BIOLOGICAL ACTVITY OF *SISYMBRIUM IRIO* AND *PETRANTHUS DICHOTOMUS* AND THEIR ISOLATED COMPOUNDS AGAINST MICROBES CAUSING URINARY TRACT INFECTIONS

Nawal H. Mohamed[1*], Gamal O.O. Hassan[1], Hesham M. Mahdi[2], Gamal A. Soliman[3] and Esam H. Abdel shakour[2]

[1]Department of Aromatic and Medicinal Plants. Desert Research Center, Egypt

[2]Department of Plant and Microbiology, Faculty of Science, Al Azhar University

[3]Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Egypt

*E-mail: nawal_h70@yahoo.com

The antimicrobial activity of two Egyptian plants; *Sisymbrium irio* and *Pteranthus dichotomus* were studied against urinary tract infections (UTIs) bacteria. One hundred clinical material samples from urinary tract of patients were collected from Egyptian hospitals and laboratories. Different methods were used for the identification of the isolated bacteria: conventional biochemical methods, cultural characterization and Analytical Profile Index test (API 10S strep test systems). Eight gram-negative (G-ve) bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Proteus mirabilis*, *Acinetobacter baumannii* and one gram-positive (G+ve) bacterium were isolated from patients with UTIs. The extracts of the two selected plants were tested for their effect against isolated bacteria. *Sisymbrium irio* extract greatly inhibited the growth of bacteria more than that of *Pteranthus dichotomus*. Successive extracts of *Sisymbrium irio* were prepared and their effects were studied against bacteria. The most effective extract was ethyl acetate extract. Three flavonoid compounds were isolated from it. Their activity was tested against isolated bacteria. The most active one was isolated and identified as apigenin-7-*O*-β-D-galactoside. Experimental infection of mice was done with three selected isolated bacteria that were sensitive to successive extracts (*Enterococcus faecalis, Citrobacter freundii* and *Acinetobacter baumannii*). In control positive group, bacteria remained in urine for more than five days. While treating mice with ethyl acetate extract, the bacteria decreased in the urine and disappeared and the number of pus cells was greatly decreased.

Keywords: *Sisymbrium irio, Pteranthus dichotomus,* UTIs bacteria, gram-positive and negative bacteria

INTRODUCTION

Extracts from medicinal and aromatic plants for diseases treatment were used in earlier times. In recent decades, a renewed interest has been found among the public in the use of phytotherapy. However, evaluating the antimicrobial phytotherapeutic properties remains a very interesting and useful task. These medicinal plants become a new source of active constituents as substitutes for currently marketed chemical compounds that have notable side effects (Labib, 2005). Due to the side effects of antibacterial medications and the resistance that has limited the use of these molecules; many studies on plant extracts antibacterial activity have been conducted to find natural alternatives to conventional antibacterial drugs.

The aim of this research is to study the antibacterial activity for selected plants against urinary tract infections (UTIs) bacteria and chemical composition of the most active extract against isolated bacteria. Among the most common infectious diseases, UTIs are one of them and during lifetime about 10% of people will experience them. Several different microorganisms can cause UTIs which include fungi and viruses, the major causative organisms are bacteria which are responsible for 95% or more of UTI cases (Zahan, 2013).

About 85% of community acquired UTIs and more than 80% of cases of uncomplicated pyelonephritis are caused by *Escherichia coli*. *Klebsiella pneumoniae* is also accounts for important cause of about 6-17% of all nosocomial urinary tract infections and *Proteus* is a common cause of UTIs in individuals with complicated urinary tract infections (Noor et al., 2004).

Gram-negative (G-ve) bacteria are associated with multiple antimicrobial resistances, posing a significant and well-recognized challenge in the long-term management of urinary tract infections Resistance has been observed across multiple genera, including *Escherichia*, *Enterobacter*, *Klebsiella*, *Proteus*, *Serratia*, and *Pseudomonas* (Noor et al., 2004).

In developing countries, UTIs are represented to be one of the most common bacterial infections encountered by clinicians. Result revealed that *Escherichia coli* (33.3%), *Klebsiella pneumoniae* (19%) and *Staphylococcus saprophyticus* (14.3%) are the most common pathogens isolated (Getnet and Wonderson, 2011).

Two plants widely distributed in Egypt (Boulos, 2000) were chosen for this study; *Sisymbrium irio* L. which belong to family Brassicaceae and *Pteranthus dichotomus* Forssk. which belong to family Caryophyllaceae.

Previous studies on *Sisymbrium irio* revealed that its ethanol extract exhibited significant antibacterial activity at a concentration of 100 mg/ml against five strains of gram-positive (G+ve) and five strains of G-ve bacteria (Vohora et al., 1980). Also, the methanol extract and isolated compounds of the roots of this plant showed high antimicrobial activity (Khan and Saeed, 2000). The plant is considered highly safe, with a median lethal dose (LD_{50})

exceeding 4000 mg/kg body weight. Biological evaluations have demonstrated that it possesses moderate anti-inflammatory activity and significant diuretic effects (Ahlam et al., 2008). *Sisymbrium irio* is known for its high nutritional value and its use in folk medicine. However, there is a lack of published data on the Egyptian species. This highlights the importance of conducting further pharmacological and phytochemical studies on this plant.

The aqueous extract of *Pteranthus dichotomus* exhibits strong cytotoxicity above 97% against cultured melanoma cell line. The alcoholic extract of the plant exhibits anti-inflammatory, diuretic, and anti-tumor activities while showing no adverse effects on liver or kidney functions. Besides that, this plant is considered highly safe (Emad et al., 2013).

MATERIALS AND METHODS

1. Plant Material

Aerial parts of *Sisymbrium irio* were collected from the western Mediterranean coastal region. *Pteranthus dichotomus* was collected from Wadi Sadr El Hetan, South Sinai

Both plants were identified by Prof. Dr. Abd-Elmagid Ali, Professor of Plant Taxonomy, Flora Research Department, Agricultural Research Center, compared to the Flora of Egypt's plant descriptions (Tâckholm, 1974 and Boulos, 2000).

