ISSN 2449-8947 MicroMedicine *Original Article*

**DOI:** http://dx.doi.org/10.5281/zenodo.3473381

# Antibacterial activity of methanol extract of *Lawsonia inermis* against uropathogenic bacteria

**Salim Shahabinejad, Ashraf Kariminik***

Department of Microbiology, Kerman Branch, Islamic Azad University, Kerman, Iran

* Correspondence: a.kariminik@iauk.ac.ir

**Received:** 15 May 2019; **Revised submission:** 28 August 2019; **Accepted:** 23 September 2019

http://www.journals.tmkarpinski.com/index.php/mmed



**ABSTRACT:** Urinary tract infections one of the most common bacterial diseases caused by microbes such as bacteria overcoming the body's defenses in the urinary tract. Extensive studies are needed to identify the bacteria of the infectious agent and to determine the pattern of drug resistance and the identification of effective drugs for proper treatment. Therefore, the purpose of this study was to determine the antibiotic resistance pattern of urinary pathogens and compare it with methanol extract of *Lawsonia inermis*. Urine samples were collected using the mid-stream "clean catch" technique and examined bacteriologically using standard procedures. The antibiotic resistance pattern of each uropathogen isolated was carried by Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute guidelines. Methanol extract of the plant was prepared by maceration method and its inhibitory effect on uropathogen isolates studied using well diffusion assay. Based on obtained results, the most common uropathogen isolated was *Escherichia coli* and the most frequent uropathogens were Gram negative rod bacteria. Most resistant antibiotics were Cefazolin, Ampicillin, Vancomycin and Nitrofurantoin. Methanol extract of *Lawsonia inermis* showed good antibacterial potential against all uropathogen bacterial isolates. The results suggest that the *Lawsonia inermis* possess antibacterial properties that support the folk medicinal use of this plant.

**Keywords:** Urinary tract infection; Antibacterial activity; Antibiotic; *Lawsonia inermis*.

## 1. INTRODUCTION

Urinary tract infections are one of the most common infections that affect every human being several times throughout life [1]. Bacteria infecting urinary tract attack urethra and bladder with a compromised body defense mechanism and reduced urine flow. Some of the most important clinical symptoms are pain, frequent urination, burning, lack of transparency of urine, nocturia and haematuria [2]. Despite the presence of appropriate antibiotics, the prevalence of antibiotic resistance is steadily increasing.The traditional medicinal methods, specially the use of medicinal plants, still play an important role to cover the basic health needs in the whole world. Therefore, research in order to find new sources of natural medicine is necessary. Hence, an attempt has been made to evaluate antibacterial activity of henna folklore medicinal plant in Kerman province, Iran, against urinary tract pathogen. *Lawsonia inermis* (Lythraceae family) is a much branched glabrous plant or small tree (2-6 m in height), cultivated for its leaves although stem bark, roots, flowers and

seeds have also been used in traditional medicine [3]. *Lawsonia inermis* is known to have therapeutic properties [4]. This plant is one of the native plants of Kerman province in Iran. In the present investigation, we studied the effect of *Lawsonia inermis* methanol extract on several bacteria causing urinary tract infections.

## 2. MATERIALS AND METHODS

### 2.1. Bacterial isolation, identification and antibiogram

Urine specimens obtained from adult patients via the clean-catch midstream method. Urine culture and isolation of uropathogens was performed by a surface streak technique on both blood and Eosin methylene blue agar (Merck Company, Germany) using calibrated loops for semi-quantitative method [5].These cultures were incubated aerobically at 37°C for 24 hours. If the concentration of bacteria in the urine was $\geq 10^5$cfu/ml, It was considered positive for UTI [6]. Identification of bacteria was made using biochemical tests, i.e. glucose, lactose, sucrose and mannitol fermentation, $H_2$, $CO_2$ and $H_2S$ production, indole, citrate utilization, oxidase, lysine decarboxylase, urea hydrolysis, catalase and coagulase [7]. The bacteria were grown in the nutrient broth at 37°C for 24 hoursand were placed at 4°C until the tests were carried out. Antibacterial susceptibility of all bacterial isolates was tested by the disk diffusion according to Clinical Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute) on Muller-Hinton media (Merck Company, Germany). The antibiotic discs were: Ceftizoxime, Cefazolin, Co-trimoxazole, Tobramycin, Tetracyclin, Ampicillin, Vancomycin, Kanamycin, Ciprofloxacin, Carbenicillin, Gentamycin, Nitraofurantoin and Nalidixic acid.

