

Comparative study on the Effect of Antibiotics and Non Antibiotics Therapy (Phytotherapy) on Some Bacteria Isolated from Urinary Tract Infection

Ikram Amin Khakil*, Ban Auday Abdul sattar**, Zahraa Qasim Ali***

ABSTRACT:

BACKGROUND:

Phytotherapy is an alternative for antibiotics therapy in these days since resistance to many antibiotics used in treatment are increasing along with high cost of some antibiotics and side effect on patients. *Punica granatum* and *Vaccinium macrocarpon* have antimicrobial properties. this paper suggest that *P. granatum* and *Vaccinium macrocarpon* may be more effective than some antibiotics used in the treatment of some Gram negative urinary tract pathogens.

OBJECTIVE:

Punica granatum juice, cranberry juice and antibiotics were used to investigate the antimicrobial activity by using well diffusion method.

MATERIALS & METHODS:

A total of four different urinary tract causing pathogens collected from patients with urinary tract infection (UTI).

RESULT:

The result indicate that extract obtained from *Punica granatum* pericarp exhibited antimicrobial activity against all organisms almost similar to the effect of cranberry which is not grown in Iraq and that *Punica granatum* and *Vaccinium macrocarpon* are more effective than some antibiotics used in the treatment of urinary tract infection.

CONCLUSION:

The antibacterial activity of crude extract of *P. granatum* against urinary tract causing organisms is reported for the first time and it showed similarity with the effect of cranberry juice that are not found or imported to the local market. Further phytochemical elucidations are required to determine the nature of compound(s) responsible for the antibacterial effects. This study is generally considered an effective approach in the discovery of new antibacterial agents from *P. granatum*.

KEY WORDS: urinary tract pathogens, punica granatum, antimicrobial susceptibility.

INTRODUCTION:

Punica granatum Linn (Pomegranate) belonging to family punicaceae, has long been esteemed as food and medicine, and is a diet in convalescence after diarrhea⁽¹⁾. Pomegranate aril juice provides about 16% of an adult's daily vitamin C requirement per 100 ml serving, and is a good source of vitamin B₅ (pantothenic acid), potassium and antioxidant polyphenols⁽²⁾. The most abundant polyphenols in pomegranate juice are the hydrolyzable tannins called punicalagins which have free-radical

scavenging properties in laboratory experiments. Punicalagins are absorbed into the human body and been shown^(3,4). In preliminary laboratory research and human pilot studies, juice of the pomegranate may have dietary value as antioxidants, but conclusive proof of efficacy in human has not yet been effective in reducing heart disease risk factors, including LDL oxidation, macrophage oxidative status, and foam cell formation^(5,6,7), all of which are steps in atherosclerosis and cardiovascular disease. Pomegranate juice has also been shown to reduce systolic blood pressure by inhibiting serum angiotensin-converting enzyme⁽⁸⁾, may inhibit viral infections⁽⁹⁾, and may have antibacterial effects against dental plaque⁽¹⁰⁾. Containing polyphenols which inhibit estrogen synthesis, pomegranate seed

*Professor Department Microbiology/ College of Medicine/ Baghdad University

**Assistant Lecturer of microbiology- Department of Biology / College of Science / Al-Mustansiriya University

***Assistant Lecturer of microbiology- Department of Anatomy, College of Medicine, Baghdad University.

BACTERIA ISOLATED FROM URINARY TRACT INFECTION

oil was effective against proliferation of breast cancer cells in vitro⁽¹¹⁾. Extract of different parts of the fruit exhibited antibacterial activity. Extracts of the whole fruit were highly active against *Micrococcus pyogens*, *S. aureus*, *E.coli*, and *Pseudomonas aeruginosa*. They were also very effective against intestinal pathogenic bacilli such as *Salmonella paradysenteriae* III-Z, *S. typhi*, *S. monetideo*, *S. scholtmuelleri* and *Shigella paradysentriae* B.H.⁽¹²⁾. The aim of the study is to compare antibacterial activity of *Punica granatum* & *Vaccinium macrocarpon* since cranberry are not found in Iraq as an alternative for cranberry and some antibiotics used for treatment for urinary tract infection.

MATERIALS & METHODS:

Preparation of crude extract

Fresh fruits were collected from the local market. Taxonomic identification of the plant was established. Pericarp of ripened fruit was collected and washed with sterile distilled water. Samples were crushed into parts and squeezed to remove the crude extract. The crude extracts were filtered through filter paper and stored in sterile vials⁽¹³⁾.

