International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Volume 3, Issue 3, March - 2016

Research Article

SOI: http://s-o-i.org/1.15/ijarbs-2016-3-3-35

In vitro Evaluation the effect of Natural Products against *Vibrio cholerae*

Khulood M. Alsaraf, B.Sc., M.Sc., Ph.D.[(1)] Suha T. Abd, B.D.S.,M.Sc.[(2)*], Nada S. Hussein,B.Sc., M.Sc.[(3)] Aseel A. Jabor, B.Sc.[(4)]

[(1)]Assistant Professor, Department of Basic Science, College of Dentistry, University of Baghdad, Iraq.
[(2)] Assistant Lecturer, Department of Basic Science, College of Dentistry, University of Baghdad, Iraq.
[(3)] Biologist in Teaching Laboratories of Medical City.
[(4)] Department of Basic Science, College of Dentistry, University of Baghdad, Iraq.
*Corresponding author: *suhatalal_abd@yahoo.com*

Abstract

Background: Cholera remains a public health threat affecting vulnerable populations living with unreliable water supply and sub-standard sanitary conditions. Cholera is an infection of the small intestine by some strains of the bacterium *Vibrio cholerae*. The aim of this study was to evaluating the antibacterial action of some natural substitutes for treatment of *Vibrio cholerae.* **Materials and methods:** Twelve tested bacterial specimens were isolated from patients affecting by cholera disease in medical city. Identification of *V. cholerae* was done by different laboratory diagnosis tests. *In-vitro* antibacterial activity of fresh lemon juice and vinegar in addition to use the Tetracycline antibiotic s a positive control was performed by agar well diffusion method on Muller Hinton agar medium. **Results:** both the fresh lemon juice and vinegar have antibacterial effect on *V.cholerae* the inhibition zone of both agents less than tetracycline antibiotic. Highly significance difference between inhibition zone both agents when compared with tetracycline antibiotic and also highly significant difference between fresh lemon juice and vinegar. **Conclusion:** Both the fresh lemon juice and vinegar may be considered natural substitutes for treatment cholera disease.

Keywords: *Vibrio cholerae*, Natural Substitutes, Inhibition zones.

Introduction

Cholera remains a public health threat affecting vulnerable populations living with unreliable water supply and sub-standard sanitary conditions. Every year, there are an estimated 3 - 5 million cholera cases and 100,000 - 120,000 associated deaths worldwide [(1)]. Cholera is an infection of the small intestine by some strains of the bacterium *Vibrio cholerae*[(2)]. Cholera is an acute intestinal infection caused by the bacterium, *Vibrio cholerae.* It is a gram-negative, rod-shaped waterborne bacterium that carries a single polar flagellum. It grows rapidly in optimum temperature at 37°C, with a range of 10 to 43°C. The organism can be inactivated at pH values less than 4.5 at room temperature and it grows in optimum pH of 7.6, with a range of 5.0 to 9.6 [(3)] . The pathogenesis of *V. cholerae* involves both the colonization of the intestine and the production of cholera toxin (CT) which acts locally to stimulate excessive electrolyte and fluid secretion, primarily from the crypt cells of the small intestine [(4)]. Symptoms may range from none, to mild, to severe.[(5)] The classic symptom is large amounts of watery diarrhea that lasts a few days [(6)].Vomiting and muscle cramps may also occur [(5)]. Diarrhea can be so severe that it leads within hours to

severe dehydration and electrolyte imbalance [6]. This may result in sunken eyes, cold skin, decreased skin elasticity, and wrinkling of the hands and feet [7]. The dehydration may result in the skin turning bluish[8]. Symptoms start two hours to five days after exposure [5]. the Ministry of Health of Iraq, in consultation with WHO, declared a cholera outbreak in governorates of Najaf, Diwaniya, and parts of west Baghdad on 15 September 2015, and announced a stepping up of measures to stop transmission and prevent further spread of The disease. The declaration came after a sudden increase in the number of acute diarrhea diseases cases. Cholera subtype 01 Inaba in 38 out of a total of 106 stool samples tested. Vinegar is a liquid consisting of about 5-20% acetic acid (CH3COOH), water and other trace chemicals, which may include flavorings. The acetic acid is produced by the fermentation of ethanol by acetic acid bacteria [9].The juice of the lemon is about 5% to 6% citric acid, which gives a sour taste. The distinctive sour taste of lemon juice makes it a key ingredient in drinks and foods and the low pH of juice makes it antibacterial [10].

