



# Antibacterial Activity of *Prunus Scoparia* Root Methanol Extract against Most Common Burn Wound Pathogens

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## What's Known

- Plants are a good natural source for the development of novel, safe, and cost-effective antibacterial agents. In southern Iran, *Prunus scoparia* (*P. scoparia*) root is a commonly used herbal medicine to treat burn wound infections.
- Studies on the antibacterial activity of *P. scoparia* root are scarce.

## What's New

- For the first time, we demonstrated the presence of various secondary metabolites and the antibacterial activity of the methanolic extract of *P. scoparia* root against the most common burn wound pathogens.
- Prunus scoparia* root showed potent anti-Methicillin-resistant *Staphylococcus aureus* activity.

## Abstract

**Background:** Burn wound infection and sepsis are serious medical conditions requiring prompt intervention. Plants are a good natural source for the development of novel, safe, and cost-effective antibacterial agents. The objective of the present study was to assess the antibacterial potential of aqueous, chloroform, and methanol extracts of the *Prunus scoparia* (*P. scoparia*) root against the most common burn wound pathogens.

**Methods:** The present experimental study was conducted at Shiraz University of Medical Sciences (Shiraz, Iran) during 2018-2019. The antibacterial activity of the total plant extract was assayed using the broth microdilution method. Fractionation was performed using a separation funnel and solvents with different polarities. Broth microdilution and agar well diffusion assays were performed to determine the antibacterial potential of the obtained fractions. Quantitative and qualitative phytochemical analyses were performed to confirm the presence of secondary metabolites in both the total extract and the fractions.

**Results:** Methanolic extract of *P. scoparia* root exhibited antibacterial activity against all tested bacterial strains, especially against Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. This extract, compared to the aqueous and chloroformic extracts, exhibited the presence of active antibacterial compounds. The quantitative and qualitative results of phytochemical screening showed that phenols and flavonoids were the main antibacterial compounds in the methanolic extract of the plant.

**Conclusion:** For the first time, we demonstrated the antibacterial activity of the *P. scoparia* root against MRSA isolates and other common burn wound pathogens.

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**Keywords** • Herbal medicine • Anti-bacterial agents • Wounds and injuries • Phenols • Flavonoids

## Introduction

Major burn wounds can be a traumatic injury, and the affected patients need immediate treatment to limit potential complications and even death.<sup>1</sup> Burn injuries initially damage the skin; the first barrier against various pathogens. Skin damage along with diminished local and systemic immune responses creates an environment for pathogens to colonize and grow rapidly on the

burn wound surface. Infections and sepsis are the leading causes of death in patients with burn injuries.<sup>1, 2</sup> The burn wound surface provides a favorable environment for microbial colonization and proliferation.<sup>3</sup> Typically, *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pyogenes* (*S. pyogenes*) are the first bacterial invaders to colonize the burn wound surface, followed by various bacteria from normal gut flora and infections during hospitalization.<sup>4</sup> The mortality rate due to sepsis is high in patients with burn injuries. This is a type of body reaction that can eventually lead to death by triggering a syndrome known as multiple organ dysfunction syndromes.<sup>5</sup> The discovery of multidrug-resistant organisms has encouraged researchers to seek novel drugs with higher efficiency, higher safety, and particularly lower costs.<sup>6</sup> Plants are a vast natural source to develop novel antibacterial agents and a cost-effective treatment option for many human diseases.<sup>7</sup>

To date, several studies have been conducted to utilize herbs and plants to develop alternative therapeutics against bacterial pathogens.<sup>7-9</sup> *Prunus scoparia* (*P. scoparia*) is a wild almond species native to Iran, Turkey, Turkmenistan, and Afghanistan.<sup>10</sup> This plant has been widely used as herbal medicines to treat respiratory and cardiovascular diseases, headaches, rheumatism, and wounds. Previous studies have shown the antidiabetic,<sup>11</sup> antioxidant, and antifungal<sup>12</sup> properties of the shoot extract of *P. scoparia*. However, as concluded from our literature review, no studies have investigated the antibacterial activities of the root extract of *P. scoparia*. In southern Iran (Fars Province), *P. scoparia* root is a commonly used herbal medicine in the treatment of burn wound infections. Hence, The present ethnobotanical study aimed to assess the *in vitro* antibacterial activity of the methanolic extract of *P. scoparia* against the most common burn wound pathogens.

