



## Does Citrus Fruit Juice Have Antibacterial Effect Against Enteric Pathogenic Bacteria?

### KEYWORDS

Citrus fruit juice, enteric pathogens, antimicrobial activity

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**ABSTRACT** The present study was carried out to determine the antimicrobial effect of three different fruit juices against six enteric pathogens named *Vibrio cholerae*, *Salmonella Typhi*, *Salmonella Paratyphi A*, *Shigella flexneri*, *Shigella sonnei*, *Escherichia coli*. The study was done by time kill assay method where the antimicrobial activity of the fruit juices was checked in different time intervals. The use of different concentrations (100%, 75%, 50%, 25%) of citrus juice extracts had an effective antibacterial activity against. Lemon juice was the most effective against the test organisms in both undiluted and diluted concentration. Orange juice and sweet lemon juice showed antimicrobial activity only against *Vibrio cholerae*.

### Introduction

An antibacterial agent is a substance that kills or inhibits the growth of bacteria. Antibacterial substance can be of two types - bactericidal and bacteriostatic. Bacteria are becoming resistant to present day antibiotics. The increase in antibiotic resistance bacteria is largely due to the widespread use of antibiotics in medicine in animal care and agriculture (Bansode et al 2012). Bacteria have the genetic ability to transfer and acquire resistance to drugs, which are utilized as therapeutic agents (Khushwaha et al 2012). Therefore alternatives to these chemical antibiotics have become necessary. The side effect associated with the available antibiotics is alarming too.

Antibacterial properties of plant extract have been a hot topic for the researchers. Besides plants, fruits also have been studied by the researchers for the presence of bioactive compounds close related to herbs, commonly referred as phytochemicals such as tannins, carotenoids, polyphenols and anthocyanins (Khushwaha et al 2012). The fruit juices exhibit significant antibacterial effect, the bioactivity being associated with mineral content and biologically active constituents. The term citrus fruit include different types of fruits and products. Although oranges are the major fruit in the citrus fruit group, the group also includes small citrus fruits lemon and lime and grape (Bansode et al 2012).

*Escherichia coli*, *Salmonella Paratyphi B.*, and *Shigella sonnei* are of major cause of intestinal infections (Bansode et al 2012). The previous workers have been demonstrated the antibacterial activity of citrus fruit juices against *S. sonnei* and *E.coli*. (Bansode et al 2012). In another study it was shown that lime lemon and sudachi juice have strong inhibitory activity against *Vibrio cholerae* (Tomotake et al 2006). The current research focuses on the extraction and assay of antibacterial component from citrus fruit which are easily available at very low cost. However reports on antibacterial effect of citrus fruit juices against *Salmonella Typhi*, *Salmonella Paratyphi A.*, *Shigella flexneri* is lacking. Therefore our study focuses on antibacterial activity and future prophylactic antibacterial effect of citrus fruit juice against enteric pathogen.

### Materials and methods

#### Citrus fruits

Fresh lemon, orange and sweet lemon were obtained from market (Mangalore, India).

#### Taking of extract from the fruits:

Surface of the fruit was disinfected using surgical spirit. Fruit was pierced with sterile 12 gauge needle and juice was aspirated and collected in sterile McCartney bottle.

#### Sterility check:

The juice (0.5 ml) was inoculated to 5ml Mueller- Hinton broth and it was incubated at 37°C for 24 hours to check sterility.

#### Microorganisms used

*E.coli*, *S. Typhi*, *S.ParatyphiA*, *S.flexneri*, *S. sonnei*, *V. cholerae* were the isolates from stool samples of patients.

#### Preparation of inoculum:

Bacteria were grown on MacConkey agar at 37°C for 24 hours. 2 to 3 identical colonies were inoculated into 5ml sterile Mueller Hinton broth and it was incubated at 37°C for 6 hours. This inoculum was adjusted with McFarland 0.5 standard (bacterial count  $1.5 \times 10^8$  CFU/ml).

#### Preparation of different concentration of fruit juice:

Different concentrations were made by adding sterile physiological saline into the juice. To prepare 1ml of 25% concentration of fruit juice, 0.25ml juice was added to 0.75 ml of saline, 1ml of 50% concentration 0.5ml juice was added to 0.5 ml of saline, 1ml of 75% concentration 0.75ml of juice was added to 0.25 ml of saline and for 1ml of 100% concentration 1ml of juice was used.

