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Antimicrobial activity of *Azadirachta indica and Moringa oleifera* extracts against some pathogenic bacteria isolated from urinary tract infections

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ABSTRACT

Bacterial resistance to antibiotics poses a serious challenge to clinicians and the pharmaceutical industry. Efforts are ongoing to counteract this issue, including extensive screening of medicinal plants from traditional medicine systems to find safer and more effective antimicrobial agents. This study involved collecting 120 clinical urine samples from patients aged 10-60 years with urinary tract infections (UTIs) at Kirkuk Hospital between December 2023 and April 2024. Bacterial isolates were identified using microscopy, morphological analysis, and biochemical tests. Agar diffusion tests were performed to evaluate antimicrobial activity. Results indicated that *E. coli* was the most prevalent bacterium (45.9%), followed by *S. aureus* (20.3%), *K. pneumonia* (14.9%), *P. aeruginosa* (10.8%), and *P. mirabilis* (8.1%). *E. coli*, *K. pneumonia*, and *P. aeruginosa* showed 100% resistance to Ampicillin, while all studied bacteria were highly sensitive to Imipenem and Amikacin. Extracts of *Azadirachta indica* and *Moringa oleifera* demonstrated significant antibacterial activity, with inhibition zones varying based on concentration. At 100 mg/mL, *A. indica* and *M. oleifera* showed inhibition diameters ranging from 20.6 mm to 38.3 mm against different bacterial isolates.

Keywords: A. indica; Opuntia ficus; M. oleifera UTI; E. coli.

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النشاط المضاد للميكروبات لمستخلص نبات الأزاديراشتا إنديكا والمورينجا أوليفيرا ضد بعض البكتيريا المسببة للأمراض المعزولة من عدوى المسالك البولية

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الملخص

تمثل مقاومة البكتيريا للمضادات الحيوية تحديًا خطيرًا للأطباء وصناعة الأدوية. ويجري العمل على معالجة هذه المشكلة من خلال فحص النباتات الطبية من الطب التقليدي للحصول على عوامل آمنة وفعالة كمضادات للميكروبات. جمعت الدراسة 120 عينة إدرار سريرية من مرضى تتراوح أعمارهم بين 10–60 عامًا مصابين بعدوى المسالك البولية في مستشفى كركوك بين ديسمبر 2023 وأبريل 2024. تم تحديد البكتيريا باستخدام الفحص المجهري والتحليل المورفولوجي والاختبارات الكيميائية الحيوية، مع تقييم النشاط المضاد للبكتيريا بطريقة الانتشار في الأجار. أظهرت النتائج أن *E. coli* كانت الأكثر شيوعًا بنسبة 45.9%، تليها *S. aureus* (20.3%)، *K. pneumonia* (14.9%)، *P. aeruginosa* (10.8%)، و *P. mirabilis* (8.1%). أظهرت *E. coli*، و *K. pneumonia*، و *P. aeruginosa* مقاومة كاملة للأمبيسلين (100%)، بينما أظهرت جميع البكتيريا حساسية عالية للإيميبينيم والأميكاسين. أظهرت مستخلصات *Azadirachta indica* و *Moringa oleifera* تأثيرًا مثبطًا للبكتيريا بقطر تثبيط تراوح بين 20.6 ملم إلى 38.3 ملم بناءً على التركيز.

INTRODUCTION

There are numerous physiologically active substances found in plants that may one day be developed into therapeutics. Although the use of herbal medications is already useful [1, 2]. In the quest for novel therapies, plants provide an alternative strategy. In traditional medicine, several different plants are thought to have medicinal and protective qualities [3]. Plants will probably always be a valuable source of novel compounds that, with a little bit of chemical tinkering, could result in brand-new, better medications [4]. Known by its common name, Neem tree, *Azadirachta indica* (Family: Meliaceae) is indigenous to Asia. India has always utilized it as a medical medicine[5]. Medicinal qualities are found in the stem, leaves, bark, and root[6, 7]. Often used as an environmentally benign and economical way to control agricultural pests, neem (*Azadirachta indica*) extract works wonders. A diverse range of chemicals, including glycosides, alkaloids, terpenoids, phenolic compounds, and regular hydrocarbons, make up neem extract [8]. Additionally, harmful microbes can be managed by neem extract. Neem extract may possess antibacterial properties against pathological bacteria when it is cultivated in vitro [9, 10]. *Moringa oleifera* L. leaf exhibits antibacterial, antifungal, antihypertensive, antihyperglycemic, antitumor, anticancer, and anti-inflammatory properties in pharmacological contexts [11]. Antioxidant effects are among the most notable. Tines, steroids, triterpenoids, flavonoids, saponins, interquinones, and alkaloids are among the antioxidants found in *Moringa oleifera* L. leaves, according to phytochemical assays [12]. According to a Fuglie study, the antioxidant power of fresh *Moringa* leaves is seven times greater than that of vitamin C[13]. Quercetin is a kind of flavonoid found in *Moringa*. In comparison to vitamin C and vitamin E, it has antioxidants four to five times greater [14]. So, the aim of this study is elevated the role of *A. indica* and *M. oleifera* extract against certain pathogenic bacteria.