### 2.2. Collection of plant and extract preparation

The test plant, *Lawsonia inermis* from tropical regions of Kerman city, Iran was collected. Plant fresh leaves was washed with water thoroughly, air dried in room temperature and then homogenized to fine powder and stored in airtight containers [8]. For preparation of methanol extract by maceration method, 50 gram of the powdered plant was soaked separately in 500 ml of methanol (Merck Company) for 7 days at room temperature in shaking conditions. Obtained extracts were filtered by Whatman paper No. 1 and then concentrated by rotary evaporator system (Heidolph, Germany) at 42$^o$C [9]. In vitro antibacterial activity of the extract was screened for the antibacterial activity by Agar well diffusion assay [10]. For all the bacterial isolates, overnight cultures grown in broth were adjusted to an inoculum concentration of $1.5x10^8$ CFU/ml for inoculation of the agar plates as described by Nalubega et al. A lawn of the bacterial isolates obtained was made on Mueller-Hinton agar (Merck Company, Germany) plates with depth of 4 mm by sterile cotton swabs [11]. 20 mg/ml concentration of the crude methanol extract in DMSO:Methanol (1:1 V/V) solvent was prepared [12]. Wells in 6 mm diameter were punctured in the media by sterile cork borers and filled with 20 µl of the methanol extract of *Lawsonia inermis*. The DMSO:Methanol (1:1 v/v) solvent solution has no antibacterial properties and therefore considered as a negative control in the tests.The plates were then incubated at 37˚C for 24 hours. Following incubation, antibacterial activity was evaluated by measuring the inhibition zones around each of the wells in mm [13]. All tests were done in triplicate.

## 3. RESULTS

A total of 50 bacterial isolates were obtained from patients with urinary tract infections referring to Kerman hospitals, which include *Staphylococcus aureus* (1 isolate), *Escherichia coli* (24 isolates), *Klebsiella pneumoniae* (5 isolates), *Proteus mirabilis* (8 isolates), *Proteus vulgaris* (1 isolate), *Pseudomonas aeruginosa*

(9 isolates), *Enterobacter aerogenes* (1 isolate) and *Acinetobacter* (1 isolate) based on diagnostic and biochemical identification tests. Antibacterial activity of methanol extract of *Lawsonia inermis* was compared with Ceftizoxime, Cefazolin, Co-trimoxazole, Tobramycin, Tetracyclin, Ampicillin, Vancomycin, Kanamycin, Ciprofloxacin, Carbenicillin, Gentamycin, Nitrofurantoin and Nalidixic acid as broad spectrum antibacterial agent. A comparison of plant extract with the antibiotics and bacterial pathogens causing urinary tract infection was given in Table 1. The results were represented as average inhibition zone in mm of all the isolates of individual species. Variance analysis of used antibiotics and herbal extract against bacteria isolated from urine specimenswas presented in Table 2.

**Table 1.** Comparison of resistance pattern of bacterial isolates from urine culture and *Lawsonia inermis* methanol extract.