Materials and Methods

Laboratory diagnosis:

Specimen: Stool and swab samples collected in the acute stage of the disease (before antibiotics have been administered) are the most useful specimens for laboratory diagnosis.

Collection of fecal specimens:

Fecal specimen should be collected in clean dry wide mouth container .The container should not sterilized by chemical disinfectant .The specimen should not contaminated with water or urine.

The collection stool should be processed as soon as possible upon receipt in the laboratory and no longer than 2 hour after collection. If it is not possible to process the specimens within 2 hours a small amount should be collected on a swab inserted in to the stool and rotated this should then be inoculated in to a suitable transport media (caryblair).

In some instances, the collection of a rectal swab rather than feces may be necessary, particularly in new borne. Rectal swabs may be more effective than feces for recovery of certain strains of Shigella, because these organisms are susceptible to cooling and drying.

Microscopic examination:

Direct microscopy of stool is not recommended as it is unreliable. Motility (dark field microscopy), but the vibrios can also be seen using transmitted light microscopy.

Culture:

V.cholerae can be selectively cultured out of bacterial mixtures at pH 9.

A number of special media have been employed for the cultivation for cholera vibrios. They are classified as follows:

1-Transport media:

Cary-Blair medium: This the most widely-used carrying media. This is a buffered solution at pH 8.4.

2-Enrichment media

Alkaline peptone water: At pH 8.6, *V.cholerae* grows rapidly, producing growth (turbidity) on and just below the surface of the medium, usually within 4-6 hours. It is an enrichment medium and its alkalinity suppresses the growth of intestinal commensals.

3-TCBS medium: (Thiosulphate- citrate bile salt sucrose agar)

This is an excellent selective medium for the primary isolation of *V.cholerae*. This medium contains thiosulphate, citrate, bile salts and sucrose. Cholera vibrios produce flat 2-3 mm in diameter, sucrose-fermenting yellow colonies after overnight incubation or after 4-6 hours at 35-37° C.

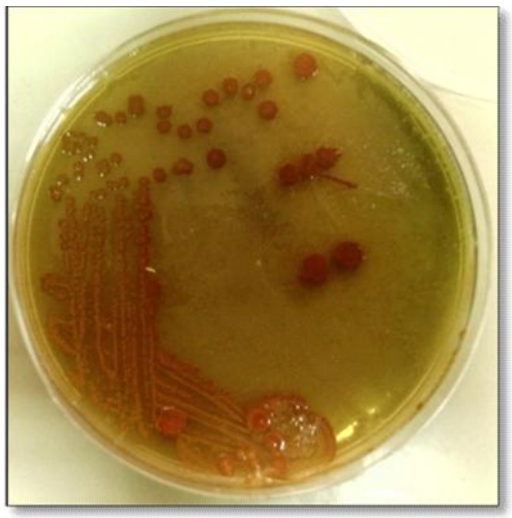

Figure 1: Growth of *V. cholerae* on TCBS

4-Mac Conkey agar: Small non-lactose fermenting colonies with prolonged incubation lactose may be fermented.

5-Blood agar: *V. cholerae* 01 and 0139 grow on blood agar (subcultured from alkaline peptone water) often produce *beta*-haemolytic colonies.

Biochemical tests:

Kligler iron agar: Acid / Alk, no gas, no H_2S,
Oxidase test : positive.
Peptone water: Indol positive (red ring).
Urea: negative.
Simmon citrate: Positive.
Semi solid mannitol: Motile, Mannitol ferment.

