Effect Of Pomegrante Peels And Bay Leaves On Multidrug Resistant Bacteria Isolated From Urinary Tract Infection Patients

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ABSTRACT

Background: Alternative natural therapy by plants extracts had opened wide door for the use of natural products as an alternative therapy instead of many antibiotics and drugs , which had many harmful side effects.Also, an increased interest has been centered on the industrial wastes, especially plant raw materials which contain phenols (e.g. Pomegranate peel and Bay leaves) which is a sources of natural antioxidants ,which are on the contrary of synthetic antioxidants that had restrict use due to their health risks , carcinogenesis and toxicity .

Objectives : This study was done to find out the etiology and sensitivity pattern of uropathogenic bacteria isolated from patients with urinary tract infection , in order to select the bacterial isolates that had multidrug resistance .

Type of the study: Cross-sectional study.

Methods: preparation of Hot water extract , Cold water extract , Methanol extract and Ethanol extract of both Pomegranate peels and Bay leaves and test there antibacterial activity against these uropathogenic bacteria which are multidrug resistant .

Results :All extracts from pomegranate fruit peels exhibited inhibitory activity against all tested bacteria . Hot , Cold water extract and Ethanol extract of pomegranate peels recorded the highest inhibition zones compared with the Methanol extract. On the other hand, there were no antibacterial activity of any extract of Bay leaves on any of tested bacteria. The medicinal plants antibacterial activity was well documented and those plants are new potential alternative antibacterial agent especially against multi resistant bacteria which had been the center of focus globally.

Conclusions: Pomegranate have antibacterial activity against multidrug resistant pathogenic bacteria isolated from UTI patients , So it emerged as alternative therapy for humans leading to reduction the cost and the risk of using antibiotics .

Keywords: bacteria,leaves,urinary.

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The second most common infections after respiratory tract infection is the Urinary Tract Infection (UTI) [1]. At the start of antibiotics use , there were higher reduction in many infections globally , But due to random wide use of antibiotics led to develop different resistant bacteria [2], also they were developed as a result of undirected use of antibiotics especially without prior specialist prescribing [1]. Patients suffering from UTI as the most common nosocomial infection[2]. Women are more susceptible to UTI infection especially during pregnancy [3,4]. About twenty percent of the women had a single infection of UTI during their life, and three percent of women had more than one infection of UTI each year [5]. Men also subjected to UTI dueto prostate enlargement and neurogenic bladder with older age [6]. Before getting results about causes of nosocomail UTI, treatment must be strated[1]. *Staphylococcusaureus*is considered as one of the major cause of nosocomial infectionsand food poisoning [7]. Antibiotic resistance among Staphylococcal strains represent important concern, so there were need to discover new effective antimicrobial agents for the treatment of infections causedby *Staphylococcus aureus* [8]. Plant extracts had antibacterial, antifungal, and antioxidant properties like bay leaf were used for various infections world wide[9 , 1 0] . Pomegranate (*Punicagranatum*) peel had many promoting activities for health and healing properties for wounds [11], besides most pomegranate fruit parts have antioxidant activity [12]. Study the inhibitory effects of pomegranate peel extract and Bay leaves extract on multidrug resistant pathogenic bacteria isolated from urinary tract infection patients was the goal of this study.

Methods:

1- Samples collection and Culture: Midstream urine specimen were collected in sterile cups [13]. After centrifugation at 3000 rpm /15 minutes, one drop of the sediment was spread on a glass slide to detect the

presence of significant pus cell in both male (3-5/HPF) and female (≥5/HPF)[1]. The samples were inoculated on MacConkey agar and Blood agar , incubated at 37° C /24 hours. The bacterial isolates were identified by usual Gram staining and biochemical tests [13]. Culture results were recorded depending on the standard criteria by the presence of growth ($\geq 10^5$ CFU/ml) as significant bacteriuria [14]. Different bacterial species were isolated distributed between G+ re and G- re bacteria .

2- Preparation of Pomegranate fruit peels Extracts: Pomegranate fruits at the maturity stage were peeled, washed so well and air dried [15]. Peels were powdered , then separately mixed with 100 ml of each of Hot water , Cold Water, Ethanol and Methanol. Each mixture was filtered after one day leave in the dark, at room temperatures. Sets of extracts were dried separately in a oven (50 ºC) to gain powder [15].

