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DIAGNOSIS OF PROTEUS MIRABILIS BACTERIA ISOLATED FROM URINARY TRACT INFECTIONS AND TESTING THE INHIBITORY EFFECTIVENESS OF THE AQUEOUS AND ALCOHOLIC EXTRACT OF THE URTICA CANNABIA PLANT. ITS DIRECTION

Omar falah hammed, Mahmoud Khalaf Saleh, Measer Abdullah Ahmed
College of Educatio for pure Sciencse, University of Tikrit

Abstract: 237 samples were collected from patients lying in the teaching hospital in Tikrit, between males and females and of various ages. The Proteus mirabilis bacteria were diagnosed based on the cultural characteristics (of the colonies) and the microscopic characteristics (of the bacterial cells). The diagnosis was made using the Vitek 2 device technology. The results showed that 196 had a positive result for the bacterial culture. The rate of (82.7%) and the rest (17.2%) were negative for bacterial culture. The results showed that the number of isolates of the Proteus mirabilis bacteria diagnosed was (11) isolates, with a rate of (4.6%). The antibiotic susceptibility of the isolates of the Proteus mirabilis bacteria was tested using the disk method for (11) types Among the antibiotics, most of the isolates appeared resistant to the antibiotics Azithromycin, Chloramphenicol, Amikacin, Ciprofloxacin, Amoxicillin-Clavulanic acid, Piperacillin, Ciprofloxacin, and Amoxicillin, in varying proportions, and the highest percentage of sensitivity was to the antibiotics Imipenem and Meropenem at 100%, respectively. The effectiveness of aqueous and alcoholic extracts of Urtica cannabia leaves against it was tested using the method of diffusion in holes and at different concentrations. The concentration of 50% for the aqueous extract of the nettle plant was the highest inhibitory among the extracts, and the lowest inhibitory concentration of the extracts was 25% for the alcoholic extract of the nettle leaf.

Key words: Proteus mirabilis, UTI, antibiotics, Urtica cannabia, nettle leaf, the aqueous extract, the alcoholic extract.

Introduction; The bacteria Proteus spp. It is part of the natural flora in the intestines of humans and animals, as well as its spread in water and soil as a result of pollution (Gelich *et al*., 2020). It is a rod-shaped, gram-negative bacterium that belongs to the intestinal family Enterobacteriaceae. It relies on flagella for its movement and is characterized by the phenomenon of swarming. There is more than one species belonging to this genus, and the most important of these species is P.mirabilis, which is a common cause of urinary tract infections. This P.mirabilis bacterium has many Virulence factors such as adhesion by fimbriae, motility, enzymes such as urease, toxins such as hemolysin, and immune evasion factors, which are opportunistic pathogens responsible for many hospital infections, especially in immunocompromised patients[1]. This bacteria causes the development of various diseases such as respiratory system infections, skin and soft tissue infections such as wounds, especially after surgical operations, and burn infections, in addition to urinary tract infections[2]. Urinary tract infections (UTIs) are one of the most common infections that affect humans, both in hospitals and consulting clinics, which include a group of clinical conditions that vary in severity, such as infections that occur without symptoms, to prostatitis, acute cystitis, nephritis,

pyelitis, and urethritis. They have become Urinary tract infections are among the diseases that most require specialist doctors to see for the purpose of treatment, because they do not affect a specific group of people, but rather are considered a threat to all ages, from birth to the elderly, and the bacterial species that settle in the intestines, which are considered a natural flora, are the Enterobacteriaceae family, including *Proteus*. It is one of the most common bacterial species responsible for urinary tract infections.[3]. The use of antibiotics has reduced child mortality rates and increased life expectancy. However, the number of infections caused by multidrug-resistant bacteria has begun to increase globally and the specter of untreatable infections has become a reality[4]. The incorrect use of antibiotics has led to the emergence of antibiotic-resistant bacteria[5]. Despite the magnitude of the achievements achieved by modern medicine, the side effects caused by medical drugs led to a return to the use of medicinal plants as therapeutic alternatives to manufactured medicines, as they are safer It is considered a raw source in the manufacture of many medicines and treatments. The nettle plant, Urtica cannabia, is considered a medicinal plant belonging to the Urticaceae family, and the nettle genus is the most widespread among the species of this family. The nettle plant possesses highly effective water-resistant chemicals for the growth of many microbes, and ethanol, aqueous, alcoholic, and phenolic extracts separated from the nettle plant have proven to be of high quality as water-resistant therapeutic alternatives for the growth of Gram-negative and Gram-positive bacteria[6].

