MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

The effect of herbal medcinal plants on the *proteus* species resistance against antibiotics

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Received:23/1/2023
Accepted:7/3/2023

Abstract: *Proteus mirabilis* is a gram-negative member of the Enterobacteriaceae family of bacteria. Numerous infections, including those of the respiratory tract and urinary tract, are caused by it. High antibiotic resistance in *Proteus mirabilis* may result in multidrug resistance and the failure of antimicrobial therapy. **Objective**: The current paper's objective is to investigate the possible antibacterial properties of medicinal plants as well as their value as a source of new anti-infection chemicals. **Results:** Twenty-five *proteus* isolates, recorded resistance to different antibiotic categories. The highest resistance was shown to trimethoprim-sulfamethoxazole (96%), the highest intermediate was shown to imipenem (28%) and the highest susceptibility was shown to Piperacillin-tazobactam (88%). The diameter of the inhibition zone of ethanolic extracts was tabulated for all extracts, the ethanolic exteract of green tea was the most active with an inhibition zone diameter (mm) ranged between 25 and 40, followed by clove with inhibition zones diameter ranged between 23 and 31. Followed by lemon and cinnamon respectively but the ginger was the most inactive one with inhibition zone diameter (mm) ranging between 0 and 14. Based on the results obtained during this study, we recommend the application of some medicinal plants like *Camellia sinensis* (green tea) against antibiotic resistant *Proteus* species isolated from patients admitted to Mansoura University Hospitals.

keywords: Ginger, Moringa, *Clarias gariepinus*, serum collection, biochemical analysis.

Introduction

Many of the gram-negative bacteria in the *Proteus* genus infect people and cause illnesses **(1)**. For example, *P. mirabilis* may be the primary culprit in urinary tract infections **(2, 3, 4, 5)**, respiratory tract infections, wound infections, burn infections, and digestive tract infections. *P. mirabilis* can secrete a variety of substances, some of which are known as virulence factors and have been associated with the capacity to cause disease **(8, 9)**. These substances include swarming, fimbriae, urease, hemolysin, iron acquisition systems **(10)**, protease, and lipopolysaccharides **(11)**. Additionally, *P. mirabilis* is well known for its capacity to colonize the airways and produce biofilms that resist antibiotic therapy. Antibiotic resistance *is more* frequently detected in strong biofilm formers than in weak biofilm formers **(12, 13)**.

Antibiotic resistance among *P. mirabilis* strains has spread globally, complicating medical management. Resistance to -lactams (both penicillins and cephalosporins), fluroquinolones, nitrofurantoin, fosfomycin, aminoglycosides, tetracyclines, and sulfonamides has also been documented in addition to the resistance to SXT that has been described **(14, 15, 16)**. Treatment failures may result from this organism's propensity to embed itself in urinary stones or crystalline biofilms on urinary catheters, which can act as a bacterial shield. Defensin, polymyxin B, protegrin, LL-37, and other antimicrobial peptides are all extremely resistant to *P. mirabilis* **(17, 18)**.

For hundreds of years, plant extracts, oils, and substances derived from them have been used to treat pathogens like bacteria, fungi, and viruses because they are effective against a wide range of microorganisms **(19)**. Historically, man has employed plants to treat common infectious diseases, and some of these folk remedies are still used regularly to treat a variety of ailments. For instance, various manuals of phytotherapy report the use of Arctostaphylos bearberry (Arctostaphylos) and cranberry juice (Vaccinium macrocarpon) to treat urinary tract infections, while broad-spectrum antimicrobial agents like *Melissa* officinalis (lemon balm), Allium sativum (garlic), and Melaleuca alternifolia (tea tree) are described as species **(20, 21)**.

Materials and methods

Bacterial strains

Clinlical samples (wound, urine, stool, nose swap, sputum) were collected from patients admissible to different Mansoura University Hospitals. Samples were collected under sterilized conditions, then cultivated in nutrient agar media to ensure they are *Proteus*, after 24 hours of incubation in an incubator at 37 ^{0}C, a smear of each sample was taken to ensure completely that these samples are *Proteus*. They were cultivating on McCKonkey agar media and blood agar media then incubated in incubator at 37 ^{0}C for 24 hours. Also, to make sure this samples were *proteus*, we applied biochemical identification and Gram staining. For the initial isolation and diagnosis of bacterial isolates, all specimens were planted on several cultures, including blood agar base and macconkey agar. subsequent identification was then done using cultural traits and biochemical testing **(22)**. Only 25 isolates were used for antibiotic susceptibility and susceptibility to different medicinal plant extracts.

