dilution in distilled water at different concentrations (1.25, 2.5, and 5) μ g/mL. DPPH was prepared by dissolving 0.004mg in 100mL of absolute methanol to reach a concentration of 400 μ g/mL. Ascorbic acid was prepared by adding 0.05g to 100mL solution of 50% ethanol and water (1:1; v/v). Approximately 750 μ L were added to 750 μ L of various concentrations of *P. domestica* extract in a final volume of 1500 μ L. The reaction mixture was mixed and incubated for 30minutes at room temperature in the dark. The absorbance was evaluated at 517 nm (Jawad, 2023). The samples' ability to block the DPPH radical was estimated using the formula equation below

DPPH scavenging activity

$$= \text{Abs. of control} - \text{Abs. of sample} \times 100 / \text{Abs. of control}$$

2.4.5. Anticancer activity

The anticancer efficacy of *P. domestica* peel extract was evaluated through the application of the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide [MTT] cell proliferation assay.

2.4.5.1. Culture of cells. RPMI-1640 medium was supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μ g/mL streptomycin to support MCF-7 cells. Trypsin-EDTA was employed for

cell passage, followed by reseeding at 80% confluence biweekly, and cultured at 37°C (Jawad et al., 2021).

2.4.5.2. MTT assay. The cytotoxic potential of *P. domestica* peel extract was assessed utilizing the MTT assay on 96-well plates (Dawidowicz et al., 2012), with 1×10^4 cells/well employed to seed the cell lines. Following a 24-h incubation period or until a confluent monolayer was established, the cells were treated with the extract at various concentrations. After a 48-h treatment duration, the medium was discarded, and 28 μ L of a 2 mg/mL MTT solution was introduced. The cells were incubated for 2.5 h at 37°C to evaluate cell viability. Upon removal of the MTT solution, 130 μ L of dimethyl sulphoxide (DMSO) was added to the wells to solubilize the residual crystals. This was done for 15 min while shaking the container at 37°C (Dawidowicz et al., 2012; Mohammed et al., 2024). The experiment was conducted in triplicate, and the absorbance was measured at 492 nm on a microplate reader. Equation was used to calculate the percentage of cytotoxicity or the inhibition rate of cell growth (Jawad et al., 2021).

$$\text{Inhibition rate} = A - B / A * 100$$

where A is the optical density of control, and B is the optical density of the samples.

2.5. Effect of *P. domestica* peel extract on rat body function tests

White male rats (weighing 120–125 g) were acquired from the Animal House-Veterinary Medicine Center/ Toxicology Unit-Industrial Research and Development Authority-Ministry of Industry-Baghdad, Iraq. The rats were fed regularly, given access to water as needed, and maintained in a typical environmental setting (25°C with a 12-h cycle of light and dark). The Institutional Animal Ethical Committee approved all experimental protocols (26 February, 2024; Ref. BCSR8). Two groups of three rats each received daily orally administered 100 μ g/Kg/day gavage treatment for 4 weeks on the rats. Standard pellet food and normal saline were given to the first group as a negative control, while orally administered 100 μ g/Kg/day of *P. domestica* peel extract was given to the second group. Animals were seen and evaluated in the 4th-week. The rats were anesthetized with sodium pentobarbital, killed, and blood samples were obtained by cardiac puncture. The blood samples were put in dry, clean centrifuge tubes and left to clot at room temperature for one to two hours. Next, sera were extracted using a Pasteur pipette before being centrifuged at $1100 \times g$ for 20 min. the collection and storage of serum at (–20°C) were carried out (Figure 2).

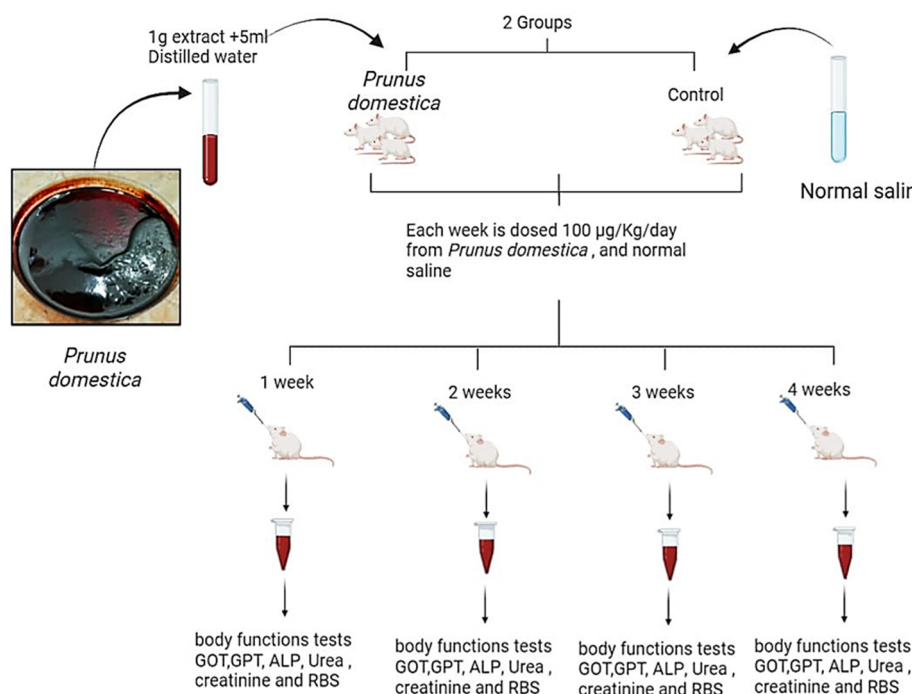


Figure 2. Schematic diagram of the experimental design for body functions test of rats.

