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Antibacterial, Antioxidant Activity and Phyto-Chemical Screening of *Prunus armeniaca* (L.) var. (Hari & Khobani) Leaf Extracts

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Abstract

Antibacterial and antioxidant properties of different varieties (Hari and Khobani) of *Prunus armeniaca* L. in methanol, water, ethyl acetate and chloroform were studied. Antibacterial activities of various leaf extracts were tested against *Staphylococcus aureus*, *Klebsiella pneumonia, Proteus vulgaris* and *Escherichia coli.* Ampicillin was used as standard antibiotic. Aqueous and methanol extracts showed significant activities whereas ethyl acetate and chloroform extracts showed least activity. Methanol extracts of *Prunus armeniaca* L. Hari and Khobani showed IC_{50} value of 0.21mg/ml and 0.01mg/ml respectively. Qualitative phyto-chemical study of aqueous and methanol extracts of *Prunus armeniaca* showed the presence of saponins, glycosides and flavonoids in both varieties. The comparison between antibacterial activities showed that both varieties of *Prunus armeniaca* L. (Hari and Khobani) were significantly different from each other. Hari showed highest antibacterial activities than Khobani. Both varieties could be used for therapeutic purpose.

Keywords: Antibacterial, Antioxidant, Phyto-chemical screening

1. Introduction

Prunus Armeniaca, popularly known as "the golden egg of the sun," grows well in colder and drier temperate climates across the world (Leccese et al., 2007). According to certain estimates, the plant has a historical heritage dating back to the reign of Emperor 5000 years (Al-Rubaei et al., 2019). Apricot fruit is high in sugar and includes a wealth of minerals and vitamins. It is medicinally important plants abundant in chemical compounds that are used for health applications (Akhone et al., 2022). The fruit is regarded as a nutritional food as it comprises a high proportion of bioactive components including carotenoids, flavonoids, phenolics, and antioxidants. The fruit has a delicious taste, a strong fragrance, and a pleasing yellow to orange appearance with a crimson topping (Leccese et al., 2012). The fruit is normally eaten fresh, but it may also be sun dried to make jam, juice, and dried fruits (Schmitzer et al., 2011). It acts as an emollient, stomach chiller, blood stimulant, reducing dehydration, disintegrates gravel, and insect repellent worms (Mosilli, 2000). The goals of this study were to look at the antibacterial properties, antioxidant capacity, and Phytochemical of two different varieties of *P. armeniaca* L.

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2. Material and Methods

2.1 Preparation of Crude Extract

In District Kotli, AJK, fresh leaves of *Prunus armeniaca* (L.) commonly known as Khobani and Hari were harvested. There are 33.32° of latitude north and 73.53° of longitude east at its location. To further investigate the leaves of plants, they were collected and shade dried in the months of May & June (2022). During the first two weeks following harvesting, leaves were stored in airtight containers to eliminate moisture content. Afterward, twenty grams of powder were dissolved in several solvents (100 mL). As part of the extraction procedure, the extracts were dehydrated and filtered (Saleh et al., 2021).

2.2 Phyto-chemical Screening of Secondary Metabolites

2.2.1 Test for Flavonoids

The plant's aqueous extract was made, and 3 mL of NH3 was carefully added to it. Following that, 1 mL of conc. (H_2SO_4) was added. The presence of flavonoids was detected by the yellowish color that developed (Sofowara, 1993).

2.2.2 Test for Saponins

0.5g pulverized plant sample was simmered and processed in 10 mL of dist. water. The filtrate (5 mL) was combined with 2.5 mL of distilled water and aggressively shaken to achieve steady, sustained foaming. The foaming was blended with few drops of saturated oil and violently agitated once more. The availability of saponins was suggested by the formation of an emulsion (Edeoga et al., 2005).

2.2.3 Test for Alkaloids

2g of plant extract was first mixed with a few drops of 5% tetraoxosulphate (VI) acid (H_2SO_4) in 5ml of (50%) ethanol before being filtered using Whatman filter paper number 42 (125 mm). In a flask, the solution was alkaline with 5ml of (NH_3) ammonia and diluted with 2.5ml of chloroform. Later, 0.5 mL of Dragendorff's reagent (Bismuth potassium iodide solution) was added with 2 mL of acid extract, and the presence of alkaloid was inferred by the precipitated orange color (Hikino et al., 1984).

2.2.4 Test for Tannins

The protocol for tannins test was described by Loman and Ju, (2017). 2 mL of 5% ferric chloride is added to 1 mL of aqueous extract. The appearance of a dark-bluish or greenish-black color was indication for presence of tannins.

