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

Antimicrobial activity of some herbal plant extract against urinary tract microorganisms

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Received:15/2/2021
Accepted:24/2/2021

Abstract: Urinary tract infection (UTI) is the second well- known cause of infection, which invades the human body. Globally, an estimated number of 150 million infections are found in the urinary tract yearly. UTI is a disease caused by bacteria such as *E. coli* and fungi such as *Candida*, which impacts the urinary tract. UTI can be treated by different antimicrobial agents. Unfortunately, the evolution and spread of antibiotic and antifungal resistance have become a threat to public health, compromising the treatment of bacterial and fungal infections. Thus, it is crucial to find alternative treatments such as herbal medicine to cure the human infectious diseases caused by *E. coli* and *Candida*. Medicinal plants proved high effectiveness in treating UTI, with no side effects especially cinnamon. Antimicrobial activity of cinnamon was probably due to its oil as well as the pure cinnamaldehyde. There are ten chemical components of cinnamon oil. The main components were eugenol and eugenyl acetate It was reported that eugenol and eugenyl acetate were the major bioactive constituents of the cinnamon oil. These volatile phenolic compounds could disrupt the membranes of microberial cells, leading to the cell death.

keywords: Antibacterial, herbal plants, UTI, *E. coli*, *Candida*

1.Introduction

It is recorded that UTI is the most habitual nosocomial infection which accounts for almost 35% of people from different age categories. The prevalence of UTI in females is more in males, as *E. coli* is considered the prime cause of UTI in humans.

Gram- negative bacteria such as *E. coli*, *klebsiella*, *Enterobacter* and *Proteus* are the habitual causes of urinary tract infection as well as the Staphylococcus sp. which is a gram-positive bacterium [1].

Cystitis is a bladder infection caused by E. coli which occurs in the lower part of the urinary tract, and Pyelonephritis is a kidney infection that happens in the upper urinary tract. It is recorded that UTI is the most habitual nosocomial infection which accounts for almost 35% of people from different age categories. The prevalence of UTI in females is more in males, as *E. coli* is considered the prime cause of UTI in humans [2].

The World Health Organization reported that medicinal herbs are used efficiently in preparing herbal remedies. Herbal medicine is extensively used in developed nations by almost 80% [3]. Globally, folk medicine is being used to produce antibiotics and modern drugs as some medicinal plants have antimicrobial activity in curing disease for thousands of years, astonishingly [4].

Escherichia coli commensally occupies the intestinal tracts of humans and many animal species, but it can also be the cause of a large spectrum of diseases from diarrhea to extraintestinal infections [5]. Itis a non-spore-forming, fimbriated facultative anaerobic gram-negative bacilli that belongs to the family Enterobacteriaceae [6].

Candida is a fungal organisms that can grow in three main cellular morphologies: yeast, pseudohyphae, and hyphae. Such organisms can cause a broad spectrum of human diseases, known as candidiasis, and can typically grow in

either yeast or filamentous form. Yeasts are single oval-shaped budding cells, whereas filaments consist of elongated cells connected end-to-end. There are two types of filamentous morphologies: pseudohyphae and hyphae [7]. Many fungal organisms possess the ability to change their physical shape or morphology. These organisms include a wide variety of fungal pathogens, like *Candida albicans*. *Candida* types are normally found as commensals in the oral cavity, gastrointestinal tract, skin, and/or vagina of healthy individuals, are a major reason for both systemic and mucosal diseases in a wide variety of immunocompromised sick people [8].

2. Materials and methods

Sampling

Fifty isolates of *E. coli*, Proteus, *Klebsiella, Staphylococcus* and *Candida* that were randomly selected and isolated from cultured plates of different urine samples from patients who were admitted to different Mansoura University Hospitals from April 2020 to December 2020.

Disc diffusion method

Antimicrobial susceptibility testing of *E. coli and Candida isolates* was carried out by Kirby-Bauer disk diffusion technique **.** Muller-Hinton agar was used for testing the sensitivity of the experimental isolates to the antibiotics. The technique was done by the standard method of inoculation; an inoculating needle was touched to a single well-isolated colony of *E. coli* and *Candida* and inoculated to 5 ml of sterile nutrient agar and incubated at 37°C for 24 hours. The turbidity of inoculated agar was then adjusted by saline to obtain a turbidity equivalent to 0.5 McFarland (0.05 ml barium chloride 1%+ 9.95 ml sulphuric acid1%).