Materials and Methods;

Blood Agar : It was prepared by adding 5% of human blood to the basic blood agar medium prepared according to the instructions of the producing company Himedia, sterilized and cooled to a temperature below 45ºC. The blood agar medium was used to identify the type of hemolysis caused by bacteria, as well as being a enriching medium to prepare the bacterial culture for tests. Biochemical

MacConkey agar: Prepare according to the manufacturer's instructions by dissolving 53.51 grams of MacConkey agar in 1000 milliliters of distilled water, sterilizing it in an autoclave, then pouring it into sterilized dishes and leaving it to cool, then storing it in the refrigerator until use.

Brain-Heart Infusion broth: It was prepared according to the manufacturer's instructions by dissolving (37) grams of BHI broth in (1000) milliliters of distilled water and distributing it into sterile bottles in the amount of (5) milliliters for each, sterilizing them in an autoclave, then storing them at a temperature of (4) degrees Celsius until use.

Vitek-2 Compact System: The compact system 2 -Vitek was adopted to definitively diagnose the isolates:

1- The bacterial suspension was prepared by inoculating the physiological solution with single pure colonies at 18-24 hours of age. Then the suspension was diluted until a suspension whose density ranged from (0.63-0.5) was obtained using a spectrophotometer with a wavelength of 625 nanometers.

2- A card cassette for diagnosing bacterial species was placed in each of the test tubes, then the tubes were placed in the Vitec device, then the results were read according to what was stated in Fritsche and his group (2011) and then the isolates were diagnosed.

Antibiotic sensitivity test by disc diffusion method: Antibiotic susceptibility testing was performed according to the standard Kirby Bauer Disk method, where ten antibiotics were used, as shown in Table 4-3) according to Brown (2007).

1. (3-5) colonies were transferred from a nutrient agar plate using a sterile loop from a young culture at 24 hours of age to test tubes containing 5 milliliters of physiological solution, thus forming a bacterial suspension. They were shaken well to homogenize the solution, and then the turbidity of the bacterial suspension in the tube was compared. The standard turbidity of the Macfarland tube is 0.5, as the turbidity of this tube represents an approximate number of 1.5 x 108.

2. A sterile cotton swab was immersed in the bacterial suspension, and the excess of the suspension was removed by rotating it. The swab was applied to the inner walls of the tube, then the bacterial suspension was spread on Mueller-Hinton medium, and the dishes were left at room temperature for (5-10) minutes for the culture to be absorbed and dried. Then the antibiotic tablets were placed using sterile forceps in equal dimensions, 5 tablets for each dish. Incubate the dishes at 37°C for 24 hours.

3. The results were read by measuring the diameters of the inhibitory zones around each disc using a ruler, then the results were compared to standard tables for the diameters of the inhibitory zones for antibiotics according to what was stated in the clinical and laboratory standards approved and updated for the year 2018 by the Clinical and Laboratory Standards Instiute CLSI.

Preparation of plant Extracts

Preparation of aqueous extract: Preparation of aqueous extract: Aqueous extracts of the plant used in the study were prepared by mixing 40 g of the plant model in 160 ml of distilled water, i.e. a ratio of 4:1 (wt/vol), and placing the plant model in the crushing device and stirring it with a magnetic stirrer for at least one hour. This is to dismantle and tear the plant cell walls. The mixture is then left in the refrigerator for 24 hours for the purpose of soaking. It is then filtered through several layers of gauze and then filtered again using Whatman No1 filter papers and emptied using a vacuum device.

Preparation of alcoholic extract: The method of the researcher, modified from the basic method of the researcher[7], was adopted in preparing alcoholic extracts by crushing 20 grams of leaves in 200 ml of 95% ethyl alcohol in an ice water bath, and then shaking the mixture. Mix well with a magnetic stirrer, leave it in the refrigerator for 24 hours, then filter the mixture through several layers of gauze. In order to get rid of the alcohol, the mixture was placed in a rotary evaporator device, which works on the basis of evaporation under rarefied pressure, at a temperature not exceeding 40°C. After evaporating the alcohol from the mixture, a thick layer of the extract was formed, which was dried by cooling under rarefied pressure in a drying device, and the samples were preserved. Then freeze until used.

