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Assessment of certain plant extracts' antibacterial efficacy in reducing urinary tract infections

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Abstract

Background and Objective: Urinary tract infections are infection that requires continuous treatment. Therefore, bacterial isolates have become resistant to a group of antibiotics, so alternative natural methods like plant extracts has become important. This study aim to study the effective of some plant extracts as clove against resistant bacterial isolates.

Methods: 90 urine samples were taken from patients where 75 had urinary tract infections, and 15 were negative. All the isolates were identified according to Bergey's Manual of Determinative Bacteriology, various antibiotics were used in the experiment. Plant extracts were tested against clinical bacterial isolates, protein analysis for bacteria were performed. The effective material were identified.

Results: The most common bacteria in clinical samples were *E. coli* (40%), followed by *P. mirabilis*, *K. pneumoniae*, *S. saprophyticus*, and *P. aeruginosa*. Ofloxacin has 52% sensitivity against isolated pathogenic bacteria, followed by amikacin, azithromycin, chloramphenicol and norfloxacin, on other hand Penicillin-G was 100.0% resistant to isolated pathogenic bacteria, followed by ampicillin and cefotaxime sodium. The extracts of cloves, rosemary, mint, hibiscus, thyme and cinnamon inhibited extremely resistant microorganisms. There is synergism between the combination of clove extract and antibiotics ofloxacin and amikacin. Bacterial isolates proteins with control and ofloxacin clove extract stress have unique resistance protein domains.

Conclusion: The diversity of plant extracts and their effectiveness provide new hope for treating urinary tract infections, which provides continuous protection from antibiotic-resistant bacteria.

Keywords: Antibiotic Resistance, Clove, Plant extracts, Urinary Tract Infections.

Introduction

The most common urinary tract infection (UTIs) in humans is one that needs to be treated right away and continuously (1,2). Most UTIs are bacterial, affecting 150 million individuals worldwide (3,4). UTIs affect 50% of women and 12% of males, with 27%–48% of women recurring. Women get more UTIs (5,6). The most vulnerable patients to UTIs are those with urinary tract anomalies, weakened immune systems, long-term catheter use, and recent urological treatments (7,8).

Pathogens that penetrate the urinary tract and produce 10^5 cfu/mL or more cause infections (9,10). Enterobacteriaceae microorganisms, including *E. coli*, *K. pneumoniae*, *Citrobacter*, *P. mirabilis*, and *Enterobacter*, cause urinary tract infections. *P. aeruginosa*, *A. baumannii, S. aureus, saprophyticus, E. faecalis, S. bovis*, and *C. albicans* are common (5,11). UPEC is spread by infected food, sexual contact, and feces (12). A higher range of pathogens is involved in these infections, particularly *Staphylococcus aureus*, *Pseudomonas aeruginosa, Streptococcus, Klebsiella pneumoniae, Acinetobacter baumannii, Proteus mirabilis*, and *Enterococcus faecalis* instead of viral or fungal infection (13,14).

Acute and persistent UTIs benefit from antibiotics. In contrast, antibiotic overuse causes bacterial pathogen resistance (2,15). Ipenem, ertapenem, amikacin, and nitrofurantoin treat UPEC (11). Multidrug-resistant UPEC has become a significant clinical concern in recent decades due to the overuse of broad-spectrum antibiotics, extended hospital stays, and expensive treatment expenses, particularly in developing nations (6).

Antibacterial drugs take over ten years to develop, and antibiotic resistance is expected (16). Since 80% of people and 30% of pharmaceutical companies use medicinal plants, a global trend is towards them (17). According to numerous research, many medicinal plants can combat a wide range of bacterial illnesses and reverse antibiotic resistance (18). Folk medicine uses several plant components to treat urinary tract infections, and the dose depends on age, gender, and health. Most therapeutic herbs are taken alone or with mixed extracts with other plants, meals, or beverages (17).

This study investigates ways to prevent antibiotic resistance in bacteria infected urinary tract, the tested extracts showed antibacterial activity. Thus, further structural elucidation of the microbial growth-inhibiting bioactivity of the above mentioned plants could be used as precursors for the synthesis of new antibiotics in the future.