Antibiotic disks

The antibiotic tested were Gentamicin, Amikacin, Amoxicillin-Clvulanate, Piperacillin-tazobactam, Ceftizoxime, Ceftriaxone, Imipenem, Trimethoprim, Sulfamethoxazole, Ciprofloxacin, and Levofloxacin. These antibiotic disks were purchased from Oxoid, England . The name of these antibiotic disks, potency, symbol and the standard evaluation of inhibition zones **(23)** was represented in **Table (1).**

The results in Table (3) and (4) indicated that the highest no. of isolates were multidrug resistant (MDR).

Table (1): The evaluation of inhibition zone diameter (nm) in antibiotic.

Antimicrobial categories	Antimicrobial Agents(Antibiotics)	Symbol	Potency (µg/ disk)	Resistance (R)	Intermediate (I)	Susceptible(S)
Aminoglycosides	Gentamicin	CN	10	≤ 12	13-14	≥ 15
	Amikacin	AK	30	≤ 14	15-16	≥ 17
Combinations	Amoxicillin-Clvulanate	AMC	20/10	≤ 13	14-17	≥18
Penicillins/β-Lactamase inhibitors	Piperacillin-tazobactam	TPZ	11	≤ 14	15-20	≥ 21
Cephalosporins/Cephamycins	Ceftizoxime	CTX	30	≤ 21	22-24	≥ 25
	Ceftriaxone	CRO	30	≤ 13	14-20	≥ 21
Carbapenems	Imipenem	IPM	10	≤ 19	20-22	≥ 23
DHFRInhibitor/Sulfonamides(DHPS Inhibitor)	Trimethoprim Sulfamethoxazole	SXT	1.25/23.75	≤ 10	11-15	≥ 16
Fluoroquinolones	Ciprofloxacin	CIP	5	≤ 20	21-30	≥ 31
	Levofloxacin	LEV	-	≤ 15	16-20	≥ 21

Disk Diffusion Method

According to Bauer et al. **(24)**, the Kirby-Bauer disc diffusion technique was used to assess the proteus species' susceptibility to antibiotics using Mueller-Hinton agar. This procedure was carried out using the inoculation standard method, which involved inoculating 5 ml of sterile nutrient broth medium before incubating it at 37 °C for 24 hours. To get the infected broth's turbidity equal to half the Mcfarland standard, distilled water was added to the mixture.The *Proteus* species suspension was inoculated by sterile cotton swab on Mueller-Hinton agar plates in 3 directions on the agar plates surface to get a uniform inoculum. Then, the plates were allowed to dry

for 3-5 minutes before adding antibiotic disks. Then the plates were incubated into an incubator at 37ºC for 24 hours. At the end of incubation period, the diameter of the zones of inhibition was measured in one direction using a ruler, including the diameter of the disk.

Medicinal plant extracts (Detection of proteus species susceptibility to different medicinal plant extracts)

The following medicinal plants, which are tabulated in **Table (3)** were collected to prepare ethanolic plant extracts. The plant samples were collected from an herbalista in Mansoura, Egypt.

Table (2): Scientific and Arabic names and parts from each medicinal plants used in preparing extracts

English Name	Scientific name	Arabic name	Family	Usedpart
Lemon	Citrus limon	الليمون	Rutaceae	Cortex
Ginger	Zingiber officinale	الزنجبيل	Zingiberaceae	Rhizomes (roots)
Cinnamon	Cinnamomum spp	القرفة	Lauraceae	Bark
Green tea	Camellia sinensis	الشاي الاخضر	Theaceae	Leaf
Clove	Syzygium spp	القرنفل	Myrtaceaea	Flower buds

Prepration of plant extract

Ten grams of dried plant material *(Citrus limon*, *Zingiber officinale*, *Cinnamomum spp.*, *Camellia sinensis* and *Syzygium spp*) were soaked in 100 ml of ethanol (ethyl alcohol 99%) for 7 days at room temperature. After that, the resulting extract was filtered through Whitman filter paper (No.1). The filtration residue was reextracted twice using the same procedure. Then the filtrates obtained were evaporated using a rotary evaporator to dryness at 40 ^{0}C. Stock solutions of the extract were obtained by dissolving in 10% dimethylsulfoxide (DMSO). All extracts were sterilized by filtration through the bacterial filter using positive pressure, and then the filtrate was kept at 4^{0}C in the refrigerator till use.

