Study And Evaluation of the Inhibitory Efficacy of Extracts of *Sumbucus Nigra* Flower and *Tribulus Terrestris* Fruits Against The Growth of Bacterial Species Isolated from Patients with Urinary Tract Infection

Noor Mudher Younis[1], Mujahid Khalaf Ali [2]

[1]Researcher, [2]Lecturer, Tikrit University - College of Sciences, Tikrit University, College of Medicine

Abstract

This study was conducted to isolate and diagnose the most important bacterial types causing urinary tract infection from the inpatients and outpatients to Salah El Din General Hospital using routine isolation and diagnosis methods. 70 samples were isolated, and the results showed two types of growth: Gram positive bacteria and Gram negative bacteria, where 10 bacterial types were isolated as follows:

E. coli 42.85%, *Kleibsiella. pneumonia* 21.42% *roteus mirabilis* (11.42%), *Staphylococcus aureus* (7.14%), *Enterobacter cloaca* (5.71%), Serratia marcescens (2.85*%), Pseudomonas aeruginosa* (2.85%), *Staphylococcus Saprophyticus*. 2.85%, *Micrococcus luteus* 1.42%, and *Citrobacter freundii* 1.42%.

The results of the susceptibility test for 9 types of antibiotics using the discs method showed a change in the level of resistance to different antibiotics. The most effective antibiotics were Imipenem, Ciprofloxacin and Sulfamethoxaole. Plant extracts (both aqueous and alcoholic) of Sambucus nigra and Tribulus terrestris fruits were used, and tested for their ability to inhibit the most isolated bacteria (*E. coli , K. pneumonia P. mirabilis and S. aureus*). All alcoholic extracts were effective in the direction of Bacterial isolates, while aqueous extracts, have little or no effect.

Keywords*: Urinary Tract Infection, Antibiotics, Plant Extracts, Sumbucus nigra, Tribulus terrestris*

Introduction

Urinary tract infections are defined as an inflammatory response to the urinary system as a result of the process of invasion and settlement that occurs by pathogenic microorganisms. Urinary Tract Infection (UTI) is one of the most common bacterial infections in human, as urinary tract infections include many pathogenic cases, including cystitis, urethritis, and pyelonephritis [(1)].

Urinary tract infections occur with or without symptoms, and infection may cause serious complications if neglected and not treated [(2) (3).] Symptoms of a UTI depend on age, gender, the affected part of the urinary system and on the health and physiological condition [(4)]

Scientific progress and continuous researches for a antibiotics that has the best effect on the pathogen has contributed to the use of antibiotics in the treatment of urinary tract infections, and despite the widespread of anti-bacterial drugs in recent times, the effectiveness of these antibacterial drugs has begun to be affected by the increase in the ability of bacteria to resist these drugs. Which may lead to side effects causing significant damage to tissues and cells of the body such as acute renal insufficiency[(5)].

Many researchers have recently turned to the use of natural plant and herbal extracts by using them as effective natural sources in the manufacture of medicines and medicinal drugs [(10)]. because they are natural, have limited side effects, are cheap and available locally, as many studies have shown that the conclusions of these

Plants produce important active compounds such as phenols, alkaloides and tannins, and these substances are effective against pathogenic microbes and have antimicrobial activity[6].

In our current study, the inhibitory effect of Sumbucus nigra, Tribulus terrestris plant extract fruit on bacterial isolates was studied. Sumbucus nigra leaves contain cyanogenic glucosides, while the flowers contain flavonoids, the most important compound in which is the rutin complex, as well as phenolic acid, triple terpenes, citrulls and volatile oils, while the Tribulus terrestris fruits contain flavonoids, anthocyanins, and vitamin A, C [7]. Mashed fresh leaves are used to treat inflammation of the base of the nails and pyorrhea, and ointment of the leaves is used to treat gout pain in the joints of the fingers.

As for the Tribulus terrestris fruits, many researchers have studied the chemical components of the electrode, which contain many types of compounds such as saponins, alkaloids, flavonoids, glycoconols, oils, calcium, and unsaturated acids, which participate in enhancing many physiological responses[8]. The Tribulus terrestris plant are used in the treatment of impotence and infertility for both sexes and to protect against bladder cancer. It is also used as a tonic, appetizer, pain reliever, astringent, and in treating intestinal disorders, in cirrhosis, treating blood pressure and diuretics [9].

Materials and methods

130 mid-stream urine samples were taken from patients with acute and chronic urinary tract infection from Salah AI din General Hospital, from October 2019 - January 2020, and when these samples were cultured , 70 samples gave a growth when cultivated by 54.6% and the isolates were given Two types of growth are negative for Gram stain (87.4%), and positive for Gram stain(12.6%), and no bacterial growth was shown for all samples because the pathogen may be fungal or viral or from anaerobic bacteria that cannot be isolated by the normal cultural methods that were used in the study and which require culture media of their own or special conditions for growth [10] .

