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Comparison of Antimicrobial Effect of *Pistacia atlantica* with Antibiotics in Urinary Tract Infection

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Abstract

Background: *Enterobacteriacea* are able to cause urinary tract infections, and *Escherichia coli* (*E. coli*) and *Klebsiella pneumonia* are the most frequently isolated pathogens from urinary samples. Untreated urinary tract infections can cause upper and lower urinary tract infections. These are among the most common infectious diseases in children. Septicemia and antibiotic resistance of urinary bacteria are the most important concerns in the developed and developing countries. One of the solutions for antibiotic resistance is herbal therapy, which as a branch of traditional medicine, has served an important role in disease treatment during the past century. The advantages of herbal therapy include minimum complications, cost-effectiveness, and high popularity. Herbalism has had a significant role in disease treatment. In this study, the antimicrobial effect of *Pistacia atlantica* was investigated in 2014.
Methods: This cross-sectional study was performed on 150 patients with positive urinary cultures in Imam Ali Hospital of Zahedan, Iran. All the cases of positive culture during three months were investigated. Extraction of ethanolic extract of *Pistacia atlantica* was performed by a rotary device. Firstly, the antibacterial effect of *Pistacia atlantica* was assessed with the disk diffusion method at four concentrations, and then for bacteria that were sensitive to this extract, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Antibacterial effect of *Pistacia atlantica* ethanolic extract on urinary tract infection was compared with some conventional antibiotics, and the data were analyzed using Chi-square test in SPSS.
Results: *E. coli*, *Klebsiella pneumonia*, and *Staphylococcus aureus* were isolated from urinary samples. Ethanolic extract of *Pistacia atlantica* created maximum non-growth halo at the concentration of 100 mg/mL, but at the 12.5 and 25 mg/mL concentrations the extract created minimum non-growth halo, indicating the bacteria were resistant in these concentrations. Overall, 27 of 150 bacteria showed sensitivity to *Pistacia atlantica* at the 50 and 100 mg/mL concentrations. The most sensitive bacteria to this extract were *E. coli* and *Staphylococcus aureus*, respectively.
Conclusions: The results presented the antibacterial effect of ethanolic extract of *Pistacia atlantica* is higher at higher concentrations.

Keywords: *Pistacia atlantica*, Urinary Tract Infections, Antibiotics, *E. coli*

1. Background

Urinary tract infections are among the most common infectious diseases in children (1, 2). It is also the second most prevalent infection, and about 150 million patients annually contract urinary system infections, and 6 billion dollars is annually spent due to this infection in the United States (3, 4). This infection is a common cause of fever and hospitalization in the pediatric population (5). *Enterobacteriaceae* (i.e., *Citrobacter*, *E. coli*, *Enterobacter*, *Klebciella*, *Proteous*, *Salmonella*, *Shigella*, and *Yersinia*) *Enterococcus*, *Streptococcus* groups A, B, and *Staphylococcus saprophyticus* can cause urinary tract infections (6-8).

E. coli is the most common etiology of urinary system infections (40%) in hospitalized patients, and the prevalence rates of *Enterococcus*, *Pseudomonas*, and *Candida* are increasing (9). Treatment of primary infection in newborns is intravenous ampicillin and gentamycin, while cephalosporins (cefotaxime) are used for children (10). Fluoroquinolones are the preferred drugs in urinary system infections, but there is concern regarding the antibiotic resistance of this drug (11-14).

A solution for resistance to chemical drugs is herbal therapy. Herbal therapy as a branch of traditional medicine has played an important role in disease treatment during the past century. Herbal therapy has minimum complications and is cost-effective and widely accepted.

One of the useful herbal medicines is *Pistacia atlantica*, which includes phenolic compounds. *Pistacia atlantica* is a tree growing to a height of 2 - 7 meters in mountains. In fall, it is orange and the name of its fruit is Baneh in Persian, Chatlangoosh in Turkish, and Hobb al khazra in Arabic. The blossom of this tree is red and used as a dye. Trunk of this tree has a kind of gum that is named *saghez* in Persian, and it is used as chewing gum. *Pistacia atlantica* is just like small pistachio and it is mostly found in Iran's mountains (Kohkolieh and Boyerahmad, Ilam, Kordestan, Fars, Kerman, Balouchestan, Yazd, Semnan, and Lorestan provinces and Alamot mountains) (11). *Pistacia atlantica* has medical and industrial applications.

Antibacterial effects of ethanolic extract of *Pistacia atlantica* against *E. coli*, *Klebsiella pneumonia*, *Pseudomonas*, and *Staphylococcus epidermis* have been established.

2. Methods

This cross-sectional study was conducted over a period of three months (2014) at the Biochemical Laboratory of Imam Ali Hospital, Zahedan, Iran. The study was approved by the Ethics committee of Zahedan University of Medical Sciences, Zahedan, Iran.