2.6. Statistical analysis

The data were analyzed with one-way analysis of variance (ANOVA) using SPSS v.24 software at a 0.05 level of statistical significance. All experiments were carried out in triplicate and the data are presented as mean $\pm$ SE.

3. Results and discussion

3.1. Extraction and characterization of anthocyanins

Anthocyanins were extracted from *P. domestica* peels by the traditional method, which is the use of the Soxhlet device, because it is an easy method, does not require complex and unavailable devices and equipment, and is safe when used. Ethanol was used, which is inexpensive, and the compounds obtained from extraction may be high purity (Chemat et al., 2012). For the extraction of anthocyanins, a

temperature of 40°C was used (Qasim and Naji, 2022). It has also been shown that the concentration of extracted anthocyanins is directly proportional to the duration of constant temperature, because a limited increase in time increases the extraction efficiency of the anthocyanins by increasing their dissolution rate and thus extracting the largest amount of anthocyanins (Vega Arroy et al., 2017). The results shown in Figure 3 indicated that the anthocyanins extracted from the peel of *P. domestica* were within the visible wavelength (510 nm), and this result was similar to that previously reported (Akbar, 2019; Mattioli et al., 2020).

According to the FTIR results revealed the functional groups of anthocyanin extracted from *P. domestica* peels and the spectrum was recorded (500–4000/cm). The absorption peaks observed have been shown in (Figure 4). O–H group in sugar vibrations and phenol, C–H in alkane, aromatic carbon groups C=C, –CO, C–O–C, and aromatic compound

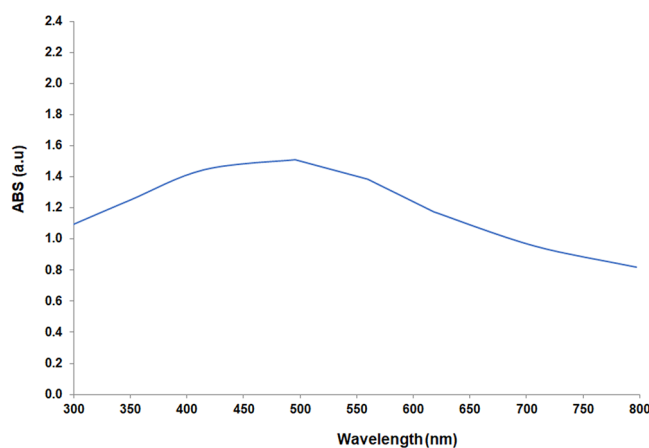


Figure 3. UV-Vis spectrum of anthocyanins dye extracted from *P. domestica* peels.

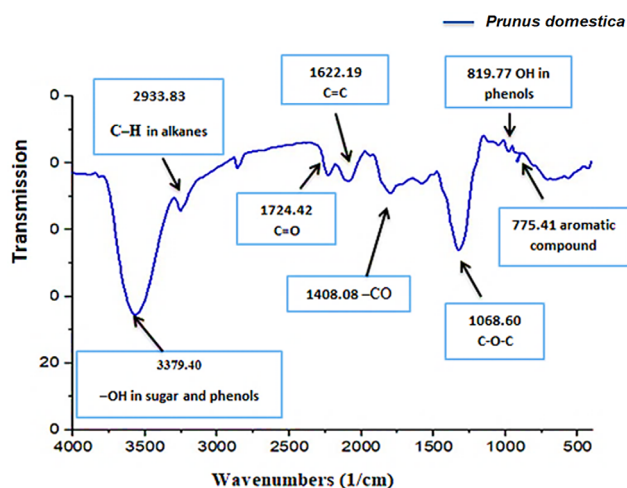


Figure 4. FTIR spectrum of anthocyanin dye extracted from *P. domestica* peels.

are detected at 3379.40, 2933.83, 1724.42, 1622.19, 1408.08, and 775. 41/cm, respectively. As a result, the concentrated extracts' spectrum examination reveals the existence of the anthocyanin-specific functional groups O–H, C=O, C=C, and C–O–C. These results confirm findings of previous studies (Avula et al., 2023; Mattioli et al., 2020; Swer et al., 2018).

3.2 Biological activities of anthocyanins extracted from *P. domestica* peels

3.2.1. Gas chromatography mass spectrometer (GC-MS)

The GC-MS findings have demonstrated the primary bioactive compounds present in the extract of *P. domestica* peels, each with distinct biological properties outlined in Table 1. The evaluation indicates that the peel of *P. domestica* exhibits potential for serving as an agent with antibacterial, anticancer, antioxidative, antipathogenic, antitumoral, antimicrobial, anti-inflammatory, antiallergenic, and antidiabetic properties. These findings align with those of Jawad et al. (2022a).

