Evaluation of Antibacterial Activity of Aqueous Extracts of Pomegranate Peels, Green Tea Leaves and Bay Leaves against *Vibrio cholera*

Zainab A. Al-Dhaher
Department of Basic Sciences, College of Dentistry, Baghdad University, Iraq
zainaba.aldhaher@gmail.com
Accepted on 24/6/2012

Summary

The antibacterial activity of aqueous extracts of Pomegranate peels, Green tea leaves, Bay leaves were evaluated in this study. Agar diffusion technique was applied. The three extracts were found to have inhibitory effect against *Vibrio cholera* and the mean of the diameter of inhibition zones ranges from (11-23 mm) for pomegranate peels, (9-20 mm) for Green tea leaves and (7-18mm) for Bay leaves extracts and statistically analyzed. There were significant differences between pomegranate peels, tea leaves and bay leaves extracts at each concentration used in this study. The pomegranate peels were found to have greater effects against *Vibrio cholera* followed by tea leaves, Bay leaves. Minimum bactericidal concentration (MBC) was also determined and it was shown that 10mg/ml was the MBC for pomegranate peels, 10-15mg/ml the MBC for tea leaves and 20-25mg/ml the MBC for Bay leaves.

Keywords: Aqueous extracts Pomegranate peels, Green tea leaves, Bay leaves, *Vibrio cholera*.

Introduction

Cholera is an acute diarrheal disease which is characterized by voluminous rice water stool caused by *Vibrio cholera*. The severe intestinal infection in cholera leads to rapidly progressing dehydration, which without appropriate intervention kills half of affected individuals (1). Electrolyte replacement therapy through oral and /or intravenous rehydration has proven extraordinarily effective in treating patients from deadly episodes of cholera. In addition, clinically typical cholera patients are routinely treated with 1-3 day courses of antibiotics to shorten the illness. But antibiotics therapy in recent years has faced difficulties due to the rapid emergence of multidrug resistance among the cholera bacteria. Therefore, the future effective treatment of cholera is becoming uncertain (2).

For centuries; nature has been an enormous source of agents of medical importance and the beneficial health effects of many plants, used for seasoning agents in food and beverages, have been claimed for not only preventing food deterioration but also acting as antimicrobials against pathogenic microorganisms (3). Scientists are searching for natural products that can be used in large scale to reduce diarrhea caused by vibrios (4).

*Punica granatum*L. (Punicaceae) referred to in English as Pomegranates, is a native shrub of Asia and Mediterranean Europe that has a rich history of traditional use in medicine (5). The phytochemistry of pomegranate has also been widely studied by many researchers and this fruit was found to be a rich source of polyphenolic compounds (Phenolic rings bearing multiple hydroxyl groups) (6). Peels of the fruit also include wide variety of phytochemical compounds including gallotinnins, ellagic acid, gallagic acid, punicalins, punicalagins and ellagitannins (7). Pomegranate have been highlighted in many studies as having antimicrobial activity against a wide range of microorganisms including bacteria (Gram positive, Gram negative), fungi, yeasts and viruses (8).

The beverage known as tea is an infusion of variously processed leaves of one of the varieties of an evergreen shrub, *Camellia sinensis*L. Tea is the most widely drunk beverage in the world (9). Green tea, popular in the Far East, differs from the black tea familiar in the West in that an oxidation step (called ‘‘fermentation’’) occurs in the processing of the

latter compound but not the former compound (10).

Green tea contains relatively large amounts of polyphenols, mainly catechins and their derivatives, considered to exert a protective effect against cancer and cardiovascular diseases. As tea is one of the most popular beverages, it could be a tremendously important source of polyphenolic constituents. Green tea leaves also contains small amounts of flavonols, such as quercitin, kaempferol, and myricetin (11). Several researchers reported that green tea has broad spectrum antibacterial activity and it is also antioxidant, antimutagenic, lowering plasma cholesterol levels and protecting from the effect of radiation (12).

Laurusnobilis L. Commonly known as El-Gharor Bay leaves and member of the Lauraeae family (13). It is traditionally used in food flavoring, herbal teas and in cosmetics industry for its Oclsand pleasant scent. Bay leaves have many properties that make them useful for treating high blood sugar, migraine headaches, bacterial and fungal infections and gastric ulcers. Bay is generally regarded as safe by the FDA (14). The aims of this study were to evaluate the antibacterial activity of aqueous extracts of pomegranate peels, green tea and bay leaves.