Methods

Study design and samples

Inpatient and outpatient UTI screenings at Zagazig University Hospitals and Clinics. The Urology Department contributed 90 urine samples for this study. These samples were obtained from January 2023 to August 2023 at Zagazig, Egypt, 80 km from Cairo.

Macroscopic, microscopic and biochemical tests

UTI was diagnosed by CLED, MacConkey, nutritional agar, and urine culture with 10^5 CFU/ml (9,10). In unspun urine, pyuria is defined as more than five pus cells per high power field in males and ten in females. Urine with higher numbers of pus cells is infected. Bergey's Manual key found bacterial isolates (19).

Antibiotic susceptibility test (AST)

All isolated bacteria were evaluated for antibiotic sensitivity using disk diffusion (20). Each isolated bacterial isolate should inject one colony into 5 ml sterile nutrient broth via the sterile loop, incubate for 24 hours at 37 °C, and adjust turbidity to 0.5 McFarland standard saline (21). Sterile swabs transferred broth inocula to Muller-Hinton agar plates, two per isolate, with antibiotic discs at constant distances. Various antibiotics were used in the experiment were the antibiotics utilised in the experiment, plates were incubated at 37 °C for 24

hours, all inhibitory zone diameters including the disc were measured in millimeter. The reading sought complete naked eye development suppression (22). Based on bacterial isolate sensitivity, ofloxacin and amikacin were tested for MIC and MBC against bacterial isolates (23).

Plant extracts and volatile oils susceptibility test (AST)

The effective part of plant samples were dried thoroughly and pestled to form smooth powder by a grinder. The cold water, hot water and ethanolic extracts of all medicinal plants were prepared by dissolving 10 g of fine powder of each plant in 50 ml of cold water, hot water (distilled water) and ethanol respectively. The contents were kept in a rotary shaker for 48 h. Then the extracts were filtered and dried in a hot air oven at 40°C. Then the extracts were refrigerated at 4°C for further studies (24,25).

Protein analysis for bacterial isolates

Protein content was extracted and assessed in treated (ofloxacin MIC for each organism and *Syzygium aromaticum* extract) and control (non-treated) bacteria (26).

Results

Men and women of all ages were given urine samples. Men and women were 20-60. The samples included 41 male and 34 female from 46 outpatients and 29 inpatients. In (Table 1), 75 of 90 urine samples had UTIs, while 15 were negative.

Table 1: Patient urine samples from various ages of male and female

Age of samples	Total urine samples	Positive urine samples	Negative urine samples
Age of males (years)			
20-30	10	8	2
31-40	9	8	1
41-50	22	18	4
51-60	8	7	1
20-60	49	41	8
Age of females (years)			
20-30	19	16	3
31-40	3	1	2
41-50	6	5	1
51-60	13	12	1
20-60	41	34	7
Total samples	90	75	15

Positive urine samples included 15 - 18 x 10^5 cfu/ml bacteria and 60 – over 100 hpf/ml pus cells. As illustrated in (Table 2), Most Gram-negative isolates (88.0%) are Enterobacteriaceae (77.33%), while Gram-positive isolates (12.0%) are *S. saprophyticus*.

Table 2: The distribution number of pathogenic bacterial isolate from positive collected samples

Pathogenic Bacterial isolates	Total positive samples	Isolates No.	Percentage of distribution, (%)
E. coli	75	30	40.00
P. mirabilis		17	22.66
K. pneumoniae		11	14.66
S. saprophyticus		9	12.00
P. aeruginosa		8	10.66

According to (Figure 1 a,b), Ofloxacin has 52.0% sensitivity against isolated pathogenic bacteria, followed by amikacin, azithromycin, chloramphenicol and norfloxacin. On other hand the pathogenic bacteria that have been isolated exhibit resistance to penicillin-G by 100%, followed by cefotaxime sodium and ampicillin.