3.2.2 Antimicrobial activity

The antimicrobial activity against three bacterial strains of Gram-negative bacteria (*S. marcescens*, *P. mirabilis*, *P. aeruginosa*) and Gram-positive bacteria (*B. cereus*, *S. aureus*) and one fungus (*C. albicans*) using anthocyanin compounds extracted from *P. domestica*

peels was examined by the diffusion method in an agar well. The treatment with (1.25, 2.5, and 5 µg/mL) of anthocyanin extract was more successful in eliminating Gram-negative bacteria than Gram-positive ones (Figure 5). It was found that the effectiveness of antibacterial compounds increased with an increase in concentration of peel extract. Three concentrations (1.25, 2.5, and 5 µg/mL) were used, and the highest inhibition was detected against the bacterium *S. marcescens*, and there was no inhibition for the lowest concentration against the bacterium *P. mirabilis*. When 5 µg/mL concentration was applied, the size of inhibition zones was 26.166 ± 0.42 , 23.766 ± 0.42 , 19.46 ± 0.50 , 15.5 ± 0.42 , 8.4 ± 0.42 , and 22.26 ± 0.42 mm for *S. marcescens*, *B. cereus*, *P. aeruginosa*, *S. aureus*, *P. mirabilis*, and *C. albicans*, respectively. At 2.5 µg/mL concentration, the area of inhibition zones was 21 ± 0.45 , 17.80 ± 0.45 , 17.2 ± 0.52 , 11.06 ± 0.45 , 6.2 ± 0.45 , and 18.56 ± 0.45 mm for *S. marcescens*, *B. cereus*, *P. aerogenes*, *S. aureus*, *P. mirabilis*, and *C. albicans*, respectively. The peel extract applied at a concentration of 1.25 µg/mL resulted in the size of inhibition zones 16.5 ± 0.38 , 15.33 ± 0.38 , 14.46 ± 0.38 , 5.53 ± 0.38 , and 14.33 ± 0.38 mm for *S. marcescens*, *B. cereus*, *P. aeruginosa*, *S. aureus*, *P. mirabilis*, and *C. albicans*, respectively.

The decrease in growth rate can be attributed to the fact that compared to Gram-positive bacteria and Gram-positive bacteria lacking *P. domestica*, Gram-negative bacteria have thinner cell walls with a

Table 1. GC-MAC analysis for *P. domestica* peel extract and their biological activity.

Peak	R. T. Min	% of total	Compounds	Molecular formula	Biological activity
1.	4.219	1.519%	Oxime-methoxy-phenyl 4-Ethylbenzoic acid	$C_8H_9NO_2$	Antipathogenic
2.	4.461	1.519%	3,4-dihydro-2H-pyran	C_6H_8O	Antibacterial
3.	4.859	1.366%	5-methyl- 2-Furancarboxaldehyde	C_6H_6O	Antimicrobial
4.	5.556	1.094%	Butanoic acid	C_4H_8O	Antibacterial
5.	6.580	1.032%	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	Antimicrobial
6.	6.809	0.601%	4-Hydroxymandelic acid	$C_8H_8O_4$	Antibiotic
7.	7.089	1.545%	Cyclopropyl carbinol	C_4H_8O	Antitumor
8.	7.820	0.499%	Ethyldiethanolamine	$C_6H_{15}NO_2$	Antioxidant
9.	7.976	9.288%	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C_6H_8O	Antioxidant, Anticancer
10.	8.798	0.545%	Oxalic acid, hexyl propyl ester	$C_{11}H_{20}O_4$	Antimicrobial
11.	8.948	0.903%	Cyclotetrasiloxane, octamethyl	$C_8H_{18}O_4Si_4$	Antibacterial, Antioxidant
12.	9.150	0.967%	4(1H)-Pyrimidinone, 2-(propylthio)	$C_7H_{10}N_2OS$	Antimicrobial
13.	9.437	22.816%	5-Hydroxymethylfurfural	C_6H_6O	Antioxidant, Antiallergenic, Antidiabetic
14.	12.900	4.341%	2-Propenoic acid, 3-phenyl	$C_9H_8O_2$	Antitumor
15.	20.140	0.565%	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Antimicrobial
16.	20.759	6.566%	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Antioxidant, Antibacterial
17.	22.455	0.911%	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	Antimicrobial, Antioxidant, Anti-Inflammatory
18.	23.081	31.572%	Oleic acid	$C_{18}H_{34}O$	Antimicrobial
19.	23.348	4.600%	Octadecanoic acid	$C_{18}H_{36}O_2$	Antimicrobial
20.	23.590	2.677%	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	Antimicrobial
21.	24.027	2.195%	Methyl 9,12-heptadecadienoate	$C_{18}H_{32}O_2$	Antioxidant, Antimicrobial
22.	28.579	0.542%	1,2-Cyclobutanedicarboxylic acid	$C_6H_8O_4$	Antitumor, Antimicrobial
23.	28.840	1.150%	4(1H)-Pyrimidinone	$C_4H_4N_2O$	Antibacterial

