



# Antimicrobial Activity of Some Common Fruits in Bangladesh against Six Different Pathogenic Bacteria

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## Abstract

This study was conducted aiming at determining the antimicrobial activity of some citrus and non-citrus fruits juices against some pathogenic bacterial species. The antimicrobial activity of these fruit extracts were examined against six bacterial strains including *Staphylococcus aureus*, *E. coli*, enter pathogenic *E. coli* (EPEC), Enter toxigenic *E. coli* (ETEC), *Vibrio parahaemolyticus*, *Salmonella* species using agar well diffusion method. The MIC values of the juices against these bacterial species were determined by measuring the OD via spectrophotometer as well. The lime (*Citrus aurantiifolia*) fruit juice at 100% concentration showed the highest effect against test organism EPEC, ETEC, *E. coli*, *V. parahaemolyticus*, *S. aureus*, *Salmonella* with maximum zone of inhibition (mm) 18.333±0.577, 17.667±0.577, 18.00±1.00, 20.33±0.577, 21.33±1.155, 14.00±1.00 respectively. At the same concentration the Kaffir lime (*Citrus hystrix*) had a higher antimicrobial activity on EPEC, ETEC, *E. coli*, *V. parahaemolyticus*, *S. aureus*, with 23.00±1.00, 16.667±0.577, 16.667±1.528, 19.33±1.154, and 19.67±0.577 zone of inhibition (mm) respectively. Pomelo (*Citrus maxima*) showed antimicrobial activity against EPEC only. Pineapple (*Ananascomosus*) had a higher antimicrobial activity on EPEC, ETEC, *E. coli* with zone of inhibition (mm) 18.00±1.00, 11.33±1.527 and 16.33±0.577 respectively. The *Salmonella* spp. was less sensitive to all fruit juices. 75% concentration of lime juice showed as MIC against *E. coli*, EPEC, *V. parahaemolyticus*, *S. aureus* and *Salmonella* spp. and 50% against ETEC. Same concentration of kaffir lime juice observed as MIC against EPEC and *V. parahaemolyticus*, 50% concentration against *E. coli* and *S. aureus*, and 25% concentration against ETEC. Pineapple had MIC at 75%, 50%, 25% concentration against *E. coli*, EPEC and ETEC respectively. Among four fruit juices lime showed most potential antimicrobial activity even than penicillin and erythromycin against *E. coli* and EPEC respectively. The present study suggested that, the citrus fruits and pineapple fruit juices have great potential as antimicrobial agents against different pathogenic bacteria.

**Keywords:** Antimicrobial activity; Antimicrobial agents; *E. coli*; MIC; Pathogenic bacteria and Spectrophotometer

## Introduction

In late time, the interest for the restorative plants is expanding in both creating and created nations because of developing acknowledgment of common items as being non-poisonous, without reactions, effortlessly accessible, and of reasonable costs. Conventional medication, being a huge component in the social patrimony, still remains the fundamental plan of action for a vast dominant part of individuals for treating medical issues like cancer and so on [1]. Roughly 80% of the populace utilizes it as a

cure. To manage the advancement of wellbeing and treating illnesses generally use of bioactive organic build-ups has profound roots in man's history and is all-encompassing which is increasing more prominent consideration and prominence in numerous locales of the world [2, 3]. Plants and plant items are utilized in treating jungle fever, looseness of the bowels, consumption, gonorrhoea, stomach issue, and different irresistible sicknesses. Endeavours of researchers in building up biogenic fixings with promising antimicrobial movement are yielding productive outcomes step by step [4]. Regular antimicrobials

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contain dynamic mixes of bioactive mixes to battle against the obstruction of microbes and save probiotic species in this way, they are alluring choices in numerous malady models [5,6]. The citrus and non-citrus natural products are such a potential specialist where non-citrus organic products incorporate banana (*Musa sharpen*), pomegranate (*Punicagranatum*), sapodilla (*Manilkarazapota*), apple (*Malusdomestica*), pineapple (*Ananascomosus*), cucumber (*Cucumissativus*), guava (*Psidiumguajava*) and so forth and the citrus organic products incorporate lime (*Citrus aurantiifolia*), pomelo (*Citrus maxima*), kaffir lime (*Citrus hystrix*) et cetera. Several health benefits of citrus fruits have been described in part to Vitamin C, which is the most common vitamin found in fruits and vegetables [7]. A plethora of Citrus species are having potential antimicrobial activity followed by different secondary metabolites or phytochemicals [8]. These phytochemicals are believed to be responsible for wide range of protective health benefits including anti oxidative, anti-infl ammatory, antitumor, and antimicrobial activities [9, 10]. Pineapples are the most recent and weirdest weapon to develop in the worldwide fight against superbugs which are anticipated to slaughter up to 10 million individuals per year by 2050. Moreover, potential enzyme named Bromelain, obtained from pineapple, is a strong anti-enter pathogenic agent that showed most efficacies against several pathogenic bacteria. Diminishing effectiveness and opposition of pathogens to antimicrobial medications made the inquiry of another antimicrobial operator as a vital system for the foundation of elective treatments is troublesome dealing with contaminations. *E.coli*, Enteropathogenic *E. coli*, Enterotoxigenic *E. coli*, *S. aureus*, *V. parahaemolyticus*, and *Salmonella* spp. are the real reasons for intestinal diseases. Pineapples, Lime, Kaffir lime, and Pomelo are heavenly tropical natural products that have been praised for quite a long time for their particular and exceptional taste, as well as for their marvelous medical advantages. Because of the report of expanding advancements of medication obstruction in human pathogen and in addition unfortunate symptoms of certain antimicrobial specialists, it is important to scan for new operators which are better, less expensive, and without reaction for treating irresistible sicknesses particularly in creating healthy nations. On the other hand as the wellbeing parts of substance or manufactured sustenance added substances are being scrutinized, the interest for normally happening additives has been expanded around the world [11]. The investigation of novel antimicrobial specialists from regular assets comprehensive of plant or plant-based items (flavor or herbs) and others has been utilized chiefly to cure disease, nourishment security, and sustenance preservation reason since numerous years back [12,13]. Overall aimless employments of anti-infection agents to control pathogenic microscopic organisms have prompted the spread of the safe strains of microorganisms