Figure 2: Biochemical tests of *V. cholerae*

String test:

Can be used to separate *Vibrio cholerae* from *Aeromonas* spp. (organism are emulsified in 0. 5% sodium deoxcholate which lyses the vibrio cells. but not those *Aeromonas* spp. Within 60 sec the cells lyse (loss of turbidity) and DNA strings when a loopful is lifted (up to 2 to 3 cm) from the slide.

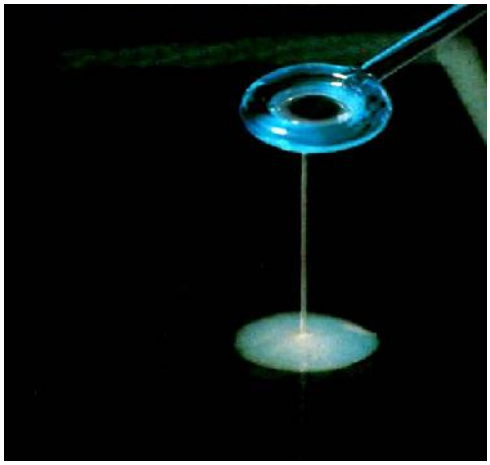

Figure 3: A positive *V. cholerae* string test

Identification by O129 disk:

V.cholerae sensitive, some strains of *V.cholerae* O1 are resistant to O\129, so the dependability of this test may be waning.

Identification by serological tests :

Diagnosis can be confirmed as well as serotyping done by slide agglutinating with specific sera: Separate antisera are required to identify *V. cholerae* 01 (Inaba and Ogawa) and *V. cholerae* 0139. Isolates from TCBS cultures require subculturing to nutrient agar before carryout serotyping.

Bacterial sensitivity media (Agar Media)

Muller Hinton Agar prepared according to manufacturer's instruction which involved the suspension of 38 gm. in one liter of de-ionized water, after being completely dissolved with boiling, it was sterilized by autoclave at 15 lb. pressure for 15 minutes, then left to cool at 45- 50^{o}C, poured and left to solidify then put them in incubator at 37°C for 24 hours then stored in refrigerator until being used.

Antimicrobial screening (*in vitro*)

The antimicrobial activity of the fresh lemon juice, vinegar by well diffusion method [11, 12]. In comparison to tetracycline antibiotic discs as a positive control were measured the prepared culture plates were inoculated with swab from diagnosed *Vibrio cholerae* bacteria using spreading method. Wells were made on the agar surface with 6 mm cork borer. The position of the wells for each extract was marked at the outside walls of plates before application of fresh lemon juice and vinegar. The two solutions were poured into the well. Each well was filled with 100µl with corresponding solution with the help of a micropipette. The plates were incubated at37±2 ºC for 24 hours. The plates were observed for the zone clearance around the wells. The resulting inhibition zones were uniformly circular. The diameters of the zones of inhibition were measured, including the diameter of the well. Inhibition zones are measured to the nearest millimeter, using a ruler, which is held on the back of the inverted petriplate.

Results

This study revealed the important antibacterial activity of the fresh lemon juice and vinegar in inhibition of growth of twelve samples *V.cholerae*.

Tetracycline antibiotic disc has the greater inhibition zones (30.833 ± 0.389) ,followed by fresh lemon juice (18.500 ± 0.522) and the least inhibition zone was by vinegar (17.833 ± 0.937). This result shown in table 1. In figure 4 see the mean and standard deviation of inhibition zones for the three studied agents.