2.2.5 Test for Glycosides

Keller-Kiliani test was used to analyze plant extracts for cardiac glycoside analysis (Gul *et al.,* 2017). Initially, 4 mL of glacial acetic acid was added into a 25 ml graduating cylinder with 1 drop of 2.0% Ferric chloride. After then, added 10ml of plant extract solution to solution mixture and concentrated the mixture with adding few drops of sulphuric acid. A brownish ring developed between layers revealing presence of cardiac steroidal glycosides.

2.3 Bacterial Strains for Study (Sample Collection)

Various bacterial strains, such as (S. aureus, K. pneumoniae, P. vulgaris, and E. coli) were tested for antibacterial activity in the Microbiology Division of the Joint Military Hospital Muzaffarabad, AJK. Fresh bacterial cultures were generated and stored at 37 degrees Celsius to ensure that each bacterium grew at a consistent pace. The disc diffusion technique was used for antibacterial screening (Mouloud et al., 2020).

2.4 Antibacterial activity

Plant extract-impregnated with discs, and then deposited aseptically on bacteria-seeded plates. The extract was dissolved using the appropriate solvents. The inhibitory zones were evaluated after 24 hours of incubation in an erect position at 30°C. The extract's zone of inhibition against test microorganisms was contrasted to that of the conventional antibiotic, Ampicillin (Kobus-Cisowska et al. 2019).

2.5 Antioxidant Activity

The non-enzymatic (DPPH) antioxidant activity was evaluated of various solvent extracts of plants. Note that, the absorbance of each sample was measured at (517nm) utilizing spectrophotometer. A reduction in DPPH solution absorbance suggests increased DPPH radical scavenging activity (Alzahrani et al. 2020). The test was carried out in triplicates. The following equation was used to compute free radical scavenging activity:

DPPH Activity (%) = $Abs._{(517)}$ of Control - $Abs._{(517)}$ of Sample/ $Abs._{(517)}$ of Control * 100

2.6 Statistical Analysis

The tests were carried out in triplicate, and data were analyzed using an Excel spreadsheet. The findings were presented as mean ± standard error. Graph Pad Prism was used to create the graph.

3. Results and Discussions

Many qualitative tests were carried out in the current investigation to determine the presence or absence of secondary metabolites. The phyto-chemical screening of *P. armeniaca* (Hari and Khobani), aqueous and methanol extracts demonstrated the presence of Glycosides, Saponins, and Flavonoids, but negative results was observed for alkaloids or tannins (See Table 1). The earlier study outcomes of Ibibia, (2016) reported that flavonoids, saponins, and glycosides are in abundance. They carried their investigation in an ethanolic extract for *P. amgydalus*. The comparison was made; it was found that both studies used different varieties of family Rosaceae, which differ to each others in secondary constituents. Whereas our study was when compared with Ben et al., (2013) and Gurib-Fakim, (2013) reported similar that results that support the outcomes of this study. Plants' medicinal value is mostly due to the presence of numerous bioactive compounds such as alkaloids, flavonoids, glycosides, saponins, tannins, and many others.

Table 1 Phytochemical screening of plants extracts of *P. armeniaca*

Phyto-chemicals	***P. armeniaca* (Hari)**		***P. armeniaca* (Khobani)**	
	Aqu. Extract	Methanolic Extract	Aqu. Extract	Methanolic Extract
Saponins	+	+	+	+
Alkaloids	-	-	-	-
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Tannins	-	-	-	-

Key: Positive sign showed the presence and negative sign showed absence

3.1 Antibacterial Activity

P. armeniaca (Hari) antibacterial activities revealed that methanol extracts exhibit high activity against *K. pneumoniae* (12.11 ± 0.67). It was found that least inhibition zone was seen against *S. aureus, P. vulgaris*, and *E. coli* (9.67 ±1.00, 8.67 ± 0.19, and 8.00 ± 0.33, respectively. Methanol extracts of *P. armeniaca* (Hari) exhibited substantial activity against *K. pneumoniae* (12.11 ± 0.67) and the least activity against *S. aureus, P. vulgaris,* and *E. coli* (9.671.00, 8.670.19, and 8.00 ± 0.33, respectively). The highest activity was seen against *S. aureus* (11.44 ± 1.06), accompanied by *E. coli* (10.33 ± 1.06), *K. pneumoniae* (10.33 ± 1.64), and *P. vulgaris*

(8.22 ± 0.22). Apart from that, *S. aureus* (7.67 ± 0.89), ethyl acetate extract was ineffective against certain microorganisms (Figure 1a). Only chloroform extracts demonstrated effectiveness (8.55 ± 0.40) towards *K. pneumoniae*. Both methanol and water extracts of *P. armeniaca* (Hari) were exhibited substantial antibacterial activity against all test microorganisms in this investigation. Yigit et al., (2009) validated our findings for both methanol and aqueous extracts of *P. armeniaca* kernels against *S. aureus* and *E. coli*, and Ahameethunisa and Hopper, (2010) in methanol extract of *Artemisia nilagirica* against *E. coli, P. vulgaris*, and *P. aeruginosa*. Several bacteria were not inhibited by leaf extracts in chloroform or ethyl acetate. Mehmood et al., (2013) discovered that a chloroform leaf extract of *Dodonaea viscosa* was ineffective against *S. aureus* and *E. coli*.