2. Phytochemical Study

2.1. Preparation of plant extracts

The aerial parts of the plants under investigation (*Sisymbrium irio* and *Pteranthus dichotomus)* were collected from their natural habitats, dried in air and shade, grinded to powder and 2 kg of dried powder of both plants were percolated separately with 70% alcohol several times (three times for 24 hours) till exhaustion. The alcoholic extracts of the plants under study were evaporated till dryness under reduced pressure. The residues obtained were dissolved in hot water (200 ml). Aqueous extracts were then concentrated under reduced pressure and pooled drop wise on excess of methanol with continuous stirring. The solutions were then filtered to remove precipitated salt, concentrated, and re-dissolved in a small amount of water. Different extracts were kept for further antibacterial and phytochemical investigation

Different extracts of *Sisymbrium irio* and *Pteranthus dichotomus* were tested for their activity against isolated bacteria. The bacterial species were subcultured in isolation medium separately supplemented with each tested plant after incubation. Diameter of the inhibition zone in mm was measured.

2.2. Preparation of successive plant extracts of *Sisymbrium irio*

The aqueous extract of *Sisymbrium irio* was subjected to fractionation using the following solvents: petroleum ether, diethyl ether, chloroform, and

ethyl acetate. Finally, the solvents were evaporated under reduced pressure. These successive extracts were tested for their activity against isolated bacteria (Eid et al., 2023).

3. Collection Area of UTIs Bacteria

One hundred clinical material samples from urinary tract of patients admitted to all specimens used in this study were collected from Egyptian hospitals and laboratory as following; 49 specimens from Almokhtabar laboratory, 29 specimens from El Qasr El Aini, 6 specimens from Assembly legitimacy, 5 specimens from Mohammed Bakhit hospital, 4 specimens from El-Rahma (Heliopolis) hospital, 3 specimens from Cairo Specialized hospital, 2 specimens from Petroleum hospital, and 2 specimens from Al-Afghani Heliopolis. A total of 100 clinical samples were collected from the urinary tracts of patients presenting with UTI symptoms.

4. Sample Inoculation and Purification

The clinical material samples were inoculated onto the surface of Petri dishes which contained different media (Blood, Chocolate, and CLED and Mac Conkey agar) using sterilized loop for the isolation of microorganisms. All plates were incubated for 18-24 hours at 37^{o}C, whereas plates were incubated at 37^{o}C for chocolate agar under anaerobic conditions for 18-24 hours. Purified colonies were then obtained by streaking repeatedly of single colony on nutrient agar fresh plates. Pure culture colonies were sub-cultured on agar medium. Colonies parameters: size, shape, texture, odor, pigmentation and hemolysis were observed. Gram staining to differentiate the bacterial isolates into G+ve and G-ve groups was carried out (Cheesbrough, 2005 and Christiana and Omolola, 2017).

5. Identification of Bacteria

The isolated microorganisms were differentiated and identified by various conventional biochemical methods (biochemical analysis) such as triple sugar iron (TSI), lysine decarboxylase (LDC)**,** motility indole ornithine **(**MIO) tests, citrate utilization, urease, oxidase, coagulase testes (Cheesbrough, 2005). Catalase activity analysis was carried out to distinguish among *Streptococcus* and *Staphylococcus* species (Cappuccino and Sherman, 2002), a deoxyribonuclease (DNase) activity test was performed to differentiate between *Staphylococcus aureus* and *Staphylococcus epidermidis* and bile-esculin test to distinguish between *Streptococcus* and *Enterococcus*. Then Analytical Profile Index (API); API 10S, API 20 strep test systems were used as well.

6. Antibiotics Sensitivity

Some commonly used antibiotics were tested for their inhibitory activity against the isolated bacterial species. The bacterial strains were subcultured on isolation media, each supplemented with one of the tested antibiotics. The antibiotics used (Bio analyse) included Netilmicin (30 µg),

Ciprofloxacin (5 µg), Imipenem (10 µg), Norfloxacin (10 µg), Gentamicin (10 µg) and Ampicillin (10 µg).

7. Isolation and Purification of Flavonoids from Active Ethyl Acetate Extract of *Sisymbrium irio*

Twenty-five grams of ethyl acetate fraction were dissolved in 40 ml methanol and mixed with 10 g silica gel G for column chromatography. The solvent was evaporated to form a dry powder. Then powdered sample was loaded onto the top of a glass column (100 × 5 cm) containing 500 g of silica gel G for column chromatography. The column was packed using the dry method and eluted with chloroform, followed by ethyl acetate and methanol, with a gradual increase in polarity. Elution was done at a rate of 30 drops/ minute; each fraction (200 ml) was concentrated using rotary evaporator under reduced pressure at 45°C, then subjected to Thin Layer Chromatography (TLC) using system ethyl acetate/Me OH/H_2O (30/5/4)). Similar fractions were collected. Then fractions were subjected to preparative TLC using the same system. The bands were investigated under UV, scratched then eluted with alcohol. After that, the eluted bands were dried and finally purified using a Sephadex LH 20 column (Al-Jaber, 2011). Three compounds were isolated namely A1, A2 and A3.

8. Sensitivity Test for Active Fraction

The direct variant of the bioautographic method (chromatogram layer) was carried out as follows: (1) Preparation of the active compound and its application onto TLC plates (silica gel G F254, Merck). (2) Preparation of the bacterial inoculum and its application onto the TLC plates. (3) Incubation of the plates to allow bacterial growth. Measurement of growth inhibition diameters using a colorimetric assay (INT) to detect the growth.

9. Identification of Pure Active Compound

Ultraviolet (UV) and Nuclear Magnetic Resonance (NMR) spectroscopy were employed to analyse the active compound. UV spectra were recorded using a UV-Visible Spectrophotometer (Thermo Spectronic, Unicam UV-300 Spectrophotometer) to measure absorbance in the UV range. A UV lamp was used for the localization of spots on TLC and PC. For NMR analysis, both ^{1}H and ^{13}C-NMR were performed using a Varian 400 MHz NMR spectrometer to study the active compound.

10. Antimicrobial Activity

10.1. Test organisms

Representatives of G-ve bacteria; namely, *Enterobacter aerogenes, Pseudomonas aeruginosa, Enterobacter cloacae, Citrobacter freundii, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae,* and *Acinetobacter baumannii* and G+ve bacteria namely *Enterococcus faecalis* were used.

10.2. Antimicrobial screening

The agar well diffusion and disc diffusion methods were used for the determination of antimicrobial activities and minimum inhibitory concentration (MIC) of the plant extracts investigated.