Table 1: Mean and Standard Deviation of Inhibition zones of the three agents against *Vibrio cholerae*

	Inhibition zones in
fresh lemon juice	18.500 ± 0.522
vinegar	17.833 ± 0.937
tetracycline antibiotic	30.833 ± 0.389

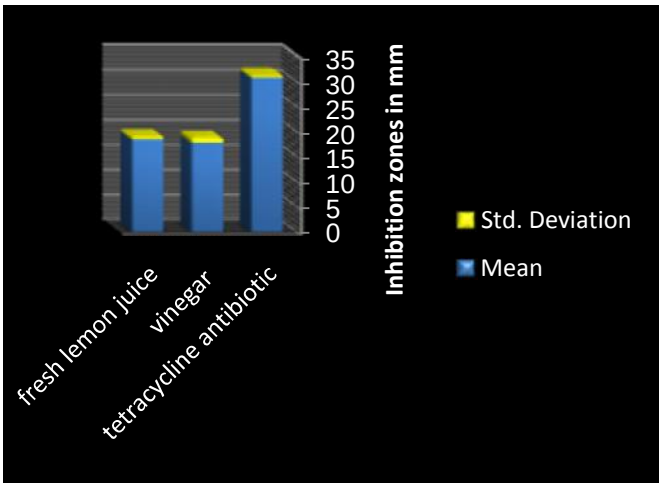

Figure 4: Inhibition zones in mm of the three agents against *V. Cholerae*

While figure 5 show inhibition zones of tetracycline antibiotic disc ,fresh lemon juice and vinegar on the inoculum of *Vibrio cholera* swabbed on Muller Hinton.

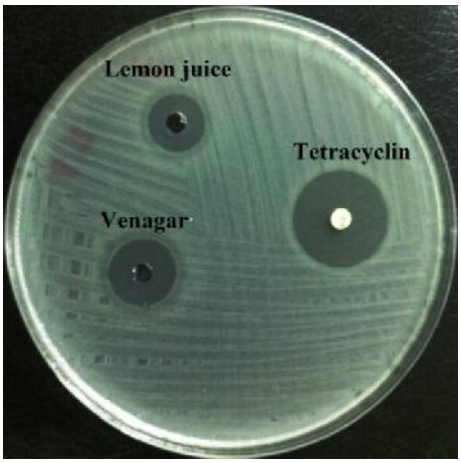

Figure 5: Inhibition zones of the three agents against *V. Cholerae* on Muller Hinton agar

Table 2 reveled statistical difference between inhibition zones for the three agents against *Vibrio cholera*e. Highly significance difference between inhibition zone both agents when compared with tetracycline antibiotic and also highly significant difference between fresh lemon juice and vinegar.

Table 2: Statistical difference between Inhibition zones for the three agents against *Vibrio cholerae*

	t	df	Sig.
lemon - tetracycline	-86.773	11	.000
vinegar - tetracycline	-52.806	11	.000
lemon – vinegar	2.966	11	.013

Discussion

For the treatment of cholera in many cases antibiotics are used: Tetracyclin, Ciprofloxacin, Erythroycin, Imipenem , Cefteriaxon , Trimethoprime but all these antibiotecs have many side effects. Resistance to tetracycline and other antimicrobial agents among *V. cholerae* has been demonstrated in both endemic and epidemic cholera settings. Resistance can be acquired through the accumulation of selected mutations over time, or the acquisition of genetic elements such as plasmids, introns, or conjugative elements, which confer rapid spread of resistance. A likely risk factor for antimicrobial resistance is widespread use of antibiotics, including mass distribution for prophylaxis in asymptomatic individuals [13,14]. For all these causes and other causes it was necessary to find natural substitutes to ordinary antibiotics for treatment of *V.cholerae* bacteria. The availability and low cost with no or minimum side effects were main causes for choosing lemon juice and vinegar in addition to many uses of lemon itself in treatment of many diseases. Lemon juice possesses antibacterial, antiviral and immune-building properties. It fights disease and infection with high levels of bioflavonoids, pectin, limonene, citric acid, magnesium, calcium and vitamins. It stimulates digestion, promoting weight loss[15]. Vinegar also have many clinical benifitswith other constituents of vinegar include vitamins, mineral salts, amino acids, polyphenolic compounds (eg, galic acid, catechin, caffeic acid, ferulic acid), and nonvolatile organic acids (eg, tartaric, citric, malic, lactic)[16,17]

Conclusion

In conclusion both the fresh lemon juice and vinegar may be considered natural substitutes for treatment cholera disease, also may be used as alternative or assistance treatment.

