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Comparative Evaluation of Antioxidant, Antimicrobial, Thrombolytic, and Hemolytic Activities of *Aerva persica* Leaf Extracts Obtained Using Different Solvents

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Abstract

This study investigates the phytochemical profile and in vitro biological activities of *Aerva persica* leaf extracts obtained using solvents of varying polarity, including *n*-hexane, chloroform, 80% ethanol, *n*-butanol, and 80% methanol. Quantitative analysis revealed that the 80% ethanol extract contained the highest total phenolic and flavonoid contents, indicating superior extraction efficiency for antioxidant compounds. Among all tested samples, the 80% ethanol extract exhibited the strongest antioxidant potential, with the highest DPPH radical-scavenging activity (82.77%), notable reducing power, and inhibition of lipid peroxidation. Antimicrobial screening demonstrated significant antibacterial and antifungal effects, particularly in 80% ethanol extracts, with measurable zones of inhibition against both Gram-positive and Gram-negative bacteria and fungal strains. The 80% ethanolic extract also showed the highest thrombolytic activity (45.72%), suggesting moderate clot-dissolving potential. Hemolytic assays indicated generally low cytotoxicity across extracts, with chloroform showing comparatively higher hemolysis. Overall, *A. persica* leaves represent a promising source of bioactive phytochemicals with antioxidant, antimicrobial, and thrombolytic potential for further pharmacological exploration.

Keywords: Biological activity, Thrombolytic activity, Hemolytic activity, Phenolics, Flavonoids, Antioxidant

Highlights

- The leaves of *Aerva persica* contain bioactive compounds such as polyphenols, flavonoids, and alkaloids, which exhibit therapeutic benefits.
- The leaves of *Aerva persica* demonstrated efficient free radical scavenging activity.
- The leaf extracts demonstrated significant antibacterial activity against a variety of gram-positive and gram-negative bacteria.
- In vitro thrombolysis revealed significant clot-dissolving activity, suggesting it could serve as a natural thrombolytic agent.

1. Introduction

Since ancient times, humans have relied on natural remedies for the prevention and treatment of various diseases, with medicinal plants playing a pivotal role in traditional healthcare systems (Kashif et al., 2026). Medicinal plants are broadly defined as plants with therapeutic properties and are widely used in the management of a wide range of health conditions. Plant phytochemistry is fundamental to the medicinal value of herbs, as their therapeutic effects are primarily attributed to bioactive compounds (Tariq et al., 2025). According to the World Health Organization, approximately 75–80% of the global population relies on plant-based medicines, either entirely or partially, for primary healthcare (Usman et al., 2025). These natural products are valued for their relatively low incidence of adverse effects and their rich content of essential minerals, vitamins, and other bioactive constituents. Phytomedicines are complex mixtures of plant-derived metabolites that contain biologically active compounds with significant therapeutic potential (Kashif, Batool, et al., 2024).

Oxidative stress and environmental factors are major sources of reactive oxygen species (ROS), which are byproducts of normal oxygen metabolism (Kashif, Naz, Younas, et al., 2024; Kashif, Naz, et al., 2025). These ROS can inactivate enzymes, damage cellular structures and biological membranes, and contribute to the development of various pathological conditions, including aging, cancer, chronic inflammation, and diabetes (Naz, Kashif, et al., 2023). Furthermore, ROS have been implicated in neurological disorders because they can disrupt neurotransmitter systems involved in the pathophysiology of depression and anxiety (Meireles et al., 2020). Notably, depression is associated with decreased levels of endogenous antioxidants, suggesting that oxidative stress plays a significant role in these conditions. Additionally,

ROS-induced inflammation can result in pain, tissue damage, and the progression of disease-related complications (Bahari et al., 2025).

Antioxidants play a critical role in counteracting the harmful effects of ROS by scavenging free radicals, inhibiting radical-producing enzymes, and enhancing the activity of endogenous antioxidant enzymes (Anwar et al., 2026; Naz, Kashif, et al., 2023). Synthetic and natural are two types of antioxidants. However, synthetic antioxidants often have limitations due to their potential toxicity in living systems compared with natural antioxidants. This has prompted growing interest in identifying and developing natural, non-toxic sources of antioxidants (Ahmad et al., 2022). Beyond oxidative stress, another major health concern is thrombotic disease, including myocardial infarction, caused by the formation of intravascular blood clots. While heparin remains a cornerstone therapy for the management of acute thrombotic conditions, its clinical use is associated with several limitations (Usman et al., 2025). Consequently, there is an increasing need for novel, safe, and effective therapeutic agents exhibiting both antioxidant and antithrombotic activities, including natural anticoagulants.

Aerva persica (*Aerva javanica*) is a dioecious shrub belonging to the family Amaranthaceae. The species is commonly distributed in the sandy soils of arid and semi-arid rangelands and is commonly known as Kapok bush, Snow bush, and Pillow bush (Fatima et al., 2024). *A. persica* has been reported to contain a diverse range of phytochemicals, including flavonoids, terpenoids, steroids, and lipids, and has been widely used in traditional medicine for the treatment of various ailments across different regions of the world (Thotathil et al., 2023). Furthermore, the ethnobotanical uses of *A. persica* have been documented, highlighting its antioxidant and antimicrobial properties (Fatima et al., 2024).