In the same way, methanol leaf extracts was prepared for *Prunus armeniaca* (Khobani) showed considerable antibacterial activity against *K. pneumoniae* (12.00 ± 1.83) but little activity against *E. coli* (9.44 ± 0.29), *S. aureus* (9.11 ± 0.78), and *P. vulgaris* (7.22 ± 0.22). Water extract was effective against *K. pneumoniae* (12.00 ± 1.02), *P. vulgaris, S. aureus*, and *E. coli* (11.55 ± 0.62, 10.55 ± 2.22, and 9.33 ± 0.33, respectively). Only S. aureus (7.78 ± 0.22) was active against ethyl acetate extract (7.780.22). Chloroform extracts have no efficacy against any bacterial strains (Figure 1b). *P. armeniaca* (Khobani) methanol and aqueous extracts were considerably potent toward *K. pneumoniae, E. coli, S. aureus,* and *P. vulgaris*. The zone of inhibition of various bacteria strains was shown in (Figure 3c). Prior investigation has shown similar outcomes (Vasantha et al., 2012). *P. armeniaca* (Khobani) ethyl acetate extract particularly shown susceptibility towards bacterial strain i.e. *S. aureus*. Even though gram-positive bacteria were far more sensitive to plant extracts (Bulbul et al., 2011). The chloroform extract proved ineffective. Raghavendra et al., (2006) also found that a chloroform extract of *Oxalis corniculata* had no antibacterial action against *Salmonella paratyphi* and *S. aureus*.

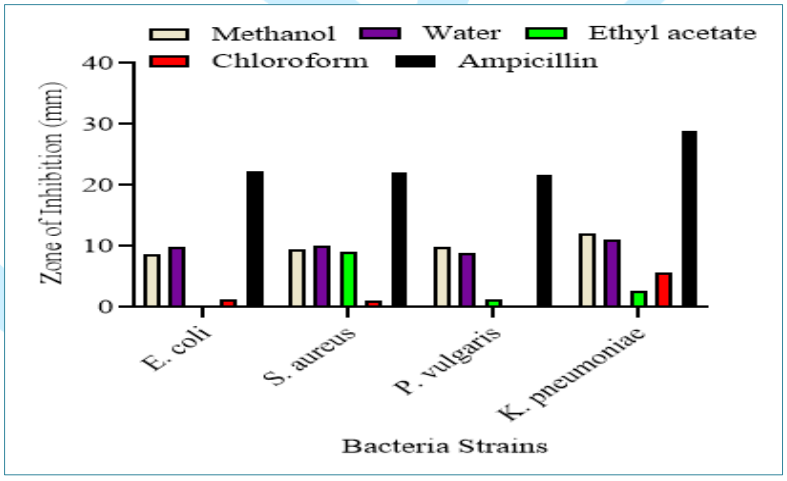

Fig. 1 Antibacterial activity of *Prunus armeniaca* (Hari)

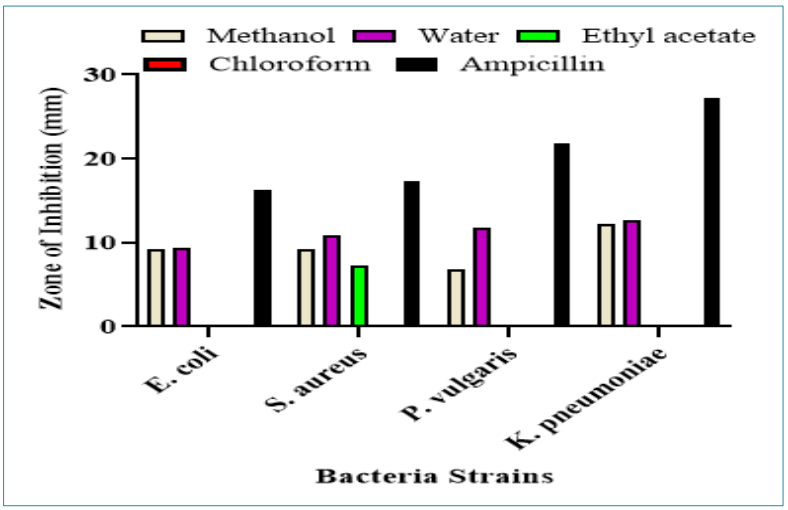

Fig. 2 Antibacterial activity of *Prunus armeniaca* (Khobani)