Materials and Methods

Six isolates of *Vibrio cholera* bacteria obtained from stool samples were isolated and diagnosed at teaching laboratories in Baghdad medical city this six isolates were used in this study to test the antibacterial activity of three plants aqueous extract; Pomegranate peels, Green tea leaves and Bay leaves). Pomegranate peels, Green tea leaves and Bay leaves were dried and powdered; 100gm of each plant were infused separately in 500ml distilled water for 2hrs to prepare the aqueous extract. The infusions were blended with electric blender for 5min. After this the infusions were filtered by filter paper (WattmanNo.1) and then residue discarded. The extracts left to dry in glass petri dishes at 37C^{o} in the incubator, the resulted powder was collected and kept in tightly closed dark glass container at room temperature until used to prepare the stock solution of the aqueous extracts. Antibacterial activity was tested by using agar diffusion technique (15).

The surface of sterile Muller- Hinton agar plates were inoculated with 0.2ml of a 24 hrs broth culture (105cfu/ml of test organisms and evenly spread using bent sterile glass rod) wells of 6mm in diameter were aseptically punched on each agar plate using a sterile cork bore. From stock solutions of each extract four concentrations (300, 200, 100, 50 and 25) mg/ml were prepared. 50µl of each concentration of each extract were loaded into wells separately. The inoculated plates were incubated at 37c^{o} for 24hrs and the antibacterial activity was evaluated by measuring the diameter (mm) of inhibition zones around the wells. All the tests were performed in triplicates.

To determine the minimum bactericidal concentration (MBC) of Pomegranate peels, Green tea leaves and Bay leaves extracts, final concentrations of 5, 10, 15, 20 and 25mg/ml were prepared from the stock solution of each extract separately each concentration was incorporate in Brain heart infusion agar (BHIA) to get 25ml of agar and extract and then poured into petri dishes and allowed to harden then every petri dish was inoculated with 0.1ml of bacterial isolates of *Vibrio cholera*.

All these Petri dishes were incubated for 24hrs 37 ^{o}c including the controls (negative control which contained BHI-A with microbial inoculums without the addition of the extract and the positive control plates which contained BHI-A and different concentrations of aqueous extracts of Pomegranate peels, Green tea leaves and Bay leaves without microbial inoculums). Each Petri dish was checked and examined after incubation. The lowest concentration of Pomegranate peels, Green tea leaves and Bay leaves extract that killed the microorganism was recorded as the MBC. All these steps were done separately for each extract.

Results were analyzed by using SPSS 15 statistical package (SPSS Ltd, working UK). ANOVA test (one way) was used to express antibacterial differences of three plants extracts against bacterial isolates.

Results and Discussion

In present study the antibacterial activity of Pomegranate peels, Green tea leaves and

Bay leaves aqueous extracts was evaluated against six isolates of *Vibrio cholera.*

Pomegranate peels seem to have strong antibacterial activity against *Vibrio cholera.* The results in table, 1 were in agreement with the results of researchers (16) whom reported that *Punica granatum* peels extracts have strong antibacterial activity against Gram positive and Gram negative bacteria. Other workers (17) also reported that *Punica granatum* extract has strong antibacterial activity against *Vibrio cholera*.Several researchers also reported that various parts of the *Punica granatum*(seed, peels, juice and whole fruit) appear to have therapeutic properties and Antimicrobial activity (18 and 19). The antibacterial activity of pomegranate peels extract might be due to the presence of various phytochemicals such as phenolic punicalagins, gallic acids, catechin, quercetin and retina (20).

Table, 1: Sensitivity of *Vibrio cholera* to aqueous extract of Pomegranate peels

Concentration of Pomegranate peels extract mg/ml	Mean diameter of inhibition zones (mm)
300	23
200	21
100	18
50	16
25	11

According to the results (table, 2) green tea leaves exhibited good antibacterial activity against *Vibrio cholera*, this was in agreement with the study of many researchers who found that Japanese green tea has been shown to inhibit the growth and toxin expression of *Vibrio cholera* (21). Other researchers (22) found that tea components inhibit the growth of *Vibrio cholera*. Green tea leaves are, a very good source of polyphenolic compounds. It contains flavonoids as well as phenolic acids which can make up to 30% of fresh leaves dry weight and only 10% of dry weight of black tea. Those constituents are referred to as bioflavonoids or vitamin B, as they show a wide spectrum of biological effects in living organisms (23).

