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Antibacterial activity of Cinnamomum zeylanicum aqueous extract against *Proteus mirabilis* isolated from Urinary Tract Infections in Kerbala province

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Abstract

Proteus mirabilis from urinary tract infections (UTIs) is to be isolated as well as detected in the presented work. *Proteus mirabilis* resistance to specific antibiotics is being studied, and the antibacterial activity regarding a cold aqueous extract of Cinnamomum zeylanicum against *Proteus mirabilis* isolated from UTIs is being tested. 150 patients with diuresis for UTIs comprise the study sample. Thus, 12 (8%) of *Proteus mirabilis* were isolated. The majority of bacterial isolates tested with such antibiotics exhibited resistance to them. The research's findings indicate that the aqueous extract of Cinnamomum zeylanicum contains active components such as tannins, glycosides, saponins, resins, and phenols in varying amounts, as well as the absence of flavones and phenols. Thus, the bacterial isolates have the greatest impact on concentration (75 mg/ml). Therefore, compared with the control factor, which contains sterile distilled water, the concentration (75 mg/ml) had the highest and lowest diameters of 30 mm and 22 mm, respectively; the concentration (50 mg/ml) had the highest and lowest diameters of 28 mm and 18 mm, respectively; the concentration (25 mg/ml) had the highest diameter of 26 mm and the least diameter of inhibition was 16 mm; and lastly, about the concentration (12.5 mg/ml), the highest and lowest diameters were 23 mm and 12 mm, respectively.

The results of the investigation of some virulence factors showed that 59(100%) isolates can form a swarm, 48(81.35%) isolates can produce biofilm, 50(91.52%) isolates can produce hemolysin, and 24(100%) isolates can produce urease enzymes. *Proteus mirabilis* is one of the pathogens of UTIs.

Keywords: *Proteus mirabilis*, urinary tract infections, virulence factors, Cinnamomum zeylanicum

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Introduction:

Microorganisms in the urinary tract are referred to as UTIs. However, it can be challenging to discern between colonization, contamination, and infection [1]. According to many works, bacteria, the most prevalent microorganism responsible for UTIs, affect people of all ages without exception, beginning with newborns, girls and boys in their adolescent years, sexually active women, and the elderly of both sexes [2]. The incidence of UTIs varies by age and gender, with females experiencing higher rates compared to males at different ages, except early childhood (< 3 months), when boys are more likely to get one than girls [3]. This is 3-5% in favour of women and 1% in favour of men. Most

UTIs are caused by gram-negative bacteria, which include E. coli, *Proteus mirabilis*, P. vulgaris, P. aeruginosa, Klebsiella spp., Serratia spp., Acinetobacter spp., and Morganella morganii. UTIs could be caused by gram-positive bacteria, such as Enterococcus spp., Streptococcus agalacticae, and Staphylococcus spp. [4].

Any part of the urinary tract, including the bladder, kidney, urethra, and ureter, could be impacted by the infection. Cystitis, pyelonephritis, and urethritis are the three most common UTIs. In cases where there are functional and structural abnormalities or the existence of foreign bodies, such as using a urinary catheter for an extended period or having stones, the infection is deemed complicated [5]. Bacteria might enter and propagate within the urinary system via three different routes: the hematogenous route, the ascending route, and the lymphatic route [6]. Predisposing factors for infection include anything that prevents the bladder from emptying, obstructs the normal flow of urine, or makes it easier for organisms to enter the bladder. Researchers believe that urine that remains in the bladder for longer than two to three milliliters increases the risk of germs multiplying [7]. Bladder catheterization contributes to the risk of UTIs and accounts for roughly 2% of hospital-acquired UTIs [8]. The length of the outer surface of the mucous membranes between the catheter and the urethral wall [9]. At the same time, other factors lead to bacterial and urinary tract infections, including changes in osmotic pressure, urea concentration in urine, and the accumulation of various urinary toxins that lead to inhibition of activities. The antigenicity of blood granulocytes and phagocytes, in addition to other immune mechanisms, also acts as a risk factor for UTIs [10]. About 95% of UTIs are diagnosed in pathological cases, as they enter through the urethra, the bladder, the ureter, and the kidneys [11]. Children's UTIs are very important because they can have serious effects on the kidneys, particularly if the child has congenital urinary system defects or if there is a condition where urine is refluxed from the bladder to the ureter and, subsequently, to the kidneys [12]. Particularly in people who use long-term catheters or have structural or functional abnormalities in the urinary tract, such bacteria are among the pathogens responsible for complex postoperative infections UTIs [13]. Due to such bacteria inside the stone mold, this form of illness is challenging to cure with antibiotics [14].