Aerva persica contains various bioactive compounds with potential medicinal value; however, limited information is available on how extraction solvents influence their phytochemical composition and biological activities. Different solvents vary in their ability to extract bioactive constituents, depending on polarity, which may affect the antioxidant and biological properties of plant extracts. Therefore, the present study was designed to evaluate the total phenolic and flavonoid contents, antioxidant potential, and the antimicrobial, thrombolytic, and hemolytic activities of *A. persica* leaf extracts prepared using solvents of varying polarities. The findings of this study may help identify suitable extraction solvents for recovering bioactive compounds from *A. persica* and provide a scientific basis for further pharmacological investigations.

2. Materials and Methods

2.1. Collection of plant material and extraction of CCEs

A. persica leaf samples were collected from the Cholistan Desert. The leaves were washed, shade-dried, and ground into a fine powder. The 30 g of powdered plant material was macerated in 30 mL of the following solvents: *n*-hexane, chloroform, 80% ethanol, *n*-butanol, and 80% methanol (v/v) and extracted for 28 h at room temperature. The extracts were then filtered through Whatman filter paper. The filtrates were concentrated using a rotary evaporator (Ecohim Ltd., St. Petersburg, Russia) at 45 ± 1°C, and the resulting concentrated crude extracts (CCEs) were stored at −4 °C for further analysis (Kashif et al., 2024).

2.2. Estimation of total flavonoid content (TFC) in CCEs

Total flavonoid content of the leaf extracts was determined according to the method described by Kashif et al. (2024). Briefly, 100 mg of CCEs was mixed with 5 mL of distilled water and 0.3 mL of 5% sodium nitrate solution and incubated at 25 °C for 10 min. Subsequently, 0.6 mL of 10% aluminum chloride solution and 2 mL of 1 M sodium hydroxide solution were added to the mixture. The absorbance of the resulting reaction mixture was measured at 510 nm using a UV–Vis spectrophotometer. Total flavonoid content was expressed as catechin equivalents (CE) and calculated on a dry-weight basis (mg/100 g dry mass).

2.3. Estimation of total phenolic content (TPC) in CCEs

Total phenolic content (TPC) of *A. persica* leaf extracts was determined according to the method described by Usman et al. (2025). Briefly, 1 mg of each extract was mixed with 7.5 mL of distilled water, 1.5 mL of sodium carbonate solution, and 0.5 mL of Folin–Ciocalteu reagent. The reaction mixture was then incubated in a water bath at 40 °C for 20 min. Subsequently, the absorbance was measured at 755 nm using a UV–Vis spectrophotometer. The TPC was expressed as gallic acid equivalents (GAE) and calculated on a dry-weight basis (mg GAE/100 g dry weight).

2.4. Estimation of antioxidant capacity using the linoleic acid system

The antioxidant capacity of recovered CCEs from the samples was evaluated by measuring their ability to inhibit linoleic acid oxidation, as described by Kashif et al. (2024). Each green extract (5 mg) was dissolved in a mixture of 10 mL of ethanol (99.8%), 0.13 mL of linoleic acid, and 10 mL of 0.2 M sodium phosphate buffer (pH 7). Deionized water was added to reach a final volume of 25 mL. The degree of oxidation was calculated from the final volume of the solution measured at 40 ℃ (Chu et al., 2000). 10 mL of ethyl alcohol (75%) and 0.2 mL of sodium thiocyanate (30%) in 0.2 mL of hydrochloric acid (3.5%), and 0.2 mL solution of ferrous chloride were sequentially added to the sample solution. The

resulting solution was incubated at room temperature for 3 minutes while stirring, then analyzed on a Spectrophotometer (Hitachi U-2001, model 121-0032) at 500 nm. A control treatment (without plant extract) was also performed, and percentage inhibition was calculated using Equation 1.

$$I(\%) = \left[\frac{Ac - A_s}{A_c}\right] \times 100 \quad (1)$$

A_c and A_s are the absorbance of the control and the sample at 350 h. and I (%) is the percentage inhibition. Positive controls will be ascorbic acid and BHT.

2.5. Assessment of Reducing Potential

The reducing power of the CCEs was determined as described by Kashif et al. (2024). Briefly, different concentrations of CCEs (10–40 mg) were mixed with 5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 5 mL of 1% (w/v) potassium ferricyanide solution. The reaction mixture was incubated in a water bath at 50 °C for 20 min. Following incubation, 5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 980 × g for 10 min at 5 °C. An aliquot (5 mL) of the supernatant was transferred to a clean test tube, followed by the addition of 5 mL of deionized water and 0.1% (w/v) ferric chloride solution. The absorbance of the resulting mixture was measured at 700 nm using a Hitachi U-2001 UV–Vis spectrophotometer (Safdar et al., 2017). All analyses were performed in triplicate, and the results were expressed as mean values of three independent determinations.

2.6. DPPH radical scavenging assay

The free radical scavenging activity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, as described by Aleem et al. (2024). Briefly, 50 µL of each extract was mixed with 5 mL of DPPH solution and incubated at room temperature for 30 min. Subsequently, the absorbance was measured at 517 nm against a blank using a UV–Vis spectrophotometer. The percentage of free radical scavenging was calculated using Equation 2.

$$I(\%) = \left[\frac{A_b}{A_s}\right] \times 100 \quad (2)$$

Where I is %age inhibition, and As and Ab are the absorbance of the sample and blank, respectively.