Determination of the antibacterial activity of medicinal plant extracts against antibiotic resistant *Proteus* species

The antibacterial activity of ethanolic medicinal plant extracts against antibiotic-resistant *Proteus* species was determined by using the agar well diffusion method. Bacterial samples were spread on Muller-Hinton agar plates by using sterilized cotton swabs, and after 5 minutes, six wells approximately 6 mm in diameter were bored by using a sterile cork borer. These six wells were filled with five plant extracts (150 µl) using a sterile Pasteur pipette. DMSO was used in the same manner in the 6th as well as negative control which did no effect on microorganisms growth. The plates were incubated at 37^{0}C for 24 hours. After the incubation period, the inhibition zone diameter was measured and tabulated.

Transmission Electron microscopic examination (TEM)

This step was applied to antibiotic-resistant *proteus* species isolate, which gives the highest susceptility to plant extract before and after plant treatment.

This section was prepared following overnight incubation, the bacterial colonies were transferred to Eppendorf tubes, then washed 3 times with sterile nanopure water, then the tubes were centrifuged to get the pellet of bacteria. Bacterial suspension fractions were fixed with (2% glutaraldehyde) in (0.1mol/L) sodium phosphate buffer at 4^{0}C for 90 minutes (pH7.4). The cells were stained with (0.25% uranil acetate) at 4^{0}C for 1 hour after being post-fixed in osmium oxide (1% OsO_4) for 90 minutes. The cells were then immersed in Spurr resin and given 24 hours in a 60^{0}C oven to polymerize. By cutting an 80-nm film at 250°C with an ultramicrotome Sorvall MT 5000 (Dupont, Boston, MA) fitted with a diamond knife, resins were sectioned. The thin segment was detected using TEM at an accelerating voltage of 80 kV while mounted on copper girds coated in carbon.

20 L aliquots of the liquid cultures were placed on carbon film-coated TEM girds. Without staining, 20 L of (5% formaldehyde) was used to fix bacterial cells. The samples were quickly looked at using TEM and SEM.

TEM Image processing

A programm for image analysis was used to digitize and store each image as 512 x 512 pixels (Digital Micrograph TM 3.7.0., Gatan Inc.). Specimens with cutting and staining

artifacts for TEM were excluded. Additionally, to process the chosen micrographs (binarization, segmentation, and extraction of local and dynamic threshold), Wayne Rasband's ImageJ 1.40g (NIH, USA) and the MBF ImageJ set of plugins and macros were utilized **(28)**. It's vital to remember that the processing was just done to make it simpler to visualize micrographs.

Results

Twenty-five of *Proteus* isolates, recorded resistance to different antibiotic categories. Plate (1) show one of the most resistant *proteus* isolates. The results in **Table (3)** and **Figure 1** show the antimicrobial activity of 25 *Proteus* to 10 antibiotics belonging to 9 antimicrobial categories. The highest resistance was shown to Trimethoprim-Sulfamethoxazole (96%), the highest intermediate was shown to imipenem (28%) and the highest susceptibility was shown to Piperacillin-tazobactam (88%).

Table (3): Antimicrbial susceptibility of 25 *proteus* species isolates

Antimicrobial categories	Antimicrobial Agents (Antibiotics)	Symbol	Resistance (R)		Intermediate (I)		Susceptible (S)	
			No.	%	No.	%	No.	%
Aminoglycosides	Gentamicin	CN	13	52	3	12	8	32
	Amikacin	AK	9	36	2	8	14	56
Combinations	Amoxicillin- Clvulanate	AMC	12	48	2	8	11	44
Penicillins/β-Lactamase inhibitors	Piperacillin-tazobactam	TPZ	0	0	3	12	22	88
Cephalosporins/Cephamycins	Ceftizoxime	CTX	18	72	1	4	6	24
	Ceftriaxone	CRO	13	52	2	8	10	40
Carbapenems	Imipenem	IPM	8	32	7	28	10	40
DHFRInhibitor/Sulfonamides(DHPS Inhibitor)	Trimethoprim Sulfamethoxazole	SXT	24	96	0	0	1	4
Fluoroquinolones	Ciprofloxacin	CIP	18	72	5	20	2	8
	Levofloxacin	LEV	6	24	5	20	11	44

Table (4): Response of the tested clinical *Proteus species* isolates against antimicrobial agents