A total of 500 urinary samples were examined, 160 of the 500 specimen were positive. Culture medium was blood agar. Isolated bacteria from positive urinary cultures were detected by gram staining and biochemical tests like triple sugar iron agar, sulfide, indole, and motility medium, and methyl red and Voges-Proskauer coagulase. Ten samples were discarded because of mix growth of bacteria and the rest of 150 samples were sent to a microbiologist for the assessment of antibacterial effect of *Pistacia atlantica*.

In this study, core of the fruit (*Pistacia atlantica*) was crushed and ground after washing with distilled water and drying of the fruits (15). Then, 50 g of *Pistacia atlantica* powder was put into a decanter, and then hot ethanol 70% was added step by step until all the powder volume was wet. The total time for the extraction procedure was 24 hours and the powder absorbed ethanol during 24 hours. Separation was performed after extraction using a rotary device. The extraction procedure was performed in Zahedan University and 5 g of *Pistacia atlantica* extract was produced. The extract was sterilized by a Millipore syringe filter with a 0.22 μ pore size.

Extract of *Pistacia atlantica* was diluted with physiological serum at the four concentrations of 12.5, 25, 50, and 100 mg/mL. An antimicrobial susceptibility test disc (Padetan Tebb Indus) was used, then 100 μL of the dilutions were added to the plates. Mac Farland standard 0.5 was considered to adjust the turbidity of all the bacterial suspensions (*Staphylococcus aureus*, *E. coli*, and *Klebsiella pneumonia*). Antibiogram discs (100, 50, 25, 12.5 mg/mL) were added to culture media, and light absorbance of the plates was read on ELISA reader at a wavelength of 630 nm after 24 hours. The plates were incubated at 37°C, and antibacterial effects were assessed by non-growth halo. Antibiogram susceptibility test was repeated in triplicate for all the bacteria.

Diameter of non-growth halo lower than 8 mm is considered resistant, 8 - 12 mm semi-sensitive, and more than 12 mm is sensitive (16). The disc containing serum was negative control and the disc with antibiotic the positive control.

At the end, the results were entered into SPSS and analyzed using Chi-square test in SPSS.

3. Results

Of 150 samples, 110 (73.3%) bacteria were *E. coli*, 33 (22%) *Klebsiella pneumonia*, and 7 (4.7%) *Staphylococcus aureus*. In this study, 129 of 150 (80.7%) samples were from women and 31 of 150 (19.3%) were from men.

Among the bacteria, *Staphylococcus aureus* showed the highest resistance to antibiotics, all the *Staphylococcus aureus* strains were resistant to nalidixic acid and gentamycin, whereas 28.6% of them were semi sensitive and 71.4% were sensitive to cefixime and ciprofloxacin, respectively. In case of *E. coli* and *Klebsiella pneumonia*, the most sensitivity was to ciprofloxacin and cefixime.

Determination of the sensitivity and resistance distribution of the isolated bacteria to ethanolic extract of *Pistacia atlantica* was based on the types of bacteria. In this study, antibacterial effect of *Pistacia atlantica* was assessed by using the perfusion method.

Totally, 27 of 150 isolates were sensitive to 100 and 50 mg/mL concentrations of ethanolic extract of *Pistacia*. *Staphylococcus aureus* was 100% resistant to *Pistacia atlantica*, *E. coli* was 51.8% sensitive to *Pistacia atlantica*, and *Klebsiella pneumonia* was 18.5% sensitive to *Pistacia atlantica*, while it was 36.4% semi sensitive and 45.5% resistant to this herb.

The greatest non-growth halo was observed at the 100 mg/mL concertation, and the most sensitive bacteria to this herb were *E. coli* and *Staphylococcus aureus*. There was no significant difference between *Pistacia atlantica* at the concentrations of 50 and 100 mg/mL and nalidixic acid (P = 0.92 and 0.36, respectively; Tables 2 and 3).

Table 1. Comparison of Antibacterial Effects on *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*[a]

	Nalidixic Acid	Cefixime	Ciprofloxacin	Gentamycin
Staphylococcus aureus				
Sensitive	0	5 (71.4)	5 (71.4)	0
Intermediate	0	2 (28.6)	2 (28.6)	0
Resistant	7 (100)	0	0	7 (100)
E. coli				
Sensitive	57 (51.8)	65 (59.1)	66 (60.9)	66 (60)
Intermediate	0	5 (4.5)	20 (17.3)	34 (30.9)
Resistant	53 (48.2)	40 (36.4)	24 (21.8)	10 (9.1)
Klebsiella pneumonia				
Sensitive	6 (18.5)	19 (57.6)	21 (63.6)	13 (39.4)
Intermediate	12 (36.4)	10 (30.3)	7 (21.2)	10 (30.3)
Resistant	15 (45.5)	4 (12.1)	5 (15.2)	10 (30.3)

[a] Values are expressed as No. (%).