3- Preparation of Bay leaves Extracts: About 100g ofdried powder Bay leaf were put in 100 ml of Hot water, Cold water, Ethanol and Methanol, then left for one day at room temperature.Theinfusions were filtered by filter paper [16], thenleft to dry in glass petridishesat the oven (50^{o}C) to get the powder.

4-Antimicrobial susceptibility testing **:** Antimicrobial susceptibility patterns were done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar with commercial antibiotic discs (Oxoid, UK) ,and results were recorded for each bacterial isolate and compared to criteria of The Clinical Laboratory Standard Institute [17]. Susceptibility to ten antimicrobial agents :Ceftazidime (30 µg), , Cephalothin (30 µg), Chloramphenicol (30 µg), Oxacillin (1 µg) ,Amoxicillin/ Clavulanic acid (20/10 µg), Nitrofurantion (300 µg), Azteronam (30 µg), Trimethoprim-Sulfamethoxazole(1.25/23.75 µg), Penicillin (10 U), Cefixime (5 µg). Those organisms which showed resistance to at least three or more antibiotics of different structural classes were considered multidrug resistant bacteria (18).

5- Determination of antibacterialactivity**:** A fresh grown culture was serially diluted , to get(1.5×10^7 CFU/ml) of bacterial cells , then 0.1 ml of it was spread onto the surface of Mueller Hinton agar platesandleft to dry at room temperature [19]. Wells weremadeinagar. Then wells were filled by 50 µl of the crude extracts of both Pomegranate peels and Bay leaves for each bacterial isolate. Plateswereincubatedat37°C/ 24 h. Inhibition zones in mm weremeasured.Theantibacterialactivity was recorded as the diameter ofinhibition zonesproducedbytheextractsagainsttestbacteria.

Results : Bacterial species were isolated from urine samples distributed between G+ re and G – re bacteria ,then were more tested biochemically to be identified as *E.coli* , *Klebsiella , Proteus,Pseudomonas,* Staphylococcus , Streptococcus , Acinetobacter and Enterococcus. Antibiotic sensitivity test was done against ten different antibiotics and results were shown in Table (1) and Figure (1). The highest sensitivity was for nitrofurantion , so it was the most effective antibiotic **.** Bacteria were tested for their sensitivity to (Hot, Cold) water extract, Ethanol extract, and Methanol extract of pomegranate fruit peels and bay leaves .Table (2) presents diameters of inhibition zones caused by the different extracts towards tested bacteria. All extracts from pomegranate fruit peels exhibited inhibitory activity against all tested bacteria as shown in Figure (2), with the lowest inhibition zones on methanol extracts , in contrary of the rest of extracts (Hot , Cold water extract and Ethanol extract) which shows higher inhibition zones. On the other hand, there were no antibacterial activity of any extract of Bay leaves on any of tested bacteria.

Discussion: Nosocomial infection is among the most huge problems dealing with it physicians and patients [20]. Almost all pathogenic bacteria have the potential to cause infection in hospitalized patients, but only limited number of both G +re and G -re bacteria cause the majority of nosocomial infections [21]. G-re bacteria bacilli cause the frequent four types of nosocomial infections(e.g. Pneumonia, Surgical Site Infections , UTI , and Blood Stream Infections) [22]. Urine culture importance for treatment of UTI came from the necessity of isolation and identification of the bacteria which cause UTI in male and female, Also the susceptibility test of these bacteria is very critical to avoid the drug resistant bacteria development [23], which is the consequence of random widespread use of common antibiotics in hospitals and cross- resistance among different bacteria [1]. Al-Zoreky [24] reported that only water-methanol extract of peels have marked inhibition (12-20 mm inhibition zones) against eleven microorganisms, such as (*Bacillus subtilis, Staphylococcus aureus, Yersinia enterocolitica, Listeria monocytogenes, Candida utilis, Saccharomyces cerevisiae*, and *Aspergillusniger*), and the water extract was not active against microorganisms tested. Braga *et al* [25] reported that the methanol extracts of *P. granatum*whole fruits were able to inhibit not only the growth of *S. aureus*FRI 722 but also the production of enterotoxins. Mele´ndez and Capriles [26] found that methanol extract of pomegranate fruit was active against *E. coli, S. aureus*, and *B. subtilis* with the diameter of inhibition zone 12, 22, and 12 mm, respectively.