| No. | Antibiotics / Bacteria | LE | CT | CZ | SXT | TOB | TE | AM | V | K | CP | CB | GM | FM | NA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *P. mirabilis* | 20 | 18 | - | - | - | 18 | - | - | 18 | 30 | 20 | 20 | 10 | 15 |
| 2 | *P. mirabilis* | 12 | 15 | | 27 | 18 | 10 | - | - | 18 | 23 | 20 | 18 | - | 13 |
| 3 | *P. mirabilis* | 20 | 20 | 10 | 18 | 30 | 12 | 15 | 22 | 15 | 30 | 22 | 30 | - | - |
| 4 | *P. mirabilis* | 20 | 12 | 10 | 18 | 20 | - | - | - | 20 | 22 | 20 | - | - | 15 |
| 5 | *P. mirabilis* | 18 | 18 | - | 18 | 18 | - | - | - | - | 18 | 10 | 22 | - | - |
| 6 | *P. mirabilis* | 30 | 12 | - | 16 | 17 | - | 15 | - | - | 20 | 12 | - | - | - |
| 7 | *P. mirabilis* | 12 | 20 | - | | 18 | - | | - | - | 18 | 17 | 17 | - | 14 |
| 8 | *P. mirabilis* | 18 | 20 | - | - | 20 | - | 10 | - | - | 27 | 25 | 22 | - | 12 |
| 9 | *E. coli* | 18 | 10 | - | 30 | 15 | 15 | 12 | 19 | 15 | - | 15 | 12 | 10 | - |
| 10 | *E. coli* | 20 | 20 | - | 30 | 20 | - | - | - | 20 | 25 | 18 | 20 | - | 18 |
| 11 | *E. coli* | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 12 | *E. coli* | 18 | 34 | 16 | 32 | 23 | 20 | - | 15 | 15 | 25 | 10 | 30 | - | - |
| 13 | *E. coli* | 15 | 18 | - | 18 | 17 | 12 | 10 | - | 15 | 25 | 23 | 15 | - | 15 |
| 14 | *E. coli* | 22 | 20 | - | - | 22 | 15 | - | - | 15 | 20 | 17 | 15 | - | 15 |
| 15 | *E. coli* | 15 | - | - | 15 | 18 | 10 | - | - | 22 | 22 | 18 | 23 | - | 10 |
| 16 | *E. coli* | 15 | 15 | - | 20 | 15 | 10 | - | - | 17 | 25 | 18 | - | - | 12 |
| 17 | *E. coli* | 20 | - | - | - | 20 | 10 | 10 | - | 18 | 30 | 25 | 18 | - | 17 |
| 18 | *E. coli* | 22 | 15 | - | 18 | 18 | - | - | - | 15 | | 19 | 18 | - | - |
| 19 | *E. coli* | 30 | 12 | - | 15 | 18 | - | - | - | 15 | 18 | 17 | 15 | | 12 |
| 20 | *E. coli* | 20 | - | - | 17 | 15 | - | - | - | - | - | - | - | - | - |
| 21 | *E. coli* | 15 | 12 | 15 | 15 | 30 | 15 | 18 | 18 | | 12 | 20 | 30 | - | - |
| 22 | *E. coli* | 20 | - | 15 | - | - | - | - | 12 | - | - | - | - | - | - |
| 23 | *E. coli* | 30 | 18 | - | - | 17 | - | - | - | 15 | 30 | 22 | 17 | - | 17 |
| 24 | *E. coli* | 15 | 20 | - | - | 18 | 10 | - | - | 15 | 25 | 18 | 17 | - | 17 |
| 25 | *E. coli* | 30 | - | - | - | 15 | - | - | 12 | - | - | - | 10 | - | - |
| 26 | *E. coli* | 25 | 20 | 12 | 12 | 17 | 18 | 12 | 17 | 18 | 23 | 18 | 15 | - | - |
| 27 | *E. coli* | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 28 | *E. coli* | 30 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 29 | *E. coli* | 18 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 30 | *E. coli* | 18 | 20 | - | - | 20 | - | - | - | 17 | 25 | 20 | 17 | - | 15 |
| 31 | *E. coli* | 18 | 20 | - | 22 | 30 | 15 | | 12 | - | 15 | | 12 | - | - |
| 32 | *E. coli* | 15 | - | - | - | - | - | - | - | - | 20 | - | - | - | - |
| 33 | *P. aeruginosa* | 15 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 34 | *P. aeruginosa* | 16 | 15 | - | 15 | 15 | - | - | - | - | - | - | - | - | - |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | *P. aeruginosa* | 18 | - | - | - | 20 | - | - | - | - | 20 | 12 | 17 | - | - |
| 36 | *P. aeruginosa* | 20 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 37 | *P. aeruginosa* | 18 | 17 | - | 20 | - | - | - | - | 25 | | 15 | 15 | - | - |
| 38 | *P. aeruginosa* | 25 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 39 | *P. aeruginosa* | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 40 | *P. aeruginosa* | 15 | - | - | 15 | 20 | - | - | - | - | - | - | - | - | - |
| 41 | *P. aeruginosa* | 15 | - | - | - | - | - | - | - | - | 20 | - | - | - | 15 |
| 42 | *K. pneumoniae* | 10 | - | - | 15 | - | - | - | - | - | - | - | - | - | - |
| 43 | *K. pneumoniae* | 12 | 17 | - | 20 | 17 | - | - | - | 20 | 30 | 22 | 18 | 10 | 16 |
| 44 | *K. pneumoniae* | 18 | 25 | - | 22 | 30 | 18 | 10 | - | 38 | 30 | 30 | 28 | 12 | 30 |
| 45 | *K. pneumoniae* | 15 | 18 | - | 22 | 18 | - | - | - | 20 | 30 | 30 | 25 | - | 17 |
| 46 | *K. pneumoniae* | 12 | 20 | - | 30 | 18 | 15 | - | - | 15 | 17 | 17 | 15 | - | 15 |
| 47 | *S. aureus* | 20 | 10 | - | 20 | 25 | - | - | 15 | 25 | 26 | 10 | 30 | - | - |
| 48 | *Acinetobacter* | 25 | 18 | - | 18 | 30 | - | - | - | 25 | 30 | 30 | 30 | - | - |
| 49 | *E. aerogenes* | 15 | - | 12 | 22 | 34 | - | - | 12 | 18 | 22 | - | 30 | - | - |
| 50 | *P. vulgaris* | 25 | 15 | - | - | 15 | - | - | - | 15 | 22 | 17 | 17 | - | 15 |