## Materials and Methods

The study protocol was approved by the Ethics Committee of AJA University of Medical Sciences, Tehran, Iran. The experiments were performed at the School of Pharmacy at Shiraz University of Medical Sciences, Shiraz, Iran, during 2018-2019.

### Reagents

Methanol, petroleum ether, dichloromethane, ethyl acetate, and all phytochemical screening reagents were purchased from Merck, Germany. Mueller-Hinton agar and Mueller-Hinton broth

were purchased from Sigma-Aldrich, USA.

### Plant Collection

The plants were collected from the mountains in southern Iran during spring 2018. The exact location was Galū Borāq village near the city of Jahrom, Fars Province, Iran (28°23'05"N 53°54'14"E). The collected plants were authenticated by an expert at Shiraz University of Medical Sciences, Shiraz, Iran (voucher number 3014).

### Preparation of Plant Extracts

The roots of the collected plants were dried and ground using a grinding apparatus (MX-110PN, Japan). The extraction process was carried using 80% methanol solvent. In brief, 200 mL of the solvent was mixed with 100 g of the powdered plant root. The mixture was properly stored for 24 hours during which it was stirred several times with a glass rod. Then, the mixture was placed in a rotary evaporator (Heidolph Co., Germany) to condense the extract until the solvent was evaporated. The extract was kept in a freezer (LG, South Korea) at -20 °C until further use.<sup>13</sup>

### Microorganisms

Bacterial strains included Methicillin-resistant *Staphylococcus aureus* (MRSA) (a clinical isolate from a patient with burn wounds at Nemazee Hospital, Shiraz, Iran), *Enterococcus faecalis* (ATCC 29212), *Acinetobacter baumannii* (NCTC 13304), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603), and *Serratia marcescens* (ATCC BAA2808).

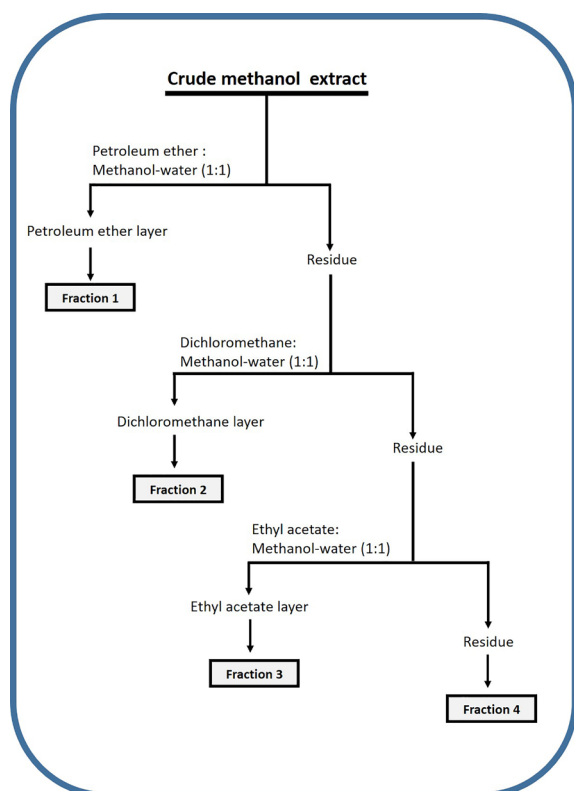
### Broth Microdilution and Agar Well Diffusion Assays

Broth microdilution assay was performed to obtain minimal inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC) of the plant root extracts against the bacterial strains.<sup>8</sup> In brief, the methanol extract was dissolved in 1% dimethyl sulfoxide and water and transferred onto 96-well plates to obtain serial dilutions (0.39-50 mg/mL). Then, the bacterial suspension with a turbidity standard of 0.5 McFarland was added into the wells to reach a bacterial concentration of  $1.5 \times 10^5$  CFU/mL in each well with a final volume of 200  $\mu$ L. The lowest concentration of the extract that showed no visible bacterial growth in the broth (after 24 hours incubation at 37 °C) was noted as MIC. Additionally, the lowest extract concentration killing 99.9% of bacterial inoculum was noted as MBC. MBC values were determined after

sub-culturing 100  $\mu$ L of the bacterial suspension on the Mueller-Hinton agar. All procedures were carried out in triplicates, and the values were presented as the mean of the three datasets. In accordance with a previous study, the antibacterial activity of the plant root was evaluated using the agar well diffusion method.<sup>8</sup>

#### Fractionation of the Crude Extracts

Fractionation was performed using a separating funnel. In brief, the extract was suspended in 150 mL of the methanol-water mixture (2:1). The separation was carried out by applying organic solvents such as petroleum ether, dichloromethane, and ethyl acetate to enhance polarity (figure 1).