Proteus species isolate No.	Diameter of Inhibition Zone (mm)												
	CN (10 µg)	AK (30 µg)	AMC (20/10 µg)	TPZ (11 µg)	CTX (30 µg)	CRO (30 µg)	IPM (10 µg)	SXT (1.25/23.75µ)	CIP (5 µg)	LEV (5 µg)	R	I	S
1	0 ®	0 ®	28 (S)	38 (S)	0 ®	30 (S)	10 ®	4 ®	4 ®	12 ®	7	0	3
2	0 ®	9 ®	25 (S)	34(S)	0®	0®	25(S)	0 ®	10 ®	0 ®	7	0	3
3	17 (S)	10 (S)	0 ®	34 (S)	0 ®	0®	32 (S)	0 ®	0 ®	0®	6	0	4
4	18 (S)	14 ®	12®	30 (S)	35(S)	26 (S)	30 (S)	0 ®	30 (I)	32 (S)	3	1	6
5	18 (S)	20 (S)	20 (S)	34(S)	18 ®	30 (S)	30 (S)	0 ®	24 (I)	22 (S)	2	1	7
6	0 ®	15 (I)	8 ®	24 (S)	0 ®	0 ®	25 (S)	0 ®	0 ®	19 (I)	6	2	2
7	5 ®	14 ®	7 ®	20 (I)	0 ®	0 ®	17 ®	0 ®	0 ®	15 (I)	8	2	0
8	15 (S)	17 (S)	17 (I)	22 (S)	24 (I)	34 (S)	21 (I)	0 ®	15 ®	19 (I)	2	4	4
9	12 ®	0 ®	10 ®	30 (S)	0 ®	0 ®	19 ®	0 ®	11 ®	20 (I)	8	1	1
10	12 ®	20 (S)	0 ®	26 (S)	4 ®	0 ®	20 (I)	0 ®	10 ®	20 (I)	6	2	2
11	12 ®	13 ®	18 (S)	27 (S)	25 (S)	20 (I)	20 (I)	0 ®	18 ®	30 (S)	4	2	4
12	0 ®	17 (S)	0 ®	22 (S)	0 ®	23 (S)	0 ®	0 ®	20 ®	23 (S)	6	0	4
13	14 (I)	25 (S)	13 ®	20 (I)	0 ®	0 ®	25 (S)	0 ®	23 (I)	23 (S)	4	3	3
14	18 (S)	20 (S)	0 ®	18 (I)	0 ®	27 (S)	20 (I)	24 (S)	30 (I)	30 (S)	2	3	4
15	0 ®	42 (S)	18 (S)	40 (S)	0 ®	0 ®	36 (S)	0 ®	6 ®	6 ®	6	0	4
16	10 ®	18 (S)	23 (S)	48 (S)	0 ®	10 ®	26 (S)	0 ®	18 ®	20 (I)	5	1	4
17	22 (S)	30 (S)	0 ®	52 (S)	36 (S)	54 (S)	22 (S)	0 ®	23 (S)	22 (S)	2	0	8
18	0 ®	20 (S)	17 (I)	27 (S)	0 ®	0 ®	0 ®	0 ®	0®	19 (I)	6	2	2
19	19 (S)	20 (S)	10 ®	22 (S)	0 ®	0 ®	20 (I)	0 ®	34 (S)	30 (S)	4	1	5
20	10 ®	10 ®	20 (S)	22 (S)	0 ®	0 ®	21 (I)	0 ®	12 ®	13 ®	7	1	2
21	14 (I)	24 (S)	20 (S)	36 (S)	22 (S)	36 (S)	16 ®	0 ®	20 ®	22 (S)	3	1	6
22	20 (S)	15 (I)	22 (S)	34 (S)	25 (S)	30 (S)	16 ®	0 ®	18 ®	46 (S)	3	1	6
23	17 (S)	14 ®	18 (S)	28 (S)	0 ®	13 ®	25 (S)	0 ®	0 ®	0 ®	6	0	4
24	14 (I)	20 (S)	0 ®	24 (S)	17 ®	30 (S)	25 (S)	0 ®	22 (I)	22 (S)	3	2	5
25	10 ®	12 ®	19 (S)	30 (S)	14 ®	8 ®	22 (I)	0 ®	14 ®	13 ®	7	1	2

CN= Gentamicin, **AK**= Amikacin, **AMC**= Amoxicillin- Clvulanate, **TPZ**= Piperacillin-tazobactam, **CTX**= Ceftizoxime, **CRO=** Ceftriaxone, **IPM=** Imipenem, **SXT=** Trimethoprim-

Sulfamethoxacin, **CIP**= Ciprofloxacin, **LEV**= Levofloxacin, , **R**= Resistant, **I**= Intermediate, **S**= Sensitive

