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Effect of Cardamom Seeds extraction on Resistant *Proteus mirabilis* to Antibiotics that Isolated from Pregnant women in Baghdad

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Ticarcillin, Cardamom, *P. mirabilis*, UTI.

ABSTRACT

Background: *Proteus mirabilis* exists in human digestive tracts and induce UTI in immunocompromised individuals. Antimicrobial resistance in *P. mirabilis* hinders clinical therapy, including proven resistance to colistin, nitrofurans, tigecycline, tetracycline, and β-lactams. **Aim of the study:** The aim of this study was isolation of *P. mirabilis* from urinary tract infections (UTI), and study antibiotic susceptibility to 14 type of antibiotics. Also, study the effect of cardamom extract on 14 isolated of *P. mirabilis* that were antibiotic resistance. **Materials and methods:** From November 2023 to February 2024, 230 patient's urine samples were collected. In Baghdad Governorate, pregnant women with urinary tract infections visited Al-Mada'in General Hospital. Negative bacteria were characteristic by Gram stain and lactose fermentation on MacConkey agar, hemolysis on blood agar, oxidase and indole tests and confirmation by VITEK-2 compact system. Result: *P. mirabilis* isolates susceptible to Amikacin were 71.4%, Gentamicin 85.7%, Imipenem 28.5%, Piperacillin 42.8%, Rifampicin 21.4%, and Tobramycin 35.7%, while *P. mirabilis* isolates were resistant to Aztreonam in 78.5 %, Amoxicillin/Clavulanic acid 85.7%, Cefepime 50%, Ceftazidime 57.1%, and Ticarcillin 57.1%. At 1000 mg/ml, the Cardamom seed alcoholic extract inhibited *P. mirabilis* bacteria best (18.67 mm). Low values (500, 250) were 14.89 and 10.6 mg/ml. **Conclusion:** This study confirm that the ethanol extracts of cardamom seeds possess strong inhibitory action against the tested pathogenic microorganisms. Based on this information, the utilization of cardamom as both an antibiotic and a dietary ingredient may have beneficial effects

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INTRODUCTION

Proteus mirabilis is primarily linked to urinary tract infections (UTIs). It ranks third in terms of frequency as a cause of complex urinary tract infections, following *Escherichia coli and Klebsiella pneumoniae*. It is also the second most prevalent cause, after *Providencia stuartii*, of catheter-associated bacteriuria in patients with long-term catheterization [1]. *P. mirabilis* exhibits numerous possible virulence factors that contribute to its pathogenesis, including the hydrolysis of urea by the enzyme urease, which leads to the formation of kidney and bladder stones [2].

In recent times, there has been a prevalent occurrence of microbes acquiring resistance to numerous commercially available antibiotics. This resistance has been a significant factor contributing to the failure in effectively treating various infectious disorders [3]. Currently, there is significant emphasis on discovering naturally occurring active chemicals that have the ability to inhibit and control certain pathogenic bacterial illnesses [4]. Medications typically offer successful antibiotic treatment for bacterial infections; however, there is a growing issue of antibiotic resistance and an ongoing requirement for innovative remedies [5].

Plant extracts have been utilized for a diverse range of applications throughout numerous millennia. There is a growing enthusiasm for natural preventive measures. Several studies have recorded the fungicidal and bactericidal properties of plants [6]. Herbs and spices are commonly regarded as safe, have been demonstrated to be useful against specific conditions [7]. The cardamom plant (Elettariacardamomum) belongs to the zingiberaceae family, and is a highly prized and old spice. It is primarily cultivated in Sri Lanka and southern India. The mature fruits of this plant produce seeds that are utilised for medical purposes as a spice, as well as a flavour enhancer in dishes such as curries, coffee, and pastries, especially in Arab nations [8]. This study intends to investigate the impact of cardamom extract on the eradication of antibiotic resistance and swarming behaviour of *P. mirabilis* strains isolated from urinary tract infections in hospitals in Baghdad.