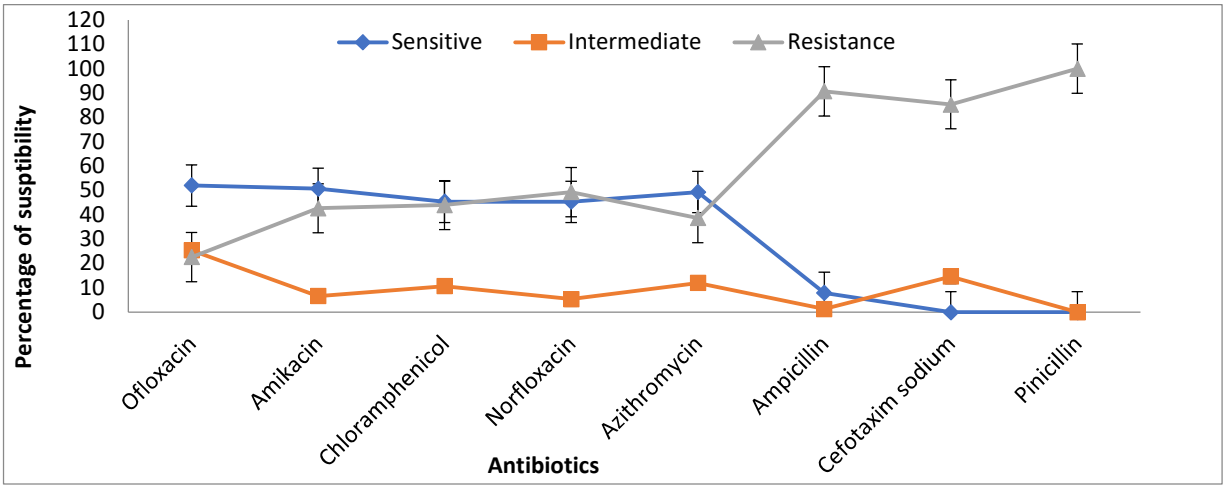
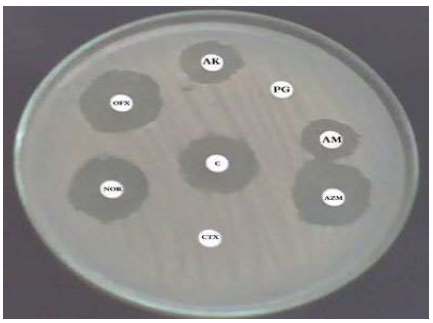
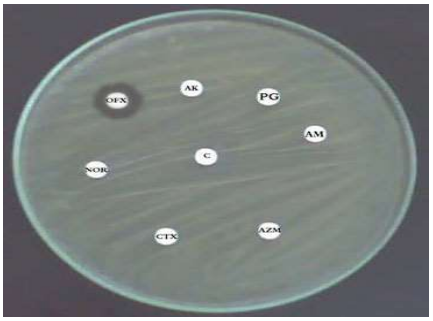


Figure 1,a: The sensitivity test of pathogenic isolates against different antibiotics drugs



Proteus mirabilis number 61



Pseudomonas aeruginosa number 71

Figure 1,b: Disc diffusion method analysis of bacterial isolates' antibiotic susceptibility AK = amikacin; PG = penicillin-G; CTX = cefotaxime sodium; OFX = ofloxacin; NOR = norfloxacin; AM = ampicillin; AZE = azithromycin & C = chloramphenicol.

Amikacin antibiotics showed the highest MBC (250 µg/ml) against *E. coli* numbers 17, 48, 52, *P. aeruginosa* 31, *K. pneumoniae* 68, and *P. mirabilis* 34, 41. And Ofloxacin had the greatest MBC against *P. aeruginosa* (31 & 71), *P. mirabilis* (41) and *K. pneumoniae* (68), as illustrated in (Table 3 a,b).