The Lauraceae family includes the small evergreen tree Cinnamomum zeylanicum. Native to Sri Lanka, Cinnamomum verum trees grow to a height of 10 to 40 meters. They are also grown in South America, Southeast Asia, and the West Indies. The fruit is small and has a clove-like appearance, the flowers are yellow, and the leaves are an alternating compound. The number of volatile oils in cinnamon peels can reach 4%. The trees typically develop to a maximum height of 3 meters as leafy brushes. The flowers are greenish and have a strong smell when they are clustered in panicles. The fruit is a single-seeded, purple, 1-cm drupe. Peels from Cinnamomum zeylanicum range in volatile oil content from 4% to 6%. The bark of Cinnamomum zeylanicum is dried, cut off, and rolled into a tube. It might be available as a powder that you could combine with other plants. For thousands of years, the bark of the cinnamon plant (Cinnamomum zeylanicum) was utilized in medicine to treat dyspeptic disorders, appetite loss, flatulence, abdominal pain, and to lower blood pressure, serum cholesterol, and blood sugar. Insulin receptor activity is increased and insulin release is stimulated with CZ. Since insulin is essential for the metabolism of fats, it's probable that elevated serum insulin levels after CZ treatment aid in the reduction of lipid levels. As a result, CZ offers numerous health advantages to people, especially in the areas of anti-inflammatory, hypertriglyceridemic, and antidiabetic effects. CZ also has several antioxidant compounds. With 0.5–1% of its components being cinnamon bark oil, "Ceylon" is regarded as one of the essential perfumes. It is a fairly oily, pale yellow to dark yellow or brownish-yellow liquid with a diffusive, strong, and warm-spicy aroma [15]

Due to the importance of *Proteus mirabilis* isolated from UTIs and to the lack of studies concerning this topic in Karbala city, which focus on the multiple resistance to antibiotics, and to seek further

information about active ingredients isolated from natural sources and use them in preventing pathogen intrusion in order to reduce the risk of antibiotics are used at present, which has great effects on patient's body health.

Materials and methods:

- **Sample collection:**

150 urine samples have been collected from Imam Hussain Teaching Hospital in the City of Karbala to investigate *Proteus mirabilis*. Then, they were placed in sterile plastic cups that could be closed tightly. The patient's information was fixed regarding name, gender, age, area of residence, and whether they had taken antibiotics.

- **Laboratory Diagnosis:**

Urine samples have been plated on MacConkey agar and blood agar and subjected to incubation at a temperature of 37°C for (18-24) hours to know the shapes and characteristics of the colonies and then diagnose them (16,17).

- **Diagnosis of bacterial isolates:**

According to [16], several biochemical tests were carried out. After the whole day, the bacteria need to grow, the diagnosis will be made through Baron. Among the biochemical tests performed were the following: Gram stain, Catalase, Oxidase, Indole, Methyl red, Voges Proskauer, Citrate Utilisation, Urease, motility, Triglyceride Screening (TSI), and gelatin liquefication tests.

The outcomes of biochemical tests have been additionally confirmed using the Vitek 2 system and the APi 20 E kit, which are known for their simplicity and speed of diagnosis.

- **Antibiotic susceptibility test:**

Twelve antibiotics (Imipenem, Ciprofloxacin, Gentamycin, Amikacin, Augmentin, Tobramycin, Cephalothin, Trimethoprim, Nitrofurantoin, Ceftazidime, Ampicillin, and Cefotaxime) have been used to treat bacterial isolates. The results have been recorded by measuring the inhibition zone and comparing it with the National Committee for Clinical Laboratory Standards (NCCLS). Muller Hinton agar was utilized, and the plate was subjected to incubation at 37 °C for 24 24-hour. The resistant and sensitive isolates of each one of the antibiotics have been noted by measuring the diameter of the inhibition zone. Also, turbidity has been put in comparison with the standard turbidity constant solution [18].