2.7. Antimicrobial activity

The antimicrobial activity of *A. persica* extracts was evaluated against selected pathogenic bacteria and fungi. The bacterial strains included *Bacillus subtilis* and *Escherichia coli*, while the fungal strains comprised *Fusarium solani* and *Aspergillus niger*. All microbial cultures were obtained from the Department of Biochemistry and Biotechnology, Government College University, Faisalabad, Pakistan. Nutrient agar Potato dextrose agar (Oxoid, UK) and medium were used to grow bacterial and fungal strains at 37 °C and 30 °C, respectively.

2.7.1. Disc diffusion method

The antimicrobial potential of the solvent extracts was evaluated using the disc diffusion method (Younas et al., 2024). Sterile discs were impregnated with each solvent extract (100 mg/mL) and placed on agar plates previously inoculated with the test pathogenic microorganisms. Negative controls (without extract) and *Amoxicillin* and *Terbinafine* are positive controls for bacterial and fungal strains, respectively.

2.7.2. Micro-broth dilution assay

The measurement of minimum inhibition concentration (MIC) was performed using the microdilution method reported by Anbumani et al. (2022). MIC represents the minimum concentration of the sample extract required to inhibit microbial growth under in vitro conditions completely. Each concentrated solvent extract from the tested wild sample was diluted to a final concentration of 5-100 mg/mL in a 96-well plate. Growth controls (without plant extract) and sterility controls (with plant extract) were also processed under similar conditions. To each well, 20 µL of the diluted wild plant extract solution was added to 160 µL of culture medium (nutrient broth for bacterial strains and Sabouraud dextrose broth for fungal strains). The inoculation of broth culture (20 µL; 5 × 10^5 CFU) for each tested microorganism was performed in 96-well plates. The 96-well plates were incubated at 37 °C for 24 h and at 30 °C for 48 h for bacterial and fungal strains, respectively. The formation of a white pellet at the bottom of the well indicated the growth of microorganisms (if any). MIC estimation was performed using a dilution at which microbial growth was inhibited.

2.8. Thrombolytic activity

The clot lysis assay was performed as described by Mohammed et al. (2014) with slight modifications. Streptokinase was used as the positive control. Human venous blood was collected from a healthy volunteer and used to evaluate thrombolytic activity. Briefly, 500 µL of blood was transferred into pre-weighed Eppendorf tubes and incubated at 37 °C for 40 min to allow clot formation. Following incubation, the serum was carefully removed without disturbing the formed clots, and the tubes containing the clots were weighed. The weight of the clot (W_3) was calculated as the difference between the weight of the Eppendorf tube containing the clot (W_2) and the weight of the empty Eppendorf tube (W_1) using Equation 3.

$$W_3 = W_2 - W_1 \quad (3)$$

Using a micropipette, 100 µL of each plant extract was added to the clot-containing Eppendorf tubes, while streptokinase and distilled water served as the positive and negative controls, respectively. All tubes were incubated at 37 °C for 90 min to facilitate clot lysis. Following incubation, the released supernatant was carefully removed from each tube without disturbing the residual clot (Tariq et al., 2025). Subsequently, each tube was weighed to determine the clot weight after lysis (W_4). The remaining clot weight after lysis was calculated as the difference between the weight of the tube containing the residual clot (W_4) and the weight of the empty tube (W_1).

The percentage of thrombolytic activity was calculated using the following Equation 4.

$$\text{Clot lysis \%} = (W_3\text{-}W_5/W_3) \times 100 \quad (4)$$

Where,

W_1= Weight of empty tube

W_2= Weight of empty tube + clot (before lysis)

W_3= Weight of a clot (before lysis) (W_2-W_1)

W_4= Weight of empty tube + clot (after lysis)

The percentage of clot lysis was calculated as the difference between the pre- and post-clotlysis weights.

2.9. Hemolytic activity

Hemolytic activity was determined as described by Naz et al. (2023). Briefly, phosphate-buffered saline (PBS) was prepared and chilled before use. Fresh human blood was collected in a Falcon tube containing heparin as an anticoagulant. The blood samples were washed twice with chilled PBS. For each washing step, 10 mL of PBS was added to the blood sample, followed by centrifugation at 850 × g for 10 min. The supernatant was discarded, and the washing procedure was repeated twice (Usman et al., 2025). The plant extracts were prepared by dissolving 0.01 g of each extract in 1 mL of its respective solvents. Subsequently, 20 µL of plant extract and 180 µL of blood suspension were added to each Eppendorf tube. The mixtures were incubated at 37 °C for 35 min. After incubation, the tubes were placed in an ice bath for 5 min and then centrifuged at 1310 × g for 4–8 min. Following centrifugation, 20 µL of the supernatant was collected and diluted with chilled PBS. The diluted samples were maintained on ice until analysis. Absorbance was measured at 576 nm using a UV–Vis spectrophotometer, with PBS serving as the blank.

2.10. Statistical Analysis

All in vitro experiments were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical analysis of the data was conducted using Student's t-test, and differences were considered statistically significant at $P < 0.001$.