MATERIAL AND METHODS

Preparation of Ethanolic extract

In order to prepare the ethanol extract, new gel was dried in an oven set to 85 0C for 48 hours, and then it was powdered. 200 milliliters of ethanol were filtered through 20 grams of Whattman filter (no. 1) paper, and the filtrate was evaporated until it was completely dry. The extract was ground into a powder and then mixed with distilled water to dissolve it. *A. indica* and *M. oleifera* gel extract were produced in different concentrations to investigate their antibacterial properties.

Preparation of Ethanolic stock solution

A stock solution, denoted by the label, was created by dissolving 0.1 gm of powder in 100 ml of distilled water to get 0.001 gm, or 1 mg/1 ml concentration. To create a 0.1 mg/ml concentration that was utilized as a stock solution, 1 ml was diluted in 100 ml of distilled water. A stock solution was used to prepare several concentrations, including 10, 20, 30, and 40 µg [(15)].

Specimen Collection

Between December 2023 and April 2024, 120 pathological samples were taken from patients who were brought to Kirkuk Hospital in Kirkuk City after seeing a specialist and being referred to the laboratory. The following was a part of the sample collection procedure: Patients with UTIs of both sexes and ages (10–60 years) had their urine samples collected; they were then sent straight to the lab to be placed on the culture media.

Bacterial Identification

Bacteria were diagnosed based on the following aspects:

Morphological diagnosis and media characteristics

Based on the culturing features of the bacteria colonies developing on blood agar, EMB, and MacConkey agar, were diagnosed after incubation for 24 hours at 37 ^{0}C.

Microscopic examination: Bacterial colonies were discovered by the use of a microscope to study the morphological features of the bacteria, particularly how the bacteria interacted the gram stain, which shows the shape and organization of the bacteria.

Biochemical reaction and motility test

Numerous biochemical tests, such as, methyl red, citrate, urease, voges-proskauer, catalase, oxidase, KIA, and indole test, were carried out in order to identify and diagnose bacteria.

Identification of bacteria isolates via VITEK2

For microbiological identification, VITEK 2, the most recent colorimetric technology generation, is the gold standard. Procedure: The subsequent steps were carried out in compliance with the instructions supplied by the manufacturer, Biomerieux.

The test of antimicrobial activity

The diffusion in agar method was used to measure the antibacterial activity on agar [(15).] A bacterial strain culture was established using the McFarland opacity standard, with a concentration of 1×106 cells per milliliter. Using a technique known as the spreading method, 0.1 ml of each bacterial isolate (1×10^6 cell per ml) was added to the Mueller Hinton agar plates. Wells were infected with 10 ml of extract as a control. Before being transferred to the incubator apparatus, petri dishes were held at 30 minutes. Every petri dish was incubated for twenty-four hours at 37°C. Measurements were made of the inhibitory zones.

Statistical Analysis

The measurements were examined using SPSS software version 17 and the one-way ANOVA test. The mean ± SE is used to express all measurements. ($P<0.05$ as the significant level) [(16)].

RESULTS AND DISCUSSION

Samples distribution

120 urine samples from patients with UTIs were included in the current investigation (Table 1). According to the data, 74(61.7%) of the total samples showed positive results for bacterial growth when cultivated in the ideal culture medium. Of the total samples, 46(38.3%) showed negative results for the development of bacteria.