The activity of ethanol extracts of peels against *S. aureus, B. subtilis* and *E. coli* (29-34 mm inhibition zones) was close to [18] (10-40 mm inhibition zones), but higher than that of [23] (13-17 mm inhibition zones). This difference in activity may be explained by many reasons such as e.g. extraction method difference, susceptibility of bacterial strain and use different procedures. The extract looks to be thermostable cause the hot- water extract (by boiling water use) still had the activity like the other extracts [23]. Peel contains huge

amounts of polyphenols such as ellagic tannins, ellagic acid, and gallic acid [27]. The phytochemical components of alcoholic extract of pomegranate are alkaloid, flavonoid, glycoside, phenol, and tannin [18].

The phenolic compounds in pomegranate juice are punicalagin isomers, ellagic acid derivatives, and anthocyanins [28].

Pomegranate peel extract was active and effective against the growth of all tested bacteria due to the presence of antimicrobial phenolic compounds, which in turn could cause degradation of cell wall , damage of cytoplasm membrane, disruption of membrane proteins and interfere with membrane-integrated enzymes, which in turn may lead to cell death [29].

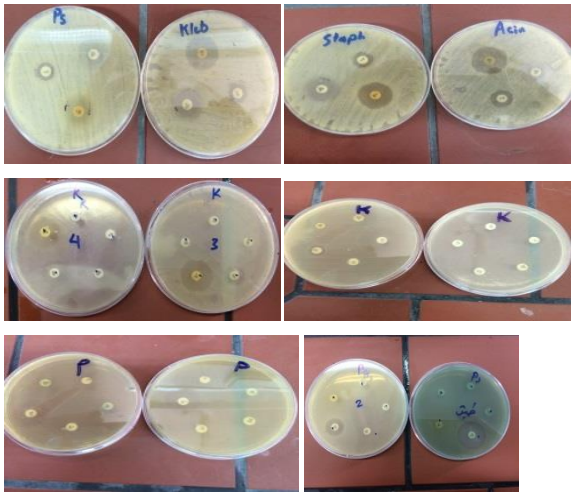

Figure (1) Antibiotic sensitivity test

Bacterial isolates :*Pseudomonas* (ps), *Klebsiella* (Kleb , K) *Staphylococcus*(Staph), *Proteus* (P). Antibiotics : Ceftazidime (CAZ), , Cephalothin (KF), Chloramphenicol (C), Oxacillin (OX) ,Amoxicillin/ Clavulanic acid (AMC), Nitrofurantion (F), Azteronam (ATM), Trimethoprim-Sulfamethoxazole (SXT), Penicillin (P), Cefixime (CFM)

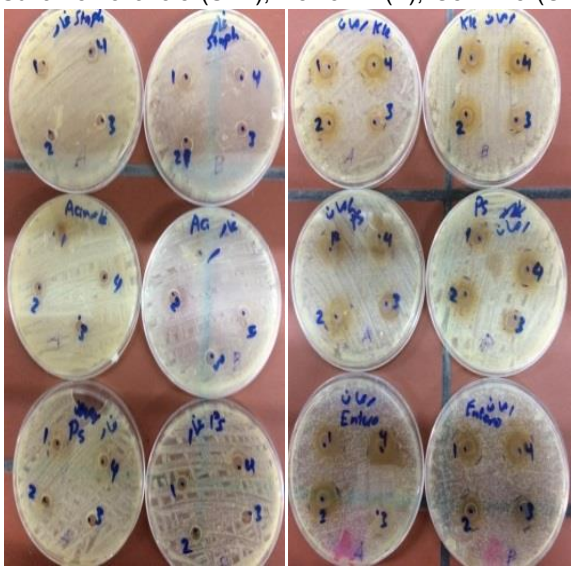

Figure (2) Antibacterial activity of Pomegranate extracts (left)and Bay leaves extracts (right)

Bacterial isolates :*Pseudomonas* (ps), *Klebsiella* (kle), *Staphylococcus* (Staph), *Enterococcus* (Entero) and *Acinetobacter* (Acin)

Table (1) Inhibition zones ofantibiotics by cm.