Table 2. Comparison of Antibacterial Effects of 100 mg/mL Concentration of *Pistacia atlantica* with Nalidixic Acid

Nalidixic Acid	100 mg/mL Concentration of *Pistacia atlantica*			P Value
	Sensitive	Intermediate	Resistant	
Sensitive	10	14	39	
Intermediate	3	1	8	0.36
Resistant	8	11	56	

Table 3. Comparison of Antibacterial Effects of 50 mg/mL Concentration of *Pistacia atlantica* with Nalidixic Acid

Nalidixic Acid	50 mg/mL Concentration of *Pistacia atlantica*			P Value
	Sensitive	Intermediate	Resistant	
Sensitive	0	4	59	
Intermediate	0	3	9	0.92
Resistant	2	4	69	

There were not significant differences between extract of *Pistacia atlantica* at 50 and 100 concentrations mg/mL and cefixime (P = 0.19 and 0.24, respectively; Tables 4 and 5). Antibacterial effect of ciprofloxacin was more significant than *Pistacia atlantica* at the concentration of 100 mg/mL (P = 0.042; Table 6), and it was not analyzable with Chi-square test. There was no significant difference between *Pistacia atlantica* (concentrations 50 and 100 mg/mL) and gentamycin (P = 0.299, 0.15, respectively; Tables 7 and 8).

4. Discussion

This study showed that the prevalence of positive cultures collected during three months was 32%, 80.7% of which were from women. The prevalence rates of *E. coli*, *Klebsiella pneumonia*, and *Staphylococcus aurous* were 73.3%, 22%, and 4.7%, respectively.

Haider et al. reported the prevalence of urinary system infection to be 17.9% (17). On the other hand, Heidari-Soureshjani et al. estimated a prevalence of 8.8% for this infection, and frequency rates of *E. coli* and *Staphylococcus aureus* infections were 70.27% and 20.27%, respectively (18). In a study by Dromigny et al. *E. coli* was responsible for 70% of cases of urinary system infection (11). In a study by Jazayeri Moghadas the prevalence of urinary system infection was 74.6% in women and 25.4% in men. The prevalence rates of *E. coli*, *Klebsiella pneumonia*, and *Staphylococcus aureus* were 75.5%, 17.4%, and 2.4%, respectively (19).

In a study by Christiaens et al., the prevalence rates of *E. coli* and *Staphylococcus saprophyticus* were 78% and 9%, re-

Table 4. Comparison of Antibacterial Effects of 100 mg/mL Concentration of *Pistacia atlantica* with Cefixime

Cefixim	100 mg/mL Concentration of *Pistacia atlantica*			P Value
	Sensitive	Intermediate	Resistant	
Sensitive	11	16	62	0.19
Intermediate	2	0	15	
Resistant	8	10	26	

Table 5. Comparison of Antibacterial Effects of 100 mg/mL Concentration of *Pistacia atlantica* with Cefixime

Cefixime	50 mg/mL Concentration of *Pistacia atlantica*			P Value
	Sensitive	Intermediate	Resistant	
Sensitive	0	6	83	0.24
Intermediate	0	2	15	
Resistant	2	3	39	

Table 6. Comparison of Antibacterial Effects of 100 mg/mL Concentration of *Pistacia atlantica* with Ciprofloxacin

Ciprofloxacin	100 mg/mL Concentration of *Pistacia atlantica*			P Value
	Sensitive	Intermediate	Resistant	
Sensitive	9	18	66	0.042
Intermediate	9	4	15	
Resistant	3	4	22	

Table 7. Comparison of Antibacterial Effects of 100 mg/mL Concentration of *Pistacia atlantica* with Gentamycin

Gentamycin	100 mg/mL Concentration of *Pistacia atlantica*			P Value
	Sensitive	Intermediate	Resistant	
Sensitive	8	15	56	0.15
Intermediate	11	7	26	
Resistant	2	4	21	

Table 8. Comparison of Antibacterial Effects of 100 mg/mL Concentration of *Pistacia atlantica* with Gentamycin

Gentamycin	100 mg/mL Concentration of *Pistacia atlantica*			P Value
	Sensitive	Intermediate	Resistant	
Sensitive	0	6	73	0.299
Intermediate	2	3	39	
Resistant	0	2	25	

spectively (20), which is in line with our findings. Similarly, in a study by Papapetropoulou et al., the prevalence rates of *E. coli* and *Klebsiella* were reported 77% and 8.7%, respectively (21).