3. Results and Discussion

3.1. Yield of extracts

The yield of different green extracts from *A. persica* leaves, expressed in g/100g with various solvents, including *n*-hexane, chloroform, 80% ethanol, *n*-butanol, and 80% methanol, is shown in Table 1. The extract yields ranged from 2.45 to 3.89 g/100 g dry matter and were influenced by both the type of solvent and the extraction conditions. Among the solvents tested, 80% ethanol produced the highest extract yield, whereas chloroform yielded the lowest extract recovery. The results of the present study demonstrated that extract yields differed significantly ($p < 0.05$) among the solvents used. The highest yield obtained with 80% ethanol suggests that this solvent was more effective in extracting bioactive constituents from *A. persica* leaves. Furthermore, the extract yields obtained in the current study were higher than those previously reported for *A. persica* (Fatima et al., 2024). These variations in extract yield may be attributed to differences in the availability of extractable phytochemicals, extraction efficiency, and prevailing agroclimatic conditions (Waqas, 2021).

3.2. Total phenolic (TPC) and flavonoid (TFC) content

There has been growing interest in the food industry in plant-derived compounds exhibiting antioxidant, anti-lipid peroxidation, and anticarcinogenic properties (Gomes et al., 2023). Natural antioxidants, particularly phenolic compounds, are widely distributed in plants. Numerous studies have demonstrated that total phenolic and flavonoid contents are major contributors to the antioxidant activity of fruits and vegetables (Kashif et al., 2025). These findings highlight the importance of screening novel plant materials for their phenolic and flavonoid constituents.

Table 1 presents the total phenolic (TP) and total flavonoid (TF) contents of *A. persica* leaves extracted using different solvents. The TP and TF values ranged from 221 to 458 mg/100 g GAE and 63 to 203 mg/100 g CE, respectively. Among the solvents evaluated, 80% ethanol extraction yielded the highest total phenolic and total flavonoid contents. The observed variations in TP and TF contents among the extracts may be attributed to differences in solvent polarity and extraction efficiency, which influence the recovery of antioxidant phytochemicals from plant tissues. The concentration of phenolic compounds in *A. persica* leaves differed significantly ($p < 0.05$) among the extraction solvents used. 80%

ethanol is widely recognized as an effective solvent for extracting phenolic antioxidants due to its relatively low toxicity and high extraction efficiency (Aleem et al., 2024).

The total phenolic content values found in this study are comparable to those reported for *A. persica*. However, compared with other studies, the *A. persica* sample segments showed higher levels of both total phenolics and total flavonoids (Fatima et al., 2024).

Table 1: Extraction yield, total phenolic (TPC), and total flavonoid content (TFC) in CCEs of *A. persica* leaves

Solvent	% Yield (g/100g)	TPC (GAE mg/100g)	TFC (CE mg/100g)
***n*-hexane**	3.14 ± 0.03	341 ± 1.67	117 ± 0.67
80% ethanol	3.89 ± 0.07	458 ± 2.37	203 ± 1.04
80% methanol	3.37 ± 0.02	379 ± 1.71	167 ± 0.91
Chloroform	2.45 ± 0.03	221 ± 1.42	63 ± 0.17
***n*-butanol**	2.79 ± 0.05	291 ± 2.09	84 ± 0.48

Values (mean ± SD) are the average of three replicates, analyzed individually.

3.3. Antioxidant activity in the linoleic acid system

Linoleic acid is an unsaturated fatty acid that undergoes oxidation, resulting in the formation of peroxides. These peroxides subsequently oxidize Fe(II) to Fe(III), which then reacts with thiocyanate ions (SCN^-) to form a colored complex (Chu et al., 2000). The absorbance of the reaction mixture is measured spectrophotometrically at 500 nm and serves as an indicator of peroxide formation (Kashif, et al., 2024). Higher absorbance values indicate greater peroxide formation, whereas lower absorbance values reflect reduced peroxide accumulation. Consequently, increased peroxide levels correspond to lower antioxidant activity, while reduced peroxide formation indicates stronger antioxidant potential (Sultana et al., 2012).

The results of the antioxidant potential of plant extracts in inhibiting lipid peroxidation are presented in Table 2. The 80% ethanol extracts had a higher concentration of phenolic compounds, resulting in significantly better protection against peroxidation (75.64%), whereas chloroform showed less inhibition (43.53%). Compared with artificial antioxidants like BHT, all tested extract samples exhibited lower inhibition of linoleic acid oxidation, with significantly lower effectiveness at 90.6%

3.4. DPPH radical scavenging assay

DPPH is an organic free radical that is purple in color and has absorption levels between 515 nm and 528 nm. Upon accepting protons from hydrogen donors, DPPH undergoes a color change from purple to yellow (Hazrati et al., 2020). The radical-scavenging activity of DPPH is directly associated with the antioxidant potential of phenolic-rich plant extracts or compounds, particularly those with higher concentrations of phenolic constituents or greater degrees of hydroxylation (Ahmed et al., 2017).

DPPH radicals have been widely used to evaluate the radical-scavenging activity of various plant extracts. In the present study, leaf extracts of *Aerva persica* demonstrated notable radical-scavenging activity, with IC_{50} values varying across solvents, as shown in Table 2. 80% ethanol extract from leaves exhibited the lowest IC_{50} value, consistent with its maximum free radical-scavenging activity. The outcomes ($p<0.05$) indicate that the extract has greater radical-capturing potential in 80% ethanol than in other solvents. When comparing the tested extracts with the artificial antioxidant butylated hydroxytoluene (BHT), the tested extracts showed lower scavenging activity. The DPPH radical-scavenging capacity of the extract correlates with the presence of phenolic content (Htay et al., 2023).