Cultivation of Samples

A drop was taken from each urine sample by means of the Loop and cultured on the blood agar, MaCconkey agar and Mannitol salt agar, then incubated at 37 ° C for 24-18 hours, after which the growing colonies were purified to obtain pure colonies for diagnosing through their phenotypic characteristics. And chemical properties [11].

Diagnosis of bacterial isolates

Cultural Characteristics

After the growing of colonies on, MacConkey, blood, and mannitol salt agar. Their size, color, and shape have been observed, and this is considered as a preliminary diagnosis. Microscopic examination: Smears of bacterial colonies were made by the sterile loop and placed on a clean slide, then examined with a Gram stain, after the slide dried, they were examined microscopically under the microscope to observe its response to the stain and the shape, size and arrangement of the bacterial cells[12].

Biochemical tests

The isolated bacteria were diagnosed the usual methods which include: Indol test, Methyl red test, Vogas Proskauer Test, citrate Utilization test, Eosine methylene blue medium Oxidase test Catalase test,Urease test ,Coagulase test and Novobiocin test.

Preparation of the plant extract and the concentrations used in the study

Taking 2 grams of the dry plant sample and dissolving it in 10 ml of sterile distilled water, then we have a storage solution at a concentration of 200 mg / ml, then sterilize the solution using sterile filter papers with diameters of 0.45 micrometers to get rid of the bacterial contaminants in it and obtain a sterile storage solution as a source for making several Concentrations of it are: (25, 50, 75, 100, mg / ml) [13].

Results and Discussion

Isolation and diagnosis of bacteria

Table (1) shows that the isolated and diagnosed germs of E. coli showed pink and dry colonies on macononi acres because they ferment lactose, while on the middle of the EMB, E. coli appeared with a bright metallic green color, which is consistent with

observations by [14], colonies that appeared in a pale pink color were diagnosed based on their cultivar properties and biochemical tests. As for Klebsiella, it appeared in the form of mucous pink colonies due to its containment of the capsule[15], while the germs of S. S. saprophyticus produces small, pink colonies with no color change to the medium[16].

Table (1): Types and numbers of bacterial isolates

Bacteria Isolated	Number of Isolates	%
Escherichia coli	30	42.85
Klebsiella pneumonia	15	21.42
Proteus mirabilis	8	11.42
Enterobacter cloaca	4	5.71
Pseudomonas aeruginosa	2	2.85
Serratia marcescens	2	2.85
S. aureus	5	7.14
S. Saprophyticus	2	2.85
Citrobacter freundii	1	1.42
Micrococcus luteus	1	1.42
Total	70	100

Suspitability test

Multiple resistance bacteria are one of the serious and major problems in the medical field, and this causes difficulty in choosing the appropriate treatment for the patient, and one of the main reasons for the emergence of resistance is the indiscriminate use of antibiotics without relying on a sensitivity test for these antibiotics [17]. The method of discs diffusion on Muller Hinton medium was used to determine the susceptibility of antibiotics against those isolated bacteria and was tested according to [18] for susceptibility , moderately sensitive and resistant. The results of the bacterial isolates showed different patterns of resistance to the antibiotics that were tested, as shown in the table. (2).

Table (2): Suspitability test

Antibiotic		S. aureus	K. pneumoniae	E. coli	P. mirbilis
Imipenem	S	0	0	0	7
	R	100	100	100	93
Ciprofloxacin	S	20	0	40	25
	R	80	100	60	75
Sulfamethoxaole	S	0	40	44	12
	R	100	60	56	88
Gentamycin	S	20	7	50	50
	R	80	93	50	50

Cont... **Table (2): Suspitability test**

Cefalexin	S	20	73.4	84	75
	R	80	26.6	16	25
Nitrofurantoin	S	0	80	17	50
	R	100	20	83	50
Oxacilin	S	40	100	100	100
	R	60	0	0	0
Vancomycin	S	40	100	96.7	100
	R	60	0	3.3	0
Tobramycin	S	66.7	66.7	87	37.5
	R	33.3	33.3	13	62.5
: sensitive ; R : Resistant					

The results of this study showed that most of E. coli isolates were resistant to antibiotics, as the percentage of resistance was high towards Oxacilin 100%, Vancomycin96.7%, Tobramycin 87%, and Cefalexin 84%. While the resistance was less in the direction of Imipenem 0%, Nitrofurantoin 13%, Ciprofloxacin 40%, Sulfamethoxaole 44%, while K. pneumoniae recorded the highest resistance against Oxacilin 100%, Vancomycin 100%, Nitrofurantoin 80%, Cefalexin 73.4%, Tobramycin 66.7%. , While the lowest resistance rate was Imipenem 0%, Ciprofloxacin 0%, Sulfamethoxaole 40%, Gentamycin 7%, while P. mirabilis had the highest resistance to Oxacilin 100%, Vancomycin 100%, Cefalexin 75%, while the lowest percentage was Resistance to the direction of Imipenem was 7%, Sulfamethoxaole 12%, Ciprofloxacin 25%, Tobramycin 37%, while S. aureus had the highest resistance to oxacilin 40%, Vancomycin 40% and the lowest resistance to Imipenem 0%, Nitrofurantoin 9%, Sulfamethoxaole 0%, Ratio 20% resistance to Gentamicin, Ciprofloxacin, Tobramycin, and Cefalexin.