Youssefi et al. reported the prevalence rates of *E. coli*, *Klebsiella pneumonia*, and *Staphylococcus* were 54.2%, 12.1%, and 15.4%, respectively (22). Further, the prevalence of *E. coli* and *Klebsiella pneumonia* were reported 67.5% and 6.6% by Mansouri et al. (23). The results of two these studies were different from ours, which could be due to difference in types of bacteria, geographic factors, lifestyle, and antibiotics use.

It is worth mentioning that 100% of *Staphylococcus* isolates were resistant to nalidixic acid and gentamycin and 71% were sensitive to cefixime and ciprofloxacin in this study. In addition, 48.2% of *E. coli* isolates were resistant to nalidixic acid, 36.4% to cefixime, 21.8% to ciprofloxacin, and 9.1% to gentamycin. Moreover, 51.8% of *E. coli* were sensitive to nalidixic acid, 59.1% to cefixime, 60.9% to ciprofloxacin, and 60% to gentamycin. What is more, 45.5% of *Klebsiella pneumonia* were resistant to nalidixic acid, 12.1% to cefixime, 15.2% to ciprofloxacin, and 30.3% to gentamycin and 57.6% of *Klebsiella pneumonia* were sensitive to cefixime, 63.6% to ciprofloxacin, and 39.4% to gentamycin.

Papapetropoulou et al. showed the sensitivity of *E. coli* to ciprofloxacin, gentamycin, cefixime, and nalidixic acid was 94.8%, 92.3%, 87%, and 75%, respectively, and sensitivity of *Klebsiella pneumonia* to ciprofloxacin, gentamycin, cefixime, and nalidixic acid was 92.6%, 92%, 77.8%, and 84%, respectively (21). Sensitivity of *Staphylococcus coagulase* negative was 86.4% to ciprofloxacin, 45.5% to cefixime, 9.1% to cefixime, and 27.3% to nalidixic acid.

In the study by Youssefi et al. 86% of all bacteria were sensitive to ciprofloxacin, 62% of them to gentamycin, and 36% to nalidixic acid (22). In a study by Urassa, sensitivity of bacteria was 80% to gentamycin and cefixime. In a study by Mansouri et al., sensitivity of bacteria was reported 35% to gentamycin and 30% to nalidixic acid (23).

Heidari-Soureshjani et al. found that pattern of *E. coli* resistance to antibiotics was as follows: 10% to gentamycin, 78% to nalidixic acid, 47% to ciprofloxacin, and 37% to cefixime (18). However, in a study by Vu-Thien, sensitivity of all bacteria was 90% (24). The most important cause of this discrepancy could be consumption of antibiotics.

We did not observe any antibacterial activity at the concentration of 12.5 mg/mL. At the 25 mg/mL concentration, 99.3% of the bacteria were resistant and 0.7% of them were intermediate. At the concentration of 50 mg/mL, 91.3% of the bacteria were resistant, 7.3% of them were intermediate, and 1.3% sensitive.

Mahdavi Meymand studied the mean diameter of non-growth halo of bacteria using *Pistacia atlantica* extract. The mean diameter of non-growth halo for *Klebsiella pneumonia* was reported 13 $\pm$ 0.3 mm, for *E. coli* 16 $\pm$ 0.6 mm, and for *Staphylococcus aureus* 15 $\pm$ 0.39 mm (15).

In a study by Benhammou et al. (25), antibacterial effects of *Pistacia atlantica* extract were proved on *Staphylococcus aureus*. The mean diameter of non-growth halo of this bacterium was 16.5 mm. Ghalem and Mohamed (26), assessed the antibacterial effect of extract of *Pistacia atlantica* on *Staphylococcus* and *E. coli* and proved the antibacterial effect of *Pistacia atlantics*.

Hanafi et al. (16), explained the antibacterial effect of *Pistacia atlantica* on *E. coli* and *Staphylococcus aureus*, the results indicated that the mean diameter of non-growth halo at the concentration of 50 mg/mL for *E. coli* was 11.6 $\pm$ 0.4 mm, and for *Staphylococcus aureus* it was 13.14 $\pm$ 0.32 mm. In this research, MIC for Staphylococcus was 0.6 mg/mL and for *E. coli* it was 5.5 mg/mL, and MBC of *Pistacia atlantica* for *Staphylococcus aureus* and *E. coli* were 20 mg/mL and 80 mg/mL, respectively. In a study by Ben, MIC of *Pistacia atlantica* for *E. coli* was 10 mg/mL.

4.1. Conclusions

Based on the results, antibacterial effect of *Pistacia atlantica* was confirmed at high concentrations, and our findings corroborates those of previous studies.

There are other health benefits to *Pistacia atlantica* as well, and it seems that antibacterial effects of *Pistacia atlantica* gum is a slightly more than in other parts. We recommend further studies on *Pistacia atlantica* because of its multiple health benefits. Assessment of antibacterial effect of other parts of this tree could complete our results.

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