#### Time kill assay

Bacterial inoculums (0.01 ml; bacterial count  $1.5 \times 10^8$  CFU/ml) was added to 1 ml of fruit juice and incubated at 37°C and 0.01 ml was subcultured on MacConkey's agar using calibrated loop at different time intervals such as 0 hour, 1 hour, 2hour, 4hour and 8hour. The culture plates were incubated at 37°C for 24 hours and the colony count was determined.

Percentage reduction of the viable count was determined by comparing with saline control.

Reduction of viable count = Colony count in saline control- colony count in test

The same procedure was followed for three different citrus fruit juice against six test organisms.

**Result and Discussion:**

In the present study anti-bacterial effect of three citrus fruit was studied by time kill assay method against seven strains of six different enteric pathogens. This study showed that lemon juice (Table 1) has great bactericidal activity against enteric pathogens whereas orange juice and sweet lemon juice is not as effective as lemon juice. A recent study in 2012 showed that fresh lemon juice is most effective against *S. Paratyphi B*, followed by *S. sonnei* and *E.coli* and fresh orange has bactericidal effect against *S. sonnei* the most followed by *S. Paratyphi B* and *E.coli* (Bansodeet al 2012) but in our study there was no bactericidal effect of orange juice (Table 2) and sweet lemon juice (Table 3) against the test organisms only except *V.cholerae*. Previous study from Tokyo in 2006 reported that lemon, lime and sudachi juices were effective in inhibiting the growth of the *Vibrio* strains (Tomotake et al 2006), the similar result is found from our study which states that lemon juice (Table 1) has excellent bactericidal effect on *V.cholerae*. Another study from Spain in 2011 reported that the antimicrobial formulations for acne based on orange and sweet basil essential oils was effective in improvement of the acne condition, which ranged between 43% and 75% clearance of lesions (Matiz et al 2011) whereas in our present study orange juice (Table 2) was not effective against the enteric pathogens. In our study we found that citrus fruit juice (Table 1) has least effect on *S. flexneri* whereas a previous study from Nigeria reported that the water based extracts with the lime juice were more effective against *S. flexneri* compared to lime oil and dried fruit powder (Aibinu et al 2006). In our present study the undiluted juice was most effective against the test organisms. This study has shown that fresh lemon juice contains potential antibiotic principles that are inhibitory to clinical isolates. It may be considered as a natural antimicrobial agent for therapeutic purposes.

**Table 1: Time kill assay result with lemon juice**

BACTERIA	100% Conc.	75% Conc.	50% Conc.	25% Conc.
<i>E. coli</i>	1 hour	1 hour	2 hour	2 hour
<i>V. cholerae</i>	0 hour	0 hour	0 hour	0 hour
<i>S. flexneri</i>	2 hour	4 hour	4 hour	4 hour
<i>S. sonnei</i>	1 hour	1 hour	2 hour	4 hour
<i>S. Typhi</i>	1 hour	1 hour	1 hour	1 hour
<i>S. Paratyphi A</i>	0 hour	0 hour	0 hour	0 hour

**Table 2: Time kill assay result with orange juice**

BACTERIA	100% Conc.	75% Conc.	50% Conc.	25% Conc.
<i>E. coli</i>	No inhibition*	No inhibition*	No inhibition*	No inhibition*
<i>V. cholerae</i>	0 hour	2 hour	4 hour	No inhibition*
<i>S. flexneri</i>	No inhibition*	No inhibition*	No inhibition*	No inhibition*
<i>S. sonnei</i>	No inhibition*	No inhibition*	No inhibition*	No inhibition*
<i>Salmonella Typhi</i>	No inhibition*	No inhibition*	No inhibition*	No inhibition*
<i>S. Paratyphi A</i>	No inhibition*	No inhibition*	No inhibition*	No inhibition*

\*No inhibition after 8 hours of exposure

**Table 3: Time kill assay result with sweet lemon juice**

BACTERIA	100% Conc	75% Conc	50% conc	25% conc
<i>E.coli</i>	No inhibition*	No inhibition*	No inhibition*	No inhibition*
<i>V.cholerae</i>	0 hour	1 hour	1 hour	2 hour
<i>S. flexneri</i>	No inhibition*	No inhibition*	No inhibition*	No inhibition*
<i>S. sonnei</i>	No inhibition*	No inhibition*	No inhibition*	No inhibition*
<i>S.Typhi</i>	No inhibition*	No inhibition*	No inhibition*	No inhibition*
<i>S. Paratyphi A</i>	No inhibition*	No inhibition*	No inhibition*	No inhibition*

\*No inhibition after 8 hours of exposure

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