Antimicrobial screening

The crude extract of the pericarp of *P. granatum* was screened against a total of four bacterial isolates *Escherichia coli*, *Klebsiella ssp.*, *Pseudomonas aeruginosa* and *Proteus ssp.*

Determination of antibacterial activity

The well diffusion method was used to screen the antibacterial activity. *In-vitro* antibacterial assay was screened by using Mueller Hinton Agar (MHA) obtained from HiMedia, India. The MHA plates were prepared by pouring 15ml of molten media into sterile Petri dishes. The Plates were allowed to solidify for 10 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. Wells (6 mm) are aseptically punched on the agar using a sterile cork borer allowing at least 30 mm between adjacent wells. Fixed volumes of the plant extract were then introduced into the wells. The plates were then incubated at 37C° for 24 hours⁽¹⁴⁾. The anti-bacterial activity was evaluated by measuring the diameter of the inhibition zone formed around the discs.

Sensitivity to antimicrobial agents

All isolates were tested against five different antimicrobial agents including: Am:Ampicillin(10mcg),CIP:Ciprofloxacin(5mcg), N.A.:Nalidixacid(30mcg),CN:Gentamicin(10mcg), NOR:Norfloxacin(10mcg)by using the Kirby-Bauer standardized single disc method.⁽¹⁵⁾

RESULT:

All four different isolates showed inhibition zone with variable diameter against cranberry juice and *P.granatum* juice but cranberry juice had wider inhibitory activity against all isolates specially *E. coli* isolate where the zone was around 23 mm as shown in table 1.

Table 1:Antibacterial activity of *P. granatum*, cranberry juice * (zone of inhibition in mm)

Test organism	*P.granatum	*Cranberry juice
<i>P.aeruginosa</i>	14	17
<i>E. coli</i>	18	23
<i>Proteus ssp.</i>	15	20
<i>Klebsiella ssp.</i>	13	18

While in table 2 the results indicates that *Pseudomonas aeruginosa* were resistant to all

antibiotics used and the diameter of inhibition to some other antibiotics were less than diameter of cranberry juice and *P. granatum* juice.

BACTERIA ISOLATED FROM URINARY TRACT INFECTION

Table 2: Antibiotics susceptibility results (zone of inhibition in mm)

Test organism	Am 10mcg	CIP 5mcg	NA 30mcg	NOR 10mcg	CN 10mcg
<i>P.aeruginosa</i>	-	-	-	-	-
<i>E. coli</i>	-	30	21	23	16
<i>Proteus ssp</i>	10	13	15	13	12
<i>Klebsiella ssp.</i>	-	14	18	15	14

Am:Ampicillin(10mcg),CIP:Ciprofloxacin(5mcg),
N.A.:Nalidixacid(30mcg),CN:Gentamicin(10mc),NOR:Norfloxacina(10mcg)

DISCUSSION:

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants and microbes⁽¹⁶⁾. The results on antimicrobial screening of the crude extracts of both cranberry juice and *P.granatium* are shown in table 1. Resulted in clear inhibition zones of at least 14mm for all the strains tested in both crude extracts this is in agreement with previous reports by the several researchers(Machado *et. al.*,2002; Voravuthikunchai *et.al.* 2005).Pomegranate fruit and derivatives, such as juice have a long history of human use dating back to ancient times⁽¹⁹⁾Acute toxicity studies of pomegranate fruit, fruit juice, extracts and derivatives in laboratory animals at concentrations and consumption levels commonly used in folk and traditional medicine noted no toxic effects⁽²⁰⁾.The polyphenol antioxidant punicalagin, which is very abundant in pomegranate juice (> 2 g/L) was evaluated for toxicity in rats. No toxic effects were observed, no significant differences were found in the treatment group in comparison to the control group and histopathological analysis of organs confirmed the absence of toxicity⁽²¹⁾.While not conclusive, case studies of allergic reactions to pomegranate have been reported in those with pre-existing allergic conditions⁽²²⁾. Voravuthikunchai *et.al.*(2004) reported that *P.granatium* contains large amount of tannins (25%) and the antibacterial activity may be indicating the presence of some secondary metabolites, on the other hand *Pseudomonas aeruginosa* showed resistance to all antibiotics as shown in table 2, while other isolates had different diameters of inhibition approximately

equal to *P.granatium* and cranberry juice.

CONCLUSION:

The antibacterial activity of crude extract of *P. granatum* against urinary tract causing organisms is reported for the first time and it showed similarity with the effect of cranberry juice that are not found or imported to the local market. Further phytochemical elucidations are required to determine the nature of compound(s) responsible for the antibacterial effects. This study is generally considered an effective approach in the discovery of new antibacterial agents from *P. granatum*.