- **Investigate the virulence factors of Proteus mirabilis:**

Hemolysin production Test: Detection of the capability of isolates for producing hemolysin through culturing such isolates on blood agar prepared using human blood type (AB.). which has this characteristic, the plate after inoculation was incubated at a temperature of 37oC for 16 hrs, after which the β-hemolysis zones have been seen as transparent areas surrounding the colonies of the producing bacteria with the development of control to compare the results [19].

- **Production of biofilm formation by Congo-red agar:**

A pure colony is transferred from MacConkey agar to a test tube containing 5 ml of physiological saline solution. After mixing it well, its turbidity is compared with MacFarland turbidity and Congo-red inoculation, and the plate is incubated. If the result is positive, the colonies appear black with dry crystal density. Meanwhile, colonies become pink if the result is negative [20].

- **Swarming test:**

This test is used to detect the susceptibility of pathological isolates to swarming formation, and this examination was conducted using the solid culture media method, following the method of [21]. The plates and when the Swarming appear, it indicates that the result is positive.

- **Urea production test:**

The procedure assessed the isolates' capacity to generate urease [16].

Collecting Plant Samples: Preparing the cold aqueous extract of Cinnamomum zeylanicum:

Collecting Plant Samples: Samples of Cinnamomum zeylanicum bark have been collected from the local markets, then washed with distilled water, dried in the shade, ground with an electric mill, and finally dried powders kept in dark sealed containers until ready to serve.

Preparing the cold aqueous extract of Cinnamomum zeylanicun: the cold aqueous extract of Cinnamomum zeylanicum has been prepared depending on the [22] method by taking 50 g. of dry powder in a 1000 ml laboratory flask and mixing it with 500 ml of distilled water by using a liquidizer and let stand for 24 hours at the usual room temperature. Consequently, the mixture was filtered using several layers of gauze to remove the plankton. After that, the mixture was centrifuged at 3000 rpm for 10 minutes, and then it was filtered using filter papers to get a pure solUTIon. The cold aqueous extract of Cinnamomum zeylanicum was then dried in an oven at a temperature of 40 degrees. Finally, it was kept in dark, sealed containers until ready to serve.

- **Chemical Qualitative Detection of the cold aqueous extract of Cinnamomum zeylanicum:**

For identifying a few of the active groups in the cold aqueous extract of Cinnamomum zeylanicum, such as tannins, glycosides, saponins, resins, flavones, phenols, and pH, a series of chemical qualitative detections of the extract were carried out [23]

- **Testing the Effect of Antibiotic on CZ Aqueous Extract:**

The well diffusion assay method has been used, depending on the method of [24], in which the Mueller Hinton agar method has been papered according to the manufacturer's instructions. Mueller Hinton agar was put in Petri dishes. After the concretion, it was vaccinated by suspension. The bacterial suspension was spread by using swabs. The dishes were left to stand for 15 minutes, and then five wells were made in each dish with a diameter of 5 mm using a sterile Cork Borer. After that, with the use of a micropipette, 10 µl of plant extract has been added to each of the four wells at the following concentrations: 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml. The fifth well was filled with distilled water and considered a control one. The Petri dishes have been incubated above 98.6 degrees Fahrenheit for more than 24 hours. To sum up, the results were marked by measuring the diameters of the inhibition zone in millimetres.

Results and Discussion:

The current study included 150 urine samples from patients suffering from UTIs, males, and females of different ages. *Proteus mirabilis* was isolated from 12(8%) and 34(22.67 %) Other types of bacteria, while 104(69.33 %) urine samples did not give any bacterial growth in this research, as shown in Table [1].

Table 1: Isolation percentage of *Proteus mirabilis* compared to other bacterial urinary tract infections

Organism	Number	Percentage
No bacterial growth	104	69.33 %
Proteus mirabilis	12	8 %
Other types of bacteria	34	22.67 %
total	150	100 %

These results converged with the study [25] in which this bacterium was isolated by (11.97%), and this is due to its being one of the members of the colon bacillus naturally present in the intestinal tract and can cause opportunistic diseases when providing the appropriate conditions after moving

from its natural place as well as the study [26] in which this bacterium was isolated by (17.6%) from UTIs.

