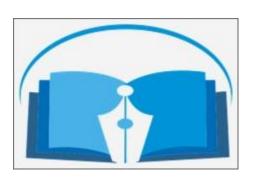


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مجلة التربوي مجلة علمية محكمة تصدر عن كلية التربية جامعة المرقب

المدرد الثالث والمشروخ يوليو 2023م

هيئة التحرير

رئيس هيئة التحرير: د. مصطفى المهدي القط مدير التحرير: د. عطية رمضان الكيلاني سكرتير المجلة: أ. سالم مصطفى الديب

- المجلة ترحب بما يرد عليها من أبحاث وعلى استعداد لنشرها بعد التحكيم .
 - المجلة تحترم كل الاحترام آراء المحكمين وتعمل بمقتضاها.
- كافة الآراء والأفكار المنشورة تعبر عن آراء أصحابها ولا تتحمل المجلة تبعاتها.
 - يتحمل الباحث مسؤولية الأمانة العلمية وهو المسؤول عما ينشر له .
 - البحوث المقدمة للنشر لا ترد لأصحابها نشرت أو لم تنشر.

(حقوق الطبع محفوظة للكلية)



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ضوابط النشر:

يشترط في البحوث العلمية المقدمة للنشر أن يراعي فيها ما يأتي:

- أصول البحث العلمي وقواعده.
- ألا تكون المادة العلمية قد سبق نشرها أو كانت جزءا من رسالة علمية .
 - يرفق بالبحث تزكية لغوية وفق أنموذج معد .
 - تعدل البحوث المقبولة وتصحح وفق ما يراه المحكمون.
- التزام الباحث بالضوابط التي وضعتها المجلة من عدد الصفحات ، ونوع الخط ورقمه ، والفترات الزمنية الممنوحة للتعديل ، وما يستجد من ضوابط تضعها المجلة مستقبلا .

- للمجلة الحق في تعديل البحث أو طلب تعديله أو رفضه .
 - يخضع البحث في النشر لأولوبات المجلة وسياستها.
- البحوث المنشورة تعبر عن وجهة نظر أصحابها ، ولا تعبر عن وجهة نظر المجلة .

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Antimicrobial Activities of Methanol Extract of *Peganum harmala* Leaves and Seeds against Urinary Tract Infection Bacteria

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ABSTRACT: The problem of microbial resistance continues to increase in all regions of the world, and even though a very large number of antibiotics have been produced in the last 30 years, microbial resistance is maintained because of the wide use of these drugs against many infectious diseases. Thus, natural products are seen as the alternative solution. Based on previous studies, Peganum harmala has been reported to exhibit several biological properties including antibacterial and antifungal. Thus, the main aim of this study is evaluating antibacterial activities of P. harmala against Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus, which isolated from infected patients in (Alassaba'a hospital, Alassaba'a, west of Tripoli). Agar disk diffusion technique was used for detecting the antibacterial activity, and the minimum inhibitory concentration was tested by serial dilution methods. The results showed that the growth of all tested bacteria were inhibited strongly by methanol extract of seeds at 1.0% with inhibition zones 14.66, 12.00 and 15.33mm against E. coli, k. pneumoniae and S. aureus respectively. Besides, MIC values ranged between 0.625 to 1.25 mg/mL. While, MBC values ranging between 1.25 to 5 mg/mL. On the other hand, methanol extract of leaves was also inhibit the growth of E. coli, k. pneumoniae and S. aureus with inhibition zones 11.00, 9.66 and 10.00mm respectively. As well as, MIC values ranging between 1.25 to 2.5 mg/mL, while MBCs value ranging between 2.5 to 5 mg/mL. In conclusion, methanol extract of P. harmala seeds exhibited strongly antimicrobial activity against E. coli, k. pneumoniae and S. aureus, thus it can be developed as anti-bacterial agent.

Keywords: Antimicrobial activity; Peganum harmala; Urinary Tract Infection.

1. Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections. About 85% of these infections are caused by *Escherichia coli*. In addition, other bacteria can cause an infection included Gram-negative species *Klebsiella* and *Pseudomonas* and Gram-positive bacterial cocci, *Staphylococcus aureus* (Fatma *et al.*, 2016). These bacteria including *E. coli*, *K. pneumoniae* and *S. aureus* are multidrug resistant bacteria that pose threats to patients in hospitals and nursing homes as well as to patients whose conditions require medical devices such as ventilators and blood catheters (Mancuso *et al.*, 2021). On the other hand, natural products are of great importance, the medicinal plants have been used in folk medicine in Libyan and other countries as diuretics, topical anti-inflammants, and other using (Alghazeer *et al.*, 2012). Recently, searching for drugs and dietary supplements derived from plants have been accelerated which have been found in many studies to have antimicrobial properties (Fatma *et al.*, 2016).