Study the effect of plant extracts on bacteria: The diffusion method used by Abdel-Sahib (2008) was followed.

1- Put 20 ml of Mellur-Hinton agar medium into each plate.

2- The plates were left at a temperature of 25°C for 5-10 minutes to solidify, after which they were pierced using a cork bone drill, and the samples were cultured after comparing them with the standard McFarland solution.

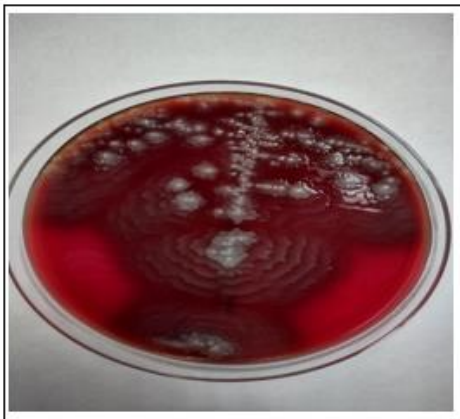
3- 1 ml of the concentrations used in the study were added to each hole, which are (25%, 50%, 75%, 100%) mg/ml.

4- The plates were incubated for 18-24 hours at a temperature of 37ºC.

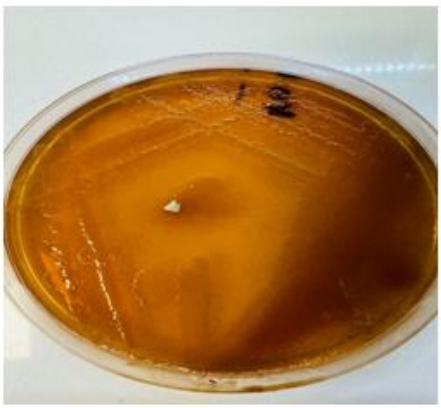
5- The inhibition zone was measured in millimeters and using a ruler.

Results and discussion;

Phenotypic diagnosis: Colonies were initially diagnosed by growing them on blood culture media and MacConkey culture medium. (11) isolates were tested as belonging to the genus Proteus, which showed the characteristic of swarming movement and fish odor on blood culture media, and also changed the color of the MacConkey culture media. To a pale color, this confirms its inability to ferment the sugar lactose and its consumption of peptone as a source of nitrogen and the production of metabolic substances that increase the value of the pH of the medium, which in turn affects the neutral red reagent, causing the colonies to turn pale [8][9]



Picture No.1



Picure no. 1 colonies ***Proteus mirabilis*** developing on the midst blood.

Picure no. 2 colonies ***Proteus mirabilis*** developing on the midst MacConkey.

Picture No.2

system Vitek 2 compact: The diagnosis of the isolates was confirmed using the Vitec device. We obtained 11 Proteus mirabilis isolates from the total clinical samples. The diagnosis confirmed that 11 isolates belong to the Proteus mirabilis type out of a total of 196. The accuracy of the diagnosis with the Vitec device reached 99%, as shown in the picture.

bioMérieux Customer: Microbiology Chart Report Printed December 23, 2023 12:08:28 PM CST

Patient Name: khadyga khadim, . Patient ID: hgre345
Location: Physician:
Lab ID: 103 Isolate Number: 1

Organism Quantity:
Selected Organism : Proteus mirabilis

Source: urine **Collected:**

Comments:	

Identification Information	**Analysis Time:**	3.85 hours	**Status:**	Final
Selected Organism	99% Probability	**Proteus mirabilis**		
	Bionumber:	0013000341542211		
ID Analysis Messages				

Biochemical Details

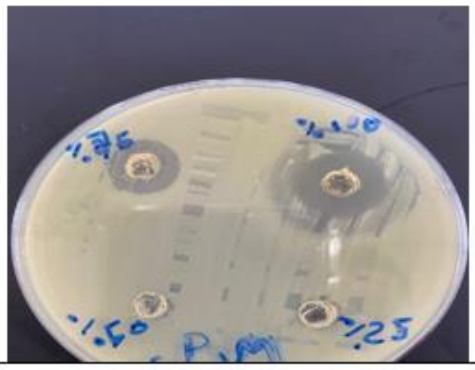
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	+	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	+	64	lLATa	-			