10.3. Disc diffusion method

Disc diffusion method was used for the determination of antibacterial activities of different plants extracts on the tested bacterial isolates. A paper discs standard blank (diameter 7 mm) was soaked separately in the different extracts (the tested extracts suspended in the vehicle (3% v/v Tween 80) and then transferred onto surface of growth media which were seeded with the tested bacteria. After incubation in suitable conditions for the tested bacteria, the diameter of zone of inhibition around the discs was measured in millimeters.

10.4. Agar well diffusion method

Agar well diffusion method was employed for determining MIC. The tested bacteria were seeded separately in the agar medium. Wells (diameter 8 mm) were cut from the agar and extract solution (0.1) with different concentrations was put into them. The plates were examined after the incubation period and zones of inhibition were measured.

Both disc diffusion and agar diffusion methods were carried out according to Clinical and Laboratory Standards Institute (2009). Performance of standards for antimicrobial disk susceptibility tests was approved by standard M2-A10, 10th Ed. Clinical and Laboratory Standards Institute, Wayne, PA.

11. Pharmacological Activity

11.1. Bacterial inoculum preparation

Three strains of most sensitive bacteria (*Enterococcus faecalis, Acinetobacter baumannii* and *Citrobacter freundii*) were selected and prepared after an incubation for 18 hours on nutrient agar medium at 37˚C and diluted in PBS buffer. Then the number of viable organism/ml was measured by the plate count method (Finegold and Martin, 1982).

11.2. Opacity tubes

McFarland No. 2 barium sulphate tubes were prepared as the following: In a test tube, two-tenth ml of barium chloride solution (1%) was mixed with 9.8 ml sulfuric acid (1%) and the mixture was shaken well before using for matching. The tube was then sealed and wrapped (in the darkness), then was used to standardize bacterial inoculum in sensitivity test. Every six months, fresh tubes were prepared (Finegold and Martin, 1982) and the opacity of the tube corresponds to 6×10^8/ml.

11.3. Experiment for mice infection

Female mice weighing 150 ±2 g and free from pathogen were used. The animals were classified as each of five animals. The mice were kept in

cages with free access to sterile water and food in a biosafety containment facility.

Three groups were used in the study: the positive control group, the negative control group, and the treatment group. These groups were tested against three species of bacteria (*Enterococcus faecalis, Citrobacter freundii* and *Acinetobacter baumannii*).

Three selected isolated bacterial species—*Enterococcus faecalis*, *Acinetobacter baumannii*, and *Citrobacter freundii*—which were sensitive to the successive extract, were injected into mice. The inoculum of 5 × 10^8 CFU per mouse for each species (*Enterococcus faecalis*, *Acinetobacter baumannii*, and *Citrobacter freundii*) was prepared following the methods of Tamura and Tanaka (1985) and Fournier et al. (1996). The inoculum was administered subcutaneously to the mice, with long exposure to ethyl acetate, at a dose of 52 ml per animal (Fournier et al., 1996).

Urine samples were collected and the way of collecting urine was with a funnel and the parameters tested in each sample presence of pus and RBCs cells. XL program (standard deviation and average) statistical analysis was carried out to compare between groups.

RESULTS AND DISCUSSION

A total of 100 specimens were collected from Egyptian hospitals for the isolation of urinary tract bacteria. The specimens were obtained from the hospitals and laboratories mentioned earlier. Of these, 65 specimens (65%) were from patients infected with bacteria, while 35 specimens (35%) were from patients without infection. Four types of media were used for isolation, MacConkey agar (MA), Cled agar, blood agar and nutrient agar media. Isolates of microorganisms have been classified by gram staining. Gram-negative (G-ve) organisms accounted for 63 (96.93%) and G+ve accounted for 2 (3.07%) out of all isolated microorganisms.

Gram-negative (G-ve) bacteria were classified by biochemical methods into 8 groups which have the same biochemical characteristics (Table 1). Gram-positive (G+ve) bacteria were grouped into 1 group which has similar criteria (Table 2).

1. Identification of Isolated Bacteria

1.1. Identification of isolates which belong to G-ve 1

These isolates were found to belong to G-ve bacteria, aerobic rod shaped, motile and non-spore former, citrate utilization and oxidase were positive. Urea was negative, other criteria are presented in Table (1). Identification with API 10S resulted in code 2402, with a very good identification as *Pseudomonas aeruginosa* as shown in Table (1, 3).

1.2. Identification of isolates which belong to G-ve 2

These isolates were found to belong to G-ve bacteria, rod-shaped and motile. Indole formation and oxidase test were negative, while catalase tests and citrate utilization were positive as shown in Table (1). Identification with API 10S resulted in code 7700, with a very good identification as *Enterobacter aerogenes* as shown in Table (1, 3).

1.3. Identification of isolates which belong to G-ve 3

These isolates were found to belong to G-ve bacteria, facultative-anaerobic, motile, rod shaped as shown in Table (1). Gelatinase, urease, oxidase and iodole formation were negative, while citrate utilization was positive. Identification with API 10S resulted in code 7404, with a very good identification as *Enterobacter cloacae* (Table 1, 3).

1.4. Identification of isolates which belong to G-ve 4

These isolates were found to belong to G-ve bacteria, rod shaped, anaerobic. *B-galactosidase*, motile, citrate utilization and indole formation were positive, while lysine decarboxylase, H_2S production, urease and oxidase were negative as shown in Table (1). Identification with API 10S resulted in code 7605, with a very good identification as *Citrobacter freundii* as shown in Table (1, 3).

1.5. Identification of isolates which belong to G-ve 5

The isolates were found to belong to G-ve bacteria, rod shaped and facultative anaerobe. Oxidase, Voges-Proskauer and H_2S production were negative while catalase, methyl red and indole formation were positive as shown in Table (1). Identification with API 10S resulted in code 7305, with a very good identification as *Escherichia coli* as shown in Table (1, 3).

1.6. Identification of isolates which belong to G-ve 6

These isolates were found to belong to G-ve bacteria, non-motile, encapsulated, rod shaped bacterium and facultative anaerobic. It was positive for citrate utilization and urease, while indole production and oxidase were negative. Other criteria are presented in Table (1). Identification with API 10S resulted in code 7524, with a very good identification as *Klebsiella pneumonia* (Table 1, 3).

1.7. Identification of isolates which belong to G-ve 7

These isolates were found to belong to G-ve bacteria, rod-shaped. indole formation, citrate utilization and oxidase test were negative, while catalase test was positive as shown in Table (1). Identification with API system 10S resulted in code 6000, with a very good identification as *Acinetobacter baumannii* (Table 1, 3).