Table 2: Inhibition (%) of linoleic acid peroxidation and IC_{50} Value of CCE of *A. persica* leaves.

Solvent	Inhibition (%)	IC_{50} Value (µg/mL)
***n*-hexane**	58.06 ± 0.34	37.11 ± 0.79
80% ethanol	75.64 ± 0.37	26.12 ± 0.31
80% methanol	66.17 ± 0.23	29.76 ± 0.46
Chloroform	43.53 ± 0.29	58.83 ± 0.56
***n*-butanol**	47.84 ± 0.11	43.77 ± 0.92

Values (mean ± SD) are the average of three replicates, analyzed individually.

3.5. Reducing power of CCEs

The antioxidant potential of plant extracts was evaluated by measuring their reducing power. In this assay, yellow ferric ions (Fe^{3+}) are reduced to blue-green ferrous ions (Fe^{2+}) in the presence of antioxidant compounds. The extent of color change reflects the reducing ability of the extracts, which serves as an indicator of their antioxidant capacity (Kayani et al., 2016). Accordingly, a higher color intensity corresponds to greater absorbance and, consequently, higher antioxidant activity. Furthermore, Tariq et al. (2025) reported a direct relationship between the reducing capacity of bioactive compounds and their overall antioxidant potential, thereby establishing a strong correlation between reducing power and antioxidant activity.

The present study demonstrated that all plant extracts exhibited an increase in reducing power with increasing extract concentration, as shown in Figure 1. To compare antioxidant activity among extracts, absorbance values were recorded using a spectrophotometer. Among all tested samples, the 80% ethanol extract showed the highest antioxidant activity, which may be attributed to its higher total phenolic content (TPC) and total flavonoid content (TFC). Therefore, reducing power assay serves as a reliable method for evaluating the antioxidant potential of plant extracts.

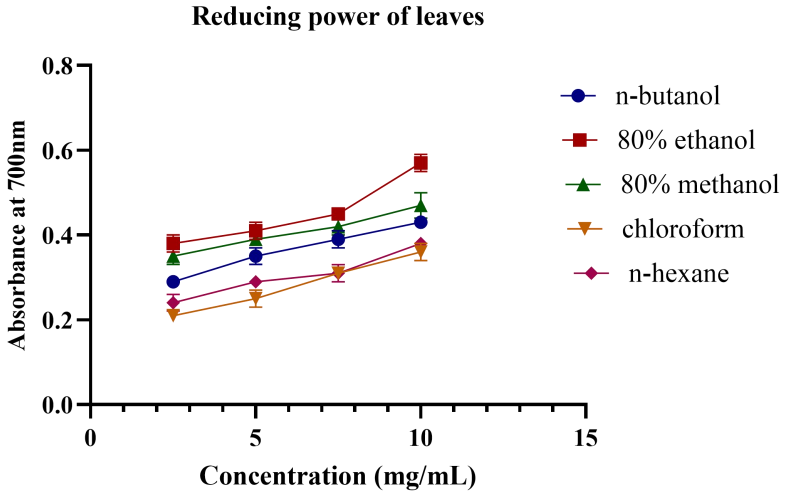

Figure 1: Reducing the potential of *A. persica*

3.6. Antimicrobial activity

Tables 3 and 4 present the antibacterial and antifungal activities of *A. persica* leaf extracts, as determined by the disc diffusion assay and minimum inhibitory concentration (MIC) analysis. The antimicrobial activity varied significantly among the different solvent extracts and microbial strains tested. 80% ethanol extracts exhibited significant zones of inhibition against *E. coli* (15 ± 0.23 mm), *B. subtilis* (12 ± 0.13 mm), *A. Niger* (11 ± 0.13 mm), and *F. solani* (8 ± 0.23 mm). Amoxicillin and Terbinafine are positive controls for bacterial and fungal strains, respectively.

The MIC results further confirmed the extracts' antimicrobial potential. 80% ethanol extracts exhibited significant MIC against *E. coli* (175 µg/mL), *B. subtilis* (215 µg/mL), *A. Niger* (121 µg/mL), and *F. solani* (163 µg/mL). Chloroform has less antimicrobial potential than other extracting solvents. Overall, *A. persica* plant extracts demonstrated promising antifungal potential.

Previous research, as noted by Fatima et al. (2024), indicates that variations in the chemical composition of extracts can lead to differences in their biological effects. In the current study, extraction of *A. persica* with various solvents yielded extracts with distinct chemical profiles and varying antimicrobial and antioxidant activities. This variation can be attributed to the diverse array of antibacterial substances found in different plant sections.

Table 3. Antibacterial activity of *A. persica* leaves extracts.

Solvent	Bacterial strains	zone of inhibition (mm)	MIC (µg/mL)
***n*-hexane**	*Bacillus subtilus*	6 ± 0.09	331
	Escherichia coli	7 ± 0.05	247
80% ethanol	*Bacillus subtilus*	12 ± 0.13	215
	Escherichia coli	15 ± 0.23	175
80% methanol	*Bacillus subtilus*	9 ± 0.01	250
	Escherichia coli	10 ± 0.13	195
Chloroform	*Bacillus subtilus*	4 ± 0.11	394
	Escherichia coli	5 ± 0.07	351
***n*-butanol**	*Bacillus subtilus*	5 ± 0.13	363
	Escherichia coli	6 ± 0.09	305
Positive control (*Amoxicillin*)	*Bacillus subtilus*	---	---
	Escherichia coli	---	---

Values are average (mean ± SD) of three replicates, analyzed individually.