The difference in the resistance rates of bacteria to antibodies may be due to several reasons, including the emergence of isolates resistant to many of the antibiotics used in this study to the presence of mutations or the presence of plasmids that carry resistance to many antibiotics, which play an important role in the spread of bacterial resistance. There is a large number of antibiotics in the bacteria, in addition to this bacterial type having the ability to form a biofilm that plays an important role in antibiotic resistance [19].

The effect of plant extracts on E. coli :

The most effective antibiotic in inhibiting E. coli bacteria was Ciprofloxacin, Imipenem, and Sulfamethoxaole, respectively, at the inhibition diameters of 32 mm, 30 mm and 11 mm. High significant differences at (P≤0.05) probability level on isolated E. coli .

The most effective extract in inhibiting the growth of E. coli bacteria was the alcoholic electrode extract at an average of 21 mm.

It was also found that there is a significant difference between the aqueous and alcoholic extract of Sumbucus nigra, where the average of the alcoholic extract was 14 mm and the aqueous extract was 7 mm. Also, significant differences were found between the concentrations used, as the most effective concentration was 100 mg / ml, followed by a concentration of 75 mg / ml while the lowest effect for both concentrations was 50 mg. / Ml and 25 mg / ml as there is no significant difference between them.

Effect of plant extracts on P.mirabilis:

The most effective antibiotic in inhibiting P.mirabilis bacteria was Imipenem, Ciprofloxacin, and Sulfamethoxaole, respectively, at inhibition diameters of 27 mm, 26 mm and 11 mm. When comparing the rates of inhibition zone for antibiotics with the rates of inhibition diameters for plant extracts, it was found Significant high differences were found at (0.05P≤) probability level on P.mirabilis isolated.

The most effective extract in inhibiting the growth of P. mirabilis bacteria was Sumbucus nigra extract at a mean of 14 mm and the alcoholic pole at an average of 16 mm. Also, it was found that there was no significant difference between the extract of aqueous Sumbucus nigra at a mean of 5 mm and aqueous olives at an average of 1 mm.

Significant differences were also found between the concentrations used, as the most effective concentration was 100 mg / ml, while there was no significant difference between the concentration of 75 mg / ml and the concentration of 50 mg / ml, as well as there was no significant difference between the concentration of 50 mg / ml and the concentration and 25 mg / ml.

The effect of plant extracts on Saureas bacteria:

The results shows that The most effective antibiotic in inhibiting P.mirabilis bacteria was Imipenem, Ciprofloxacin, and Sulfamethoxaole, respectively, at inhibition diameters of 28 mm, 25 mm and 19 mm. High significant differences at (0.05P≤) likelihood level on S.aureas isolates.

It became clear that there were no significant differences between Sumbucus nigra extract at an average of 7 mm and the alcoholic pole at an average of 7 mm and aquas Sumbucus nigra at an average of 3 mm. Also, it was found that there was no significant difference between the extract of aqueous Sumbucus nigra at an average of 0 mm.

Significant differences were also found between the concentrations used, as the most effective concentration was 100 mg / ml, while there was no significant difference between the concentration of 75 mg / ml and the concentration of 50 mg / ml, as well as there was no significant difference between the concentration of 50 mg / ml and the concentration and 25 mg / ml.

These results obtained are consistent with the results of other researchers, as they agree with [23] in the effectiveness of the electrode extract and that the higher the concentration, the better the effectiveness. It agrees with ([20] that Sumbucus nigra extract has an inhibitory effect on bacterial isolates.