CT: Ceftizoxime, CZ: Cefazolin, SXT: Co-trimoxazole, TOB: Tobramycin, TE: Tetracyclin, AM: Ampicillin, V: Vancomycin, K: Kanamycin, CP: Ciprofloxacin, CB: Carbenicillin, GM: Gentamycin, FM: Nitraofurantoin, NA: Nalidixic acid, ME: Methanol extract *of Lawsonia inermis.*

**Table 2.** Analysis of variance of used antibiotics and herbal extract against bacteria isolated from urine specimens.

ANOVA IZ

| | **Sum of squares** | **df** | **Mean Square** | **F** | **Sig** |
|---|---|---|---|---|---|
| Between Groups | 2642727 | 13 | 203.287 | 8.190 | 000 |
| Within Groups | 8017.113 | 323 | 24.821 | | |
| Total | 10659.840 | 336 | | | |

Multiple comparisons

Dependent variable: IZ, Dunett t (2-sided)[a]

| (I) antibiotic | (J) antibiotic | Mean difference (I-J) | Std. error | Sig. | 95% confidence interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| NA | ME | -3.38 | 1.30 | .103 | -7.11 | .34 |
| FM | ME | -8.86* | 2.59 | .009 | -16.30 | -1.42 |
| GM | ME | 1.08 | 1.13 | .990 | -2.16 | 4.32 |
| CB | ME | .11 | 1.13 | 1.000 | -3.13 | 3.35 |
| CP | ME | 4.62* | 1.12 | .001 | 1.41 | 7.84 |
| K | ME | -56 | 1.19 | 1.000 | -3.98 | 2.86 |
| V | ME | -3.46 | 1.73 | .394 | -8.42 | 1.50 |
| AM | ME | -6.42* | 1.80 | .005 | -11.60 | -1.23 |
| TE | ME | -4.92* | 1.43 | .008 | 09.03 | -.81 |
| TOB | ME | 1.86 | 1.09 | .621 | -1.27 | -4.99 |
| SXT | ME | 1.14 | 1.18 | .988 | -2.24 | -4.52 |
| CZ | ME | -6.00* | 2.01 | .036 | -11.78 | -.23 |
| CT | ME | -1.48 | 1.13 | .891 | -4.73 | 1.76 |

* The means difference is significant at the 0.05 level.

a. Dunnett t tests treat one group as a control, and compare all other groups against it.

## 4. DISCUSSION

Increase of resistance by the pathogenic bacteria to chemotherapeutic agents appears to be a continuous process since the discovery of antibiotics [14]. Researchers have recognized the importance and power of medicinal plants as potential sources of antibacterial herbal remedies to compete with antibiotics that may also be of lower cost and minor toxicity [15]. Urinary tract infection is one of the essential causes for seeking medical attention in the community [16]. The correct and proper treatment of patients with urinary tract infection depends on the type of organism involved in the infection and the selection of an effective antibiotic agent to the organism and the choice of appropriate antibiotics to the organism [17]. The present study investigated the antibacterial potential of medicinal plant, *Lawsonia inermis* methanol extract against common gram negative and gram-positive urinary tract pathogens. Antibacterial activity of the extract was compared with common antibiotics used in urinary tract infections. *Escherichia coli* was the main etiological agent in causing Urinary tract infection and the most frequent uropathogens were Gram negatives which made up 98% of all the isolates. Most resistant antibiotics were Cefazolin, Ampicillin, Vancomycin and Nitrofurantoin. Methanol extract *of Lawsonia inermis* showed good antimicrobial potential against all uropathogen bacterial isolates. Efforts have been devoted over the past years to the search of new antibacterial constituents from natural sources [18]. Habbal et al. showed Henna samples demonstrated antibacterial activity against all *Pseudomonas aeruginosa* isolates [4]. In this study, the well diffusion method was used. It is certainly the most suitable way of evaluating the antibacterial effects of plant extracts because the extracts can diffuse more easily into the culture media. Results of Valgas et al. showed that agar well diffusion method proved to be more sensitive than disc diffusion method [10]. *Lawsonia inermis* (Henna) is widely used in Iran, such as its application in cosmetic, fever treatment, anti-inflammatory and for treating mouth ulcers [4, 19]. In conclusion, our results showed that the plant extract of methanol extract *of Lawsonia inermis* possesses potential antibacterial activity against uropathogen bacteria and the extract can be considered as a good candidate for in vivo treatment of urinary tract infections.