UTIs represent one of the most significant infections in various nations; they affect humans in different age groups, and 95% of these infections are caused by many types of bacteria [27], in addition to possessing many virulence factors that may be enzymatic or toxins [28]. Some samples showed no growth on the culture media, and this may be due to several reasons, including some bacterial types that require special culture media for their growth or patients took some antibiotics before the bacterial culture procedure, which supports that most of the negative samples come from patients lying in the hospital [29].

Biochemical tests: Several biochemical tests were conducted according to [15], as exhibited in Table 2.

Table 2: Biochemical tests for *Proteus mirabilis* isolated from urinary tract infections

Test type	Result
Gelatin laquification test	+
Catalase test	+
Voges Proskauer test	-
Methyl red test	+
urease test	+
Citrate Utilization Test	-/+
Motility test	+
Indol production test	-
Oxidase test	-
Gram stain	-
Triple Sugar Iron test)TSI) H2S gas+	K /A + +

The ready-made Api-20E diagnostic kit was used to confirm the diagnosis, and the Vitek 2 system, characterized by ease, speed, and accuracy, was used to confirm the biochemical diagnosis results.

- **Antibiotic susceptibility test:**

The results showed a clear variance in the extent of response of the isolates under study to the used antibiotics, as shown in Table [3]. The results showed that 15(25.42%) isolates were resistant to Ciprofloxacin. On the contrary, all isolates have been resistant to Cefotaxime, Ampicillin, and Nitrofurantoin, with a percentage of 100%. The results also showed that 34(57.62%) isolates were resistant to Gentamycin, 32(54.23%) were resistant to Tobramycin, 39(66.10%) isolates were resistant to Trimethoprim, 31 (52.54%) were resistant to Cephalothin, 50(84.74%) were resistant to isolation resisted Augmentin.

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Table 3: Percentage and number of resistant *Proteus mirabilis* for each antibiotic

Antibiotics	Sensitive bacteria		Resistant bacteria	
	Number	%	number	%
Ciprofloxacin	44	89.18	15	25.42
Imipenem	50	83.78	9	15.25
Amikacin	30	54	19	32.20
Gentamycin	25	18.91	34	57.62
Tobramycin	27	18.91	32	54.23
Trimethoprim	20	8.1	39	66.10
Augmentin	22	16.2	37	62.71
Nitrofurantoin	0	0	59	100
Cephalothin	18	8.1	31	52.54
Ceftazidime	9	2.7	50	84.74
Ampicillin	0	0	100	59
Cefotaxime	0	0	100	59

The results of this work specified that most bacterial isolates have been resistant to many antibiotics, especially beta-lactam antibiotics, while their resistance to the aminoglycoside group antibiotics varied. The cause of resistance to bacterial isolates may be the misuse of such antibiotics, resulting in resistant bacterial strains [30].

The results showed the resistance of *Proteus mirabilis* to Ceftazidime, Cefotaxime, and Ampicillin, and these results agreed with the findings of [31], which showed the resistance of *Proteus mirabilis*. The result also coincided with the study [32] of *Proteus mirabilis* isolated from urine samples resistant to Ceftazidime. In contrast, the study of [33] differed from the results of this work, where the percentage of resistance of *Proteus mirabilis* to Ceftazidime was 66% and 83%, respectively.

As for the aminoglycoside group, *Proteus mirabilis* showed clear resistance to Amikacin, Gentamycin, and Tobramycin; this result was similar to a study [34] on patients with otitis externa. The percentage of resistance of *Proteus mirabilis* the Amikacin has been 4.3%. As for Gentamycin, the result was similar to what was found by [35]. The results of a study conducted by [36] differed from the results of the current study, as they indicated resistance to *Proteus mirabilis*. The current study did not agree with the study conducted by [37], as the resistance of *Proteus mirabilis* isolated from patients with UTIs in Canada to this pathogen was 3.33%.

The current study showed that Ciprofloxacin showed the best effect on the isolates under study, and the result was identical to the study [38]. *Proteus mirabilis* was resistant to Ciprofloxacin in this work, and this result was the same as the research results [39]. Last, it was resistant to Nitrofurantoin, even though the percentage of resistance to Augmentin in this work agreed with the percentage of resistance to Augmentin in the study [31], which amounted to 77.77%.