REFERENCES:

1. Nadkarni, A.K. Dr. K.M. Nadkarni's.Indian Materia Medica, 3rd ed. Mumbai, India: Popular Nutrition data for raw pomegranate, Nutritiondata.com Prakashan Private Limited 2000;I:1031-35.
2. Seeram NP, Henning SM, Zhang Y, Suchard M, Li Z, Heber D ."Pomegranate juice ellagitannin metabolites are present in human plasma and persist in urine for up to 48 hours". *J Nutr.* 2006;136: 2481–5.
3. Mertens-Talcott SU, Jilma-Stohlawetz P, Rios J, Hingorani L, Derendorf H .. "Absorption, metabolism, and antioxidant effects of pomegranate (*Punica granatum* L.) polyphenols after ingestion of a standardized extract in healthy human volunteers". *J Agric Food Chem* 2006;54: 8956–61.
4. Aviram M, Rosenblat M, Gaitini D, *et al.* "Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation". *Clin Nutr* 2004;23: 423–33.

5. Esmailzadeh A, Tahbaz F, Gaieni I, Alavi-Majd H, Azadbakht L "Concentrated pomegranate juice improves lipid profiles in diabetic patients with hyperlipidemia". *J Med Food* 2004; 7: 305–8.
6. Kaplan M, Hayek T, Raz A, *et al.* "Pomegranate juice supplementation to atherosclerotic mice reduce macrophage lipid peroxidation cellular cholesterol accumulation and development of atherosclerosis". *J Nutr.* 2001;131: 2082–9.
7. Aviram M, Dornfeld L "Pomegranate juice consumption inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure ". *Atherosclerosis* 2001;158: 195–8.
8. Neurath AR, Strick N, Li YY, Debnath AK. "Punica granatum (Pomegranate) juice provides an HIV-1 entry inhibitor and candidate topical microbicide". *BMC Infect. Dis.* 2004; 4: 41.
9. Menezes SM, Cordeiro LN, Viana GS. "Punica granatum (pomegranate) extract is active against dental plaque". *Journal of herbal pharmacotherapy* 2006;6: 79–92.
10. Kim ND, Mehta R, Yu W, *et al.* "chemopreventive and adjuvant therapeutic potential of pomegranate (Punica granatum) for human breast cancer". *Breast Cancer Res Treat.* 2002;71: 203–17.
11. Ram, A. The Wealth of India, A Dictionary of Indian Raw materials and Industrial products, Raw material. National institute of scientific communication, Council of Industrial and Scientific Research, New Delhi. 1998: VIII.
12. -B.V. Pradeep1*, M.K. Manojbabu2 and M. Palaniswamy. Antibacterial Activity of Punica granatum L. against Gastro Intestinal Tract Infection Causing Organisms. *Ethnobotanical Leaflets* 2008;12: 1085-89.
13. Mbata TI, Debiao L, Saikia A. Antibacterial activity of the crude extract of Chinese Green Tea (Camellia sinensis) on *Listeria monocytogenes*. *Internet J. Microbiol.* 2006;2.
14. Bauer A; Kirby W. Antibiotic susceptibility testing by standardized single disc diffusion method. *Am J clin patho.* 1966;45:393.
15. Erdogrul, O.T. Antibacterial activities of some plant extracts used in folk medicine. *Pharmaceutical Biology.* 2002; 40: 269-73.
16. Machado, T.B., Leal, C.R., Amerl, A, C. F., Santos, K.R.N., silva, M.G. and Kuster, R.M. Antimicrobial ellagitannin of Punica granatum fruits. *Journal of Brazilian Chemical Society,* 2002;13: 606-10.
17. Voravuthikunchai, S.P., Sririrak, T., Limsuwan, S., Supawita, T., Iida, T. and T. Honda. Inhibitory effect of active compounds from Punica granatum Pericarp on verocytotoxin production by Enterohemorrhagic Escherichia coli O157: H7. *Journal of Health Science,* 2005; 51:590-96.
18. Madihassan, S. Outline of the beginnings of alchemy and its antecedents. *Am J Chinese Med* 1984;12: 32-42 .
19. Desta, B. Ethiopian traditional herbal drugs. Part I. studies on toxicity and therapeutic activity of local taenicidal medications. *J Ethnopharm* 1995;45: 27-33
20. Cerda B, Ceron JJ, Tomas-Barberan FA, Espin JC, Repeated oral administration of high doses of the pomegranate ellagitannin punicalagin to rats for 37 days is not toxic. *J Agric Food Chem.* 2003; 51:3493-501.
21. Zoccatelli G, Olivieri M, Peruffo A. Allergy to pomegranate: a case report. *Allergy Clin Immunol Int.* 2005;1(suppl):580.
22. Voravuthikunchai, S., Lortheeranuwat, A., Jeeju, W., Sririrak, T., Phongpaichit, S. and Supawita, T. Effective medicinal plants against Enterohaemorrhagic Escherichia coli O157: H7. *Journal of Ethanopharmacology.* 2004; 94:49-54.