Testing the inhibitory activity of aqueous and alcoholic extracts of nettle

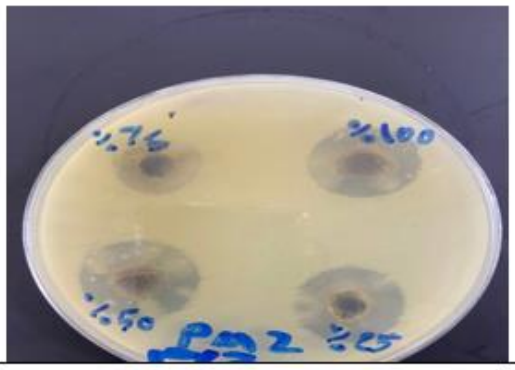
The effect of aqueous and alcoholic extracts on P.mirabilis bacteria was tested using the etching method and compared with the antibiotics (Imipenem, Gentamycin, Amikacin, Ciprofloxacin, Meropenem, Amoxicillin, Amoxicillin-Clavulanic acid, Piperacillin, Cefotaxime, Azithromycin, Chloramphenicol,) The current study showed good results of inhibition by plant extracts, with differences between them in terms of inhibition, which depends on the active substances present in each extract and the variation in concentration as well as the type of bacteria, as is clear in Table No. 1,2 and Image No. 3, in which the diameters of the inhibitor

appear clearly and in concentrations. Different concentrations are 100, 75, 50, and 25 mg/ml, respectively, and are considered to have a higher inhibitory effect when compared to some antibiotics, such asAmoxicillin, Azithromycin, Chloramphenicol, Amikacin, Ciprofloxacin, Amoxicillin-Clavulanic acid, Piperacillin, and Ciprofloxacin. The extracts showed weak inhibition compared to the two antibiotics, Meropenem and Imipenem, respectively. The diameters of inhibition for the anti-Meropenem were the highest among the antibiotics, as shown in Table 3, where the highest inhibition reached 37 mm and the lowest inhibition. 15 mm and the aqueous extract of nettle plant was more effective against Proteus mirabilis bacteria The inhibitory effectiveness of the alcoholic extract of the nettle plant and the rest of the antibiotics, except for the antibiotics Meropenem and Imipenem, was clearly evident. The 50% concentration of the aqueous extract of the nettle plant was the most effective among the four concentrations of the extract, as the maximum inhibition diameter for this concentration was 28 mm and the minimum inhibition was 18 mm, as is clear in the table. No. 1, and the concentration of 25% of the alcoholic extract of the nettle plant had the highest inhibition among the concentrations of the alcoholic extract, where the highest diameter of inhibition was 20 mm and the minimum inhibition was 17 mm, as shown in Table No. 2. This indicates that the aqueous extract of the nettle plant is the most effective against the Proteus mirabilis bacteria. From the alcoholic extract of nettle plant.

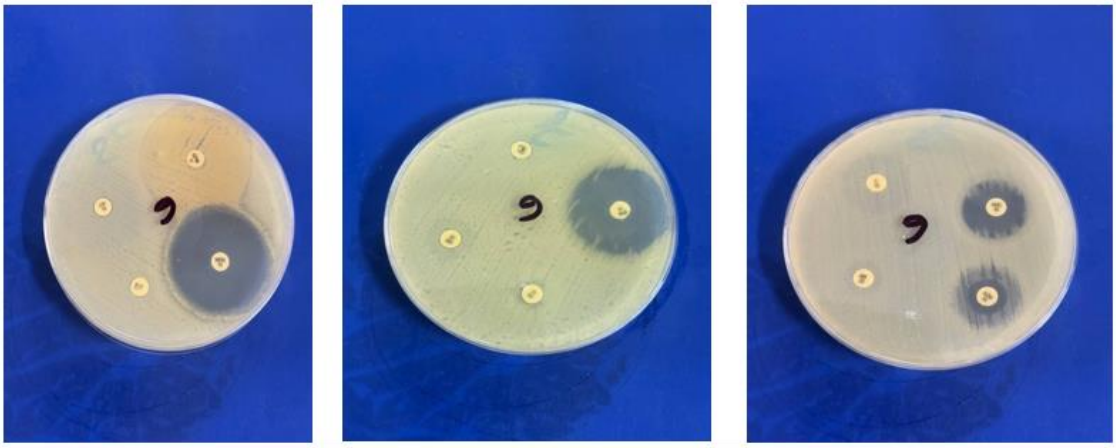
The nettle plant is one of the most effective plants with inhibitory activity, and its extracts have proven highly effective in inhibiting many negative and positive bacteria. [9]The reason for the inhibitory effect is that the nettle plant contains compounds with an effective effect in inhibiting bacteria, such as phenolic compounds and fatty acids, which have an effect on the cell wall and stops the process of building cellular molecules such as amino acids and proteins by interfering with them through hydrogen bonds. Which leads to stopping the construction process [10] Studies have shown that phenolic compounds have high inhibitory activity against pathogenic bacteria [11]The pumaric found in the nettle plant is considered to inhibit bacterial growth, and the results showed that it works to disrupt the cell membrane of the bacteria and bind to DNA, and this leads to the inhibition of cellular functions. Ultimately, the bacterial cell dies [12]Also, there is vanillic acid, which acts as an antibacterial and antifungal by affecting the plasma membrane and the cell wall, stopping the process of cell[13,14]. Likewise, the compound quercetin is considered an inhibitor and lethal to some bacteria and has a selective effect. -Ortega et al., 2020).