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Peganum harmala belongs to the Zygophyllaceae family, which commonly known as "harmal" in Libya. Extensively spread in Middle and East Asia and North Africa (Ahmed et al., 2021). It is a highly branched perennial, herbaceous, glabrous plant, which grows from 30 to 60 cm tall with short creeping roots. It possesses narrow leaves arranged alter-nately on fleshy, bright green stiff stems. The flowers are solitary, small, pale yellow or white. The fruits are capsules with 3 chambers and about 6 to 10 mm across (Shahrajabian et al., 2021). The main medicinal part of P. harmala is the seed (Niroumand et al., 2015). The seeds are digestive, hallucinogenic, diuretic, antipyretic, antispasmodic, emetic, nauseant, narcotic and a uterine stimulant (Kartal et al., 2003). In addition, the incense of seeds is used for toothache and repelling mosquitos (Tonkaboni et al., 2007). On the other hand, the leaves used in the treatment of asthma, colic, dysmenorrhea, hiccups, neuralgia, hysteria and rheumatism (Ezer & Arisan 2006).

Various studies have been reported the antimicrobial activities of the *P. harmala* such as antifungal and antibacterial activities (Nenaah, 2010). Methanol extract of the seeds and leaves of *P. harmala* showed antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* (Fatma *et al.*, 2016). In addition, ethanol extract of *P. harmala* can restrain the growth of *Streptococcus pyogenes, Staphylococcus aureus and Staphylococcus epidermidis* (Darabpour *et al.*, 2011). Moreover, *Bacillus subtilis and Proteus vulgaris* are the most sensitive bacteria to the metabolites derived from this plant (Moloudizargari *et al.*, 2013). As well as, ethanolic crude extracts of *P. harmala* seeds from Libya had higher activities against *Salmonella typhi*, *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Ahmed *et al.*, 2021). *P. harmala* also had activity against the *Candida albicans, Aspergillus flavus, Aspergillus fumigates* and *Aspergillus niger* (Saadabi, 2006). Besides, the methanol extract of leaves of *P. harmala* had a greater inhibitory effect than the seeds extract on the *Proteus mirabilis*, *E. coli*, *P. aeruginosa* and *S. aureus* (Fatma *et al.*, 2016).

The main aim of this study is evaluating antibacterial activities of *P. harmala* against *Escherichia coli, Klebsiellae pneumoniae* and *Staphylococcus aureus*, which isolated from urine culture of patients suffered from urinary tract infections. A further objective is to determine which part of plant (leaves or seeds) have a strongest activity against selected bacteria.

2. Methods and Material

2.1 Sample Collection and Processing

The leaves and seeds of *P. harmala* were collected from Umm al-Harmal area, in the city of Alassaba'a -Libya, in July 2022. A botanist (Prof. Sh-hoob Elahmir) authenticated plant identity and a voucher specimen (PH1642) was placed in the miniherbarium of the faculty of sciences, Gharyan University. Plant samples were cleaned with distilled water, then dried for 5 days in oven at $40\,\mathrm{C}^\circ$.

2.2 Preparation of Extracts

Ten grams powder of each sample (leaves and seeds) were dissolved in 150 mL of 98% methanol and placed at room temperature for 24 hours and then extracts were filtered through Whatman No 1 filter paper, concentrated on a vacuum rotary evaporator at $40~\rm C^{\circ}$. Then stored at $4~\rm C^{\circ}$ prior to further analysis.

2.3 Bacterial isolation, identification and preparation of Bacterial Inoculum

Clinical isolates of the following bacteria: E. coli, K. pneumonia and S. aureus were isolated from urine culture of patients in (Alassaba'a hospital, Alassaba'a, west of



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Tripoli) suffered from urinary tract infections during the year 2022. Samples were cultured on fresh media (CLED agar), and incubated for one day at 37C°. The colonies identified based on colonial morphology. Sub-culture on other media such as Eosin-Methylene Blue Agar (EMB) and macconkey agar were also used to confirm the *E. coli*, *K. pneumonia* bacteria. While, Mannitol Salt Agar (MSA) used for *S. aureus*.