Ethanolic plant extracts preparation (cinnamon,clove,anise,camel hay, liqurice)

The five herbal plants were dried and pulverized into a fine powder. The powdered material was stored in air tight sterile containers and protected from sunlight until required.

1. Ten grams (10g) of every dried powdered plant material was mixed with 100 ml of 95% ethanol solvent in a sterile conical flask, covered with foil paper and placed on a rotary shaker for 24 hours.
2. Then it was filtered through Whitman filter paper .
3. The supernatant was collected and concentrated in a vacuum for 15 min at 37°C using a rotary evaporator to make the final volume half of the original volume (stock solution).
4. The concentration was then dissolved in 10 ml of 1% dimethyl sulfoxide (DMSO).
5. All extracts were sterilized by filtration through the bacterial filter of pore size 0.45µm using positive pressure, then the filtrate was kept at 4°C in the refrigerator till use.

3. Results and Discussion

Table (1) and "Fig1" recorded the data of antimicrobial susceptibility of 22 *E. coli* isolates against 5 agents from 6 antimicrobial categories, and 20 candida isolates against 4 antifungals. The highest resistance in *E. coli* isolates is detected with both Aztreonam ATM (100%) and Ceftriaxone CRO (100%), followed by Amoxicillin AMC (95%), Nitrofurantoin F (95%), and the highest susceptibility was shown to Trimethoprim sulphame thoxazole SXT (91%).

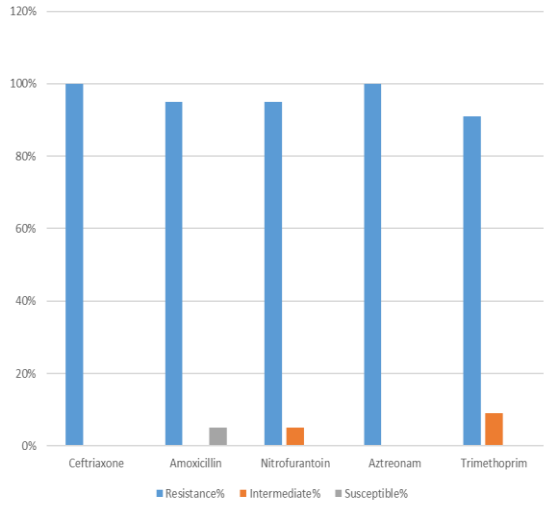

Fig1. Antibacterial susceptibility of E.coli Isolates against Aztreonam, Ceftriaxone, Amoxicillin, Nitrofurantoin, Trimethoprim sulphame thoxazole

Regarding Candida isolate, "**Fig2"** recorded that the highest resistance was to Kanamycin K (100%) followed by Amphotericin AMB (80%) and Itraconazole ITR (70%). The highest susceptibility to Nystatin NY (65%).

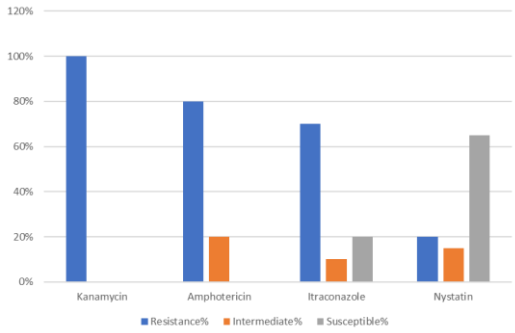

Fig2. Comparative susceptibility of Candida isolates against Nystatin, Kanamycin, Itraconazole, Amphotericin

It is interesting to note that the crude extracts of tested herbal plants showed good activity against clinical *E. coli* and *Candida* isolates while the antibacterial and the antifungals treatment had limited effect as shown in **Table 1 and Table 2**

For instance, it is found that cinnamon is active on all of the E. coli and Candida items while the antimicrobial agents didn't indicate ***CRO:*** *Ceftriaxone,* ***SXT:*** *Trimethoprim Sulphame Thoxazole,* ***F:*** *Nitrofurantotin,* ***ATM:*** *Aztreonam,* ***AMC:*** *Amoxicillin/Clavulanicacid*