**Authors Contributions:** SS: performed experiments; AK: designed and performed experiments and wrote the paper. All authors read and approved the final manuscript.

**Conflict of Interest:** The authors declare no conflicts of interest.

## REFERENCES

1. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med. 2002; 113(1): 5-13.
2. Mishra MP, Rath S, Swain SS, Ghosh G, Das D, Padhy RN. In vitro antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria. J King Saud Univ Sci. 2017; 29(1): 84-95.
3. Borade AS, Kale BN, Shete RV. A phytopharmacological review on *Lawsonia inermis* (Linn.). Int J Pharm Life Sci. 2011; 2(1): 536-534.
4. Habbal O, Hasson S, El-Hag A, Al-Mahrooqi Z, Al-Hashmi N, Al-Bimani Z, et al. Antibacterial activity of *Lawsonia inermis* Linn (Henna) against *Pseudomonas aeruginosa*. Asian Pacific J Trop Biomed. 2011; 1(3): 173-176.

5. Beyene G, Tsegaye W. Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in jimma university specialized hospital, southwest Ethiopia. Ethiopian J Health Sci. 2011; 21(2): 141-146.
6. Sahoo S, Rout B, Mekap S, Dhal N. Antimicrobial activity of *Acalypha godseffiana* against selected urinary tract infection causing pathogens. J Pharmacogn Phytochem. 2014; 3(4): 83-85.
7. Bonadio M, Meini M, Spitaleri P, Gigli C. Current microbiological and clinical aspects of urinary tract infections. Eur Urol. 2001; 40(4): 439-445.
8. Shakiba M, Kariminik A, Parsia P. Antimicrobial activity of different parts of *Phoenix dactylifera*. Int J Mol Clin Microbiol. 2011; 1: 107-111.
9. Shahidi Bonjar G. Screening for antibacterial properties of some Iranian plants against two strains of *Escherichia coli*. Asian J Plant Sci. 2004; 3(3): 310-314.
10. Valgas C, Souza SMd, Smânia EF, Smânia Jr A. Screening methods to determine antibacterial activity of natural products. Braz J Microbiol. 2007; 38(2): 369-380.
11. Nalubega R, Kabasa JD, Olila D, Kateregga J. Evaluation of antibacterial activity of selected ethnomedicinal plants for poultry in Masaka district, Uganda. Res J Pharmacol. 2011; 5(2): 21-18.
12. Shahidi Bonjar G, Kariminik A, Heidari M, Ghasemzadeh M, RashidFarrokhi P, Moein M, et al. Anti-Pseudomona and antibacilli activity of some medicinal plants of Iran. Daru. 2003; 11(4): 157-163.
13. Shahidi Bonjar GH, Aghighi S, Karimi Nik A. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran. J Biol Sci. 2004; 4(3): 405-412.
14. Bollenbach T. Antimicrobial interactions: mechanisms and implications for drug discovery and resistance evolution. Curr Opin Microbiol. 2015; 27: 1-9.
15. Joshi B, Sah GP, Basnet BB, Bhatt MR, Sharma D, Subedi K, et al. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). J Microbiol Antimicrob. 2011; 3(1): 1-7.
16. Foxman B. The epidemiology of urinary tract infection. Nature Rev Urol. 2010; 7(12): 653.
17. Dielubanza EJ, Schaeffer AJ. Urinary tract infections in women. Med Clin. 2011; 95(1): 27-41.
18. Cheesman MJ, Ilanko A, Blonk B, Cock IE. Developing new antimicrobial therapies: Are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution? Pharmacogn Rev. 2017; 11(22): 57.
19. Chaudhary G, Goyal S, Poonia P. *Lawsonia inermis* Linnaeus: a phytopharmacological review. Int J Pharmac Sci Drug Res. 2010; 2(2): 91-98.