MATERIAL

Collection of samples

Through the period from November 2023 to February 2024, 230 clinical samples of urine were collected from patients who pregnant women with urinary tract infection attending to Al-Mada'in General Hospital, in Baghdad Governorate. By using sterile equipment's and media, then samples were transferred to the lab for isolation and identification of *Proteus*.

A total of 230 urine samples isolates were obtained from Al-Mada'in hospitals in Baghdad city. The initial identification of gram-negative bacteria was based on gram stain and their ability to ferment lactose on MacConkey agar. Additionally, growth on blood agar, as well as oxidase and indol tests, were conducted. Confirmatory tests were performed using the VITEK-2 compact system [9].

Antimicrobial susceptibility test

The Kirby-Bauer method, as described by Betty et al.[10], was employed to conduct antimicrobial susceptibility testing for 25 distinct antimicrobial drugs. A sterile cotton swab was dipped into a bacterial solution that had been adjusted to have the same

cloudiness as the 0.5 McFarland turbidity standard (1.5 x 108 CFU/ml) by creating a series of diluted samples over a period of 18 hours. A Brain Heart Infusion culture of the bacteria was employed, namely the third dilution, which was selected after comparing it with the 0.5 McFarland turbidity standard. The diameter of the inhibition zones formed around the antimicrobial discs was measured in millimetres using a metric ruler, following the guidelines set by the Clinical Laboratories Standards Institute(CLSI, 2011) [11].

Plant extraction

The cardamom seeds were procured from the local market in Baghdad. The seed were peeled, cleansed with distilled water, dried and pulverised using an electric grinder. A quantity of 100 grammes of plant powder was measured and soaked in 500 millilitres of sterilised double distilled water and absolute ethanol, resulting in two different extracts depending on the solvent used. The mixture was then placed on a magnetic stirrer with a magnetic bar and left to blend for 72 hours at room temperature. Afterward, the mixture was filtered first through muslin cloth and then through filter paper. The aforementioned procedure was iterated 3-5 times to obtain a residue, until a transparent and colorless supernatant extraction liquid was acquired, signifying the absence of any further extraction from the plant material. The obtained extract was also concentrated by evaporation until all liquid had evaporated, and then stored in the refrigerator at a temperature of 5 °C until it was ready to be utilized [12].

Determine the inhibitory activity of the alcoholic extract

After making the requisite dilutions (250, 500, 1000)% from Cardamom seed extracts, it were pour in holes in agar to assess their biological inhibitory efficiency on *Proteus mirabilis* bacteria. Bacteria were distributed on Mueller-Hinton agar using a cork punch to create 5 holes per dish. Each dish included 50 lμ of each concentration, along with a drop of sterile distilled water as a control sample in the center. After incubation, the plates were in the incubator for 18–24 hours at 37°C. The diameter of inhibition rates for each extract concentration and bacteria were measured with a graduated ruler.

Minimum inhibitory concentration MIC

MIC of medicinal plant extracts was assessed by preparing serial dilutions for each plant extract within the range of 500-5000 μg/ml [13]. Furthermore, the control sample comprises 10ml of nutrient broth and 0.1ml of an overnight culture of bacterial suspension. This mixture is then incubated at a temperature of 37°C for a duration of 24 hours [14]

RESULTS

The collected samples were distributed between the prevalence of bacterial isolates showed that there was UTI in causative agents. In urine the major bacterium was *E.coli* with high occurrence frequency 124 (53.9 %) cases followed by *Pseudomonas* spp. 45 (19.5%) *Klebsiella* species in a percentage of 23 (10%), *Proteus mirabilis* 14 (6.3%). As shown in Table (1).