Table (1). Comparison between the activity of CRO, AMC, F, ATM, SXT antibiotics and different alcoholic plant extracts against clinical *E. coli* isolates

***E. coli* isolates No. (E)C**	Diameter of inhibition zone (mm)									
	CRO 30 µg S ≥26	**AMC30 µg** S ≥20	**F300 µg** S ≥17	**ATM30 µg** S ≥26	**SXT5 µg** S ≥16	**Anise**	**Liqurice**	**Camel hay**	**Cinn amon**	**Clo ve**
EC1	0	0	0	0	0	0	0	10	25	0
EC2	0	0	0	0	0	0	0	6	12	0
EC3	0	0	0	0	0	0	0	0	14	13
EC4	0	0	0	0	0	0	0	0	12	0
EC5	0	0	0	0	0	0	0	0	20	15
EC6	0	15	0	0	10	0	0	0	20	0
EC7	0	22	0	16	9	0	5	5	15	0
EC8	0	0	0	0	0	0	0	0	20	0
EC9	0	0	0	0	0	0	0	0	15	0
EC10	0	0	0	0	0	0	0	0	10	0
EC11	0	0	0	0	0	0	0	7	21	0
EC12	0	0	10	15	0	0	3	0	15	0
EC13	0	10	0	0	0	0	0	0	15	0
EC14	0	0	6	0	10	0	0	0	21	0
EC15	0	0	0	0	0	0	2	0	20	2
EC16	0	0	10	10	12	0	0	0	24	0
EC17	0	0	0	0	0	0	0	0	17	0
EC18	0	0	0	0	8	0	0	0	19	0
EC19	0	0	0	0	0	0	0	0	20	0
EC20	0	0	0	0	0	0	0	0	17	5
EC21	0	0	15	0	0	0	0	4	20	0
EC22	0	11	0	12	14	0	0	0	25	0

CRO: *Ceftriaxone,* ***SXT:*** *Trimethoprim Sulphame Thoxazole,* ***F:*** *Nitrofurantotin,* ***ATM:*** *Aztreonam,* ***AMC:*** *Amoxicillin/Clavulanicacid*

Photos 1&2 show the difference between the effects of the antibacterial and the medicinal plant on the *E. coli* isolates. The herbal plants, especially Cinnamon extract are more effective than the antibacterials.

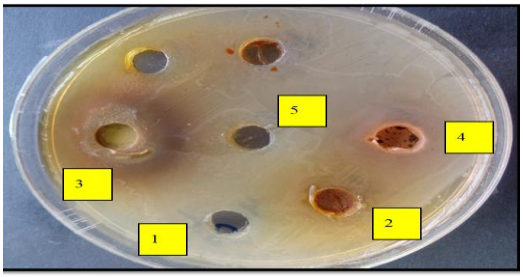

Photo 1. *Activity of different alcoholic plant extract against E. coli isolates*

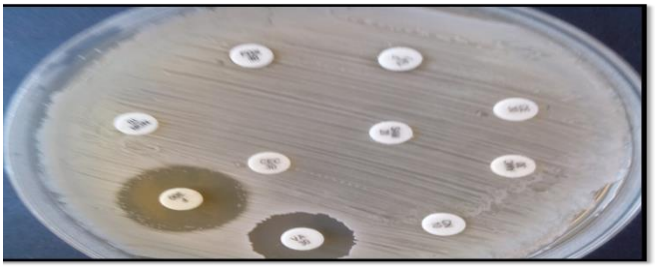

Photo 2. *Resistance of 8 antibiotics (CRO),(F),(NOR),(ATM),(AMC),(CTX),(SXT),(CIP)against E.coli isolates*

Table (2). Comparison between the activity of antifungal and different alcoholic plant extracts against clinical *Candida* isolates