Table 1: Distributed study samples according to UTI

	No. (%) +ve culture	No. (%) -ve culture	Total No. (%)	P value
Patients	74(61.7%)	46(38.3%)	120(100.0%)	0.068

Isolation of bacteria

The morphology, diameter, and forms of the bacterial isolates were determined on blood agar, MacConkey agar, and EMB. Furthermore, the outcomes of the biochemical identification were validated through the utilization of the System small Vitek-2 apparatus and microscopic and biochemical analyses, which included the specific tests for every category. The outcomes of the biochemical testing and the Vitek-2 results agreed. Table (2) shows that the highest incidence of urinary tract infection was due to *E. coli,* where the number of isolates reached 34 isolates, at a rate of 45.9% *E. coli*, followed by *S. aureus* 20.3%, *K. pneumonia* 14.9%, *P. aeruginosa* 10.8% and *P. mirabilis* 8.1%, *E. coli* accounts as one of the most prominent bacteria leading to urinary infections due to number of virulence factors like the colonization and invasion of the urethral epithelium, and microorganisms emerging from per urethral areas contaminated with fecal flora due to proximity to the anus and warm, moist environment [17]. A study in Kirkuk city, Iraq,[18] revealed that in a total of 563 urine samples, 234 (41.6%) were culture positive and *E. coli* was the predominantly (57.7%) isolated bacteria, followed by Staphylococcus aureus (17.5%), *K. pneumonia* (14.5%), and Proteus (10.3).[19]found that *E. coli* was the predominant isolates (58.57%) from the cases of UTI, followed by *S. aureus* (14.29%), *K. pneumonia* (8.57%), *S. epidermidis* (7.14%) and Pseudomonas aeruginosa (5.71%), Proteus mirabilis (2.86%), *K. oxytoca* (1.43%), *P. morganii* (1.43 %). Other study conducted in Erbil city and included 300 children with sign and symptoms of urinary tract infection found that *E. coli* (33.8%) the predominant cause of UTI.

Table 2: isolate percentages of uro-pathogenic bacteria

Bacteria	No.	%
E.coli	34	45.9
P. mirabilis	6	8.1
K. pneumonia	11	14.9
P. aeruginosa	8	10.8
S. aureus	15	20.3
Total	74	100
P value	0.093	Non-significant

The test of antibiotic sensitivity

Antibiotic susceptibility testing was performed using the following antibiotics: Ampicillin, Gentamicin, Trimethoprim, Imipenem, Ciprofloxacin, Levofloxacin, Amikacin. These antibiotics are the most commonly used against urinary tract infections. *E. coli*, K. pneumonia and P. aeruginosa showed 100.0% resistance to Ampicillin, while resistance to P. mirabilis and S. aureus towards the same drug 16.7% and S. aureus respectively. In contrast, all studied bacteria showed high sensitivity to Imipenem and Amikacin (Table 3).

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Table 3: show the effect of antibiotics on bacteria

Treatment / Bacteria	AMP (%)		CN (%)		TMP (%)		CIP (%)		LEV (%)		IMI (%)		AK (%)	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
E.coli	0	100	76.5	23.5	26.5	73.5	14.7	83.3	20.6	79.4	94.1	5.9	88.2	11.8
P. mirabilis	16.7	83.3	66.7	33.3	66.7	33.3	66.7	33.3	83.3	16.7	0.0	100	0.0	100
K. pneumonia	0	100	22.2	77.8	45.5	54.5	36.4	65.6	45.5	54.5	90.9	9.1	90.9	9.1
P. aeruginosa	0	100	75.0	25.0	62.5	37.5	62.5	37.5	62.5	37.5	87.5	12.5	87.5	12.5
S. aureus	6.7	93.3	66.7	33.3	53.3	46.7	66.7	33.3	46.7	53.3	86.7	13.3	93.3	6.7

AMP= Ampicillin, CN=Gentamicin, TMP=Trimethoprim, IMI=Imipenem, CIP=Ciprofloxacin, LEV= Levofloxacin, AK=Amikacin