Table 1: Frequancy of bacteria isolated from different clinical sources

Number of	**Microscopic**

Positive isolates (%)	organism
124 (53.9 %)	*Escherichia coli*
45 (19.5%)	*Pseudomonas spp*
23 (10%)	*Klebsiella species*
14 (6.3%)	*Proteus mirabilis*
3 (1.3%)	*Staphylococcus aureus*
1 (0.4%)	*Serratia marsecens*
20 (8.6%)	*Candida albicans*
230	Total

Isolation and diagnosis of *Proteus*

The present study showed that *Proteus* colonies are non-lactose fermentor on Macconkey agar and colourless colonies on XLD agar after incubation at 37° C for 24 hrs. In addition All isolates were appeared polymorphic Gram negative rods by using Gram stain Figure (1).

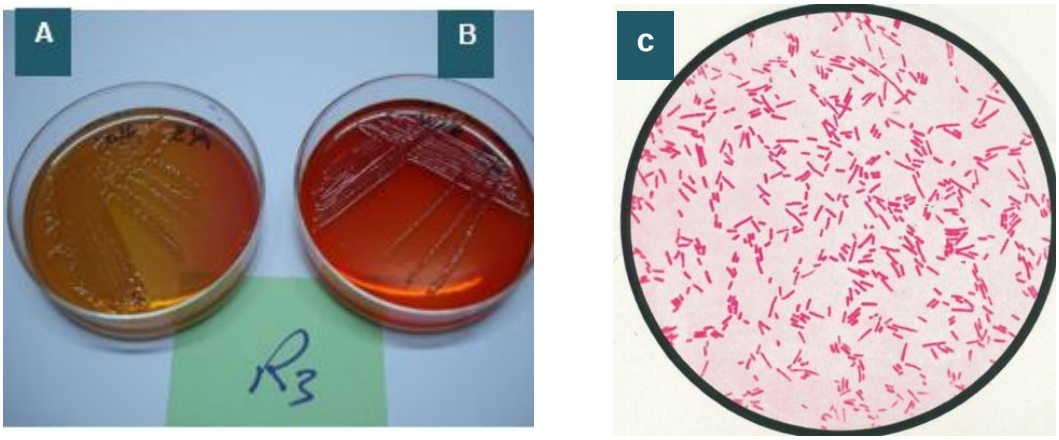

Figure 1: *Proteus* colonies are non-lactose fermentor on Macconkey agar (A); and colourless colonies on XLD agar (B) after incubation at 37° C for 24 hrs.(c): *Proteus* colonies microscopically diagnosis

The susceptibility of 14 samples of *P. mirabilis* isolates to antimicrobial agents as in (Table 2) and (Figure 2) were investigated by using Kirby-Bauer method. The results indicated that *P. mirabilis* isolates had variable degrees of resistance towards different categories of antimicrobial agents. It included *P. mirabilis* isolates were susceptible to Amikacin 71.4%, Gentamicin 85.7 %, Imipenem 28.5 %, Piperacillin 42.8 %, Rifampicin 21.4 %, Tobramycin 35.7%. While the study recorded that *P. mirabilis* isolates were resistant to Aztreonam 78.5 %, Amoxicillin/ Clavulanic acid 85.7 %, Cefepime 50%, Ceftazidime 57.1% and Ticarcillin 57.1%

Table 2: Distribution of resistance of *P. mirabilis* isolates to antimicrobial agents

Id	Antimicrobial discs	Code	Interpretation	Number of total bacterial isolates (14)	%
1	Amikacin	AK	S	10	71.4
2	Aztreonam	AZ	R	11	78.5
3	Amoxicillin/ Clavulanic acid	AMC	R	12	85.7
4	Cefepime	CPM	R	7	50
5	Ceftazidime	CAZ	R	8	57.1
6	Gentamicin	CN	S	12	85.7
7	Imipenem	IPM	S	4	28.5
8	Piperacillin	PRL	S	6	42.8
9	Rifampicin	RIF	S	3	21.4
10	Ticarcillin	TC	R	8	57.1
11	Tobramycin	TOB	S	5	35.7