**Figure 1:** The fractionation procedure to obtain pure extracts is illustrated.

#### Preliminary Phytochemical Screening

The total plant extract and the fractions were checked in accordance with a previously described protocol<sup>14</sup> for the presence of various secondary metabolites including phenols, flavonoids, alkaloids, saponins, tannins, anthocyanins, anthraquinones, and terpenoids.

#### Folin-Ciocalteu (F-C) Assay for Total Phenolic Content (TPC)

Briefly, 500  $\mu$ L of different concentrations of the plant extract fractions in water was mixed with 1.5 mL of prediluted Folin-Ciocalteu reagent, gallic acid, (40  $\mu$ L, Merck, Germany). The mixture

was then incubated at room temperature for five minutes. Sodium bicarbonate (1.2 mL, 7.5% w/v) was added, and the mixture was again incubated at room temperature for 60 minutes. Then, the absorbance of the mixture was measured at 765 nm. For calibration, several concentrations of gallic acid (10-500 mg/L) were used, and the measurements were expressed in mg of gallic acid equivalents (GAE) per g of the sample.

#### Total Flavonoid Content

The total flavonoid content (TFC) was determined based on a colorimetric assay method.<sup>15</sup> In brief, after mixing 1 mL of the extract fraction with 4 mL of distilled water in a tube, 0.3 mL of sodium nitrate (5%) and 0.3 mL of aluminum chloride (10%) were added to the mixture. After incubation at room temperature for five minutes, 2 mL sodium hydroxide (1 M) was added to the mixture to increase the volume to 10 mL. The test tube was placed on a shaker for five minutes, and the absorbance was measured at 510 nm. For calibration, several concentrations of catechin (50-100 mg/L) were used, and the measurements were expressed in mg of catechin equivalents (CEQ) per g of the sample. All the reagents were purchased from Merck, Germany.

## Results

The MIC and MBC values of the total plant root extract and fractions against the tested microorganisms are presented in table 1. The results showed effective antibacterial activity of the root extract of *P. scoparia* against the microorganisms. We observed low or no antibacterial activity from the aqueous and chloroformic extracts of the plant root. The methanol extract was used for fractionation and phytochemical analysis. In most cases, the ethyl acetate fraction of the methanolic extract showed a better antibacterial response against the tested bacterial strains.

The results of broth microdilution and agar well diffusion assays were similar and the obtained fraction quantifiably showed their bacterial growth inhibition potential (figure 2). The qualitative and quantitative results of the phytochemical screening are shown in table 2. Different secondary metabolites were observed in the methanolic extract of the plant root. Different amounts of phenolic and flavonoid contents were observed in the antibacterial activities of the selected fractions. The third fraction had more phenolic and flavonoid contents.

**Table 1:** Minimal inhibitory concentration and minimal bactericidal concentration of *Prunus scoparia* root extract and the obtained fractions against tested microorganisms (dashes indicate the growth of microorganisms)

Plant	Extract	Tested microorganisms and antibacterial parameters													
		<i>S. aureus</i> (MRSA isolate)		<i>E. faecalis</i> (ATCC 29212)		<i>A. baumannii</i> (NCTC 13304)		<i>E. coli</i> (ATCC 25922)		<i>P. aeruginosa</i> (ATCC 27853)		<i>K. pneumoniae</i> (ATCC 700603)		<i>S. marcescens</i> (ATCC BAA2808)	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>P. scoparia</i> (root)	Chloroform	25	25	50	50	25	25	12.50	12.50	50	50	50	12.50	12.50	
	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Methanol extract	1.15	1.15	6.25	6.25	6.25	6.25	12.50	12.50	12.50	12.50	25	6.25	6.25	
	Petroleum ether	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Dichloromethane	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Ethyl acetate	0.78	1.15	3.12	3.12	3.12	3.12	6.25	6.25	3.12	3.12	6.25	6.25	12.50	
	Remaining methanol extract	3.12	6.25	-	-	-	-	-	-	-	-	-	12.50	25	

