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Original Research

The Inhibitory Effect of Water and Alcoholic Extracts of Seed of *Peganum Harmala* on the Isolation of Bacterial *E. coli* and *Staphylococcus aureus*

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ABSTRACT:

This experiment was conducted to evaluate the inhibitory effects of aqueous and alcoholic seed extracts of *Peganum harmala*. The antimicrobial activity of both extracts was tested against *Staphylococcus aureus* (Gram-positive) and Escherichia coli (Gram-negative). Four concentrations were used: 2, 4, 6, and 9 mg/ml for both extracts. The results showed that the alcoholic seed extract of *Peganum harmala* exhibited stronger antimicrobial activity, with

inhibition zones of 11 mm against S. aureus and 10 mm against *E. coli*. In contrast, the aqueous extract produced smaller inhibition zones: 4 mm against S. aureus and 2 mm against *E. coli*, indicating lower antimicrobial activity compared to the alcoholic extract.

KEYWORDS: *Peganum harmal, Staphylococcus aureus, E.coli* , Inhibitory Effect.

INTRODUCTION

In recent years, the need to seek alternatives to conventional synthetic drugs has significantly increased, particularly due to the emergence of numerous bacterial strains that have developed resistance to commonly used antibiotics and chemical compounds. This resistance is largely attributed to the excessive and prolonged use of these agents in treating both human and plant diseases, as well as in food preservation (Gupta, 1994). As a result, the effectiveness of traditional antibiotics has declined, prompting a global shift toward exploring natural plant-based alternatives.

Medicinal plants have gained growing attention in this context, as they are rich in diverse bioactive compounds—many of which remain undiscovered and are not yet recognized by pathogenic microorganisms. This unique property has positioned medicinal plants as promising candidates in the search for novel therapeutic agents. The appeal of these natural remedies lies in their potent biological activity, affordability, ease of preparation, and the possibility of combining various plant extracts to enhance efficacy while minimizing toxicity.

Peganum harmala, commonly known as Syrian rue, is one of the most notable medicinal plants used in traditional and alternative medicine. It has been extensively studied for its pharmacological properties, particularly its antimicrobial potential. The plant contains several alkaloids such as harmaline, harmalol, and harmine, which have demonstrated significant antibacterial activity. Ross et al. (1980) reported that harmaline and harmalol, isolated from P. harmala growing in Egypt, exhibited inhibitory effects against Staphylococcus species and Escherichia coli. Further studies conducted by Atta et al. (1991), Saeed et al. (1993), Basher et al. (1994), and Ali (1998) reinforced these findings, confirming the plant's antimicrobial potential.

Moreover, laboratory investigations have shown that both aqueous and alcoholic extracts of P. harmala seeds possess considerable inhibitory effects against Gram-positive bacteria such as Staphylococcus aureus, and Gram-negative bacteria such as E. coli. Li et al. (1995) also demonstrated that harmaline, harmalol, and gamma-harmalol exhibit radioprotective properties, further supporting the medicinal value of this plant.

Given these findings, *Peganum harmala* represents a promising natural source of antimicrobial agents and continues to be the subject of interest in both pharmaceutical and botanical research.

MATERIALS AND METHODS

The study was conducted using the following materials and equipment*:* distilled water , 75% ethanol , petri dishes, test tubes, centrifuge, Incubator , *micropipettes,* sterile nutrient agar medium, and sterile filter paper. Clinical isolates of *Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative)* were obtained from the Medical

Analysis Centers in Derna and Umm al-Razm, Libya. Dried seeds of *Peganum harmala* were purchased from certified herbal stores and prepared for extraction.

Preparation of Plant Extracts

The seed extracts of *Peganum harmala* were prepared the following noted modifications:

To prepare the ethanolic extract, 10 grams of finely ground P. *harmala* seeds were soaked in 100 mL of 75 % ethanol and left at room temperature for 24 hours.

Similarly, the *aqueous* extract was prepared by soaking another 10 grams of the powdered seeds in 100 mL of boiling distilled water, also for 24 hours.

After the soaking period, both mixtures were filtered using the sterile filter paper, and the filtrates were collected and stored at 4°C until further use.

Antibacterial Activity Testing

The antibacterial properties of both extracts were tested using the agar well diffusion method, as outlined by Hammer et al. (2003). The process involved several key steps:

1. **Bacterial activation**:

   Bacterial isolates were first cultured on nutrient agar and incubated at 37°C for 24 hours to ensure active growth.

2. **Plate preparation**:

A uniform bacterial suspension was spread over the surface of nutrient agar plates to form a consistent lawn.

3. **Well Creation**:

Two wells were carefully punched into each plate using a sterile cork borer.

4. **Extract application**:

Four concentrations (2 mg/mL, 4 mg/mL, 6 mg/mL, and 9 mg/mL) of each extract (aqueous and ethanolic) were added to the wells using sterile micropipettes.

5. **Incubation**:

The plates were incubated at 37°C for another 24 hours.

6. **Observation and Measurement**:

After incubation, the inhibition zones (clear areas around the wells) were observed and measured in millimeters to assess the antibacterial activity of each extract.

RESULTS AND DISCUSSION

The present study investigated the antibacterial efficacy of *Peganum harmala* (harmal) seed extracts—both ethanolic and aqueous- against two bacterial strains: *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). The results revealed clear differences in the inhibition zones concentrations.

Effect on *Escherichia coli*

The ethanolic extract demonstrated significantly higher antibacterial activity against E. coli compared to the aqueous extract. The largest inhibition zone (10 mm) was observed with the ethanolic extract at a

concentration of 6 ml, while the smallest (1 mm) was recorded at a concentration of 4 ml. In contrast, the aqueous extract showed more limited activity, with the highest inhibition zone measuring 2 mm at 9 ml, and the lowest being 1 mm at 2 ml.