After identified the all bacterial strains, two to three colonies of bacteria were transferred into 1 mL of nutrient broth (using a sterile cotton swab and the bacterial suspension vortexed for 10 min and subsequently allowed for the development for one day at 37 C°. Then, 10 μ L of the bacterial suspension was transferred into 10 mL of nutrient broth. The turbidity of inoculum was diluted to approximately to above 10^6 colony-forming unit/mL (CFU/mL) concentrations, utilizing a standard broth microdilution (Rukayadi *et al.*, 2013) and inoculum quantification methods (Indira, 2014). Inoculum quantification was performed by plating 20 μ L of bacterial suspension on MHA and counting the colonies formed after incubation for one day at 37C°.

2.4 Preparation of Standards and Stock Solutions

A10% solution of dimethyl sulfoxide (DMSO) has been used as a negative control in since it has been reported to show no inhibition or killing effect on the bacterial strains used throughout the current research (Rukayadi *et al.*, 2013). The solution was prepared by dissolving 10 mL of 99.9% DMSO (R and M Marketing, Essex, UK) in 90 mL of distilled water. Meanwhile, Vancomycin 10µg (VA) and Streptomycin 5µg (S) were used as the positive standard against gram (+) and gram (-) bacteria, respectively. The stock extracts were primed by dispersing a (10 mg) crude extracts in 100% dimethyl sulfoxide (DMSO; Fisher Scientific, Leicestershire, United Kingdom) to obtain a 100 mg/mL concentration. Additional dilution of all solutions was done employing 1:10 (v/v) sterile deionized distilled water (ddH2O) to manufacture 10 mg/mL stock solution (1.0% concentration). The stocks were put in storage at 4C° until to use.

2.5 Antibacterial Assay

2.5.1 Antibacterial Disc Diffusion Assay

Antibacterial activity of the plant extracts have been evaluated using disc diffusion assay (CLSI, 2003). Briefly, the bacterial inoculum was streaked on the surface of MHA plate using sterile cotton swab. Sterile 6 mm filter paper discs (Whatman, Germany) were pre-wetted with 10 µL aliquots of the test extracts, prepared at a concentration of 10 mg/mL (1%). The discs were subsequently put on the inoculated plates at a good distance from each other. Positive 10µg (VA), 5µg (S) and negative (10% DMSO) control discs were put on the inoculated plate. The test plates have subsequently been incubated for 24 h at 37 C° and observed for clear zones, measured in millimeters, which indicated the prevention of bacterial growth by the test sample.

${\bf 2.5.2~Determination~of~Minimum~Inhibitory~Concentration~(MIC)~and~Minimum~Bactericidal~Concentration~(MBC)~Values}$

The MICs and MBCs were defined as described by CLSI (2003). The MICs and MBCs of 1% (10 mg/mL) methanol extracts of *P. harmala* leaves and seeds against *S. aureus, K. pneumoniae and E. coli* were accomplished in a 96-well microliter plate with two-fold consecutive standard stock microdilution method and bacterial concentration inoculum of approximately 10⁶ CFU/mL. Moreover, the micro titer



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plate has been incubated aerobically at 37 °C for 24 h. MIC is the minimum antibacterial agent concentration, which completely prevents visible growth. MBC for every bacterial species has been determined as outlined for MIC by eliminating the media from every well, which showed no visible growth then sub culturing them on MHA plates. Thus, the plates were later incubated at 37 °C for 24 h until visible growth was seen in control plates. Correspondingly, MBC is identified as comparable concentrations that are required for killing microorganisms completely (Rukayadi *et al.*, 2008). Both the MIC and MBC test have been made in duplicates.

2.6 Statistical Analyses

Windows Excel 2010 was intended for the analysis of antimicrobial outcome data. The results were expressed as mean \pm SD of 3 replicates.

3. Results

The colonies identified based on colonial morphology on CLED agar as follows; *E. coli* appeared with large elevated, yellow with center more intense yellow, while *K. pneumonia* extremely mucoid colonies varying in color from yellow to whitish-blue and yellowish medium. On the other hand, *S. aureus* appeared with deep yellow colonies about 0.75 mm (Figure 1). In addition, sub-culture on other media such as Eosin-Methylene Blue Agar (EMB) used to confirmed. *E. coli*, which grow with a metallic green sheen with a dark center. Whilst, *K. pneumoniae* had large mucoid dark pink colony on Macconkey agar. Furthermore, *S. aureus* on Mannitol Salt Agar (MSA) displayed a yellow (Figure 2). In addition, some chemical tests were used to identify these bacteria such as catalase and coagulase test for *S. aureus*, which gave positive results of both tests. As well as, *E. coli* gave positive results of catalase test a long with *k. pneumoniae*.