Table 4. Antifungal activity of *A. persica* leaf extracts.

Solvent	Bacterial strains	zone of inhibition (mm)	MIC (µg/mL)
***n*-hexane**	*Aspergillus Niger*	6 ± 0.09	168
	Fusarium solani	5 ± 0.05	207
80% ethanol	*Aspergillus Niger*	11 ± 0.13	121
	Fusarium solani	8 ± 0.23	163
80% methanol	*Aspergillus Niger*	8 ± 0.05	146
	Fusarium solani	6 ± 0.13	184
Chloroform	*Aspergillus Niger*	4 ± 0.11	236
	Fusarium solani	2 ± 0.07	287
***n*-butanol**	*Aspergillus Niger*	5 ± 0.13	197
	Fusarium solani	5 ± 0.09	274
Positive control	*Aspergillus Niger*	28 ± 0.29	105
(***Terbinafine***)	*Fusarium solani*	32 ± 0.33	35

Values are average (mean ± SD) of three replicates, analyzed individually.

3.7. Thrombolytic activity

The thrombolytic activity of *A. persica* leaf extracts was evaluated using human blood clots, following the method described by Mohammed et al. (2014). The percentage of clot lysis exhibited by the different extracts is presented in Table 5. All extracts demonstrated varying degrees of thrombolytic activity. The percentage of clot lysis ranged from 19.73% to 38.69%, depending on the extraction solvent. Among the tested extracts, the 80% ethanol extract exhibited the highest thrombolytic activity (38.69%), whereas the chloroform extract showed the lowest clot lysis activity (19.73%). The positive control, streptokinase, produced 77% clot lysis, indicating substantially greater thrombolytic efficacy than that of plant extracts.

3.8. Hemolytic activity

The hemolytic activity of *A. persica* leaf extracts was evaluated using human erythrocytes (red blood cells, RBCs), with Triton X-100 serving as the positive control and phosphate-buffered saline (PBS) as the negative control (Yasir et al., 2022). The positive control exhibited 97.58% hemolysis, whereas PBS showed no detectable hemolysis. In comparison, the plant extracts induced varying degrees of hemolysis. The percentage hemolysis values were 6.57 ± 0.04% for the 80% ethanol extract, 8.94 ± 0.09% for 80% methanol extract, 11.28 ± 0.14% for *n*-hexane extract, 12.45 ± 0.1% for *n*-butanol extract, and 17.35 ± 0.12% for chloroform extract. Assessment of erythrocyte membrane stability provides a useful indicator of the cytotoxic effects of chemical compounds on RBCs. Increased hemolysis reflects greater membrane damage and, consequently, higher cytotoxicity. In contrast, lower hemolytic activity suggests improved biocompatibility of the tested extracts. Similar erythrocyte membrane disruption has been reported in various pathological conditions and in response to cytotoxic agents (Usman et al., 2025).

Table 5: Thrombolytic and hemolytic activity of *A. persica* leaves extracts

Solvent	Clot lysis (%)	RBC lysis (%)
***n*-hexane**	28.82 ± 1.17	11.28 ± 0.14
80% ethanol	38.69 ± 0.97	6.57 ± 0.04
80% methanol	31.57 ± 0.74	8.94 ± 0.09
Chloroform	19.73 ± 0.37	17.35 ± 0.12
***n*-butanol**	24.51 ± 0.41	12.45 ± 0.1
Positive control (streptokinase)	77 ± 2.07	-
Positive control (Triton X-100)	-	97.58 ± 2.13

Values are the average (mean ± SD) of three replicates, analyzed individually.

Conclusion

The present study demonstrated that the extraction solvent significantly influenced the recovery of bioactive compounds and the biological activities of *Aerva persica* leaves. Among the solvents evaluated, 80% ethanol was the most effective, yielding the highest extract recovery, total phenolic content, and total flavonoid content. Ethanolic extracts also exhibited superior antioxidant activity, as evidenced by enhanced inhibition of linoleic acid oxidation, strong DPPH radical scavenging capacity, and greater reducing power. Furthermore, the ethanolic extract exhibited the most pronounced antibacterial, antifungal, and thrombolytic activities while showing the lowest hemolytic effect, indicating comparatively better biocompatibility. In contrast, chloroform extracts displayed the weakest biological activities and the highest hemolytic activity. Overall, the findings suggest that *A. persica* leaves constitute a promising source of natural bioactive compounds with antioxidant, antimicrobial, and thrombolytic properties. The results further indicate that hydroalcoholic

extraction is more suitable for recovering these phytoconstituents. Nevertheless, further investigations, including phytochemical characterization, isolation of active constituents, toxicity assessment, and in vivo pharmacological studies, are necessary to validate the therapeutic potential and potential pharmaceutical applications of this species.

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Conflict of Interest

It is declared that there is no conflict of interest among authors.

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References

Ahmad, N., Arslan, M., Kashif, A. R., & Abbas, A. (2022). *Nutraceutical and biological attributes of wild Withania coagulans fruits*. *8*(3), 106–113.