Antibiotics / UTI causes	Ceftazidime	Cephalothin	Chloramphenicol	Oxacillin	Amoxicillin/ Clavulanic acid
A	2 I	1 R	2 S	-	1 R
B	1 R	-	2 S	-	-
C	-	-	2 S	-	-
D	2 S	-	-	-	-
E	1 R	1.2 R	1 R	0.9 R	1.9 R
F	1.2 R	1.3 R	1.2 R	0.7 R	1 R
G	1.3 R	1.1 R	1.4 R	1 R	1.2 R
H	1 R	1 R	1 R	1 R	1 R

A:*E.coli* ;
B:*Klebsiella* ;
C:*Proteus*
D:*Pseudomonas* ;
E:*Staphylococcus*
F:*Streptococcus* ;
G: *Acinetobacter*
H:*Enterococcus*

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Table (2) Inhibition zones of Pomegranate Extracts by cm

Extract / Bacteria	Hot water extract	Cold water extract	Ethanol extract	Methanol extract
A	2	2.1	2	1.7
B	2	1.7	1.8	1.3
C	1.7	2	1.9	1.1
D	2	1.9	2	1.4
E	2.1	1.8	1.9	1.2
F	2	1.7	1.8	1.3

A:*E.coli*; B:*Klebsiella* ; C:*Proteus*
D:*Pseudomonas* ; E:*Staphylococcus*
F:*Streptococcus* ; G: *Acinetobacter*
H:*Enterococcus*

References

1- Pai, V. and Nair, B. (2012) Etiology and sensitivity of uropathogens in outpatients and inpatients with urinary tract infection: Implications on empiric therapy. Ann. Trop. Med. Public Health,5:181-184.

2- Mazed ,M.A. ; Hussain ,A. ; Akter, N, ; Sultan, T. and Dewanje ,A.K. (2008) Pattern of bacteria causing Urinary Tract Infections and their antibiotic susceptibility profile at Chittagong Medical College Hospital. Bangladesh J. Med. Microbiol. , 02(02):17-21.

3- Ronald ,A.R. and Puttulo ,M.S. (1991) The natural history of urinary infection in adults. Med. Clin. North. Am. , 75:299- 312.

4- Pastore ,L.M. ; Savitz ,D.A. and Thorp, J.M. (1999) Predictors of symptomatic Urinary tract infection after 20 weeks gestation. J. Perinatol, 19:488-493.

5- Gebre-Selassie ,S. (1998) Asymptomatic bacteriuria in pregnancy: epidemiological, clinical and microbiological approach. Ethiop. Med. J. , 36:185- 192.

6- Liperky ,B.A. (1989) Urinary tract infection in men: epidemiology, pathophysiology, diagnosis and treatment. Ann. Intern. Med. ,111:138-150.

7- Patterson ,J.E. andRan, L. (2005) The synergistic activity of antibiotics nosocomial infection. Curr.Opin. Dis., 13:593-8.

8- Adwan, G. and Mhanna, M. (2008) Synergistic Effects of Plant Extracts and antibiotics on *Staphylococcus aureus*strains Isolated from clinical specimens Middle-East . J. Scientific Res. ,3 (3):134-9.

9- Burt ,S.A. (2004) Essential oils: their antibacterial properties and potential applications in foods: a review. Inter. J. Food Microbiol.,94:223-53.

10- Irkin, R. and Korukluoglu ,M. (2009) Growth inhibition of pathogenic bacteria and some yeasts by selected essential oils and survival of *L. monocytogenes*and *C. albicans*in apple-carrot juice. Food borne Pathog. Dis. ,6(3):387-94.

11- Chidambara, M.K. ; Reddy, V.K. ; Veigas, J.M. and Murthy, U.D. (2004) Study on wound healing activity of *Punicagranatum*peel. J. Med. Food, 7: 256-259.