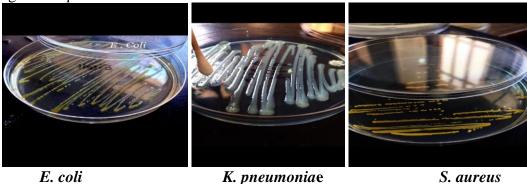
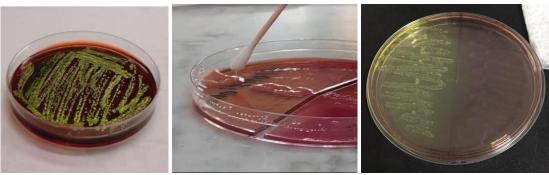


Figure 1: Isolated the bacterial tests



E. coli on(EMB) K. pneumoniae on (Macconkey agar) S. aureus on (MSA) Figure 2: identified the bacterial tests on different types of media



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Findings of the antibacterial disc diffusion test on the crude methanolic extracts of P. harmala against E .coli, K. pneumoniae and S. aureus are given in Table 1 and Figure 3 . From the results, it has been found that the seed extract more effective against the bacterial strains compared to leaves extract.

Table 1: Disc diffusion of methanol extract of *P. harmala* against *E. coli*, *K. pneumonia* and *S.aureus*.

Bacteria	Mean diameter of inhibition zone (mm)			
	Control (+)	Leaves	Seeds	
E. coli	16.5 ±0.70	11.00±1.00	14.66±0.57	
K. pneumonia	18.5 ±0.70	9.66±0.57	12.00±1.00	
S. aureus	14.00±0.00	10.00±1.00	15.33±0.57	

All extracts were tested at 1% concentration. The diameter of inhibition zones in mm (including disc). Results were expressed as means \pm standard deviation. Significant differences in means (triplicate).







Figure 3: Disk diffusion results of *P. harmala* leaves and seeds extract against tested bacteria

In general, the disc diffusion assay showed that the 1% crude methanolic extract of *P. harmala* seeds with the higher effects against all bacterial, which used in this study compared to leaves extract. The inhibition zones of seeds extract were, 14.66, 12.00 15.33mm against *E. coli*, *k. pneumonia* and *S. aureus* respectively. While the inhibition zones of leaves extract were 11.00, 9.66 and 10.00mm against, *E. coli*, *k. pneumoniae* and *S. aureus* respectively.

Based on the inhibitory zone diameters, also the susceptibility of, *E. coli*, *k. pneumoniae S. aureus*, and *P. harmala* crude extracts were assessed on the basis of their minimum inhibitory concentration (MIC) and their minimum bactericidal concentration (MBC) values. The MIC and MBC values are given in Table: 2 and figure: 4 and 5.

Table 2: Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

Type of	Leaves		Seeds	
bacteria	MIC	MBC	MIC	MBC
E. coli	2.5	5	1.25	5
K. pneumoniae	1.25	2.5	1.25	5
S. aureus	1.25	2.5	0.625	1.25



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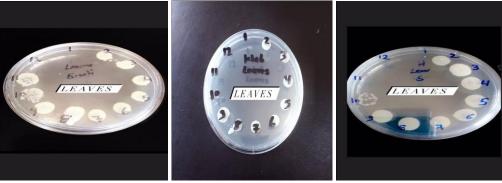


Figure 4: Minimum bactericidal concentration (MBC) of *P. harmala* leaves extract against tested bacteria



Figure 5: Minimum bactericidal concentration (MBC) of *P. harmala* seeds extract against tested bacteria

Current research demonstrated that the outcome leaves extract of *P. harmala* with MICs ranging between 1.25 to 2.5 mg/mL, while the MICs of seeds extract ranging between 0.625 to 1.25 mg/mL. However, the results of MBCs for the leaves extract ranging between 2.5 to 5 mg/mL and the MBCs of seeds extracts ranging between 1.25 to 5 mg/mL.

4. Discussion

Uurinary tract infection is one of the common infections. Recently, there has been an increase in the incidence of resistant organisms causing urinary tract infection (Fatma *et al.*, 2016). The extracts of medicinal plants are used for their antimicrobial properties in many parts of the world, and the antibacterial action of the plants is poorly understood and remains in debate (Fatma *et al.*, 2016).