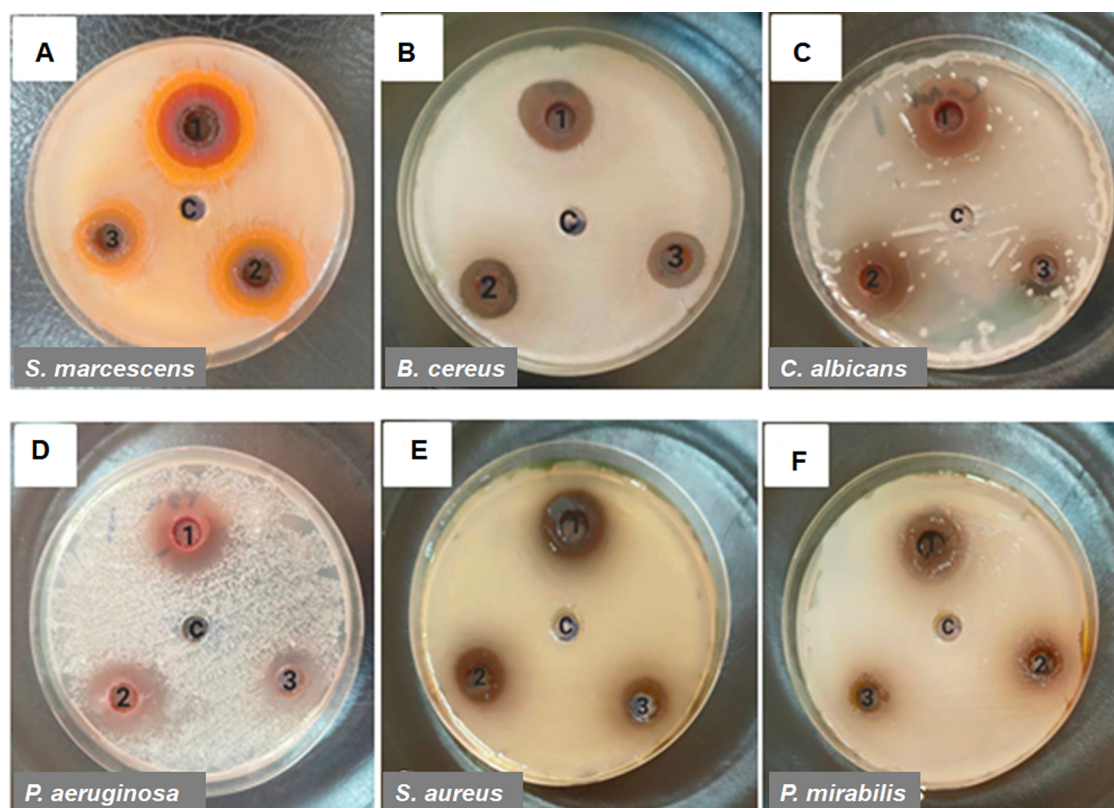


Figure 5. Inhibition zones of anthocyanin extracts from *P. domestica* peel against different pathogens (A-F). 1 = 1.25 µg/mL, 2 = 2.5 µg/mL, 3 = 5 µg/mL, C = control.

single layer of polypeptidoglycan. However, in comparison to the anthocyanin dye with the highest activity, the anthocyanin dye showed poor inhibitory action against Gram-positive bacteria. An earlier study indicated that anthocyanin dye had more potential against Gram-negative bacteria than against Gram-positive bacteria, indicating that Gram-negative bacteria may employ the outer membrane as a defense against this type of natural product, the results presented in the table agreed with the findings reported by Abdulsada et al. (2023). The key finding was that the inhibition or killing of negative bacteria was greater than the inhibition on positive bacteria. Additionally, the killing rate of the bacteria increased as the concentration of the treatment was increased. Nicotinamide adenine dinucleotide phosphate (NADPH) activates the bacterial outer membrane or cell wall through electrostatic interaction, which is one of the main mechanisms of the anthocyanin stain extraction from *P. domestica* peels extract's antimicrobial effect. This leads to the formation of reactive oxygen species (ROS), which cause oxidative DNA damage, lipid membrane oxidation, and protein denaturation (Hasan et al., 2022; Hasoon et al., 2024; Jawad, 2023; Mohammed et al., 2024; Mohanaparameswari et al., 2023; Yosif et al., 2024).

3.2.3 Antibiotic-susceptibility test

The range of antibiotic resistance exhibited by microbial isolates was assessed using the Kirby disc diffusion technique, and the results were compared with the results of the Clinical and Laboratory Standard Institute (CLSI, 2022). *S. marcescens* was highly resistant to Amikacin, Streptomycin, Cefotaxime, and Tetracycline, whereas it was sensitivity to Lincomycin and Rifampicin (Figure 6A). *P. aeruginosa* showed sensitive to Amikacin, Streptomycin, Cefotaxime, Tetracycline, and Rifampicin, whereas it was resistant to Lincomycin and Amoxicillin (Figure 6B). *B. cereus* was sensitivity to Amikacin, Streptomycin, Lincomycin, Tetracycline, and Rifampicin, and it was resistant to Cefotaxime and Amoxicillin (Figure 6C). *P. mirabilis* bacterium has sensitivity to Rifampicin and Streptomycin, and it was resistant to Amikacin, Lincomycin, Cefotaxime, Amoxicillin, and Tetracycline (Figure 6D). *S. aureus* shown sensitivity to Amikacin, Cefotaxime, and Rifampicin, and it was resistant to Amoxicillin, Lincomycin and Tetracycline (Figure 6E). *C. albicans* fungi has resistance to Nystatin, Ketoconazole, and Fluconazole at a percentage of 100% (Figure 6F).

The effectiveness of the *P. domestica* peel extract as an antibacterial compound was proven because it