MIC: Minimal inhibitory concentration (mg/mL), MBC: Minimal bactericidal concentration (mg/mL), MRSA: Methicillin-resistant *Staphylococcus aureus*, ATCC: American type culture collection, NCTC: The national collection of type cultures, *S. aureus*: *Staphylococcus aureus*, *E. faecalis*: *Enterococcus faecalis*, *A. baumannii*: *Acinetobacter baumannii*, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *K. pneumoniae*: *Klebsiella pneumoniae*, *S. marcescens*: *Serratia marcescens*

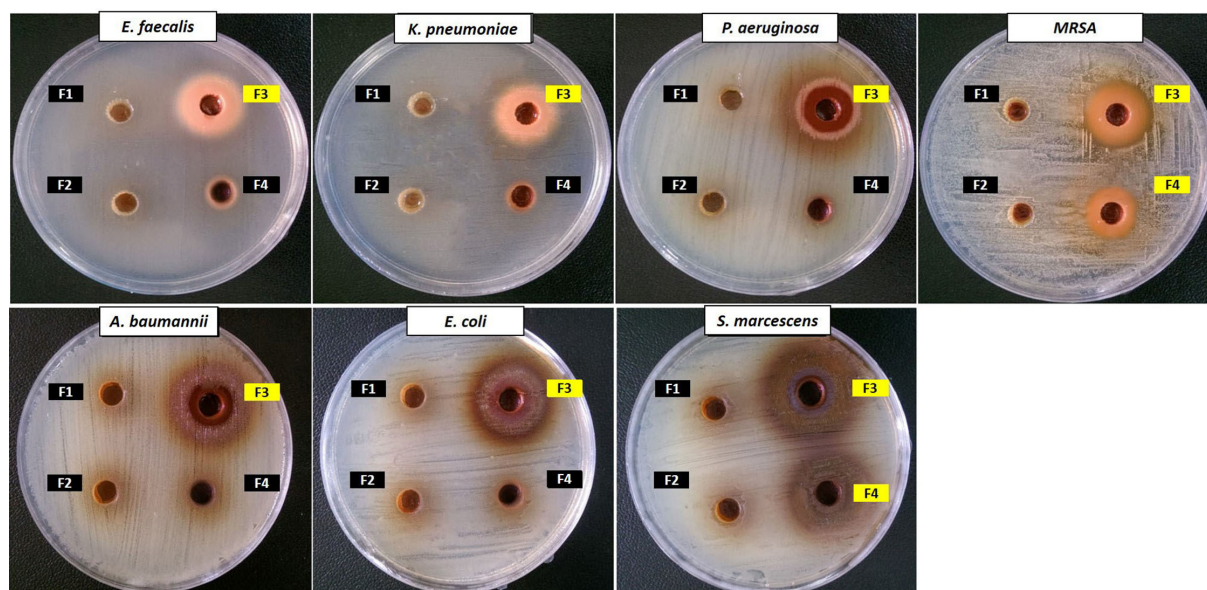
## Discussion

In the present study, the antibacterial potential of the root extract of *P. scoparia* was demonstrated. Some studies have reported the potent antibacterial activity of different plants.<sup>16, 17</sup> In terms of wound infection, Sukantha and colleagues reported the antibacterial activity of different fruit peel extracts of *Pithecellobium dulce* (*P. dulce*) against *Klebsiella pneumoniae* and *S. aureus*.<sup>13</sup> In another study, Yin and colleagues investigated the methanolic extract of *Gentiana macrophylla* as a medicinal herb and reported a significant antibacterial activity against the two most important pathogens causing burn wound infection, namely *S. aureus* and *P. aeruginosa*.<sup>18</sup>

In a preliminary screening experiment, we used the root extract of *P. scoparia* to assess the potential antibacterial activity of its chloroform, methanol, and aqueous extracts against seven different Gram-positive and Gram-negative bacterial species related to burn wounds. The results showed the presence of active antibacterial compounds in the methanolic extract of the plant contrary to its aqueous and chloroformic extracts. Based on the MIC and MBC values, the methanolic extract of the plant was designated as a potential antibacterial agent.