In this study, the antibacterial activity of 1% leaves and seeds extracts of *P. harmala* against tested bacteria mentioned previously was determined the disk diffusion, MICs and MBCs values. 1% seeds extract of *P. harmala* had a greater effects against all tested bacteria included *E. coli, K. pnemoniae* and *S. aureus* compared to leaves extract at the same concentration. In addition, the seeds extracts can inhibit the growth of all tested bacteria with a lowest MICs and MBCs value compared to leaves extract. Mostafa et al. (2018) reported that the difference in MIC of plant extracts is because due to the unstable nature of chemical components. *P. harmala* extracts are reported to contain many compounds included alkaloids, flavonoids and anthraquinones (Bensalem *et al.*, 2014). Besides, harmine, harmaline, harmalol, harmol, harmalol peganine and β-carboline have been isolated from different parts of this plant. Most of



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these compounds had antimicrobial activities against many types of bacteria (Asgarpanah & Ramezanloo, 2012). Thus, the antibacterial activity of methanol extract of *P. harmala* seeds and leaves in this study might be linked to the high quantity of different compounds in the both extracts. In addition, their antimicrobial effect can be connected to their different ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins, may also disrupt microbial membranes (Fatma *et al.*, 2016).

There were several reports concerning the susceptibility of many types of bacteria to antimicrobial of different solvent extracts of this plant.

Fatma et al. (2016) described the effect of aqueous extract of leaves and seeds of P. harmla from Algeria on urinary tract infection pathogens including E. coli, S. aureus, P. mirabilis and P. aeruginosa with inhibition zones from leaves 10.00, 5.00, 11.00 and 5.00 mm respectively, and inhibition zones from seeds 5.00 against all bacteria. Additionally, Mohsenipour et al. (2016) reported antimicrobial activity of methanol extract of P. harmla seed and stem against S. aureus, B. cereus and E. coli with inhibition zones of 12.3±1.1, 12±1.4 and 10.4±1.2 mm respectively, which were less than of current study results. On the other hand, methanol extract of P. harmala leave had low antimicrobial activity against Salmonella species and E. coli, with inhibition zones of 8.00 to 10.00 mm, while no effect against S. aureus and B. subtilis (Muhaisen et al., 2016). Furthermore, Khademalhossini et al. (2015) stated that ethanol extract of P. harmala seed was inhibited the growth of S. aureus, E. coli and K. pneumoniea with inhibition zones 13.0 mm, 13.0 and 11.5 mm respectively. Whiles, Ahmed et al. (2021) have reported that, ethanol extract of P. harmala was inhibited the growth of S. aureus, S. epidermidis and E. coli with inhibition zones of 12.33 ± 2 , 13.66 ± 3 and 10.00 ± 2 mm respectively, these results were less than of current study results.

On the other hand, Ahmed et al. (2021) have been reported antimicrobial activity of ethanolic extract of Libyan *P. harmala* against *Salmonella typhi* ATCC 14028, *E. coli* ATCC 25923, *B. subtilis* ATCC 6633 and *S. aureus* ATCC 29213 with MIC values ranging from 0.019 to 0.312 mg/mL and MBC values varied among 0.78 to 0.625 mg/mL. Methanol extract of *P. harmla* seed and stem from Iran inhibited also the growth of *S. aureus*, *B. cereus*, *E. coli* and *K. pneumoniae* with a MIC values of 0.039, 0.156, 0.156 and 0.312 mg/mL respectively, and MBC values of 0.78, 0.312, 0.625 and 1.25 mg/mL respectively (Mohsenipour & Hassanshahian, 2016). Such concentrations have been less than obtained values in the current research.

Moreover, the methanol extract of *P. harmala* seeds significantly suppressed the growth of *E. coli and S. typhi* with MIC values 0.625 mg/mL (Darbpour *et al.*, 2011). Additionally, Khademlhosseini et al. (2015) described that ethanol extract of *P. harmala* seeds inhibited the growth of *E. coli, K. pneumoniae, S. typhimurium, S. aureus, M. luteus, C. pseudotuberculosis* and *S. pneumonia*, with MIC values ranging between 0.68-1.3 mg/mL, and MBC values varied among 1.3-5 mg/mL.

In addition, ethanol extract of Libyan *P. harmala* inhibited the growth of *E. coli, B. subtilis* and *S. aureus* with MIC values of 31.25 , 1.95 and 15.62 mg/mL respectively, while the MBC values of 62.50 , 7.80 and 31.25 mg/ml, respectively (Alabeed, 2021). This study will be continued by other studies to identify the chemical compounds of *P. harmala* seeds extract .



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The results of current study showed *P. harmala* seeds extract as a potential source of antimicrobial drug against the most common urinary pathogens. This is particularly important in the fight against the recent resistant organisms with multiple drugs. These results suggest that future researches should be done to investigate the in vivo activity of this plant, toxicity and thus to determine the pharmalogical activity in the seeds of this plant which have the greater antibacterial effect.

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