Ahmed, S., Saeed-Ul-Hassan, S., Islam, M., Qureshi, F., Waheed, I., Munawar, I., Ishtiaq, S., Rasool, S., Akhtar, M. F., Chishti, S. A., Shabbir, M., Peerzada, S., Raza, M., Amir, K., & Sharif, A. (2017). Antioxidant activity of pistacia khinjuk supported by phytochemical investigation. *Acta Poloniae Pharmaceutica - Drug Research*, *74*(1), 173–178.

Aleem, A., Ahmad, N., Raza, A., Hameed, Z., Mustafa, M., Usman, A., & Ahmad, F. (2024). *Appraisal of Various Solvent Extracts from Basil Aerial Parts for their Biological Attributes*. *1*, 1–13.

Anbumani, D., Dhandapani, K. vizhi, Manoharan, J., Babujanarthanam, R., Bashir, A. K. H., Muthusamy, K., Alfarhan, A., & Kanimozhi, K. (2022). Green synthesis and antimicrobial efficacy of titanium dioxide nanoparticles using Luffa acutangula leaf extract. *Journal of King Saud University - Science*, *34*(3), 101896. https://doi.org/10.1016/j.jksus.2022.101896

Anwar, N., Nadeem, R., Usman, A., Ali, A., Usama, M., Ali, Y., & Kashif, R. (2026). Photocatalytic performance of Pistacia khinjuk -mediated zinc titanate nanocomposite for anionic dye degradation. *Chemical Papers*. https://doi.org/10.1007/s11696-025-04584-6

Bahari, Z., Saidi, A., Anwar, F., Tohidfar, M., Shojaeiyan, A., Ahmad, N., & Mnif, W. (2025). High-value phytochemicals and Nutra-pharmaceutical prospects of Althaea officinalis L. (Marshmallow). A review. *Biocatalysis and Agricultural Biotechnology*, *67*(April), 103601. https://doi.org/10.1016/j.bcab.2025.103601

Chu, Y., Chang, C., & Hsu, H. (2000). flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture*, *80*(5), 561–566. https://doi.org/10.1002/(sici)1097-0010(200004)80:5<561::aid-jsfa574>3.0.co;2-#

Fatima, K., Asif, M., Farooq, U., Gilani, S. J., Bin Jumah, M. N., & Ahmed, M. M. (2024). Antioxidant and Anti-inflammatory Applications of Aerva persica Aqueous-Root Extract-Mediated Synthesis of ZnO Nanoparticles. *ACS Omega*, *9*(14), 15882–15892. https://doi.org/10.1021/acsomega.3c08143

Gomes, S. M., Leitão, A., Alves, A., & Santos, L. (2023). Incorporation of Moringa oleifera Leaf Extract in Yoghurts to Mitigate Children's Malnutrition in Developing Countries. *Molecules*, *28*(6), 1–20. https://doi.org/10.3390/molecules28062526

Hazrati, S., Govahi, M., Ebadi, M. T., & Habibzadeh, F. (2020). Comparison and Evaluation of Oil Content, Composition and Antioxidant Properties of Pistacia atlantica and Pistacia khinjuk Grown as Wild. *International Journal of Horticultural Science and Technology*, *7*(2), 165–174. https://doi.org/10.22059/ijhst.2020.287550.316

Htay, T. M., Sann, K. K., & Haini, H. (2023). Comparative Study on Phytochemical Screening and Antioxidant Activity of Aqueous Extract from Various Parts of Bauhinia purpurea. *Bioactivities*, *1*(1), 24–31. https://doi.org/10.47352/bioactivities.2963-654x.183

Kashif, Ali Raza; Younas, Muhammad Usama; Hiader, Farasat; Usman, Adil; Ali, Mahmood; Bouaziz, Mohamed; Avilla-Villa, Luz Angelica; Rodriguez-ramirez, R. (2026). *Enhanced Biomedical and Photocatalytic Capabilities of Biosynthesized Zinc Oxide Nanoparticles Using Pistacia khinjuk Plant Extract*. 1–11. https://doi.org/10.1002/biot.70194

Kashif, A. R., Batool, K., Rabbani, S. M., Javed, N., Asadullah, M., & Yaseen, S. (2024). Comparative Analysis of Phenolic and Flavonoid Content in Mango Varieties : Evaluating Antioxidant and Antimicrobial Activity Highlight : *J. Adv. Nutri. Sci. Technol*, *4*(3–4), 52–65. https://doi.org/10.15228/ANST.

Kashif, A. R., Naz, S., Rasheed, M. N., Ghani, A., Younas, M. U., Ahmad, F., & Shahzad, Z. M. (2025). Nature's nano-factories: Pistacia khinjuk-mediated FeNPs with improved biomedical and environmental capabilities.

Biochemical and Biophysical Research Communications, *766*(April), 151884. https://doi.org/10.1016/j.bbrc.2025.151884

Kashif, A. R., Naz, S., Usman, A., Javed, N., Younas, M. U., & Zahoor, A. (2024). In vitro study of antioxidant and antimicrobial potential of Moringa oleifera leaves as a green food preservative in chicken burger. *Journal Advances of Nutrition Science and Technology*, *4*(1–2), 1–8. https://doi.org/10.15228/anst.2024.v04.i01-2.p01

Kashif, A. R., Naz, S., Younas, M. U., Usman, A., Tariq, M. U., Rehan, M., & Shakeel, N. (2024). *Nutrapharmaceutical Attributes of Different Aerial Parts of Commiphora wightii Highlight : 4*, 66–76. https://doi.org/10.15228/ANST.