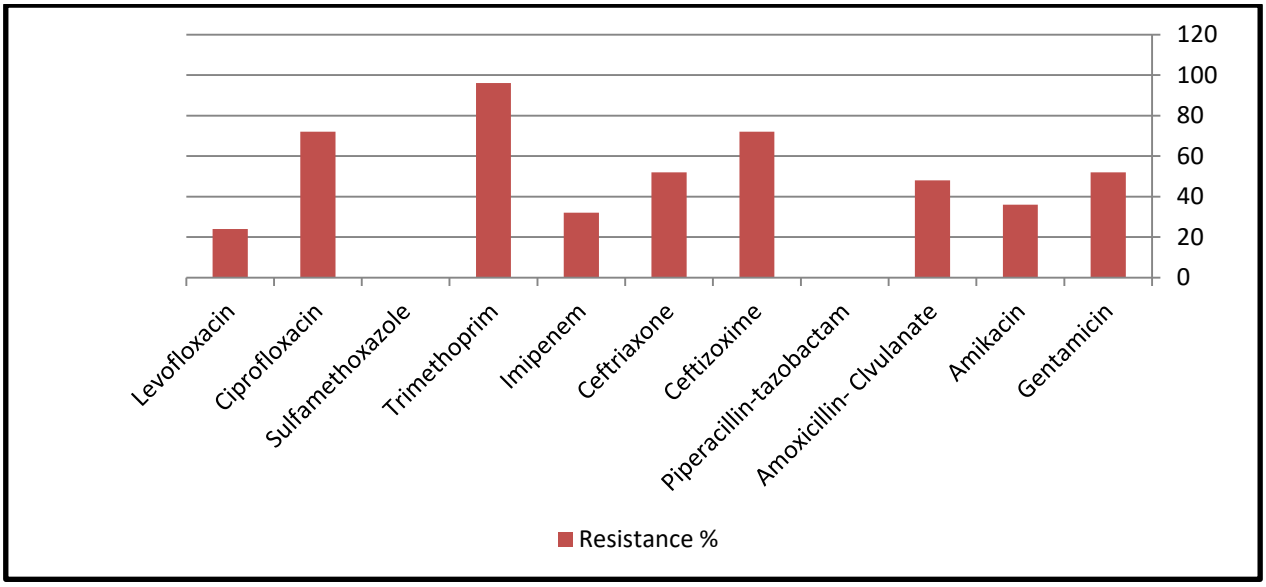


Figure (1): Comparative resistance of *proteus* isolates against antibiotics

The clear zones were measured and compared to the standard recommendation of Clinical Laboratory Standard Institute (CLSI) **(29).** The Result in **Table (4)** and **Figure(2)** showed that the maximum zone of inhibition observed for Imipenem was 25 mm in diameter, followed by Piperacillin-tazobactam (24 mm in diameter) followed by Levofloxacin (19 mm in diameter) followed by Amikacin (15 mm in diameter) and showed no zones of inhibition for Gentamicin, Amoxicillin- Clavulanate, Ceftizoxime, Ceftriaxone, Trimethoprim-Sulfamethoxacin and Ciprofloxacin, in the most resistant *proteus* species isolate no. 6

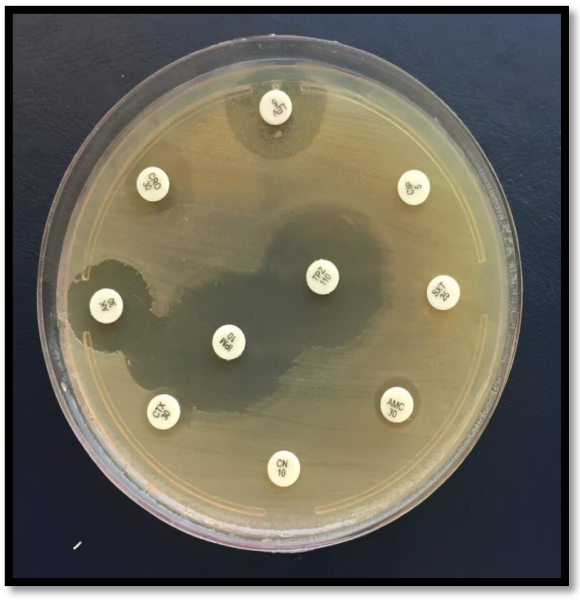


Figure (2): The most resistance proteus isolate (isolate No. 6)

LEV = Levofloxacin, CIP = Ciprofloxacin, SXT = Trimethoprim-Sulfamethoxazole, CRO = Ceftriaxone, IPM = Imipenem, CTX = Ceftizoxime, TPZ = Piperacillin-tazobactam, AMC = Amoxicillin-Clavulanate, AK = Amikacin, CN = Gentamicin.