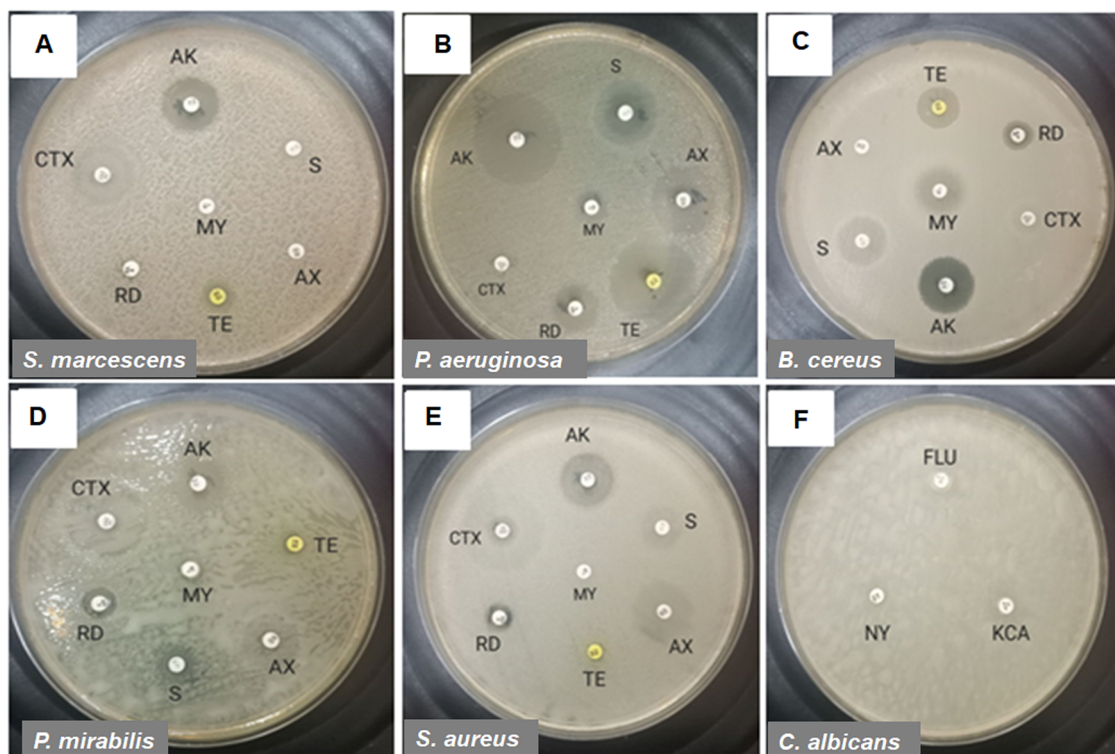


Figure 6. Antibiotic -Susceptibility test. Amikacin =AK, Amoxicillin =Ax, Fluconazole =FLU, Ketoconazole =KCA, Lincomycin =MY, Nystatin =NY, Tetracycline =TE, Streptomycin =S, Rifampicin =RD, Cefotaxime =CTX.

was effective in inhibiting the growth of bacteria in all concentrations that used, even at the lowest concentration of $1.25\mu\text{g/mL}$. In contrast, antibiotics tested in our experiments for the same bacteria were ineffective, as the bacteria gained resistance to some types of antibiotics. The bacteria may become resistant to most antibiotics over time (Llor and Bjerrum, 2014). The effectiveness of *P. domestica* peel extract against the *Candida* fungus has also been proven (Figure 5C) compared to the antifungals tested (Fluconazole, Ketoconazole, Nystatin), which showed a 100% resistance (Figure 6F). However, this result was in disagreement with those reported earlier, which showed no effectiveness of anthocyanin extracted from *P. domestica* peels against *C. albicans* fungus, although extraction conditions were different (Jawad et al., 2022a).

3.2.4 Interaction between anthocyanin and some antibiotics

Interaction between anthocyanins extracts from *P. domestica* peel, and some antibiotic discs was studied by agar well diffusion method against *S. marcescens*, *P. aeruginosa*, *B. cereus*, *P. mirabilis*, *S. aureus* and *C. albicans*. Inhibition zone diameters were measured in millimeters (mm). Results are summarized in Figure 7. Anthocyanin dye may bind

to the cell membrane, which increases the permeability of the cell membrane and may cause cell death. Furthermore, anthocyanins allow for improved contact and interaction with the microbial cell, causing the cell membrane to change structurally and then become more permeable. This process continues until the cell dies. Anthocyanins alone were more effective in the inhibition of growth in all microbes tested in this study compared to all antibiotic discs. In this study, it was found that the combined effects of anthocyanin extracts from *P. domestica* peels with some antibiotic discs resulted in an enhanced antimicrobial effect against tested microorganisms, giving the opportunity to reduce the adverse effect and the development of bacterial resistance (Abdelmawgoud et al., 2021). Differences between the cell wall structures of Gram-negative bacteria and Gram-positive bacteria may explain the reason for their different responses to the anthocyanin peel extracts from *P. domestica* peels. The exact mechanisms of anthocyanins against bacteria are yet unclear. However, some researchers believe that the action of anthocyanins on bacteria is related to their capability to invade the cell, the development of free radicals, inactivation of proteins in the cell by anthocyanins, and the production of reactive oxygen species (ROS).