Effect on Staphylococcus aureus

Similarly, the ethanolic extract exhibited superior inhibitory activity against Staphylococcus aureus. The highest inhibition zone was 11 mm at 9 ml, while the lowest was 9 mm at 2 ml.

As for the aqueous extract, the inhibition zones ranged from 1 mm (at 2 ml) to a maximum of 4 mm (at 4 ml), indicating limited but measurable antibacterial activity.

These results suggest a concentration-dependent effect, where increased extract volumes correlate with larger inhibition zones. This finding is consistent with previous studies, such as those by Taylor et al. (1996), who emphasized that higher concentrations of bioactive plant extracts tend to produce stronger antimicrobial effects.

The observed trend reflects the increasing availability of active compounds at higher concentrations, which likely enhances their ability to disrupt bacterial growth.

Comparison Between Extract Types

Across all tested conditions, the ethanolic extract consistently outperformed the aqueous extract in antibacterial activity. This difference can be attributed to the polarity of ethanol, which facilitates more efficient extraction of active secondary metabolites, such as alkaloids, flavonoids, and phenolic compounds—many of which possess known antibacterial properties. These findings align with those of Sageda and Ali (1987), who emphasized the role of solvent polarity in phytochemical extraction, and with Berenbaum (1995), who confirmed the broad-spectrum antibacterial potential of alcoholic extracts against both Gram-positive and Gram-negative bacteria.

Moreover, Adedayo et al. (2001) reported that alcoholic solvents are more effective in extracting plant-based antimicrobial agents compared to aqueous solutions. The present study supports this, as the ethanolic extract exhibited significantly larger zones of inhibition in both bacterial strains tested.

Table :(1). The Growth Inhibition Diameters of *Staphylococcus* Bacteria for Both Extracts.

Staphylococcus	0.2	0.4	0.6	0.9
Aqueous extrac	mm 1	mm 4	mm 2	mm 2
Ethanol extract	mm 9	mm 10	mm 9	mm 11

Table: (2). The Growth Inhibition Rates of *E.coli* Bacteria for Both Extracts

Staphylococcus	0.2	0.4	0.6	0.9
Aqueous extrac	mm 1	mm 4	mm 2	mm 2
Ethanol extract	mm 9	mm 10	mm 9	mm 11

Figure 1: Zone of Inhibition of Aqueous Extract of *E.coli*

Figure 2: Zone of Inhibition of the *E.coli* Alcoholic Extract.

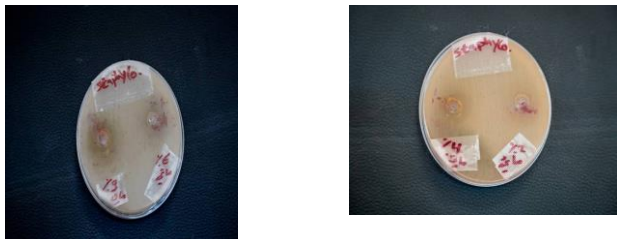

Figure 3: Zone of Inhibition of the Aqueous Extract of *Staphylo*.

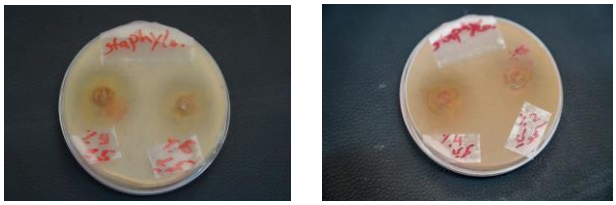

Figure 4: Zone of Inhibition of *Staphylo* Alcohol Extract.

CONCLUSION

This study demonstrated the significant antibacterial activity of Peganum harmala seed extracts, with the ethanolic extract showing a notably stronger effect against both Escherichia coli and Staphylococcus aureus compared to the aqueous extract. This difference in efficacy is likely due to ethanol's greater ability to dissolve and extract bioactive antimicrobial compounds.

A clear dose-dependent relationship was observed, where increasing the concentration of the extract led to a wider inhibition zone. This indicates that the active compounds are more abundant or more effective at higher concentrations, enhancing the overall antibacterial activity.

These findings support the potential use of ***P. harmala*** extracts especially the ethanolic form as natural antimicrobial agents. Given the global rise in antibiotic resistance, such plant-based alternatives could provide valuable contributions to future antimicrobial therapies. Further research is recommended to isolate the active components and assess their effects on a wider range of pathogens.

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الملخص

أُجريت هذه التجربة لتقييم التأثيرات المثبطة للمستخلصات المائية والكحولية لبذور الحرمل. تم اختبار النشاط المضاد للميكروبات لكلا المستخلصين ضد بكتيريا المكورات العنقودية الذهبية (موجبة الغرام) وبكتيريا الإشريكية القولونية (سالبة الغرام). استُخدمت أربعة تراكيز: 2، 4، 6، و9 ملغم/مل لكلا المستخلصين. أظهرت النتائج أن المستخلص الكحولي لبذور الحرمل يتمتع بنشاط مضاد للميكروبات أقوى، حيث بلغت مناطق التثبيط 11 مم ضد المكورات العنقودية الذهبية و10 مم ضد الإشريكية القولونية. في المقابل، أنتج المستخلص المائي مناطق تثبيط أصغر: 4 مم ضد المكورات العنقودية الذهبية و2 مم ضد الإشريكية القولونية، مما يشير إلى نشاط مضاد للميكروبات أقل مقارنةً بالمستخلص الكحولي.

الكلمات المفتاحية: الحرمل، المكورات العنقودية الذهبية، الإشريكية القولونية، التأثير المثبط.