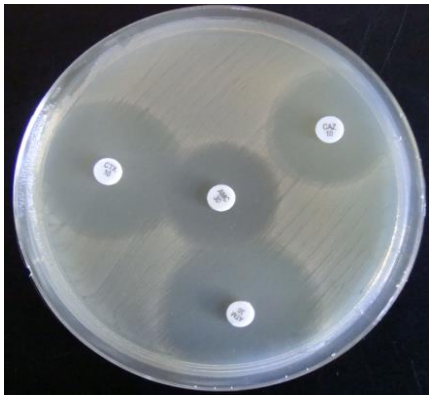

Figure 2: *P. mirabilis* inhibition zone on Muller Hitone agar

A study found that Cardamom seed alcoholic plant extracts inhibited *Proteus mirabilis*, a disease with great antibiotic resistance. Table 3 and Figures 3 showed how bacterial isolates react to Cardamom seed extract. At 1000 mg/ml, the *Cardamom* seed alcoholic extract inhibited *Proteus mirabilis* bacteria best (18.67 mm). Low values (500, 250) were 14.89, 10.6 mg/ml.

Table 3: Inhibitory effect of blow plant extracts on *Proteus mirabilis*

Concentration%	Mean (mg/dl)
1000	18.67
500	14.89
250	10.6
Control	0

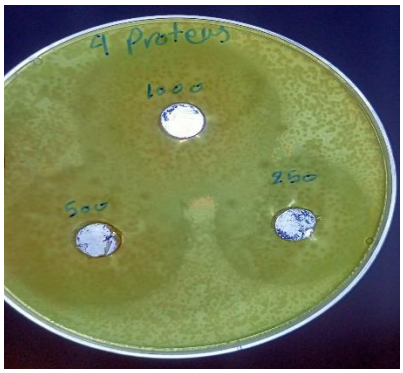

Figure 3:Inhibitory effect of Cardamom seeds extracts on *Proteus mirabilis* by using well diffusion technique

A range of concentrations was utilized (5000, 2500, 1250, 625, 312.5, 156.2, 78.125, 39)µg/ml, to calculate MIC by Micordilution method. A total from 14 samples, 6(42.9%) was inhibited in all concentration, while the MIC for 5(35.7%) isolates was (625) µg/ml. Furthermore the MIC for 3(21.4%)

isolates was (312.5) µg/ml, as shown in Table(4).

Table 4: The minimum inhibitory concentration of Cardamom seeds against *Proteus mirabilis* in different concentration.

No of isolation	5000	2500	1250	625	312.5	156.2	78.125	39
1	-	-	-	-	-	+	+	+
2	-	-	-	-	+	+	+	+
3	-	-	-	-	+	+	+	+
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	+	+	+	+
8	-	-	-	-	-	-	-	-
9	-	-	-	-	+	+	+	+
10	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-
12	-	-	-	-	-	+	+	+
13	-	-	-	-	-	+	+	+
14	-	-	-	-	+	+	+	+

DISCUSSION

The samples were analysed to determine the prevalence of bacterial isolates causing urinary tract infections (UTIs). The predominant bacterium found in the urine samples was *E. coli*, with a high occurrence frequency of 124 (53.9%). *Pseudomonas spp*. was 45 (19.5%), *Klebsiella spp*. was 23 (10%), and *Proteus mirabilis* followed this with a frequency of 14 (6.3%).

This finding closely aligned with the study conducted by Sokhn, Elie S., et al. which identified *E. coli* as the predominant bacterium, followed by *Klebsiella* species and *P. mirabilis*[15]. In line with the findings of Orji and Dike it was observed that the predominant bacterium identified in urine samples was *Pseudomonas aeruginosa*[16]. Betty *et al.* utilised three distinct types of media to separate and distinguish *Proteus spp*. from other species of Enterobacteriaceae. This primary identification method was based on the most prevalent characteristics, namely the swarming phenomena and the absence of hemolytic activity on blood agar media.The non-lactose fermenter isolates exhibited pale, convex, round, and smooth colonies on MacConkey agar, as shown in figure (4-1A), and colorless colonies on XLD agar media[10]. These isolates also had a distinct fish-like odor .