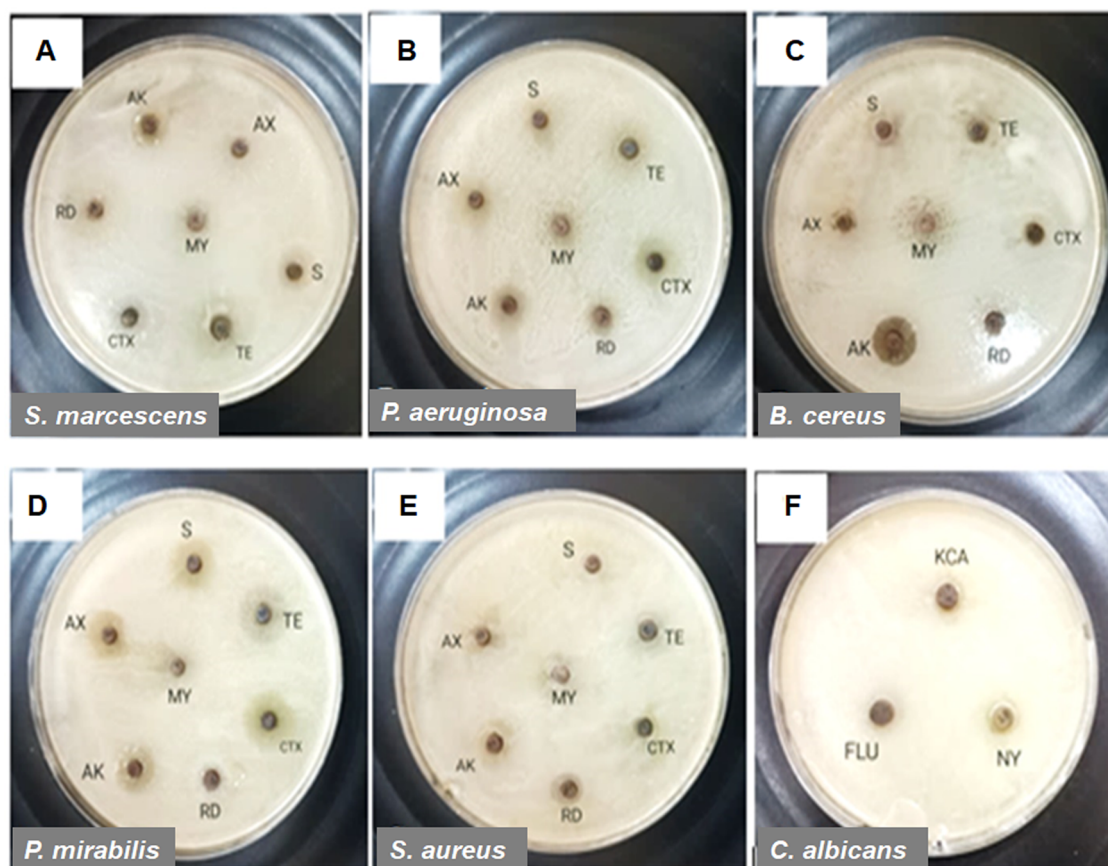


Figure 7. The combined effect of anthocyanins extracts from *P. domestica* peels with some antibiotic.

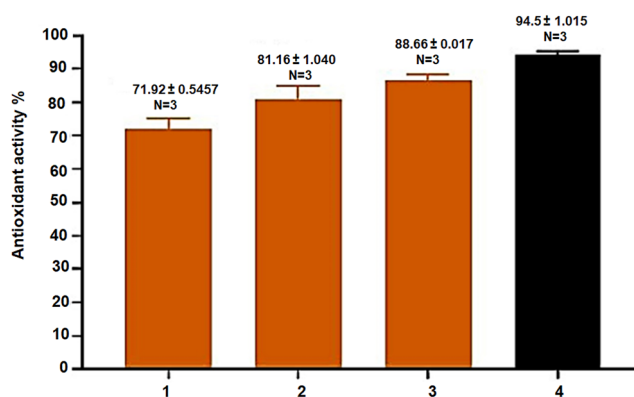


Figure 8. Antioxidant activity of anthocyanin extracted from *P. domestica* peels by using DPPH assay, 1 = 1.25 µg/mL, 2 = 2.5 µg/mL, 3 = 5 µg/mL, 4 = positive control ascorbic acid.

3.2.5 Antioxidant activity of *P. domestica* peels extracts

The antioxidant activities of anthocyanins have been evaluated in comparison to the DPPH free radical. The assays conducted for anthocyanin has revealed the amount of DPPH scavenged corresponded to the anthocyanin concentrations (1.25, 2.5, and 5 µg/mL) and the scavenging abilities of the DPPH free radicals were $71.92 \pm 0.545\%$, $81.16 \pm 1.040\%$, and $86.66 \pm 0.017\%$, respectively. At room temperature,

DPPH is a potent free radical that produces a darkening violet color when it is dissolved in organic solvents. Figure 8 illustrates the UV wavelength absorption results obtained for the synthesized samples. The decrease in absorbance at 517nm was utilized to compute the free radical scavenging percentage. A positive control in the same concentration range, ascorbic acid, was utilized. It is supposed that this effect will increase with an increase the concentration of anthocyanin extracted from

P. domestica peel, but it remains lower as compared to ascorbic acid (Basuny et al., 2012). The radical scavenging properties of the *P. domestica* peels extract can be related to the presence of bioactive components with strong antioxidant activity. Anthocyanins have a high level of cytocompatibility and antioxidant activity (Jawad et al., 2022a). Anthocyanins extracted from *P. domestica* peel were observed to reduce the stability of DPPH, and the violet color changed to yellow due to the presence of phenolic OH groups (Mohammed et al., 2020; Jawad, 2023) and presences of bioactive compounds extract by GCMS and FTIR. A DPPH methodology constitutes one of the expedited and straightforward techniques employed in the evaluation of antioxidants by quantifying the effectiveness of various substances utilized simultaneously (Al hamza Hasoon, 2024; Mohammed et al., 2024). The underlying principle of this methodology is predicated on the electron transfer involved in the generation of the violet coloration, wherein stable free radicals engage in a reaction with an antioxidant compound, thereby facilitating the transfer of electrons from the latter substance, resulting in a discernible loss of color (Al-Saadi et al., 2023; Hasan et al., 2022; Hussein et al., 2023; Jawad, 2023; Messaoudi et al., 2023; Mohanaparameswari et al., 2023; Yosif et al., 2024).