Table 3: MICs (µg/ml) and MBCs of amikacin and ofloxacin antibiotics against bacterial isolates

a) Antibiotic amikacin			
Bacterial isolates	No.	(MIC) (µg /ml)	(MBC) (µg /ml)
E. coli	17	250	250
P. aeruginosa	31	125	250
P. mirabilis	33	125	125
P. mirabilis	34	125	250
P. mirabilis	41	125	250
E. coli	48	125	250
E. coli	50	125	125
E. coli	52	125	250
S. saprophyticus	54	62.5	62.5
P. mirabilis	61	62.5	125
K. pneomoniae	68	250	250
P. aeruginosa	71	125	125

b) Antibiotic ofloxacin			
Bacterial isolates	No.	(MIC) (µg /ml)	(MBC) (µg /ml)
E. coli	17	62.5	125
P. aeruginosa	31	250	250
P. mirabilis	33	62.5	125
P. mirabilis	34	62.5	125
P. mirabilis	41	125	250
E. coli	48	125	125
E. coli	50	62.5	125
E. coli	52	62.5	125
S. saprophyticus	54	7.82	31.25
P. mirabilis	61	15.63	62.5
K. pneomoniae	68	125	250
P. aeruginosa	71	125	250

The extracts of rosemary, lemon grass, peppermint, hibiscus, clove, marjoram, thyme, cinnamon, castor plant, and chamomile were the most successful in combating pathogens, as illustrated in (Figure 2 a,b,c,d).

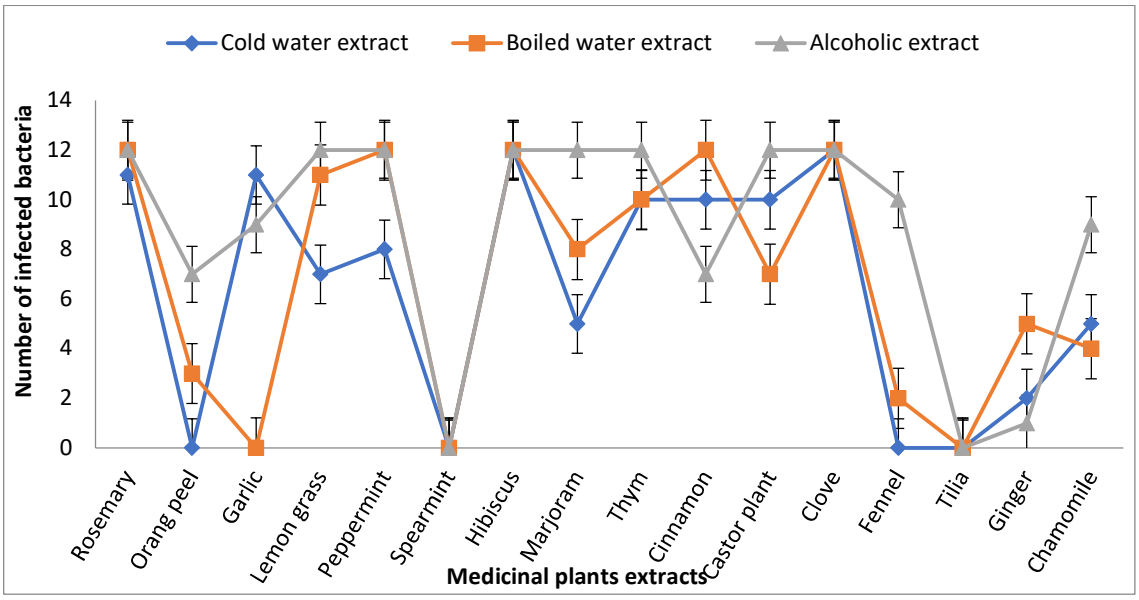
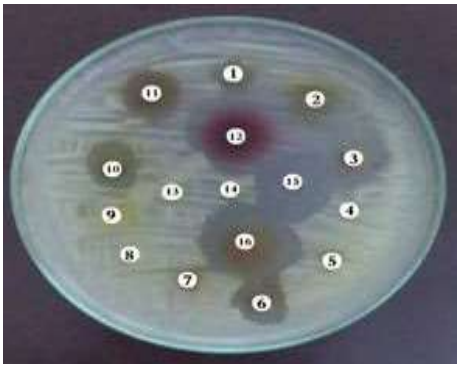
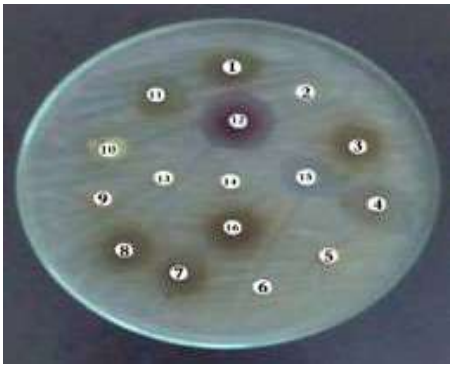