The prevalence of Ampicillin resistance was determined to be 91.7%, and the percentage of resistance to Ampicillin recorded in this study. Gram-negative bacteria can demonstrate resistance against β-lactam antibiotics due to the production of βlactamases. (ESBLs) are a class of enzymes that can hydrolyze a variety of β-lactams, including penicillin and cephalosporins like ceftazidime, cefotaxime, and ceftriaxone, but not cefoxitin [20]. However, according to [21], 82% of patients had resistance to the drug levofloxacin. The findings of the present investigation aligned well with those of the [21] investigation, which revealed that 77% of *E. coli* samples were resistant to ciprofloxacin, 10% to amikacin, and 20% to gentamicin. Ampicillin resistance was found in most P. mirabilis isolates. Conversely, the maximum sensitivity was shown against Amikacin, Levofloxacin, Imipenem, and Ciprofloxacin. P. mirabilis from Nigeria has been found to have resistance rates to ciprofloxacin, nalidixic acid, and gentamicin of 13.9%, 53.7%, 74.1%, and 26.9%, respectively[22]. 36.4% of Klebsiella isolates were resistant to ciprofloxacin, an antibiotic that is classified as quinolone[23] discovered that 20% of people have resistance to ciprofloxacin. However[24] results differed somewhat from the data. They found that 72.22% of the isolates of Klebsiella were resistant to ciprofloxacin. The outcomes demonstrated that every isolate of P. aeruginosa was highly susceptible to imipenem and amikacin. The study's obtained results, which are displayed in Table (3), indicate a notable increase in pseudomonal resistance to beta-lactam antibiotics. These findings concurred with research published by [25]. Antibiotic susceptibility test findings for Staph. aureus isolates revealed a high proportion of resistance to most Penicillin medications. The current study's findings appear to be consistent with those of [26, 27], who noted that the same antibiotic resistance existed in Iraq. However, these findings disagreed with related research by [28], who discovered that 100% of Staphylococcus spp. were entirely sensitive to gentamycin. Also, do not agree with [18] where the isolates were (75.6%) resistant to gentamycin.

The inhibitory effect of ethanol extracts of *A. indica* and *M. oleifera*

The inhibitory effects of *Azadirachta indica* and *Moringa oleifera* extract on bacteria at 100mg concentration. The results show ability of *Azadirachta indica* and *Moringa oleifera* for bacteria inhibition according to concentration. Concentration of *Azadirachta indica* were caused in inhibitory effect on *E. coli*, *P. mirabilis*, *K. pneumonia*, *P. aeruginosa*, S. aureus isolates at diameter 25.6± 4.1, 24.3±5.2, 27.1± 6.3, 20.6± 2.4 and 31.3±7.6 mm respectively. While, *Moringa oleifera* extract at 100% concentration was caused in inhibitory effect bacteria isolates at diameter 31.6±3.5, 27.5±2.2, 38.3±3.8, 35.9±5.7 and 22.8±2.4 mm respectively as shown in table (4).

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Table 4: show the effect of ethanolic extract of *Azadirachta indica* and *Moringa oleifera* at concentration 100%

Bacteria type \ Treatment	*Azadirachta indica*	*Moringa oleifera*
E.coli	25.6± 4.1	31.6±3.5
P. mirabilis	24.3±5.2	27.5±2.2
K. pneumonia	27.1± 6.3	38.3±3.8
P. aeruginosa	20.6± 2.4	35.9±5.7
S. aureus	31.3±7.6	22.8±2.4

The study's results indicate that the neem extracts exhibited different levels of inhibition against the tested microorganisms. Antibacterial activity against S. aureus, P. mirabilis, K. pneumonia, P. aeruginosa, and *E. coli*. The results of [29], who showed that neem bark is helpful in treating wounds, fever, cough, and loss of appetite, are in line with this. The zones of inhibition for each of the five examined bacteria in the agar well diffusion test revealed a substantial variation in zone diameter. Maximum zones of inhibition were seen in this approach for S. aureus and Enterococcus as well [30]. Neem leaf extracts have been shown to have therapeutic value. They have been used as endodontic irrigating fluid [31], an ingredient in floor cleaning formulations [32], and a variety of surface disinfectants. At greater concentrations, fruit extracts and seeds have also shown antimicrobial action. At a dosage of 100 µg/ml, neem bark extract (NBE) effectively prevented HSV-1 from infecting cells [33]. In the current study, microorganisms studied exhibited antibacterial activity from the *Moringa* leaf. This result is more than the 8.75 mm water extract of *Moringa* leaf that was studied [34] and the 7 mm *Moringa* leaf hexane extract that was shown to have antibacterial activity against *Staph. aureus* bacteria[34]. When[35] evaluated the antibacterial activity of *Moringa oleifera* leaf extract on *P. aeruginosa*, *E. coli, Staph. aureus*, and other bacteria, they found that only Escherichia coli showed an inhibitory zone. In the investigation conducted by [36] on the antibacterial activity of a moringa leaf water extract, only Staphylococcus aureus showed sensitivity among the tested bacteria; no activity was detected against *E. coli*, *Salmonella spp*., and *K. pneumonia*.

CONCLUSIONS

Based on the results of the current study, a high prevalence of resistance was observed in bacteria isolated from UTI patients, and the best alternative to antibiotics was *Azadirachta indica* and *Moringa oleifera* extracts, which showed suitable antibacterial activity against Gram-negative and Gram-positive bacteria, and these extracts can play a role in the therapy of infection diseases.

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