Table, 2: Sensitivity of *Vibrio cholera* to aqueous extract of Green tea leaves

Concentration of Green tea leaves extract mg/ml	Mean diameter of inhibition zones (mm)
300	20
200	19
100	16
50	13
25	9

The most important polyphenol groups in tea leaves are substances whose nomenclature is still a divergence. These compounds are often referred to as flavan-3-ols or catechins. Green tea catechins can act as inhibitors of some enzymes important to microorganisms and primarily act on damaged bacterial membrane (24).

Bay leaves extract also inhibit the growth of *Vibrio cholera* at all the concentration used but it was the third according to the potentiality of antibacterial activity against *Vibrio cholera* Table, 3.

Table, 3: Sensitivity of *Vibrio cholera* to aqueous extract of Bay leaves.

Concentration of Bay leaves extract mg/ml	Mean diameter of inhibition zones (mm)
300	18
200	15
100	13
50	10
25	7

Several researchers also recorded that bay leaves have good antibiotic properties and the oils of Bay leaves have shown antibiotic activity (25 and 26).

Essential oil of Bay leaves contains various volatile components with antimicrobial activities against bacteria, yeasts and some molds. Tannin in Bay leaves may potentiate its antibacterial activity (27). Other researchers (28) found that the *Laurus nobilis* leaves (Bay leaves) extract showed varying degrees of activities against Gram negative and Gram positive bacteria.

Table,4 demonstrates the minimum bacterial concentration (MBC) of Pomegranate peels, Green tea leaves, Bay leaves aqueous extracts and the results showed that the MBC of

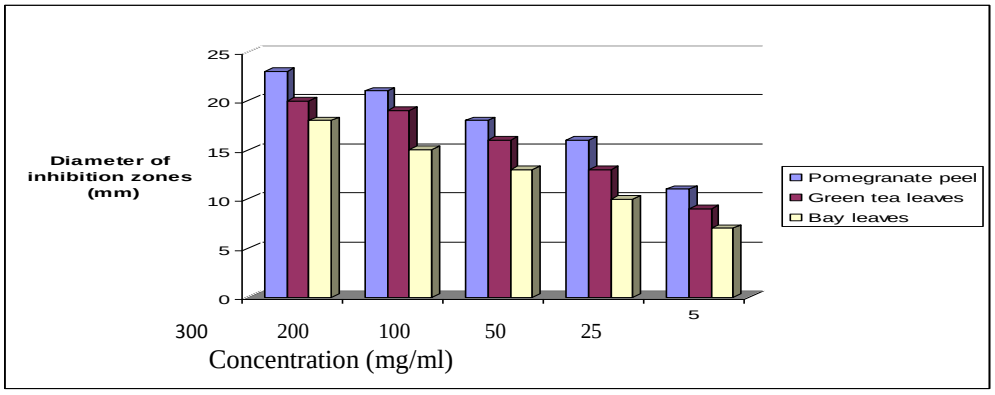
Pomegranate peels extract is 10mg/ml while the MBC of Green tea extract is 10-15 mg/ml and finally the MBC of Bay leaves is 20-25 mg/ml.

Table, 4: Minimum bactericidal concentration (MBC) of Pomegranate peels, Green tea leaves and Bay leaves aqueous extracts on *Vibrio cholera* isolates.

Type of extract	Number of extract	Concentration of extract (mg/ml)				
		5	10	15	20	25
Pomegranate peels	6		6			
Green tea leaves	6		1	5		
Bay leaves	6				1	5

The results of this study as mentioned earlier clearly demonstrate that the three aqueous extracts used exhibited antibacterial activity against *Vibrio cholera* and according to statistical analysis using ANOVA test at ($P<0.05$) Pomegranate peels extract exhibited the strongest antibacterial activity while Green tea leaves extract was the second one according to antibacterial potentiality followed by Bay leaves extract and there are also significant differences at ($P<0.05$) between the three extracts at each concentration used in this study and diameters of inhibition zones increase proportionally according to concentrations for each extract (figure,1).

In conclusion the use of infusions prepared in water of Pomegranate peels, Green tea leaves, and Bay leaves could be useful to treat diarrhea caused by *Vibrio cholera* or help in the treatment of patients infected with *Vibrio cholera*. Thus provides a scientific basis for the use of these aqueous extract as home-made remedies and their possible application in treating gastrointestinal disorders.