Picure no.4: Demonstrating the inhibitory activity of the alcoholic extract of nettle leaves



Picure no.3: Demonstrating the inhibitory activity of aqueous extract of nettle leaves.



Picure no.5 Explain the action of antibiotics.

Abstract	Inhibition zone diameter for each concentration (mm)			
Sample sequencing	%25	%50	%75	%100
P. mirabilis 1	22	28	14	17
P. mirabilis 2	22	24	16	20
P. mirabilis 3	18	24	19	15
P. mirabilis 4	21	22	13	21
P. mirabilis 5	17	19	15	17
P. mirabilis 6	19	20	12	16
P. mirabilis 7	14	18	17	19
P. mirabilis 8	18	22	14	14
P. mirabilis 9	21	20	14	16
P. mirabilis 10	19	22	17	18
P. mirabilis 11	22	24	15	15

Table No. 1 shows the inhibitory activity of the aqueous extract of nettle leaves

Abstract	Inhibition zone diameter for each concentration (mm)			
Sample sequencing	%25	%50	%75	%100
P. mirabilis 1	18	11	15	9
P. mirabilis 2	20	0	13	0
P. mirabilis 3	19	0	9	0
P. mirabilis 4	17	0	11	0
P. mirabilis 5	19	17	15	17
P. mirabilis 6	17	11	9	0
P. mirabilis 7	18	0	12	0
P. mirabilis 8	19	0	11	0
P. mirabilis 9	16	0	9	11
P. mirabilis 10	20	0	10	0
P. mirabilis 11	18	0	14	0

Table No. 2 shows the inhibitory activity of the alcoholic extract of nettle leaves

Antibiotics / Samplesequencing	Ak	AMC	CFM	MEM	IPM	AZM	CIP	C	GM	AM	PRL
P. mirabilis 1	-	-	-	15	30	12	43	-	15	22	17
P. mirabilis 2	12	24	23	32	28	15	30	-	16	-	12
P. mirabilis 3	-	-	-	35	15	-	-	-	15	-	-
P. mirabilis 4	11	14	32	32	27	15	-	-	14	-	-
P. mirabilis 5	17	13	24	33	23	14	-	22	-	-	-
P. mirabilis 6	14	22	29	29	16	-	40	-	-	-	-
P. mirabilis 7	16	-	-	27	26	11	-	-	-	-	-
P. mirabilis 8	12	-	31	37	17	13	-	-	-	-	-
P. mirabilis 9	-	19	-	35	22	16	-	-	15	-	-
P. mirabilis 10	-	16	-	30	33	14	36	-	14	-	13
P. mirabilis 11	-	22	23	33	35	12	-	-	-	23	-

Table No. 3 shows the diameters of the inhibition points for the following antibiotics: AK: Amikacin , AMC: Amoxicillin / Clavulanic acid , AM: Amoxicillin , , AZM: Azithromycin , CAZ: Ceftazidine , CIP : Ciprofloxacin, C : Chloramphenicol , CFM: Cefixime , PRL: Piperacillin ,GM: Gentanmicin, IPM: Impinem, MEM: Meropenem.

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