References

1- World Health Organization. Cholera. Fact sheet no. 107. Geneva: World Health Organization; 2010 [Available from: http://www.who.int/mediacentre/factsheets/fs107/en/index.html Accessed on May 13, 2011].

2- Finkelstein, Richard. "Medical microbiology". http://www.ncbi.nlm.nih.gov/books/NBK8407./

3- New Zealand Food Safety Authority. Microbial pathogen data sheets: *Vibrio cholerae.* 2001 [Available from: http://www.foodsafety.govt.nz/elibrary/industry/Vibrio_CholeraeScience_Research.pdf Accessed on May 3, 2011]. 3.

4- World Health Organization. Guidelines for the production and control of inactivated oral cholera vaccines. Geneva: World Health Organization, Annex (WHO Technical Report Serial, No. 924); 2004 [Available from: http://www.who.int/biologicals/publications/trs/areas/vaccines/cholera/12 9-149.pdf Accessed on May 4, 2011].

5- "Cholera - *Vibrio cholerae* infection Information for Public Health & Medical Professionals". cdc.gov. January 6, 2015. Retrieved 17 March 2015.

6- "Cholera vaccines: WHO position paper." (PDF). Weekly epidemiological record 13 (85): 117–128. Mar 26, 2010.

7- Harris, JB. La Rocque, RC. Qadri, F. Ryan, ET. Calderwood, SB (2012). Cholera. *Lancet*; 379 (9835): 2466–76.

8-Diane Bailey (2011). Cholera (1st ed.). New York: Rosen Pub. p. 7.ISBN 9781435894372.

9- Takeyoshi Nakayama (1959). Studies on acetic acid-bacteria I. Biochemical studies on ethanol oxidation. Vol 46, Issue 9, pp 1217-1225.

10-Julia F. Morton (1987). "Lemon in Fruits of Warm Climates". Purdue University. pp. 160–168.

11-Bauer , AW; Kibry, WM; Sherris, JC; Turck ,M. (1966) Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol. 45,493-496.

12-Perez C, Anesini C. (1994). Ethnopharmacol J. *In vitro* antibacterial activity of argentine folk medicinal plants against *Salmonella typhi.* 44, 41-46.

13- Weber, JT; Mintz, ED;Cañizares, R; Semiglia, A; Gomez, I; *et al*. Epidemic cholera in Ecuador: multidrug-resistance and transmission by water and seafood. Epidemiol Infect. 1994;112(1):1-11.

14- Towner, KJ; Pearson, NJ; Mhalu, FS; O'Grady, F. Resistance to antimicrobial agents of *Vibrio cholerae* E1 Tor strains isolated during the fourth cholera epidemic in the United Republic of Tanzania. B World Health Organ. 1980;58(5):747-51.

15- La Jolla Mom 10, Reasons Why You Should Drink Lemon Water in the Morning, January 11, 2011 (page 3)

16-US Food and Drug Administration. Acetic Acid - Use in Foods. Available at: http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/cpg562-100.html. Accessed March 9, 2006.

17- Natera, R; Castro, R; Garcia-Moreno, M; Hernandez, M; Garcia-Barroso, C. Chemometric studies of vinegars from different raw materials and processes of production. J Agric Food Chem. 2003;51:3345–3351.

Access this Article in Online	
Quick Response Code	Website: www.ijarbs.com
	Subject: Antimicrobial Study

How to cite this article:
Khulood M. Alsaraf, Suha T. Abd, Nada S. Hussein, Aseel A. Jabor. (2016). *In vitro* Evaluation the effect of Natural Products against *Vibrio cholerae*. Int. J. Adv. Res. Biol. Sci. 3(3): 271-275.