Figure 2,a: Bacterial isolates' sensitivity to cold, boiling, and alcoholic plant extracts.

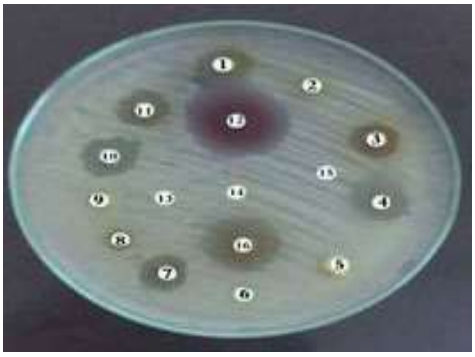


Proteus mirabilis number 61

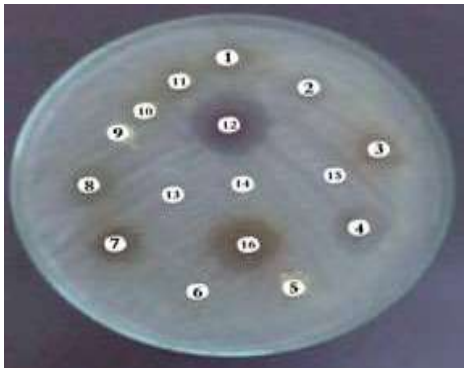


Pseudomonas aeruginosa number 71

Figure 2,b: The disc diffusion effect of cold water medicinal plant extracts on bacterial isolates

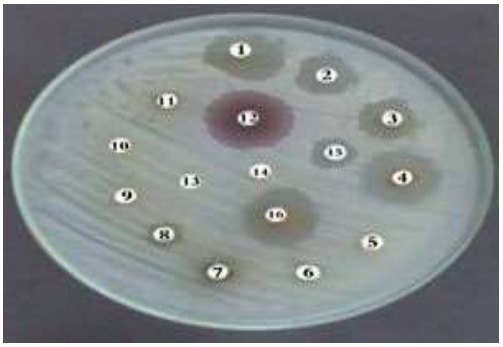


Proteus mirabilis number 61

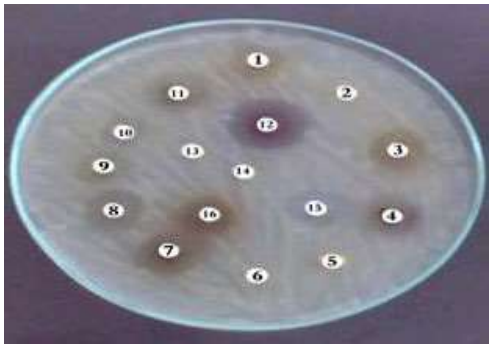


Pseudomonas aeruginosa number 71

Figure 2,c: The disc diffusion effect of heated water medicinal plant extracts on bacterial isolates



Proteus mirabilis number 61



Pseudomonas aeruginosa number 71

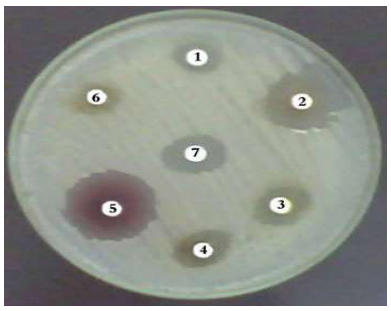
Figure No. 2,d: Disc diffusion test of alcoholic medicinal plant extracts against bacterial isolates

1- Rosemary, 2- Chamomile, 3- Thyme, 4- Cinnamon, 5- Ginger, 6- Orange peel, 7- Peppermint, 8- Marjoram, 9- Fennel, 10- Lemon grass, 11- Castor plant, 12- Hibiscus, 13- Tilia, 14- Spearmint, 15- Garlic, 16- Clove.