Antibacterial activity of medicinal plant extracts against the 25 isolates of *Proteus*

In the current study, five ethanolic extracts derived from different parts of five medicinal plants with different concentrations (*Citrus lemon* and *Syzygium aromaticum* = 200 mg/ml, *Zingiber officinale* = 80 mg/ml, *Cinnamomum spp* = 140 mg/ml and *Camellia sinensis* = 120 mg/ml) of traditionally used in Egyptian folk medicine belonged to five families and were screened for their antibacterial activity against the 25 isolates of *proteus* by the agar well diffusion method.

The Diameter of the inhibition zones of ethanolic extracts was tabulated in **Table (5)** and shown in **Figure (3)**, of all extracts, the ethanolic exteract of green tea was the most active one with inhibition zones diameter (mm) ranging between 25 and 40, followed by clove with inhibition zones diameter ranged between 23 to 31. Followed by lemon and cinnamon respectively but the giner was the most un active one with inhibition zones diameter (mm) ranging between 0 and14.

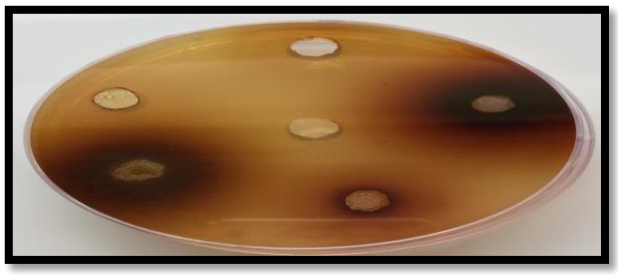


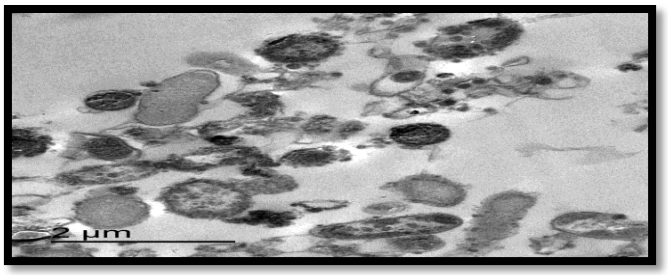
Figure (3): Inhibition zone of different plant extract against one of the most resistant *Proteus* species isolates (isolate No. 7)

Citrus lemon (200 mg/ml) and Syzygium aromaticum (200 mg/ml) had good antibacterial activity.The inhibition zones diameter (mm) ranged between 25 to 40 for Camellia sinensis, 19 to 25 for Citrus lemon and between 23 to 31 for Syzygium aromaticum.While antibiotics (gentamicin, amikacin, amoxicillin-clavulanate, piperacillin-tazobactam, ceftizoxime, imipenem, ceftriaxone, trimethoprim-sulfamethoxazole, ciprofloxacin and levofloxacin) were almost resistant.

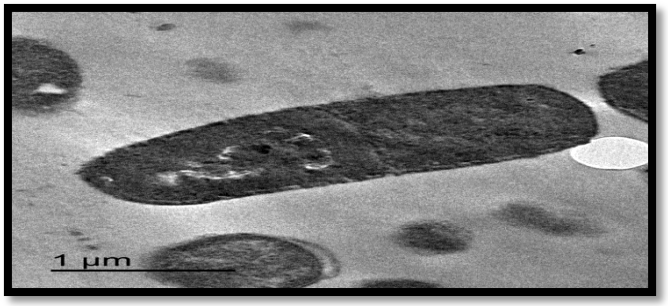
Transmissionelectronmicroscopeexamination of plant susceptible *proteus* species isolates

The most effective medicinal plant extract against antibiotic-resistant *Proteus* species isolates was green tea. One of the resistant isolates (isolate No. 7) was examined under TEM before and after treatment with green tea (at 280 mg/ml concentration).