Conclusions

It was found through the study that the alcohol extracts were effective in inhibiting the bacterial isolates under study, while the results showed that the aqueous extracts had less effect and sometimes had no effect on the bacterial isolates under study.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Not required

References

1. Adwan, G.M., Abu-Shanab, B.A. and Adwan,K.M. In vitro activity of certain drugs in combination with plant extracts against Staphylococcus aureus infections. Pak. J. Med Sci. 2014; 24.(4):22-34
2. Al-Basir, Abdul Hadi Ali. The effect of pomegranate peel extract on some types of bacteria positive and negative for cram stain. Sebha University Journal, Faculty of Science / Department of Botany / Division of Microbiology, 2017; 10 (1): 1-23**.**
3. Al-Darraji, Nawal Jassim Senior . Study of some chemical contents of tecoma stans and the effect of its crude extracts on the growth of some pathogenic bacteria. Baghdad, University of Baghdad, 2012: 135**.**
4. Arjan,d,A.Z. Effect of Aqueous and Ethanolic Extract of Aerial Parts of Parsley (*petroselinum crispum*) on Some Bacteria Isolated from Nosocomial Infections in-vitro. Res J Med Plant . 2017;13(3) :153-163.
5. Atlas , R.M..Hand Book of Microbiological Media for the Examination of food. 2006 .2nd ed. Taylor and francis Group
6. Brankovic, S.; Kitic,D.; Radenkovic., M.; Ivetic,V.; Veljkovic,S, and Nesic,M. (Relaxant activity of aqueous and ethanol extracts of parsley *Petroselinum crispum* Nymn . ex A . W . Hill , Apiaceae) on isolated ileum of rat. Medicinnsk pregled ,2010; (63): 475 - 78.
7. Clinical and Laboratory Standards Institute (CLSI) .(2014). Performance standards for antimicrobial susceptibility testing, 24th informational supplement,. 34 (1) , Pennsylvania U.S.A.
8. Devi P. ; Meera R. ; Chithambaranathan N. ; Kameswari B. and Badmanaban R. (). Diuretic

and Antimicrobial Activity of Methanolic Extract of *Petroselinum cripsum* Leaves. Int J Pharm Tech Res. 2010; 2 (1): 228-231.

9. Emamghorashi, F.; Shohreh, F.; Mehdi, K.; Shadokht, R. and Maryam, H. The Prevalence of O Serogroups of Escherichia coli strains causing acute urinary tract infection in children in Iran. Saudi. J. Kidney Dis., Transpl. 2010 ; 22(3): 597-601.
10. Fakhri al-Din Qasim, Ibrahim Hadi Muhammad. The effect of eating Tribulus terrestris on some biochemical indicators of young lifters, Journal of Sports Sciences. 2015; 7(20): 24-33..
11. Grabe, M.; Bjerklund, T.E.; Botto, H.; Çek, M.; Naber, K.G.; Pickard, R.S.; Tenke, P.; Wagenlehner, F.; Wullt, B. Guidelines on Urological Infections. 2013: 12-49.
12. Atlas,M.R.(2005).Handbook of Media for Invironmental Microbiology.2nd ed .Taylor & Francis publisher.New York.U.S.A,2005: 157-189.
13. Hearst, Caroline, Graham McCollum, David Nelson, Linda M. Ballard, B. Cherie Millar, Colin E. Goldsmith, Paul J. Rooney, Anne Loughrey, John E. Moore, and Juluri R. Rao. Antibacterial activity of elder (*Sambucus nigra L.*) flower or berry against hospital pathogens. Journal of Medicinal Plants Research 2010; 4(17): 1805-1809
14. Kwiecinska, J.; Bogiel, T. and Gospodarek, E.effects of ceftazidime and ciprofloxacin on biofilim formation in *proteus mirabilis* rods . The journal of Antibiotics,2013; (66): 593-597.
15. Mahmoud, Zaid Shaker Taji and Khalil, Muhammad Abdul-Jalil. The effect of aqueous and alcoholic extracts of cardamom on the growth of some bacteria isolated from otitis media. Ibn Al-Haytham Journal of Pure and Applied Sciences,2010; 23 (1): 8-11
16. Mazzulli T. Diagnosis and management of simple and complicated urinary tract infections (UTIs) Can J Urol. 2012;19 (suppl 1):42–48.
17. Mukherjee M. Koley S. Mukherjee S. Basu S. Ghosh B. Chakraborty S. . Phylogenetic background of *E. coli* isolated from asymptomatic pregnant women from Kolkata, India. J Infect Dev Ctries. 2015; 9(7):720-724.
18. Rubaie, Name Caesar Mazloum. . The combined effect of antibiotics and some plant extracts on the viability of some bacteria. Babylon University Journal / Pure and Applied Sciences .2016; 6 (24) 1511-1522.
19. Soomro, A.H., Arain, M.A., Khaskheii, M. and Bhutto, B.. Isolation of *Escherichia coli* from Raw milk and Milk Products in Relation to Public Health Sold under Market Condition at Tandojam, Pak J Nutr, 2002; 1(3): 151-152.
20. Verle, V.A.; Jefferson, B.M.; Arnold Joseph, M.F.; Januario, D.V. and Sonu, B. . Predict Urinary Tract Infection and to Estimate Causative Bacterial Class in a Philippine Subspecialty Hospital. Singapore.J. Neph. Ther. 2015; 5(2):2-6.