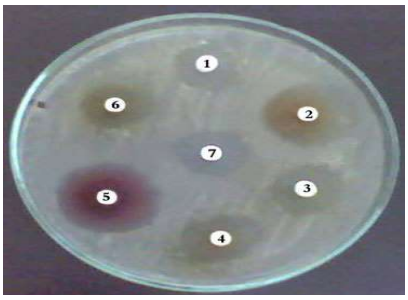
The results in (Tables 4,5) and (Figure 3,4) illustrated that there is synergism between the combination of clove extract and antibiotics (ofloxacin and amikacin), but there isn't synergism between the combination of garlic extract and antibiotics (ofloxacin and amikacin).

Table 4: Ofloxacin MICs and medicinal plant extracts against bacterial isolates

Bacterial isolates	No.	Ofloxacin MICs		Diameter of inhibition zones (mm) of medicinal plant extracts					
				Rosemary	Garlic	Clove	Castor plant	Peppermint	Hibiscus
		µg/ml	IZ	IZ	IZ	IZ	IZ	IZ	IZ
E. coli	17	62.5	12	15	ND	18	13	14	15
P. aeruginosa	31	250	15	17	15	22	11	15	17
P. mirabilis	33	62.5	12	22	15	20	ND	17	18
P. mirabilis	34	62.5	12	ND	12	18	12	13	16
P. mirabilis	41	125	15	15	12	20	11	20	18
E. coli	48	125	14	17	12	15	14	13	18
E. coli	50	62.5	11	15	ND	20	ND	15	14
E. coli	52	62.5	15	ND	16	17	11	10	12
S. saprophyticus	54	7.82	15	15	18	25	17	11	23
P. mirabilis	61	15.63	15	10	12	20	8	10	22
K. pneomoniae	68	125	13	23	10	25	23	21	20
P. aeruginosa	71	62.5	12	14	12	22	10	15	23



Proteus mirabilis number 61

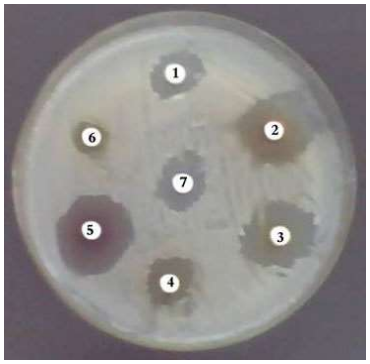


Pseudomonas aeruginosa number 71

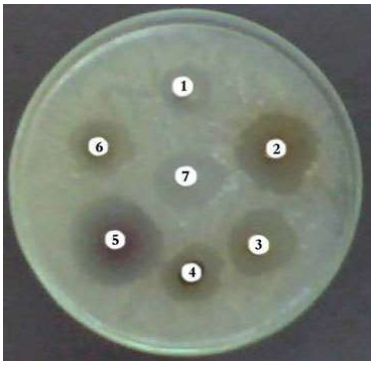
Figure 3: Combining medicinal plant extracts (1- Garlic, 2- Clove, 3- Rosemary, 4- Peppermint, 5- Hibiscus, 6- Castor plant) and (7- MIC of ofloxacin) against bacterial isolates using disc diffusion.