A study conducted by [40] on patients with urinary tract infections did not agree on the percentage of resistance of *Proteus mirabilis* to anti-nitrofurantoin. At the same time, Imipenem was ranked

second in terms of the effectiveness of the antibiotics used in the study after Ciprofloxacin, which bacteria were resistant to; these results follow the work carried out by [41]. The results showed that bacterial resistance to Trimethoprim in the current study matched the results of the study it conducted [33]. In the Tikrit district Proteus spp. cause urinary tract infections, although some studies have indicated that these bacteria are sensitive to this antibody [42].

The method of reducing permeability or genetic mutations may sometimes be resistant to Proteus spp. This antagonist, or by producing a type of enzyme that is more resistant to the action of this antagonist, is Dihydrofolate reductase, which encodes for it by genes carried on the plasmid and then neutralizes the effect of this antigen [42].

- **Investigation of important virulence factors of *Proteus mirabilis*:**

Proteus mirabilis possesses numerous important virulence factors that are prominent in its pathogenesis. These factors include undulation motility, urease production, hemolysin production, and biofilm formation. All 59 isolates of *Proteus mirabilis* were subjected to *Proteus mirabilis* to study these factors, and as shown in Table [4], the results of the swarming movement showed that 59(100%) isolates belong to *Proteus mirabilis* can form undulating movement on a normal agar ratio (1.5-2%). This conclusion was consistent with the research of Liaw and his team [20], who cultured *Proteus mirabilis* on a culture medium containing 1.5% agar and observed the phenomena of entanglement from the earlier bacterium. The results of biofilm creation also demonstrated that 48 isolates of *Proteus mirabilis* formed for biofilm (81.35%) and that bacteria that grow on biofilms can withstand a variety of bacterial medications as well as phagocytosis, palatability, and many environmental factors [43]. This study's isolation of bacteria with the ability to form biofilms is compatible with those of (44,45). The present findings revealed that 50 (91.52%) of the bacterial isolates under study can produce hemolysin when grown on a medium containing 5% blood agar. All isolations are being studied according to the present work's findings. It is distinguished by its strong capacity to make urease, as all 59 isolates (100%) produced urease. This enzyme's ability to break down urea into ammonia NH4 and carbonic acid H2CO3 makes it important [46].

The author used the urea medium containing the index phenol red, which is affected by the changes in the pH of the medium, so the resulting ammonia will increase the pH of the medium, which leads to the change of the color of the index to pink. A total of 24 (100%) isolates showed positive results for the urease test, and this study agrees with the results by [47], which found that a total of 25(100%) isolates gave a positive result for the urease production test.

Table 4: Number and percentage of *Proteus mirabilis* bacteria producing virulence factors

Number of *Proteus mirabilis*	Virulence factors	*Proteus mirabilis* produces virulent factors	Percentage
59	Urease	59	100 %
59	Hemolysin	54	91.52%
59	Swarming	59	100 %
59	Biofilm	48	81.35%

- **Chemical Qualitative Detection of some Active Groups and pH of Cinnamomum Zeylanicum Aqueous Extract:**

The results of this study show that the qualitative detection of active ingredients in the aqueous extract of Cinnamomum zeylanicum includes Glycosides, Tannins, Resins, Saponins, and Phenols in varied percentages, and the lack of Phenols and flavones. These results follow what has been mentioned by many researchers who state that Cinnamomum zeylanicum contains the compounds

mentioned above in varied percentages, whereas [48] states that the cold aqueous extract of Cinnamomum zeylanicum contained active compounds such as (tannins, phenols, glycosides, and Resins) additionally the lack of flavones and alkaloids has not been observed. The cold aqueous extract of Cinnamomum zeylanicum is characterized by its active ingredients. This is a reason why it has a high inhibitory effect against bacterial isolates in this research, as shown in table 5:

Table [5]: The Results of Chemical Qualitative Detection of some Active Groups and pH of Cinnamomum Zeylanicum Aqueous Extract:

Active Groups	The cold aqueous extract of Cinnamomum zeylanicum
Glycosides	+
Alkaloids	-
Tannins	+++
Resins	+
Saponins	+++
flavones	-
Phenols	++
pH	6.5

+ sign means that the test is positive, and - is a negative result of the test.