12- Kaur ,G. ; Jabbar, Z. ; Athar, M. and Alam ,M.S. (2006) *Punicagranatum* (pomegranate) flower extract possesses potentantioxidantactivityandabrogatesFe-NTAinducedhepatotoxicityinmice.FoodChem.Toxicol. ,44: 984-93.

13- Crichton ,P.B. (1996) Enterobacteriaceae: In: "Mackie and McCartney Practical Medical Microbiology". Collee ,J.G. ; Fraser, A.G. ; Marmion ,B.P. ; Siminous, A. (editors). 14th ed. New York: Churchill Livingston.pp: 361-4.

14- Gupta ,V. ; Yadav, A. and Joshi, R. M. (2002) Antibiotic resistance pattern in uropathogens. Indian J. Med. Microbiol., 20: 96-98.

15- Al-Zoreky, N. (2009) Antimicrobial Activity of Pomegranate (*Punica granatum* L.) Fruit Peels. Inter. J. Food Micro, 134(3): 244-248.

16- Cowan ,M.(1999) Plant products as antimicrobial agents. Clin. Microbiol. Rev. , 12 (4):564-82.

17- Clinical and laboratory standards institute (CLSI) performance standards for antimicrobial susceptibility testing. (2006) 16th Informational Supplement. M100-S16. CLSI, Wayne, PA, 29(3): 22.

18- Ahmad ,I. and Beg, A.Z. (2001) Antimicrobial and phyto-chemical studies on 45 Indian medicinal plants againstmulti-drug resistant human pathogens. J. Ethno. pharmacol. , 74:113-23.

19- Simner ,P. J. ; Zhanel ,G. G. ; Pitout ,J. ; Tailor, F. ; McCracken, M. ; Mulvey, M. R. ; Lagace-Wiens, P. R. ; Adam, H. J. and Hoban, D. J. (2011) Prevalence and characterization of extended-spectrum beta-lactamase and AmpC beta-lactamase producing *Escherichia coli*.

results of the CANWARD 2007-2009 study. Diagn. Microbiol. Infect. Dis., 69(3):326-334.
20- Jain, A. and Singh, K.(2007) Recent advances in the management of nosocomial infection. J. Med. Edu. Res. (JK Sciences), 9(1): 3-8.
21- Bereket, W. ; Hemalatha, K. ; Getenet, B. ; Wondwossen, T. ; Solomon, A. and Zeynudin A. (2012) Update on bacterial nosocomial infections. Eur. Rev. Med. Pharmacol. Sci. , 16(8): 1039-44.
22- Gaynes, R. and Edwards, J.R. (2005) Overview of nosocomial infections caused by gram-negative bacilli. Clin. Infect. Dis. , 41(6): 848-54.
23- Behzadi, P. ; Behzadi, E. ; Yazdanbod, H. ; Aghapour ,R. ; Cheshmeh, M.A. and Omran, D.S. (2010) A survey on urinary tract infections associated with the three most common uropathogenic bacteria. Maedica, 5(2):111-115.
24- Al-Zoreky, N.S. (2009) Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. Int. J. Food Microbial., 134: 244-8.
25- Braga, L.C. ; Shupp, J.W. ; Cummings, C. ; Jett, M. ; Takahashi, J.A. ; Carmo, L. S. ; Chartone-Souza, E. and Nascimento, A.M. (2005) Pomengranate extract inhibits Staph. Aureus growth and subsequent enterotoxin production. J. Ethno. Pharmacol., 96: 335-9.
26- Mele´ndez, P.A. and Capriles, V.A. (2006) Antibacterial properties of tropical plants from Puerto Rico. Phytomedicine ,13: 272-6.
27- Negi, P.S. ; Jayaprakasha, G.K. and Jena, B.S.(2003) Antioxidant and antimutagenic activities of pomegranate peelextracts. Food Chem., 80: 393-7.
28- Li, Y. ; Guo, C. ; Yang, J. ; Wei, J. ; Xu, J. and Cheng, S. (2006) Evaluation of antioxidant properties of pomegranate peelextract in comparison with pomegranate pulp extract.Food Chem. 96, 254-60.
29- Shan, B. ; Cai, Y. ; Brooks, J. and Corke, H. (2007) The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. Int. J. Food Microbiol., 117: 112-119.