Table 5: Combination of medicinal plant extracts and amikacin MICs against bacterial isolates

Bacterial isolates	No.	Amikacin MICs		Diameter of inhibition zones (mm) of medicinal plant extracts					
				Rosemary	Garlic	Clove	Castor plant	Peppermint	Hibiscus
		µg/ml	IZ	IZ	IZ	IZ	IZ	IZ	IZ
E. coli	17	250	12	11	11	20	13	14	13
P. aeruginosa	31	125	10	14	12	19	11	14	18
P. mirabilis	33	125	10	12	14	20	13	12	15
P. mirabilis	34	125	11	12	11	18	13	14	22
P. mirabilis	41	125	13	13	10	18	22	15	12
E. coli	48	125	13	16	ND	18	17	15	15
E. coli	50	125	10	13	ND	22	10	12	16
E. coli	52	125	12	15	ND	16	ND	10	14
S. saprophyticus	54	62.5	13	22	26	28	15	20	22
P. mirabilis	61	62.5	12	16	12	19	8	12	19
K. pneomoniae	68	250	13	15	ND	22	15	12	20
P. aeruginosa	71	125	13	15	12	19	12	12	21



Proteus mirabilis number 61



Pseudomonas aeruginosa number 71

Figure 4: Disc diffusion of medicinal plant extracts (1- Garlic, 2- Clove, 3- Rosemary, 4- Peppermint, 5- Hibiscus, 6- Castor plant)and (7- MIC of amikacin) antibiotic against bacterial isolates.

Clinical bacterial isolate protein analysis under clove extract and ofloxacin stress. *P. aeruginosa* 31, *P. mirabilis* 41, and *K. pneumoniae* 68 were evaluated with antibiotic ofloxacin concentrations of 125, 62.5, and 62.5 µg/ml, respectively. As illustrated in (Figure 5 and Table 6) some protein bands disappear and some new protein bands were formed.

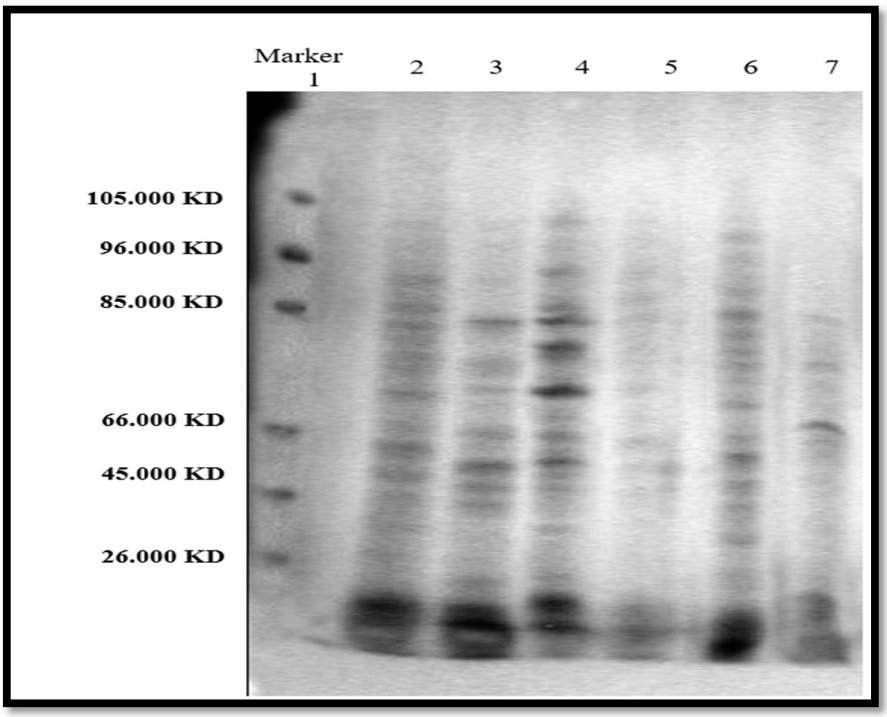


Figure 5: SDS-PAGE of protein banding patterns for clinical bacterial isolates as control and under stress of clove extract with ofloxacin antibiotic.

1- Marker.

2- *Pseudomonas aeruginosa* number 31 (control).

3- *Pseudomonas aeruginosa* number 31 after stress.

4- *Proteus mirabilis* number 41 (control).

5- *Proteus mirabilis* number 41 after stress.

6- *Klebsiella pneumoniae* number 68 (control).