2. Antibacterial Activity of Cinnamomum Zeylanicum Aqueous Extract against *Proteus mirabilis* isolated from UTIs

The results show that with the use of the agar-well diffusion method, Cinnamomum zeylanicum's aqueous extract has an effect against *Proteus mirabilis* isolated from UTIs. So, 12 bacterial isolates have been used after diagnosis. Hence, four different concentrations (12.5, 25, 50, and 75 mg/ml) have been studied, taken from the cold aqueous extract of Cinnamomum zeylanicum using five wells (the first one was a control). Consequently, four of them were compared to investigating the inhibitory efficiency of all concentrations mentioned above. However, it is clear from the results that the concentration (75 mg/ml) is the most affected by the bacterial isolates. As a result, the lowest diameter of the concentration (75 mg/ml) was 22 mm at its lowest and 30 mm at its highest. The lowest diameter of concentration (50 mg/ml) was 18 mm and 28 mm at the highest. The highest diameter was 26 mm at the concentration of 25 mg/ml, whereas the lowest diameter of inhibition measured 16 mm. The concentration (12.5 mg/ml) resulted in the highest diameter of 23 mm and the lowest diameter of 12 mm when compared to the control factor that contained sterile distilled water, as indicated by the table [6].

The above results show that the concentration of 75 mg/ml gave the best rate of inhibition diameters, and the diameters of the inhibition zones increased with increasing concentration of the extract because the cold aqueous extract of Cinnamomum zeylanicum has a high inhibitory effect against the pathogenic bacteria currently under consideration. The inhibitory effect of the aqueous extract of Cinnamomum zeylanicum could be due to its effective inhibitory compounds.

Table [6]: Inhibition zone (mean diameter of inhibition in mm) of different concentrations of the cold aqueous extract of Cinnamomum zeylanicum against *Proteus mirabilis* isolated from UTIs

Con.(mg/ml) Isolates	75	50	25	12.5	p-value
	Mean±S.D				
1	28±3.1	26±1.9	24±2.2	20±1.6	0.030*
2	28±3.1	24±2.2	18±0.9	15±1.0	≤0.0001**
3	24±2.2	20±1.6	17±1.8	14±0.8	≤0.0001**
4	26±1.9	20±1.6	16±1.2	12±1.1	≤0.0001**
5	30±4.3	27±1.1	25±1.4	22±1.7	0.011*
6	28±3.1	24±2.2	21.5±3.3	17±1.8	≤0.0001**
7	25±1.4	20±1.6	16±1.2	12±1.1	≤0.0001**
8	22±1.7	18±0.9	16±1.2	12±1.1	≤0.0001**
9	30±4.3	28±3.1	26±1.9	23±1.5	0.043*
10	30±4.3	27±1.1	24±2.2	22±1.7	0.021*
11	26±1.9	21.5±3.3	18±0.9	14±0.8	≤0.0001**
12	28±3.1	26±1.9	24±2.2	22±1.7	0.086

* Refer to significant difference at p≤0.05.
** Refer to significant difference at p≤0.01.

The antibacterial activity of the cold aqueous extract of Cinnamomum zeylanicum increases with the concentration of Cinnamomum zeylanicum, which is likely due to its inhibitory effects of active compounds that could inhibit bacterial growth; this may be because of the ability to inhibit several bacterial species because it contains some Phenolic compounds such as Thymol to be useful as an antifungal against bacteria, which has a great effect on the bacterial cell wall by the dissolving the fats of the cell wall and make H-bond with water molecules as well as to nitrogen-containing amino acids in the bacterial cell, which may effect on the working of Cell membrane of living organisms and therefore inhibiting the growth of the microorganism.

Besides, the effect of the inhibitory activity regarding the extract varies depending on the concentration used and the bacterial isolate, as well as the environmental conditions needed for bacteria to grow. This effect has been compared with the antibiotics that have been mostly used. The results show that most bacterial isolates (belonging to *Proteus mirabilis*) isolated from UTIs greatly affect the extract's inhibitory activity compared with the antibiotics used in this study. Finally, it is essential that extensive studies be conducted on Imipenem and how it can be used as an effective treatment for patients suffering from *Proteus mirabilis*. Therefore, encourage using effective compounds that are isolated from natural sources as well as using these natural sources to help in eliminating the growth of some pathogens, to reduce the side effects of antibiotics that are being used at present, which may cause many severe problems to the patient's body health.

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