Figure (4) showed denaturation of bacterial internal shape where vacuolation, cyst formation and cell wall rupture of the isolate after treatment with plant **(B)**



A Before treated



B After treate

Figure (4) (A&B) : Transmission electron microscope examination of green tea susceptible *proteus* species isolate (isolate No. 7)

Table (5): Antibacterial activity of ethanolic plant extracts against the 25 isolates of *proteus* species

Proteus species isolates No.	Diameter of inhibition zone (mm) of different ethanolic plantExteracts				
	Citrus lemon (Lemon)	Zingiber officinale (Ginger)	Syzygium aromaticum (Clove)	Cinnamomum spp (Cinnamon)	Camellia sinensis (Green tea)
1	20	0	30	14	30
2	25	0	26	16	35
3	20	0	30	20	30
4	19	0	25	15	38
5	19	14	26	18	32
6	23	0	29	16	37
7	22	0	27	20	40
8	19	0	30	15	35
9	23	0	30	10	39
10	25	0	26	16	35
11	21	0	26	20	35
12	20	0	23	19	30
13	24	0	30	11	35
14	20	0	25	16	35
15	23	0	28	13	35
16	23	0	30	16	35
17	20	0	30	15	35
18	20	0	27	13	35
19	20	0	30	15	29
20	20	0	25	15	40
21	20	0	25	12	30
22	25	0	28	15	34
23	23	0	25	13	35
24	20	0	30	13	35
25	20	0	31	18	25

1= Citrus lemon (Lemon), 2 = Zingiber officinale (Ginger), 3 = Syzygium aromaticum (Clove), 4 = Cinnamomum spp (Cinnamon), 5 = Camellia sinensis (Green tea), 6 = Blank (DMSO)

Discussion

potential infection-causing agents as well as possible sources of resistance genes that might spread to other bacterial diseases. The isolates' high levels of lactamase synthesis and multi-drug resistance are signs that the threat from resistance in Ghana is getting worse, according to past investigations **(31)**. This resistance depends on changes to lipopolysaccharide (LPS) and extracellular proteases like ZapA. **(32, 33, 34)**. The development of biofilms, which are connected structures containing microbial cells and populations embedded in a polysaccharide layer, is a key component of *Proteus*' pathogenicity. Better bacterial cell adaptability to the external environment is made possible by the biofilm, which also facilitates **(35).**

According to Feglo et al. **(36)**, resistance to ampicillin, sulphamethoxazole-trimethoprim, and chloramphenicol was shown to be 77-82%. According to Newman et al. **(31)** the resistance rates to ampicillin, chloramphenicol, and cotrimoxazole were 76%, 75%, and 73%, respectively. In our investigation, the tested cephalosporins, fluoroquinolones, and gentamicin isolates of P. mirabilis exhibited moderate resistance (31%–53%). Kamel et al. reported higher resistance rates to ciprofloxacin (51%). (37). Ceftazidime and ceftotaxime resistance rates were greater (44.7% and 51.1%, respectively) than those reported by Al-Bassam and Al-Kazaz **(38)** who found resistance rates of 40% and 30%, respectively. Cefepime resistance was also higher than that documented by Ahmed **(39)** who discovered that just 20% of isolates exhibited resistance.

Isolates of *Proteus mirabilis* exhibit moderately low resistance (> 30%) to piperacillin, amikacin, aztreonam, and imipenem. Among the examined drugs, imipenem and meropenem had the lowest rates of resistance (8.5% and 6.4%, respectively). This conclusion was shared by Adamus-Bialek et al **(40)**. But according to Serry et alresearch .'s **(41)** *P. mirabilis* isolates had reduced resistance rates to amikacin, levofloxacin, ciporofloxacin, and gentamicin (2.2% -17.8%), but P. mirabilis isolates were 100% responsive to imipenem. High antimicrobial resistance was discovered in the isolated *Proteus* species against tetracycline (85%), chloramphenicol (82.5%), co-trimoxazole (81%) and ampicillin (77%).

Recently, biologically active chemicals isolated from well-known plant species have received a lot of attention. Phytochemicals found in plant extracts include alkaloids, flavonoids, ouramin, psoralens, and carotenoids as well as essential oils and other compounds. The ability of groups of phenol compounds to denaturize bacterial cell proteins and harm bacterial cell membranes gives flavonoid compounds the ability to effectively suppress the growth of bacteria, fungi, and viruses **(42)**.

The utilization of medicinal plants is essential in meeting the fundamental healthcare needs of underdeveloped nations, and the plants may provide a new supply of antibacterial, antifungal, and antiviral compounds that have potent activity against infectious germs **(43,44)**.

Different green tea leaf extracts have demonstrated anti-UTI bacterial activity. Tea's active ingredient is thought to inhibit the growth and development of germs. Due to the presence of particular antioxidant catechins and polyphenols that harm bacterial cell membrane, tea has the strongest antibacterial activity **(45)**. The presence of diverse secondary metabolites like hydroxyl groups on the active ingredients affects the antibacterial action of plant extracts.