7- *Klebsiella pneumoniae* number 68 after stress.

*Stress = Bacterial cultivation with clove extract and ofloxacin (MIC) antibiotics.

Table 6: Protein analysis for clinical bacterial isolates under stress of clove extract and ofloxacin antibiotic

Band No.	Marker MW (KD)	*P. aeruginosa* number 31		*P. mirabilis* number 41		*K. pneumonia* number 68	
		Control	Treated	Control	Treated	Control	Treated
	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
1	107.811	1	0	1	0	1	0
2	97.613	0	1	0	0	1	0
3	85.594	1	0	1	0	1	0
4	75.545	1	0	1	1	1	0
5	70.785	1	1	1	1	1	1
6	63.901	0	0	1	0	1	0
7	60.323	1	1	0	0	1	1
8	57.365	0	0	0	1	1	0
9	52.362	1	1	1	1	1	0
10	46.419	0	0	1	0	0	1
11	44.08	0	1	0	0	1	0
12	41.405	1	0	1	1	1	0
13	38.233	1	1	1	0	0	0
14	35.677	0	1	1	0	1	0
15	34.06	0	0	1	0	0	0
16	32.438	0	1	1	0	1	0
17	29.644	1	0	0	0	0	0
18	28.218	0	0	1	0	1	0
19	25.765	1	0	1	0	0	1
20	24.321	0	0	0	0	1	0
21	23.241	0	1	1	0	0	1
22	21.921	1	0	0	0	0	0
23	20.683	1	1	1	0	1	1
24	19.364	0	0	0	1	0	0

25	18.525	0	0	1	1	1	1

Discussion

Urinary tract infection is a problem, in most cases related to bacterial infections, in this study, 83.1% of isolates are Gram-negative. Enterobacteriaceae comprised 79.0% of isolates, and *P. aeruginosa* comprised 4.1%. 15.2% of isolates were Gram-positive, including 9.0% *Enterococcus spp*. *Candida sp*. comprised 1.7% of isolates (29).

Pathogenic isolated UTI-causing bacteria evaluated with commercial antibiotics. Urine isolates were resistant to trimethoprim-sulphamethoxazole and ampicillin but susceptible to nalidixic acid, nitrofurantoin, and ofloxacin (29). Meronem (76.19%), amikacin (70.27%), nitrofurantoin (66.60%), norfloxacin (64.28%), and gentamicin (58.33%) were the most susceptible urine isolates(30). These findings confirm that all urine isolates were ampicillin-intolerant but sensitive to ofloxacin, amikacin, and norfloxacin.

Plant extracts play an important basis in combating bacterial isolates. According to the study, thyme, rosemary, caraway and dianthus extracts work well against *P. aeruginosa, K. aerogense*, and *E. coli*. both distilled water and alcohol extract. All investigated strains of bacteria, including *S. aureus, Enterococcus cloacae, Salmonella paratyphi, Klebsiella pneumoniae, E. coli, Citrobacter spp*., and *Candida albicans*, were eliminated by clove extract (31).

Combination between plant extracts and antibiotics, increase or decrase antibiotics effectivity, so this study support our discovery that clove extract synergizes with ofloxacin but not garlic (32).

Clove has different compounds but not all are effective against bacteria only eugenol. The present study illustrated that the compound responsible for the clove aroma is eugenol, it is the main component in the essential oil extracted from cloves, comprising 72-90%. Eugenol has pronounced antiseptic, antimicrobial and anaesthetic properties (33).

Conclusion

The tested extracts showed antibacterial activity, it showed promising antimicrobial effect on multidrug resistance bacteria. Thus identifying and isolating secondary metabolites of those medicinal plants will helping pharmaceutical companies to the synthesis of new antibiotics used for the treatment of diseases caused by pathogens including polypharmacy resistant bacteria. Hence further structural clarification the biological activity that prevents the growth of microbes in the above-mentioned plants, especially clove which contain eugenol, can be used as a precursor for the creation of new antibiotics in the future.

Acknowledgments
None

Conflicts of Interest
The authors declare no conflict of interest

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