3.2.6. Anticancer activity of *P. domestica* peels extracts

The cytotoxic effect of *P. domestica* peels extract against MCF-7 cells was studied. The anti-proliferative activity of the *P. domestica* peels extract was tested by studying its ability to inhibit the proliferation of the MCF-7 cell line. Cells were grown in the presence of *P. domestica* peel extract at concentrations ranging from 6.25 to 200 µg/mL to measure the inhibitory effect on proliferation. Treatment with *P. domestica*

peel extract dramatically inhibited cell growth, according to the results of an *in vitro* MTT experiment (Figure 9). The results demonstrated the ability of prepared *P. domestica* peels extract to destroy and kill breast cancer cells. With concentration-dependent cytotoxicity, the anthocyanin's extracted from *P. domestica* peels also showed greater anti-cancer efficacy against MCF-7 breast cancer cell lines when applied at higher concentrations (Figure 10). The highest cytotoxicity was recorded at a concentration of 89% at 200 µg/mL, while the lowest cytotoxicity was observed at a concentration of 5.33% at 6.25 µg/mL. The same amounts of extract were examined to confirm that the inhibition shown in MCF-7 cells was attributable to the anthocyanin pigment from *P. domestica* peels. The inhibitory concentration value (IC_{50}) of anthocyanins extracted from *P. domestica* peels was 34.92 µg/mL. the results of this study are superior to those reported by other researchers (Al-Majedy et al., 2024; Hussein et al., 2023; Jawad et al., 2021; Jawad et al., 2022b; Mohammed et al., 2024) because it produced superior outcomes for the treatment of breast cancer on the same cell line (MCF-7) at the same dosages of copper oxide nanoparticles (CuO NPs) (Jawad et al., 2022b). The fact that chemical-based treatments are very hazardous, in contrast to plant-based treatments, which are less toxic, safe when utilized, easy to handle, affordable, and widely available in nature, leads us to propose the application of plant-based treatments more widely than chemical-based treatments. Human epidermal growth factor receptor 2 (HER2)+ tumors make up 30% of breast cancer cases in people. Compared to normal cells, the morphological properties of affected cells showed differences in cell size, shape, and quantity. When compared to untreated cells, treated cells had significantly lower densities, which indicate that use *P. domestica* extract has a negative impact on the MCF-7 cells. The results were

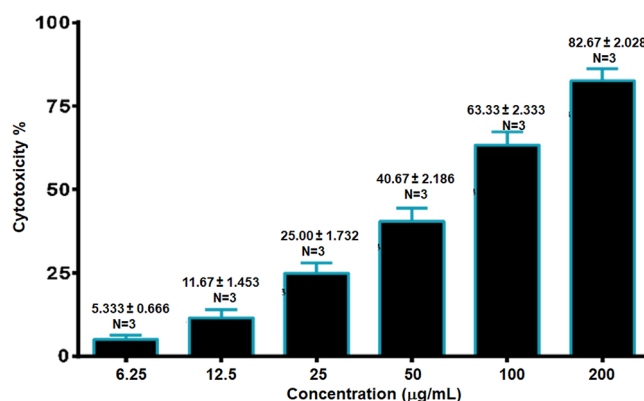


Figure 9. Cytotoxicity of anthocyanin extracted from *P. domestica* peels in MCF-7 cells. IC_{50} = 34.92 µg/mL.

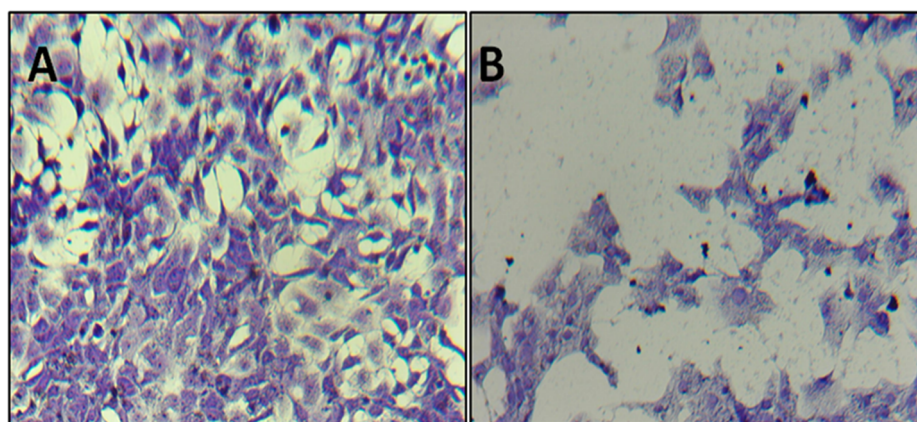


Figure 10. Photomicrographs of histological study of normal breast cells (A), control untreated MCF-7 cells (B). Morphological changes in MCF-7 cells after 72h of treatment with different concentration of anthocyanin extract from *P. domestica* peels.