1.8. Identification of isolates which belong to G-ve 8

These isolates were found to belong to G-ve bacteria, rod-shaped. Indole formation, citrate utilization and oxidase test were negative, while catalase test and H_2S were positive as shown in Table (1). Identification with API 10 S resulted in code 2074, with a very good identification as *Proteus mirabilis*.

1.9. Identification of isolates which belong to G+ve 9

These isolates were found to be G+ve, cocci-shaped. Bile esculin, hemolysis gamma (γ) and growth w/ Tellurite hydrolyze PYR (pyrrolidonyl-β-naphthylamide) were positive, while catalase test was negative with a very good identification as *Enterococcus faecalis* as shown in Table (2).

The nine selected bacterial species were chosen from other similar isolates. The representative bacteria would be *Pseudomonas aeruginosa, Enterobacter aerogenes, Enterobacter cloacae, Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis and Acinetobacter baumannii.* Table (4) summarizes the distribution of different species among the total 65 microbial isolates from clinical materials taken from 65 patients with UTIs.

The most abundant bacteria were *Escherichia coli* 29 (44.61%) and *Klebsiella pneumoniae* 14 (21.53%). In contrast, *Acinetobacter baumannii* was the least isolated bacterial species; it was 1 (1.53%).

Table (1). Characteristics of isolated gram-negative (G-ve) bacteria by conventional biochemical methods.

Characteristics	Gram negative (G-ve) groups of isolates							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
TSI	K/N	A/A	A/A	A/A	A/A	A/A	K/N	
LDC	-	+	-	-	+	+	-	-
MIO	+	+	+	+	+	-	-	-
Citrate utilization	+	+	+	+	-	+	-	-
Urease	-	-	-	-	-	+	-	+
Oxidase	+	-	-	-	-	-	-	-

(+): positive reaction results; (-): negative reaction results, K: Alkaline; A: acid; LDC: lysine decarboxylase, TSI: tri sugar agar media., MIO: motility indole ornithine.

Table (2). Characteristics of isolated gram-positive (G+ve) bacteria by biochemical methods.

Characteristics	Gram positive (G+ve) groups of isolates
	G+ve 9
Gram stain	+
Spore formation	-
Cell shape	Cocci-shaped
Catalase	-
DNase	-
Bile-esculin	+
Coagulase	-

(+) positive reaction results; (-) negative reaction results.

Table (3). Identification of isolated gram-negative (G-ve) bacteria with API 10S test.

Character	Gram negative (G-ve) groups of isolates							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
GLU	+	+	+	+	+	+	+	+
ONPG	-	+	+	+	+	+	-	-
LDC	-	+	-	-	+	+	-	-
ARA	+	+	+	+	+	+	+	-
CIT	+	+	+	+	-	+	-	-
ODC	-	+	-	+	+	-	-	-
H_2S	-	-	-	-	-	-	-	+
URE	-	-	-	-	-	+	-	+
TDA	-	-	-	-	-	-	-	+
IND	-	-	-	+	+	-	-	-
NO_2	-	-	+	+	+	+	-	+
OX	+	-	-	-	-	-	-	-

GLU: glucose, ONPG: o-nitrophenyl-b-D-galactopyranoside, LDC: lysine decarboxylase, ARA: arabinose, CIT: citrate, ODC: ornithine decarboxylase, H_2S: hydrogen sulfide, URE: urease, TDA: tryptophan deaminase, IND: indole, NO_2: nitrite OX: oxidase.

Table (4). Distribution of total microorganisms isolated from urinary tract infections patients.

No.	Species	Numbers of isolates	Percentage (%)
1	*Pseudomonas aeruginosa*	9	13.85
2	*Enterobacter aerogenes*	2	3.07
3	*Enterobacter cloacae*	4	6.15
4	*Citrobacter freundii*	2	3.07
5	*Escherichia coli*	29	44.61
6	*Klebsiella pneumoniae*	14	21.53
7	*Acinetobacter baumannii*	1	1.53
8	*Proteus mirabilis*	2	3.07
9	*Enterococcus faecalis*	2	3.07
Total		65	100

2. Antibiotics Sensitivity

As shown in Table (5), the antibiotic activity on the isolated bacteria was evaluated based on the diameter of the clear zone and the number of isolated bacteria. The antibiotics tested included Ciprofloxacin, Norfloxacin, Imipenem, Gentamicin, Netilmicin, and Ampicillin.

3. The Effect of Antibiotics on Growth of Nine Selected Urinary Tract Bacterial Isolates

The effect of Ciprofloxacin on the growth of 9 tested urinary bacterial isolates varied according to type of bacteria. Some bacterial species were affected greatly by Ciprofloxacin **(**Table 5**)**, for instance, *Enterobacter*

aerogenes, Citrobacter freundii and *Escherichia coli* recorded clear zone with diameter (20 mm, 19 mm and 18 mm, respectively), while the inhibition zone diameter of *Enterobacter cloacae* was 18 mm. On other hand, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were resistant to Ciprofloxacin.

Table (5). The effect of antibiotics on growth of nine selected urinary tract bacterial isolates (Inhibition zone measured in mm).

Bacteria	Ciprofloxacin	Netilmicin	Imipenem	Norfloxacin	Ampicillin	Gentamicin
Pseudomonas aeruginosa	-ve	-ve	18	-ve	-ve	7
Acinetobacter baumannii	-ve	9	-ve	-ve	-ve	-ve
Enterobacter aerogenes	20	12	7	17	-ve	13
Enterobacter cloacae	18	12	12	17	- ve	11
Enterococcus faecalis	-ve	7	-ve	7	7	7
Citrobacter freundii	19	11	13	16	8	10
Proteus mirabilis	13	9	9	11	8	7
Escherichia coli	18	12	14	17	-ve	13
Klebsiella pneumoniae	15	9	12	13	-ve	

Netilmicin inhibited the growth of *Enterobacter aerogenes*, *Enterobacter cloacae* and *Escherichia coli* and the inhibition zone diameter was 12 mm and for *Citrobacter freundii* was 11 mm. On the other hand, *Enterococcus faecalis* inhibition zone was 7 mm and *Pseudomonas aeruginosa* was resistant to Netilmicin, as shown in Table (5).