The methanolic extract of *P. scoparia* root exhibited significant activity against all tested bacterial strains, especially MRSA isolates. Compared to other studies, we additionally performed the fractionation procedure to obtain pure extracts. After the *P. scoparia* crude extracts were fractionated, and the MIC and MBC values were measured for each fraction, all tested bacterial strains showed sensitivity to the ethyl acetate fraction (third fraction). Compared with the crude extract, the third fraction showed better antibacterial activity against almost all tested bacterial strains. However, of the fractions, petroleum ether fraction (first fraction) and dichloromethane fraction (second fraction) represented no antibacterial activities. Similar to the third fraction, the fourth methanolic extract fraction also showed antibacterial activity against MRSA and *Serratia marcescens*. But, the antibacterial activity of the third fraction was higher than the fourth fraction.

Several previous studies indicated a direct association between the presence of some secondary plant metabolites (especially alkaloids, phenols, and flavonoids) and antibacterial activities.<sup>19-22</sup> Based on the qualitative analysis, we demonstrated the presence of phenols, flavonoids, and alkaloids in the third and fourth fractions of *P. scoparia* root extract. Phenolic and flavonoids compounds are



**Figure 2:** The results of agar well diffusion assay for different fractions obtained from the crude methanol extract of *P. scoparia* root are illustrated. F1: First fraction, F2: Second fraction, F3: Third fraction, F4: Fourth fraction, Yellow boxes: Fraction per sample responsible for bacterial growth inhibition

**Table 2:** Preliminary phytochemical analysis of the *Prunus scoparia* root extract and the fractions, as well as total phenolic content (mg GAE/g sample) and total flavonoid content (mg CEQ/g sample) in the third and fourth fractions

Fractions	<i>P. scoparia</i> root extract				
	Crude extract (yield: 29.2%)	F1	F2	F3	F4
Alkaloids	+	-	-	+	+
Anthocyanins	-	-	-	-	-
Antraquinones	+	-	-	+	+
Saponins	-	-	-	-	-
Tannins	+	-	-	+	+
Terpenoids	+	-	+	+	-
Flavonoids	+	-	-	+(35.44±4.12)	+(8.31±2.12)
Phenols	+	-	-	+(123.06±6.43)	+(57.62±5.23)

F1: First fraction (petroleum ether), F2: Second fraction (dichloromethane), F3: Third fraction (ethyl acetate), F4: Fourth fraction (remaining methanol extract)

suggested to possess antibacterial activity.<sup>23</sup> To confirm this, the TPC and TFC of the third and fourth fractions were assayed. In line with our qualitative data, the results of TPC and TFC assays also revealed the presence of phenolic and flavonoids compounds in both fractions, although the amount was higher in the third fraction. Therefore, it was deduced that the antibacterial activity of the *P. scoparia* root extract could be attributed to these compounds, but primarily in the third fraction. However, since the two tested bacterial strains also showed sensitivity to the fourth fraction, the role of other secondary metabolites (e.g., anthraquinones, saponins, and terpenoids) should not be ignored. Further detailed investigations are required to specify the exact role of these secondary metabolites in the antibacterial activity of *P. scoparia* root. In line with the findings of two previous studies,<sup>13, 18</sup> we demonstrated the

potential of the secondary plant metabolites against burn wound pathogens. Note that the above-mentioned studies did not perform the fractionation procedure to obtain pure extracts, whereas we performed liquid-liquid separation and obtained different fractions out of the total plant root extract to achieve a high degree of purification.

The main limitation of the present study was related to the separation step, which could have been performed using advanced techniques such as high-performance liquid chromatography. Obtaining a highly pure fraction is key to successful content analysis.

## Conclusion

For the first time, we demonstrated the presence of various secondary metabolites and the antibacterial activity of the methanolic extract of

*P. scoparia* against the most common burn wound pathogens. *P. scoparia* root is a potential source of antibacterial agents acting against MRSA and other common burn wound pathogens. It is recommended to use more advanced techniques to accurately separate plant fractions.

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**Conflict of Interests:** None declared.

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