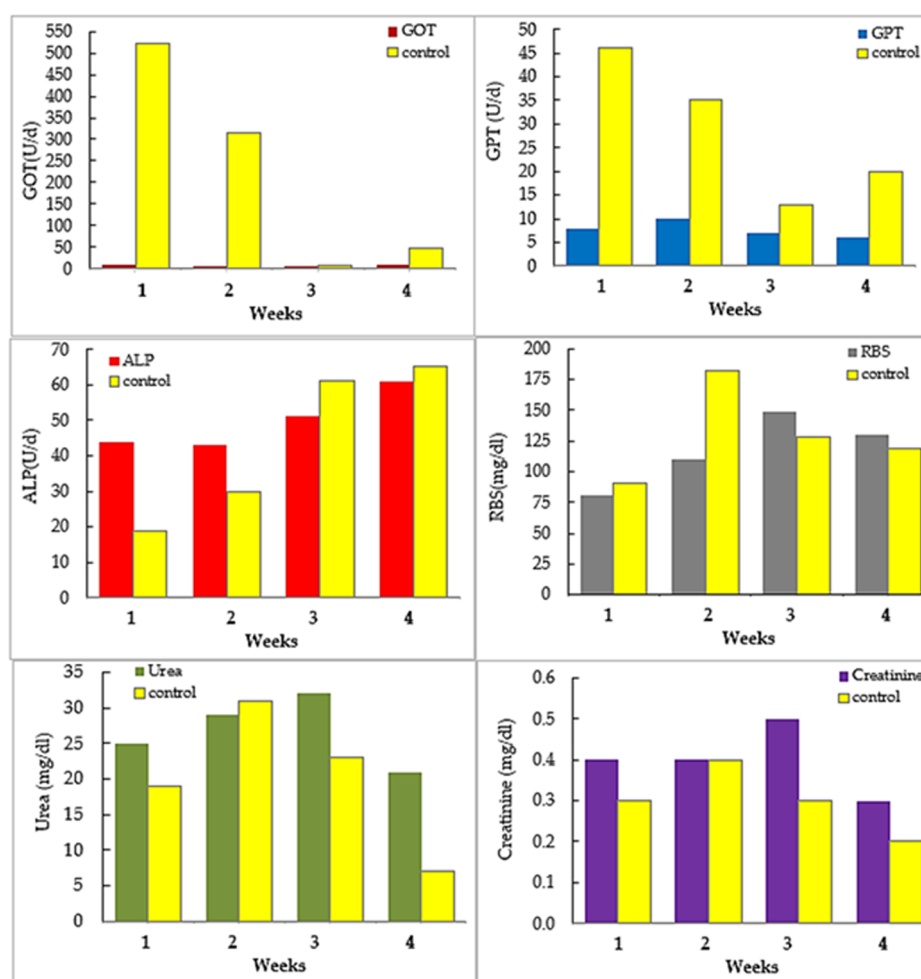


Figure 11. The effect of anthocyanins extracted from *P. domestica* peels on liver function (GOT, GPT, ALP) kidney function (Urea and Creatinine) and sugar RBS in the blood serum of rats in different periods (first week, second week, third week, and fourth week).

compatible with those reported earlier (Al-Majedy et al., 2024; Jawad et al., 2022b).

3.3. Effect of *P. domestica* peel extract on rat body function tests

Experiments were conducted to reveal the role of anthocyanins extracted from *P. domestica* peels and the extent of their effect on the functions of the human body when anthocyanins are taken in a certain quantity daily. Previous studies have proven that anthocyanins play a role in the regulation of fat metabolism and blood sugar. Thus, the *P. domestica* peels extract was administered daily and orally to rats in a dose of 100 µg/Kg/day for different periods (one week, two weeks, three weeks, and four weeks). Each of the liver functions (GOT, GPT, and ALP), kidney function (urea and creatinine), and random blood sugar (RBS) were tested. The results are shown in Figure 11 shown how anthocyanin extract affects the body. Liver function (GOT) was within the normal range during four weeks compared with control, which was very high in the first and the second weeks, since anthocyanin extract decreased the GOT within the normal range. Moreover, the GPT was also reduced by anthocyanin extract to keep it within the usual range over the four weeks, while the control group had very high GPT levels in the first and second weeks. ALP has been shown within the normal range during the four weeks, and kidney function (Urea and creatinine) have been shown within the normal range during the four weeks. Anthocyanin extract regulated the RBS in the second week because the control was higher than normal results, and in the third week anthocyanin extract resulted in a slight rise in RBS. These results are similar to other published results (Basuny et al., 2012).

However, in order to lessen oxidative stress, anthocyanins dye has indirectly activates particular detoxifying enzymes as glutathione reductase, glutathione peroxidase, and glutathione S-transferase. Numerous research have shown that anthocyanins dye is effective in reducing insulin sensitivity and hyperglycemia, protecting β cells, and increasing insulin production in diabetic mice. Furthermore, it has been discovered that anthocyanins dye has renal protective effects in diabetic mice, lowering urine microalbumin levels and lessening renal lipotoxicity (Liu et al., 2018). The elevation of serum liver enzymes may be associated with hepatocellular damage resulting from various factors, including oxidative stress, inflammatory processes, or augmented hepatic lipid accumulation following insulin resistance (Gonçalves et al., 2021).

4. Conclusions

Anthocyanins were extracted from the peels of *P. domestica* utilizing a conventional approach, enabling the provision of substantial quantities commercially owing to the simplicity of the extraction procedure. This method does not necessitate costly or inaccessible materials, machinery, or instruments; it is also deemed a secure technique. Additionally, it is the only practice available in Iraq that is used in the pharmaceutical industry. One of the disadvantages of this method was eliminated by condensing the largest amount of solvent through the condenser because it requires large quantities of solvent. Our study demonstrated the favorable efficacy of anthocyanins extracted from *P. domestica* peels in terms of their antibacterial, antifungal, antioxidant, and anti-cancer properties; further, they exhibit the potential to modulate liver and kidney enzymes while regulating blood glucose levels throughout the day. Consequently, the findings of this study recommend the consumption of meals rich in anthocyanins as a preventive measure against various ailments.

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Authors' Contributions

Conceptualization, H.J.O. and B.A.H; methodology, A.A.H; software, Y.H.D.; validation, H.J.O, B.A.H and A.A.H; formal analysis, B.A.H; investigation, H.J.O.; resources, H.J.O.; data curation, A.A.H; writing—original draft preparation, H.J.O, B.A.H; writing—review and editing, Y.H.D., K.M.T. and N.M.D; visualization, Y.H.D., K.M.T. and N.M.D; supervision, B.A.H, and A.A.H; project administration, B.A.H. All authors have read and agreed to the published version of the manuscript.

Disclosure Statement

The authors declare no conflicts of interest.

Data availability

Derived data supporting the findings of this study are available from the corresponding author [B.A.H. and Y.H.D.] on request.

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