Imipenem inhibited the growth of *Pseudomonas aeruginosa* with inhibition zone of 18 mm diameter, while the inhibition zone diameter of *Escherichia coli* and *Citrobacter freundii* were 14 and 13 mm. On the other hand, *Enterococcus faecalis* and *Acinetobacter baumannii* were resistant to Imipenem, as shown in Table (5).

Enterobacter aerogenes, *Enterobacter cloacae* and *Escherichia coli* were the organisms that were most inhibited by Norfloxacin with inhibition zone diameter of 17 mm. While *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were resistant to Norfloxacin, as shown in Table (5).

Citrobacter freundii and *Proteus mirabilis* were inhibited by Ampicillin (8 mm) and *Enterococcus faecalis* was 7 mm in diameter, while the other organisms were resistant to Ampicillin as shown in Table (5).

Gentamicin inhibited the growth of *Escherichia coli* and *Enterobacter aerogenes* with inhibition zone of 13 mm in diameter, while the inhibition zone diameter of *Enterobacter cloacae* and *Citrobacter freundii* were 11 and 10 mm. On the other hand, *Acinetobacter baumannii* was resistant to Gentamicin.

4. Antimicrobial Screening

The effect of *Sisymbrium irio* and *Pteranthus dichotomus* extracts on the growth of selected urinary tract bacteria is shown in Table (6). It shows that *Sisymbrium irio* and *Pteranthus dichotomus* extracts had different activity towards some strains of bacteria. The diameter of clear zones is presented in Table (6).

Table (6). Antimicrobial activity of *Sisymbrium irio* and *Pteranthus dichotomus* extracts on the growth of selected urinary tract bacteria.

Bacteria	Inhibition zone diameter (mm)	
	Sisymbrium irio	***Pteranthus dichotomus***
Pseudomonas aeruginosa	12	10
Acinetobacter baumannii	15	15
Enterobacter aerogenes	- ve	11
Enterobacter cloacae	10	- ve
Enterococcus faecalis	12	10
Citrobacter freundii	11	11
Proteus mirabilis	- ve	- ve
Escherichia coli	- ve	- ve
Klebsiella pneumoniae	11	10

-ve= no Inhibition zone appeared, showing that the test organism was resistant.

5. The Effect of Successive Extracts of *Sisymbrium irio* on the Growth of Selected Urinary Tract Bacteria

Table (7) shows that the most effective extract in inhibiting bacterial growth, based on the diameter of the clear zones was ethyl acetate extract.

6. Isolation and Purification of Active Constituents from Ethyl Acetate Extract of *Sisymbrium irio*

Three major compounds namely A1, A2, A3 were isolated from ethyl acetate extract of *Sisymbrium irio,* these compounds were subjected to study their effect on growth of isolated bacteria.

7. The Effect of Isolated Active Material from Ethyl Acetate *Sisymbrium irio* on the Growth of Selected Urinary Tract Bacteria

As shown in Table (8), the growth of *Acinetobacter baumannii* and *Citrobacter freundii* was inhibited and recorded inhibition zone with diameters of 11 and 10 mm, while the other organisms were resistance to A1, and the growth of *Acinetobacter baumannii, Enterococcus faecalis* and *Citrobacter freundii* was inhibited and recorded inhibition zone with diameters of 11, 10 and 10 mm, while the other organisms were resistance to A2.

Growth of *Acinetobacter baumannii, Enterococcus faecalis* and *Citrobacter freundii* was inhibited and recorded inhibition zone with diameters of 15, 14 and 10 mm, while the other organisms were resistance to

A3. Results revealed that compound A3 is the most active one against the growth of bacteria.

Table (7). The effect of successive extracts of *Sisymbrium irio* on the growth of selected urinary tract bacteria.

Bacteria	Inhibition Zone Diameter (mm)				
	Petroleum ether	**Ether**	**Chloroform**	**Ethyl acetate**	**Ethanol**
Pseudomonas aeruginosa	-ve	9	10	12	- ve
Acinetobacter baumannii	10	10	- ve	10	- ve
Enterobacter aerogenes	- ve	- ve	- ve	10	- ve
Enterobacter cloacae	- ve	- ve	- ve	- ve	9
Enterococcus faecalis	10	9	9	11	10
Citrobacter freundii	-ve	10	10	10	- ve
Proteus mirabilis	- ve	- ve	- ve	- ve	- ve
Escherichia coli	- ve	- ve	- ve	- ve	- ve
Klebsiella pneumoniae	- ve	- ve	- ve	- ve	10

-ve= no Inhibition zone appeared, showing that the test organism was resistant.

Table (8). The effect of isolated active material from ethyl acetate extract of *Sisymbrium irio* on the growth of selected urinary tract bacteria.

Bacteria	Inhibition Zone Diameter (mm)		
	A1	A2	A3
Pseudomonas aeruginosa	-	-	-
Acinetobacter baumannii	11	11	15
Enterobacter aerogenes	-	-	-
Enterobacter cloacae	-	-	-
Enterococcus faecalis	-	10	14
Citrobacter freundii	10	10	10
Proteus mirabilis	-	-	-
Escherichia coli	-	-	-
Klebsiella pneumoniae	-	-	-

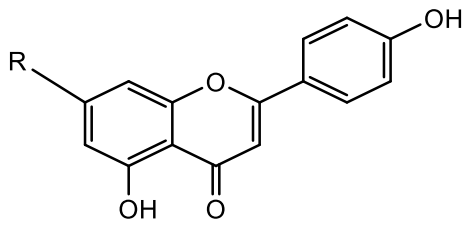
8. Identification of Isolated Compounds

From data shown in Table (9), it can be concluded that by comparing the above data with published ones (Mabry et al., 1970; Harborne and Mabry, 1982 and Agrawal, 1989) it can be concluded that compound A1 could be identified as Apigenin, compound A2 identified as Apigenin-7-O-gluco(6``-1```) rhamnoside and compound A3 identified as apigenin-7-*O*-β-D-galactoside.

Table (9). Data of isolated flavonoid compounds A1-A3.