Figure, 1: Antibacterial activity of Pomegranate peels, Green tea leaves, Bay leaves on *Vibrio cholera*.

References

1. Sack, D.A; Sack, R.B.; Nair, G.B. and Siddique, A.K. (2004). Cholera. Lancet, (363): 23-33.
2. Chatterjee, S.; Ghosh, K.; Roychoudhuri, A.; Chowdhury, G.; Bhattacharya, M.K. and Mukhopadhyay, A.K. (2009). Incidence virulence factors and clonality among clinical strains of non-O1, non-O139 Vibrio cholerae isolates from hospitalized diarrheal patients in Kolkata India. J. Clin. Microbiol., (47): 1087-1095.
3. Iwu, M.W.; Duncan, A.R. and Okunjii, C.O. (1999). New antimicrobials of plant origin. In: Alexandria Janick J editor Perspectives on new crops and new uses. Virginia ASHS Press, PP: 457- 462.
4. Hasegawa, N.; Matsumoto, Y.; Hoshino, A. and Iwashita, K. (1999). Comparison of effects of *Wasabia japonica* and allylisothiocyanate on the growth of four strains of *Vibrio parahaemolyticus*in lean and fatty tuna meat suspensions. Int J Food Microbiol., (49): 27-34.
5. Lansky, E.P. and Newman, R.E. (2007). *Punica granatum* (Pomegranate) and its potential for prevention andtreatment of inflammation and cancer J. Ethno. Pharmacol., 19(2): 177-206.
6. Dahham, S.S.; Ali, M.N.; Tabassum, H. and Khan, M. (2010). Studies on

antibacterial and antifungal activity of pomegranate (*Punica granatum* L.*)* Amer. Eurasian J. Agri. Environ. Sci., 9(3): 273-281.

7. Machado, T.B.; Pinto, A.V.; Pinto, M.C.; Leal, I.C. and Silva, M.G. (2003). *In vitro* activity of Brazilian medicinal plants, naturally occurring napthoqu- inones and their analogues, against methicillin – resistant *Staphylococcus aureus.* Int. J. Antimicrob., (21): 279-284.
8. Naz, S.; Siddiqi, R.; Ahmed, S.; Rasool, S.A. and Sayeed, S.A. (2007). Antibacterial activity directed isolation of compounds from *Punica granatum*. J. Food Sci., (72): 341- 345.
9. Ahn, Y.J; Kawamura, T.; Kim, M.; Yamamoto, T. and Mitsuoka, T. (1991). Tea polyphenols: selective growth inhibitors of *Clostridium* spp. Agric. Biol. Chem., (55): 1425–1426.
10. Acker, S.A.; Vanbalen, G.P.; Vandenberg, D.J.; Bast, A. and Vandervijgh, W.J. (1998). Influence of iron chelation on the antioxidant activity of flavonoids. Biochem. Pharmacol., (56): 935–943.
11. Amarowicz, R. and Shahidi, F. (1995). Antioxidant activity of green tea catechins in a b-carotene-linoleate model system. J. Food Lipids, 2: 47–56.
12. Mbata, T.I. (2007). Preliminary studies of the antibacterial activities of processed Kenyan and Nigerian tea. African J. Biotech., 6 (3):278-279.
13. Fiorini, C.; Fouraste, I.; David, B. and Bessiere, J. (1997). Composition of the flower leaf and stem essential oils from L. nobilis L. FlaVour Fragr. J., 12: 91-93.
14. Biljana, K.A.; Mira, P.O. and Sanja, V.L. (2010). *In Vitro* and *in Vivo* Effects of *Laurusnobilis*L. Leaf Extracts. Molecules, 15: 3378-3390.
15. National Committee for Clinical Labotratory Standards (NCCLS) (1993). Performance Standards for Antimicrobial Disc Suspectibility tests. Approved Standard NCCLS Publication M2-A5 Villanova, Pennsylvania, USA M2-A5.
16. Vijayanand, S. and Hemapriya, J. (2011). *In vitro* Antibacterial Efficacy of Peels and Seed Extracts of *Punicagranatum*L against Selected Bacterial StrainsInt. J. Medicobio. Res., 1(4): 231-234.
17. Vasudha, P.A.; Thangjam, R.C.; Rituparna, C.H.; Bangar, R.A.; Richard, L.O. and Mamatha, B.A. (2011). Evaluation of the antimicrobial activity of *Punicagranatum* peels against the enteric pathogens: An *invitro* study. Asian J. Plant Sci. Res., 1(2):57-62.
18. Vidal, A.; Fallarero, A.; Pena, B.R.; Medica, M.E. and Gra, B. (2003). Studies on the toxicity of *Punica granatum* L. (Punicaceae) whole fruit extracts. J. Ethno. Pharmacol., 89: 295-300.
19. Jurenka, J.S. (2007). Therapeutics application of pomegranate (*Punica granatum* L.*)* A review Altern. Med. Rev., 13:128-144.
20. Reddy, M.K.; Gupta, S.K.; Jacob, M.R.; Khan, S.I. and Ferreira, D. (2007). Antioxidant antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum* L. Planta Med., 73: 461-467.
21. Yamasaki, S.H.; Asakura, M.A.; Basu, N.S.; Hinenoya, A.T.; Iwaoka, E.M and Aoki, S.H. (2011). Inhibition of virulence potential of *Vibrio cholerae*by natural compounds. Indian J. Med. Res., 133:232-239.
22. Tiwari, R.P.; Bharti, S.K.; Kaur, H.D.; Dikshit, R.P. and Hoondal, G.S. (2005). Synergistic antimicrobial activity of tea and antibiotics Indian J. Med. Res., 122: 80-84.
23. Wang, H.; Provan, G.J. and Helliwell, K. (2000). Tea flavonoids their functions utilization and analysis Trends Food. Sci. Tech., 11: 152–160.
24. Okamoto, M.; Leung, K.P.; Ansai, T.; Sugimoto, A. and Maeda, N. (2003). Inhibitory effects of green tea catechins on protein tyrosine phosphatase in Prevotella intermedia Oral. Microbiol. Immunol., 18: 192–195.
25. Kloucek, P.; Polesny, Z.; Svobodova, B.; Vlkova, E. and Kokoska, L. (2005). Antibacterial screening of some Peruvian