3.2 Comparative study of antibacterial activities in genus Prunus armeniaca (Hari & Khobani)

The antibacterial properties of *P. armeniaca* variants were compared using a completely randomized (CRD) with three replicates. The data analysis demonstrated that variety Hari was substantially more active (10.72 mm) as compared to Khobani (8.87 mm) at $p = 0.05$. K. pneumoniae (12.08 mm) proved most sensitive to leaf extracts in various solvents, followed by S. aureus (10.52 mm) and E. coli (8.42), whereas P. vulgaris was the least resistant with an inhibition zone of 8.17 mm. Leaf extracts in various solvents shown varying antibacterial activity. Aqueous and methanol extracts were the most active (10.35 and 9.57 mm, respectively), whilst ethyl acetate (3.24 mm) and chloroform (2.11 mm) extracts were the least active. Plant extracts' antibacterial activity in all solvents were lower than that of conventional antibiotics.

3.3 Antioxidant Activity

P. armeniaca (Hari) methanol extract exhibits substantial antioxidant activity in doses of 80µL (87.04%), 40µL (86.83%), and 20µL (72.62%). The IC50 concentration was 0.206 mg/mL (See Table 2). The antioxidant potential of *P. armeniaca* (Khobani) extract was highest at 40µL (85.23%), followed by 80µL (71.23%), and 20µL (67.02%). The IC50 concentration was 0.010 mg/mL. Both *P. armeniaca* cultivars (Hari and Khobani) show high antioxidant activity. It was found that both varieties are rich in flavonoid which is the highly involved bioactive compound serves as scavengers. The presence of flavonoids was responsible for the antioxidant action. The current results outcome was compared to other studies of Deb et al., (2010) and Priya et al., (2011) which validated the finding (2012).

Table 2 Non-enzymatic (DPPH) activity of *Prunus armeniaca*

Selected plants	Percentage scavenging activity			IC_{50}
	20 µL	40 µL	80 µL	
P. armeniaca (Hari)	72.6200	86.8333	87.0367	0.206679287
P. armeniaca (Khobani)	67.0233	85.2300	71.2333	0.010154643

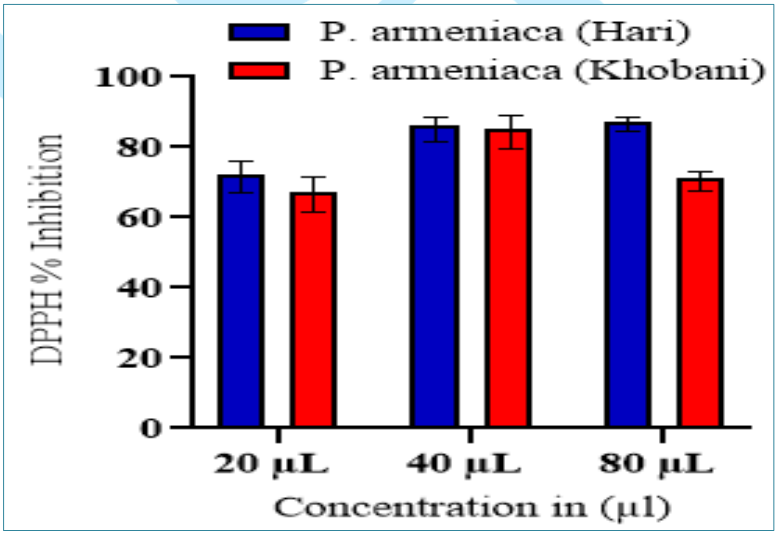

Fig. 3 Antioxidant activity of *Prunus armeniaca* (Hari & Khobani)

4. Conclusions

A comparison of *P. armeniaca* (Hari and Khobani) antibacterial activities found that Hari had higher antibacterial activity than Khobani. Antibacterial activity of aqueous and methanol extracts is substantial. The antibacterial activity of ethyl acetate extracts was lower, whereas chloroform extract was essentially non-existent. The antibacterial activity of leaf extracts in all solvents was significantly lower than those of conventional antibiotics. Khobani has the highest antioxidant activity compared to Hari. Flavonoids, saponins,

and glycosides were also found in the extracts. Plants can be employed in pharmaceutical businesses due to the presence of these active chemicals. Some plants may also include antibacterial and antioxidant compounds. It is suggested that more research be conducted on the extraction of chemical ingredients so that these chemicals can be employed to treat various viral disorders.

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Declaration of Conflict

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

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