	Compound A1	**Compound A2**	**Compound A3**
R_f	0.9	0.82	0.71
U.V. λmax	MeOH: (nm) 266, 335; **(**$AlCl_3$) 277,302,348,384; ($AlCl_3$/ HCl) 277, 302, 338, 384; (NaOAc) 272, 300, 373; (NaOAc/H_3BO_3) 270, 300, 340; (NaOMe) 275, 321, 390.	in MeOH: 265, 387; (NaOAc) 229, 265, 388; (NaOAc/H_3BO_3) 266,339; ($AlCl_3$) 274, 297, 342, 378; ($AlCl_3$/HCl) 274, 296, 339, 376	in MeOH: (nm) 269, 335; **(**$AlCl_3$) 276,302,348,388; ($AlCl_3$/ HCl) 277,300,342,384; (NaOAc) 256sh, 269, 355, 388; (NaOAc/H_3BO_3) 265,342; (NaOMe) 245sh,303sh, 390.
^{1}H-NMR (DMSO-d_6): (ppm)	7.92 (1H, d, *J* = 8 Hz, H2`& H6``), 6.90 (2H, d, *J* = 8 Hz, H3` and H5`), 6.76 (1H, d, *J* = 2.5 Hz, H8), 6.46 (1H, S, H3), 6.20 (1H, d, *J* = 2.5 Hz, H6)	δ 7.91 (2H, d, *J* = 8.8, H-2', 6'), δ 6.7 (2H, d, *J* = 8.8, H-3', 5'), δ 6.81 (1H, s, H-3), δ 6.74 (1H, d, *J* = 2.4, H-8), δ 6.37 (1H, d, *J* = 2.4, H-6), δ 5.2 (1H, d, *J* = 7.2, H-1"), δ 4.30 (1H, d, *J* =2.0), δ 3-4 (remaining sugar proton, m), δ 1.17 3H, s, of rhamnose);	7.97 (1H, d, *J* = 8 Hz, H2` & H6``), 7.05 (2H, d, *J* = 8 Hz, H3` and H5`), 6.96 (1H, d, J = 2.5 Hz, H8), 6.56 (1H, S, H3), 6.32 (1H, d, *J* = 2.5 Hz, H6). 5.7 (1H, d, *J* = 2.5 Hz, H6), 5.4 (1H, d, anomeric sugar proton), 3-4 (m, remaining sugar protons).
^{13}C NMR (DMSO)	182.20 (C-4); 164.18 (C-2); 163.30 (C-7); 161.94 (C-5); 161.78 (C-4`); 157.87 (C-9); 128.97 (C-2` & C-6`); 121.62 (C-1`); 116.50 (C-3` & C-5`); 105.82 (C-10); 10 3.31 (C-3); 99.88(C-6); 94.56 (C-8).	δ 182.4 (C-4), δ 164.2 (C-2), δ 103.5 (C-3), δ 160.9 (C-5), δ 100.4 (C-6), δ 162.6 (C-7), δ 94.11 (C-8), δ160.21 (C-9), δ 105.20 (C-10), δ 121.31 (C-1'), δ128.8 (C-2'), δ 116.30 (C-3'), δ 159.51 (C-4'), δ116.3 (C-5'), δ 128.82 (C-6'), δ 102.31 (C-1"), δ 73.6 (C-2"), δ 74.1 (C-3"), δ 69.8 (C-4"), δ 81.20 (C-5"), δ 61.0 (C-6"), δ 100.1 (C-1'"), δ 70.2 (C-2'"), δ68.2 (C-3'"), δ 73.8 (C-4'"), δ 65.7 (C-5'"), δ 17.21(C-6'").	182.45 (C-4); 164.24 (C-2);163.83 (C-7); 162.03 (C-5); 161.99 (C-4`); 158.00 (C-9); 129.24 (C-2`& C-6`); 122.13 (C-1`); 116.76 (C-3` & C-5`); 106.21 (C-10); 103.73 (C-3); 100.04 (C-6); 95.15 (C-8). 102.5 (C-1``); 71.2 (2``); 73.2 (3``);68.5 (4``); 75.5 (5``); 5 9.8 (6``).
	Apigenin	Apigenin-7-O-gluco(6``-1```) rhamnoside	Apigenin -7-*O*-β-D-galactoside

9. Structure of Isolated Compounds



Compound A R = OH

Compound A2 R = O- gluco (6``-1```) rhamnoside

Compound A3 R = O- galactose

10. Pharmacological Activity

10.1. Experiment for mice infection

Using ethyl acetate fraction of *Sisymbrium irio* in this experiment results are as follows:

1. The positive group was mice with bacteria, without treatment and, the dose was used according to the weight of mice (about 5 ml for each one).
2. The negative group was mice without bacteria and without treatment (normal groups) and the dose was used according to the weight of mice (about 5 ml).
3. The treatment group was mice with bacteria, and with treatment, and the dose was used according to the weight of mice (about 5 ml for each one).

In control positive group 10^{6-7} cfu/ml bacteria remained in urine until after 5 days, control positive appear infections, with a median of 10^5 cfu with *Enterococcus faecalis,* 10^6 cfu with *Citrobacter freundii* and 10^7 cfu with *Acinetobacter baumannii*. Treated mice had sterile urine. The bacteriuria declined and disappeared, the cfu decreased from 10^8 cfu to 10^3 after one dose with *Enterococcus faecalis* and *Acinetobacter baumannii,* whereas decreased to 10^4 cfu with *Citrobacter freundii* and bacterial counts remained with a median of 10^{3-4} cfu. Also, the number of pus cells is greatly decreased as shown in Tables (10, 11 and 12). The xl program (standard deviation and average) of statistical analysis was carried out to discuss the significant differences between groups.

Drug resistant bacteria appeared due to excessive use of several antibacterial drugs. Moreover, synthetic drugs have side effect to patient (Tomin and Tomasz, 1986). To solve this, new antibiotics from fungi, algae, and higher plants must be discovered. Higher plants have important roles by

producing a lot of organic compounds which are bioactive compounds, bactericidal, bacteriostatic, and chemotherapeutic agents (Evans et al., 1986 and Purohit and Bohra, 1998).

Table (10). Effect of ethyl acetate extract of *Sisymbrium irio* on mice infected with *Enterococcus faecalis*.

Groups	Days of experiment				
	1st day	2nd day	3rd day	4th day	5th day
Control negative	P (1-2) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)
Control positive	P (15-20) R (1-2)	P (15-20) R (1-2)	P (15-20) R (1-2)	P (15-18) R (1-2)	P (13-15) R (1-2)
Treatment	P (4-6) R (1-2)	P (1-3) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)

P= pus cells (normal 0-2 cells), R =red blood cells (0-2 cells).