medicinal plants used in Callería District. J. Ethno. Pharmacol., 99:309-312.
26. Prabuseenivasan, S.; Jayakumar, M. and Ignaciuthu, S. (2006). In vitro Antibacterial activity of some plants essential oils. mbc compel. Altern. Med., 6(11):147.
27. Kone, W.M.; Atindehon, K.K.; Terreaux, C.; Hostettmann, K.; Traore, D. and Dosso, M. (2004).Traditional medicine in north cote-dIvoire: screening of so plants for antibacterial activity. J. Ethno. Pharmacol., 93:43-49.
28. Al-Hussaini, R.I. and Mahasneh, A.M. (2009). Antimicrobial and antiquorum sensing activity of different parts of *laurusnobilis*. Extracts. J. Med., 43(4): 286 -298.

تقييم الفعالية ضد البكتيريا للمستخلصات المائية لقشور الرمان ,اوراق الشاي الاخضر و اوراق الغار ضد بكتريا الكوليرا

زينب عبدالجبار الظاهر
فرع العلوم الاساسية- كلية طب الاسنان- جامعة بغداد - العراق

الخلاصة

تم التحري عن الفعالية الضد بكتيرية للمستخلصات المائية لقشور الرمان, اوراق الشاي الاخضر واوراق الغار لاجل تثمينها باستخدام تقنية الانتشار بواسطة أل(اگار) وتم التوصل الى ان المستخلصات الثلاثة لها فعالية تثبيطية ضد بكتريا الكوليرا وتراوحت اقطار التثبيط مابين (11-23) لقشر الرمان ,(9-20) لورق الشاي الاخضر و (7-18) لورق الغار وكانت هناك فروق معنوية مابين المستخلصات الثلاثة ولكل تركيز وكان قشر الرمان هو الافضل بينهم يتبعه اوراق الشاي الاخضر ثم اوراق الغار كما تم التحري عن التركيزالقاتل الادنى لهذه المستخلصات ضد بكتريا الكوليرا وكانت قيمته 10ملغم/ملم لقشر الرمان و15-20ملغم/ملم لورق الشاي الاخضر و20-25ملغم/ملم لورق الغار.

الكلمات المفتاحية: المستخلص المائي، قشور الرمان، الشاي الاخضر، اوراق الغار، الكوليرا.