***Candida* isolates No.(C)**	Diameter of inhibition zone (mm)								
	NY100 µg S ≥15	**K30 µg** S ≥18	**ITR10 µg** S ≥15	**AMB 10 µg** S ≥15	**Anise**	**Liqurice**	**Camel hay**	**Cinn amon**	**Clove**
C1	16	0	18	0	0	0	0	25	20
C2	11	0	0	0	0	15	0	20	0
C3	20	0	0	0	0	0	0	20	13
C4	15	0	0	0	0	20	0	15	0
C5	0	0	0	0	0	0	0	13	0
C6	0	0	0	0	0	20	0	13	15
C7	20	0	0	10	0	0	0	20	0
C8	0	0	0	0	0	20	0	20	0
C9	20	0	0	10	0	0	0	20	0
C10	15	0	0	0	0	0	0	20	0
C11	20	0	13	0	0	0	0	21	14
C12	12	0	13	0	0	12	0	15	0
C13	15	0	0	0	0	0	0	18	0
C14	20	0	0	0	0	0	0	17	0
C15	14	0	0	0	0	0	0	20	12
C16	15	0	19	0	0	15	0	24	0
C17	15	0	18	8	0	0	0	17	0
C18	15	0	15	10	0	0	0	19	0
C19	0	0	0	0	0	0	0	20	10
C20	16	0	0	10	0	0	0	25	0

***NY:** Nystatin, **K:**Kanamycine, **ITR:** Itraconazole, **AMB:** Amphotericin*

Photos 3&4 show the difference between the effects of the antifungal and the medicinal plants on the *Candida* isolates. It is obvious that the herbal plants, especially Cinnamon extract is more effective than the antifungals.

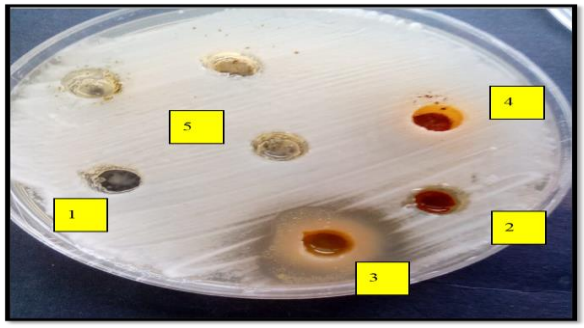

Photo 3. Activity of different alcoholic plant extract against Candida isolates

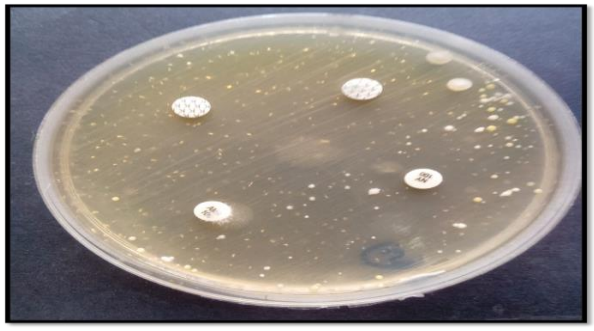

Photo 4. Resistance of 4 antifungal (NY), (K), (ITR), (AMB) against Candida isolates

Discussion

E. coli and *Candida* are considered the prime cause of UTI in humans, which is the most habitual nosocomial infection, and it accounts for almost 35% in people from different age categories.[9] Moreover, The *E. coli* and *Candida* were the most dominant infections in the collected human samples, as the *E. coli* was present in twenty-two isolates accounting for 44% of the total samples while *Candida* was present in twenty isolates accounting for 40%. study. Cinnamon extract was effective. It was reported that eugenol and eugenyl acetate were the major bioactive constituents of cinnamon oil. These volatile phenolic compounds could disrupt the membranes of microbial cells, leading to cell death. [10]

In the present study, 22 *E. coli* urine isolates include 18(82%) from females and 4 (18%) from males, and from the 20 *Candida* urine isolates there were 15(75%) and 5(25%), respectively. This result indicated that female patients had a higher prevalence of UTI than males. Several factors are associated with a high prevalence of infection in females such as shorter and wider urethra in females than in

males., [11]. lack of antimicrobial properties of prostatic fluid that contain zinc which acts as bactericidal substance as in males[12]

Conclusion

The uncontrolled use of antibiotics and antifungals arises microbial resistance. Globally, folk medicine is being used to produce antibiotics and modern drugs as some medicinal plants have antimicrobial activity in curing disease for thousands of years, astonishingly. Contrary to synthetic drugs, antimicrobial of plant extracts is not associated with many side effects and have a great therapeutic potential to the treatment of infections caused by resistant microbes.

4. References

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