Table (11). Effect of ethyl acetate extract of *Sisymbrium irio* on mice infected with *Citrobacter freundii*.

Groups	Experiment Days				
	1st day	2nd day	3rd day	4th day	5th day
Control negative	P (1-2) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)
Control positive	P (6-8) R (1-2)	P (15-20) R (1-2)	P (15-20) R (1-2)	P (15-20) R (1-2)	P (13-15) R (1-2)
Treatment	P (1-2) R (1-2)	P (4-6) R (1-2)	P (6-8) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)

P= pus cells (normal 0-2 cells), R =red blood cells (0-2 cells)

Table (12). Effect of ethyl acetate extract of *Sisymbrium irio* on mice infected with *Acinetobacter baumannii.*

Groups	Experiment Days				
	1st day	2nd day	3rd day	4th day	5th day
Control negative	P (1-2) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)
Control positive	P (15-20) R (1-2)	P (6-8) R (1-2)	P (15-20) R (1-2)	P (15-20) R (1-2)	P (15-18) R (1-2)
Treatment	P (1-3) R (1-2)	P (1-2) R (1-2)	P (6-8) R (1-2)	P (1-2) R (1-2)	P (1-2) R (1-2)

P= pus cells (normal 0-2 cells), R =red blood cells (0-2 cells)

Urinary tract infections (UTIs) are among the most common and significant health problems in Egypt and many African countries, ranking second after respiratory infections. Approximately 10% of individuals

experience a UTI during their lifetime, with bacteria responsible for more than 95% of the cases. The control of UTIs is frequently difficult because of the problem connected with the identification of etiological agents and frequent cases of self-medication. Identification of microorganisms associated with UTIs by more than one method is very important to determine the appropriate therapy based on the testing of antibiotic sensitivity for the true causative agents.

In this study, 100 bacterial isolates were obtained from patients at Egyptian hospitals and laboratories. The bacteria were subjected to purification and identification using biochemical and API methods. Nine bacterial isolates were identified: *Pseudomonas aeruginosa, Enterobacter aerogenes, Enterobacter cloacae, Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis, and Acinetobacter baumannii*. The effect of six antibiotics on the isolated bacteria was measured, and it was found that the bacteria were most sensitive to Ciprofloxacin (based on the clear zone diameter) compared to Norfloxacin, Imipenem, Gentamicin, Netilmicin, and Ampicillin. Additionally, according to the diameter of the clear zone and the number of isolated bacteria Ampicillin was the antibiotic to which the organisms showed the highest resistance.

The effect of the medicinal plants under study; *Sisymbrium irio* and *Pteranthus dichotomus* on the isolated bacteria under study was evaluated. Recorded results revealed that *Sisymbrium irio* had more activity against isolated bacteria than *Pteranthus dichotomus.* Successive extraction for *Sisymbrium irio* using petroleum ether, diethyl ether, chloroform and ethyl acetate were conducted. Isolated bacteria showed sensitivity for ethyl acetate fraction (according to clear zone) more than any other successive extractions. This is because it contains biologically active compounds including flavonoids compounds. Three flavonoids were isolated from ethyl acetate extract of *Sisymbrium irio*; A1, A2 and A3. Isolated bacteria were more sensitive to A3 which identified as apigenin-7-*O*-galactoside.

Isolated bacteria: *Citrobacter freundii, Enterococcus faecalis* and *Acinetobacter baumannii,* which were sensitive for successive extracts were injected in mice. The negative group was mice without bacteria and without treatment (normal groups), the positive group was mice with bacteria and without treatment and treatments groups were those of mice with bacteria and with treatment. The dose was used according to the weight of mice (about 5 ml).

In the control (positive) group, the bacteria remained 10^{6-7} cfu / ml in urine even after 6 days and the treated mice had sterile urine. The bacteriuria decreased and disappeared from urine after 6 days. The cfu decreased from 10^5 cfu/bladder after one dose to 10^3 cfu/bladder in the treated mice, on day 4

after the sixth dose. The bacterial counts became 10^{3-4} cfu/bladder even on day 6 at the end of ethyl acetate extract treatment.

CONCLUSION

The present study showed that these plants can be administered and used in the ethnomedical practice pathogens bacteria. As a conclusion, according to the present study both plants are safe and contain active compounds and can be used as treatment agents. The presence of flavonoid compounds in the plants extracts contributes to a promising biological activity. Biological activities and the active agents from medicinal plants reported in traditional medicine must be studied as it is of interest as they may play also as templates for drug discovery.

REFRENCES

Agrawal, P.K. (1989). In: 'Carbon-13 NMR of Flavonoids'. Elsevier Sci. Publishing Co. 581 p.

Ahlam, M.E., E.M. Atta, A.S. Gamal, M. Jaspars, H.M. Nawal and E.T. Hala (2008). Phytochemical and biological studies of *Sisymbrium irio* L. growing in Egypt. Journal of Saudi Chemical Society, 12 (4): 477-488.

Al-Jaber, N.A. (2011). Phytochemical and biological studies of *Sisymbrium irio* L. growing in Saudi Arabia. Journal of Saudi Chemical Society, 15 (4): 345-350.

Boulos, L. (2000). In: 'Flora of Egypt VII'. Al Hadara Publishing. Cairo, Egypt.

Cappuccino, J.G. and N. Sherman (2002). In: 'Microbiology: A Laboratory Manual'. 6th Edition, Pearson Education Inc., San Francisco, pp. 15-224.

Cheesbrough, M. (2005). In: 'District Laboratory Practice in Tropical Countries'. 2nd Edition, Cambridge University Press, New York, pp. 38-39.

Christiana, E.A., Omolola O. (2017). Microbiological quality assessment of pupuru and plantain flours in an urban market in Akure, Ondo State, South Western Nigeria. Open Access Library Journal, 4 (8): 1-11.

Eid, N.A., N.H. Mohamed and A. Elsharawy (2023). Effect of *Heliotropium arbainense* extracts and bioagents on controlling bacterial canker of tomato plant. Egyptian Journal of Desert Research, 73 (2): 469-488.

Emad, M.A., A.N. Adel, M.H. Nawal and R.H. Ahmed (2013). New flavonoid glycoside and pharmacological activities of *Petranthus dichotomus* Forssk. Records of Natural Products, 7 (2): 69-79.