The presence of oxygenated mono- and sesquiterpenes, phenolic chemicals (shogaol, gingerol), which are lipid-soluble phenol compounds principally extracted from the root of ginger **(48)**, is what gives ginger its antibacterial activity. **(46, 49)**. These substances influence cells in a variety of ways, including by damaging their permeability and causing the release of intracellular substances like ribose and Na glutamate, as well as by interfering with membrane activities (electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity). Thus, these substances may have a variety of invasive

targets that could suppress microbial infections **(50)**.

Cinnamon, clove, and rosemary oils demonstrated potent and reliable inhibitory effects against a variety of pathogens, according to research **(51,52)**. The presence of various active elements in the oils has been linked to the antibacterial action. Cinnamaldehyde was found to be the main component of cinnamon oil according to our GC-MS investigation. The most active ingredient present in cinnamon oil was cinnamonaldehyde **(53, 54)**. Because cinnamon aldehyde is a natural antioxidant, animal experiments have shown that taking an oral extract of cinnamon bark may prevent stomach ulcers **(55)**.The antimicrobial activity of clove essential oils could be associated with Eugenol (2 methoxy-4 allyl-phenol)25, the main component of clove oil, which is already known to exhibit antibacterial and antifungal activity30. Clove antimicrobial activity also due to high tannin content (10-19%) 26.The main compounds present in rosemary essential oil were 1,8-cineole, α-pinene, camphor, etc., which have been evaluated for their antimicrobial effects **(56 - 58)**.

. Numerous phytochemicals, such as alkaloids, flavonoids, ouramin, psoralens, and carotenoids are also present in citrus fruits. Citrus fruits offer a variety of pharmacological effects, including those that are antibacterial, antioxidant, anticancer, and respiratory, according to prior pharmacological investigations **(59)**. In addition, lemon (Citrus limon) juice has vitamin C, a helpful antioxidant. The primary component of lemon (Citrus limon) juice is organic acid, namely citric acid, which makes up the majority of the juice. The amount of citric acid in the fruit determines how acidic (in terms of pH) it becomes. One of the elements that can prevent bacterial development is an acidic pH, which can lower the internal pH of bacterial cells and prevent bacterial cell growth **(60)**.

Bacterial suspensions must be maintained on a thin layer of plastic, carbon, or a combination of the two applied to the surface of an electron microscope specimen grid for microscopic inspection under TEM **(61)**. Organelles like nucleoids, ribosomes, cell membranes, and cell walls may all be seen clearly under a microscope. We were able to get crisp and detailed TEM photos of P. mirabilis. Proteus bacteria were sufficiently dispersed throughout our protocol to avoid touching one another, yet they were also close enough to allow for the observation of a few of them in each frame.

The bacterial lipid bilayer cell membrane is damaged as a result of the catechins' adhering to it, which accounts for many of the direct actions of green tea catechins **(62, 63)**. Damage to the bacterial cell membrane prevents the bacteria from adhering to host cells and from adhering to one another to form biofilms, both of which are important for pathogenicity **(64, 65).** Damage to the bacterial membrane also prevents the bacteria from secreting toxins **(66, 67)**.

Conclusion

The study documented that *P. mirabilis* isolated from different clinical specimens have high resistance to antibiotics where, he highest resistance was shown to Trimethoprim-Sulfamethoxazole (96%) , The highest intermediate susceptibility to imipenem was found to be 28%, and the highest susceptibility to piperacillin-tazobactam was found to be 88%. The herbal medicinal plant extracts, oils, and their derivative substances have been used for hundreds of years to fight pathogens like bacteria, fungi, and viruses. They are recognized to be effective against a wide range of microorganisms. In this study, some plants were identified which have been traditionally used for the treatment of bacteria such as *Rutaceae, Zingiber officinale, syzyginum spp, Cinnamomum and Camellia sinensis*. The ethanolic exteract of green tea was the most active one with an inhibition zones diameter (mm) ranging between 25 and 40, followed by clove with an inhibition zones diameter ranged between 23 to 31. Followed by lemon and cinnamon respectively but the giner was the most inactive one with inhibition zones diameter (mm) ranged between 0 to 14. Based on the result obtained during this study, we recommend the application of some medicinal plants like *Camellia sinensis* (green tea) against antibiotic-resistant *Proteus* species isolated

from patients admitted to Mansoura University Hospitals.

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