Based on the aforementioned findings, it is evident that *P. mirabilis* isolates exhibited susceptibility to antimicrobial agents that hinder protein synthesis, namely aminoglycosides (amikacin, gentamycin, tobramycin), as well as antimicrobial agents that impede nucleic acid synthesis, such as rifampicin. Conversely, a greater number of isolates demonstrated resistance to Aztreonam and Amoxicillin/Clavulanic acid. The data clearly showed that cefepime, an antibiotic drug, is a protein synthesis

inhibitor. It was particularly effective against *P. mirabilis*, but the isolates showed significant resistance. Furthermore, *P. mirabilis* isolates exhibited a notable degree of vulnerability to antimicrobial drugs that impeded cell wall production, such as imipenem. The study conducted by Moosavian confirmed that various isolates of Enterobacteraceae showed a high susceptibility to Imipenem. However, they exhibited moderate to high resistance to certain first-generation cephlaosporins. In contrast, they displayed low resistance to most of the third-generation cephlaosporins used in the study. Although the rate of resistance to ceftazidime was increasing, it is evident that cefepime exhibited high activity and resistance to fourth-generation cephalosporins [17]. The study conducted by [18] revealed that the minimum inhibitory concentration (MIC) for proteus mirabilis was 2500 µg/ml. Additionally, it indicated the inhibitory impact of alcoholic cardamom on proteus mirabilis. The MIC's lower concentration value signifies the extraction's effectiveness[19]. The methanolic extract of cardamom demonstrated the highest inhibitory activity against the investigated isolates of *Proteus mirabilis*. This can be attributed to the presence of active components that are most effectively extracted by methanol solvent [20]. The antimicrobial properties of herbs are attributed to a range of chemical compounds found in their tissues, such as volatile oils, alkaloids, tannins, and lipids. Cardamom seeds, for example, contain 4% volatile oil, which is primarily composed of terpinyl acetate and cincole, along with small amounts of other monoterpenes, including alcohols and esters[21]. In line with our research, a study [22] found that the extract from Cardamomum seeds had varying levels of antibacterial action against different microorganisms. The chemical makeup of Cardamomum varies significantly depending on the variety, locality, and age of the product. The concentration of volatile oils in the seeds is highly influenced by the storage conditions [23]. Tannins are efficient against microorganisms due to many mechanisms, such as enzyme inhibition, reduction of essential nutrients and mineral ions required for microbial growth, blockage of the phosphorylation process, and oxidative stress, which ultimately hinders cellular metabolism [24]. Tannins possess the capacity to create complexes with many molecules, including large molecules like proteins and polysaccharides [25]. Recently, multiple writers have documented the antibacterial, antifungal, anti-cancer, antioxidant, and gastroprotective properties of cardamom [26]. The antibacterial action of the ethanolic extract of E. cardamomum was seen at a concentration of 512 µg/ml[27]. The seeds of E. cardamomum exhibit antibacterial properties against gram-negative bacteria [28]. According to JEBUR et al., cardamom exhibits strong antibacterial effects against some Gramme positive and Gramme negative bacteria, as indicated by the zone of inhibition [29]. The ethanolic extract of large cardamom, administered at a concentration of 100mg/ml, and the aqueous extract, administered at a concentration of 200mg/ml, both demonstrated anti-inflammatory properties . [30].

CONCLUSION

Conclusively, our investigation determined that the ethanol-based

extracts of cardamom seeds possess potent inhibitory effects on the tested pathogenic bacteria. Based on this information, the utilisation of cardamom as antibiotics and in food could potentially be beneficial.

CONFLICT OF INTEREST

Non.

ACKNOWLEDGEMENTS

Non.

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