Evans, J.S., E. Pattison and P. Moris (1986). Antimicrobial agents from plant cell culture, in secondary metabolites in plant cell culture (Moris, P.A., A. Scraggs. Stafford and M. Flower Eds.) Cambridge University, London.

Finegold, S.M. and W.J. Martin (1982). In:'Diagnostic Microbiology'. 6 th Edition. The C.V. Mosby Company, London.

Fournier, J.L., F. Ramisse, A.C. Jacolot, M. Szanik, O.J. Petitjean et al. (1996). Assessment of two penicillins plus β-lactamase inhibitors versus cefotaxime in treatment of murine *Klebsiella pneumonia* infections. Antimicrobial Agents and Chemotherapy, 40: 325-330.

Getnet, B. and T. Wondewosen (2011). Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in Jimma University specialized hospital, southwest Ethiopia. Ethiopian Journal of Health Science, 21 (2): 141-146.

Harborne, J.B. and T.J. Mabry (1982). In: 'The Flavonoids: Advances in Research'. Chapman and Hall, London, New York, 744 p.

Khan F.Z. and A. Saeed (2000). Antimicrobial potentials of the constituents of *Sisymbrium irio* L. Hamdard Medicus, 43 (1): 22-28.

Labib, S.M. (2005). Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. International Immunopharmacology, 5: 1749–1770.

Mabry, T.J., K.R. Markham and M.B. Thomas (1970). The systematic identification of flavonoids. Springer-Verlag, Berlin, 354 p.

Noor, N., M. Ajaz, S.A. Rasool, and Z.A. Pirzada (2004). Urinary tract infections associated with multi-drug resistant enteric bacilli: characterization and genetical studies. Pakistan Journal of Pharmaceutical Sciences, 17 (2): 115-123.

Purohit, P. and A. Bohra (1998). Effect of some plants extracts on conidial germination of some important phytopathogenic fungi. Geobios New Report, 17: 183-184.

Tâckholm, (1974). In: 'Student's Flora of Egypt'. Published by Cairo University, Printed Cooperative Printing Co., Beirut, Lebanon.

Tamura, Y. and S. Tanaka (1985): Effect of calcium chloride on experimental infection of mice with *Pseudomonas aeruginosa*. Infection and Immunity, 48: 648-651.

Tomin, E. and A. Tomasz (1986). Betalactam specific resistant mutants of *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy, 30: 377-383.

Vohora, S.B., S.A. Naqvi and I. Kumar (1980). Antipyretic, analgesic and antimicrobial studies on *Sisymbrium irio*. Planta Medica, 38 (3): 255-259.

Zahan, R.A. (2013). Prevalence of urinary tract infection and antibiotic susceptibility among women in childbearing age in Northern area of Bangladesh. MSc Thesis. The Institute of Biological Sciences University of Rajshahi, Bangladesh.

النشاط البيولوجي لمستخلصات الشلياط والبترنشس دايكوتومس ومركباتهم المفصولة ضد الميكروبات المسببة لالتهابات المسالك البولية

نوال هداية محمد[1*]، جمال عثمان عثمان[1]، هشام محمد مهدي[2]، جمال عبد الحكيم سليمان[3] وعصام حسين عبد الشكر[2]

[1]قسم النباتات الطبية والعطرية، مركز بحوث الصحراء، القاهرة، مصر
[2]قسم النبات والميكروبيولوجي، كلية العلوم، جامعة الأزهر، مصر
[3]قسم الفارماكولوجي، كلية الطب البيطري، جامعة القاهرة، مصر

ظهرت البكتيريا المقاومة للأدوية بسبب زيادة استخدام العديد من الأدوية المضادة لها، علاوة على ذلك ظهور الآثار الجانبية لهذه الأدوية. لذلك لابد من البحث عن مصادر طبيعية آمنة لعلاج الأمراض الناتجة عن الإصابات البكتيرية ومنها مرض التهابات المسالك البولية. تم عمل دراسة تأثير مستخلصات نباتي الشلياط ووالبترنشس دايكوتومس (الناميان في الصحاري المصرية) على البكتيريا المسببة لالتهابات المسالك البولية، حيث تم عزل ٨ أنواع من البكتيريا السالبة لصبغة جرام ونوع واحد من البكتريا الموجبة لصبغة جرام. وأوضحت الدراسة أن مستخلص الكحول لنبات الشلياط له نشاط أعلى من نبات والبترنشس دايكوتومس ضد البكتيريا المعزولة. تم عمل اختبار الحساسية لسلالات البكتيرية بالمستخلصات المتتابعة لنبات الشلياط وهي كالآتي: الايثير البترولي – الايثير – الكلوروفورم – الاسيل أسيتات – الكحول الايثيلي. وجد أن أكثر المستخلصات التتابعية تأثيرًا على أكبر عدد من الأنواع البكتيرية المعزولة هو مستخلص اسيتات الايثيل، ثم تم فصل المركبات الفعالة لهذا المستخلص. تم عمل اختبار الحساسية لسلالات البكتيرية بالمركبات المفصولة من مستخلص أسيتات الايثيل عن طريق البيواوتوجراف وذلك لمعرفة أعلى المركبات من حيث التأثير على أكبر عدد من السلالات البكتيرية المعزولة، ووجد أن المركب ج ٣ هو أكثر المركبات تأثيرًا على أكبر عدد من السلالات البكتيرية وتم تعريفه وهو أبيجينين -٧- جلاكتوزيد. تم عمل إصابة للفئران عن طريق الفم ببعض السلالات البكتيرية المسببة لالتهابات المسالك البولية حيث تم عمل ثلاث مجموعات ١- مجموعة تحكم سالبة وهي غير محقونة بالبكتريا، ٢- مجموعة تحكم موجبة وهى محقونة بالبكتريا فقط، ٣- المجموعة المعالجة وهي التي حقنت بالبكتيريا وبمستخلص أسيتات الايثيل لنبات الشلياط. وكانت النتائج كالاتي: مجموعة التحكم الموجبة ظلت الإصابة البكتيرية كما هي حتى نهاية التجربة (لم يقل عدد البكتريا)، مجموعة التحكم السالبة وهي خالية من الإصابة بالبكتيريا المسببة لالتهابات المسالك البولية ومجموعة المعالجة وبها عدد البكتيريا بدأ في الاضمحلال إلى أن اختفت وهو ما يعنى أن السلالات البكتيرية لها حساسية لمستخلص أسيتات الايثيل داخل حيوانات التجارب.