Kashif, A. R., Younas, M. U., Usman, A., Haider, F., Maqsood, A., Aiman, V., & Safeen, A. (2025). Enhanced Biomedical and Photocatalytic Capabilities of Biosynthesized Titanium Dioxide Nanoparticles Using Pistacia Khinjuk Leaf Extract. *ChemistrySelect*, *10*(47), 1–12. https://doi.org/10.1002/slct.202504107

Kayani, W. K., Dilshad, E., Ahmed, T., Ismail, H., & Mirza, B. (2016). Evaluation of Ajuga bracteosa for antioxidant, anti-inflammatory, analgesic, antidepressant and anticoagulant activities. *BMC Complementary and Alternative Medicine*, *16*(1), 1–13. https://doi.org/10.1186/s12906-016-1363-y

Meireles, D., Gomes, J., Lopes, L., Hinzmann, M., & Machado, J. (2020). A review of properties, nutritional and pharmaceutical applications of Moringa oleifera: integrative approach on conventional and traditional Asian medicine. *Advances in Traditional Medicine*, *20*(4), 495–515. https://doi.org/10.1007/s13596-020-00468-0

Mohammed, A. S., Mohammad, M. U. R., Mohammad, F. K., Rashedul, A., Md., S. I., Rana, D., & A., T. M. Y. (2014). In vitro anti-arthritic and thrombolytic activities of methanolic extract of Protium serratum leaves. *Journal of Medicinal Plants Research*, *8*(16), 615–618. https://doi.org/10.5897/jmpr2014.5418

Naz, S., Kashif, A. R., Tawab, A., Rasool, M. Z., Rauf, A., Hussain, S., & Khan, U. (2023). Assessment of the Antidiabetic Properties of Essential Oil from Cannabis sativa. *Journal Advances of Nutrition Science and Technology*, *3*(3–4), 78–86. https://doi.org/10.15228/anst.2023.v03.i03-4.p09

Naz, S., Rasheed, M. N., Saeed, J., . A., Ghuffar, A., Kashif, A. R., & Waheed, R. (2023). Isolation of Bioactive compounds from the essential oil of Jambolan (Syzygium cumini) and Invitro evaluation of biological potential. *Journal Advances of Nutrition Science and Technology*, *3*(1–2), 15–23. https://doi.org/10.15228/anst.2023.v03.i01-2.p03

Safdar, M. N., Kausar, T., & Nadeem, M. (2017). Comparison of Ultrasound and Maceration Techniques for the Extraction of Polyphenols from the Mango Peel. *Journal of Food Processing and Preservation*, *41*(4). https://doi.org/10.1111/jfpp.13028

Sultana, B., Hussain, Z., Asif, M., & Munir, A. (2012). Investigation on the Antioxidant Activity of Leaves, Peels, Stems Bark, and Kernel of Mango (Mangifera indica L.). *Journal of Food Science*, *77*(8), 849–852. https://doi.org/10.1111/j.1750-3841.2012.02807.x

Tariq, M. U., Kashif, A. R., Usama Younas, M., Usman, A., Bibi, H., Yaseen, S., Haider, F., & Raza, A. (2025). Exploring the Therapeutic Potential of Colocasia esculenta Leaves: A Study on Antioxidant, Antimicrobial and Thrombolytic Activities. *Research Article J. Adv. Nutri. Sci. Technol*, *5*(2), 1–11. https://doi.org/10.15228/ANST

Thotathil, V., Sidiq, N., Fakhroo, A., & Sreerama, L. (2023). Phytochemical Analysis of Anastatica hierochuntica and Aerva javanica Grown in Qatar: Their Biological Activities and Identification of Some Active Ingredients. *Molecules*, *28*(8). https://doi.org/10.3390/molecules28083364

Usman, A., Kashif, A. R., Asadullah, M., & Bibi, H. (2025). Therapeutic potential of Mangifera indica leaves : Antioxidant , cytotoxic , and thrombolytic. *Journal of Pharmacognosy and Phytochemistry*, *February*. https://doi.org/10.22271/phyto.2025.v14.i1e.15260

Waqas, M. (2021). Comparative study of biological activities of different extracts of root and whole plant materials of Ajuga bracteosa from Bhimber Azad Kashmir Pakistan. *Pure and Applied Biology*, *10*(4), 1303–1311. https://doi.org/10.19045/bspab.2021.100135

Yasir, M., Jamil, N., Nazir, A., Kanwal, Q., Mehr-un-Nisa, Athir, N., Mustafa, R., Al-Mijalli, S. H., Iqbal, M., & Ahmad, N. (2022). Hemolytic activity and protective potential of Lamiaceae plants seed extracts and their bioactive components profiling having potential for functional foods and nutraceutical formulations. *Biocatalysis and Agricultural Biotechnology*, *46*(August), 102556. https://doi.org/10.1016/j.bcab.2022.102556

Younas, M. U., Usman, A., Kashif, R., & Zahoor, A. (2024). *Green Synthesis of TiO 2 NPs Using Agave americana Leaves : Antioxidant , Cytotoxicity , and Photocatalytic Activity*. *202404050*. https://doi.org/10.1002/slct.202404050

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