making treatment of bacterial diseases by these medications troublesome. In this way, researchers are looking for safe elective treatments against these hazardous and even perilous pathogens. Plants create numerous phytochemicals having antimicrobial properties. These might be utilized as normal medications which are for the most part non-toxic and more affordable than the manufactured medications. In this investigation, four distinctive antimicrobial characteristics contain assets, for example, non-citrus natural products incorporate pineapple (*Ananascomosus*), and the citrus organic product incorporates lime (*Citrus aurantiifolia*), pomelo (*Citrus maxima*), kaffir lime (*Citrus hystrix*) were broken down for antimicrobial adequacy. Finally, in this endeavor, we tried to discover the antimicrobial properties of the juices of some basic eatable Citrus and non-citrus organic products against six pathogenic microbes.

## Material and Methods

The study was designed with the attention to antimicrobial activity of some citrus & non citrus fruit juices against on different pathogenic microbes.

## Sample Collection and Processing

Samples were collected from different districts of Bangladesh such as Jashore, Tangail (Modhupur Bazar), Mymensingh and Gazipur. Used distilled water for sample cleaning.

## Fresh Fruit Juice Preparation

Fresh fruit were purchased from market of Jashore, Tangail, Mymensingh and Gazipur. The outer hard portion of these fruits was removed by using autoclaved knife and squeezing by autoclaved percolator and fresh juice was collected by using a sterile sample bottle following filtration.

## Bacterial Strains and Cultural Conditions

Six strains of pathogenic bacterial cultures were used in this study. These are among one is Gram-positive *S. aureus* and other is Gram-negative *E. coli*, Enteropathogenic *E. coli* (EPEC) , Enterotoxigenic *E. coli* (ETEC), *V. parahaemolyticus*, and *Salmonella* spp. The pure pathogenic bacterial strains were collected from Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh and Department of Microbiology, Jashore University of Science and Technology, Jashore. The cultures were maintained on apendrop tube at 4°C and subcultures for 24 hours before use.

## Determination of Antimicrobial Activities of Fruit Juices

Cultures of six pathogenic bacteria namely- Gram-positive *S. aureus* and other is Gram-negative *E. coli*, Enteropathogenic *E. Coli* (EPEC), Enterotoxigenic *E. Coli*

(ETEC), *V. parahaemolyticus*, *Salmonella* spp. were obtained from the institutional microbiology laboratory and were stored in a refrigerator. Antimicrobial properties of fresh fruit juices were evaluated by agar well diffusion method. Briefly, petriplates containing nutrient medium composed of peptone, beef extract, sodium chloride, agar and distilled water were prepared for the evaluation of antimicrobial activities of fruit juices. 100µl of inoculum of each bacterium under our study was uniformly spreaded on the nutrient agar lawn in separate petriplates with the help of a sterilized glass spreader and the plates were then incubated for 30 minutes in the room temperature inside a laminar hood. Following this, with the help of a sterile cork, wells having the diameter of 6mm were bored in the plates. 50 µl of fresh crude and diluted fruit juice samples, distilled water and antibiotic disc erythromycin & penicillin were poured separately into the wells. Distilled water and antibiotic acted as negative and positive controls respectively. After 10 minutes of incubation at room temperature all the plates were transferred to an incubator and kept inside it at 37°C for 24 hours. Following the incubation, plates were checked for the presence of zones of inhibition (ZOI). Diameters of the observed ZOIs were measured in mm and the results were recorded by taking photographs of the ZOIs on the plates.

### Determination of the Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that inhibits or kills the visible growth of microorganisms. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against microorganisms. Four different concentrations i.e. 100%, 75%, 50%, 25% of these juices were prepared by diluting with normal saline. The nutrient broth was prepared. From this broth 9ml broth was added in each of four test tubes labelled as 100%, 75%, 50%, 25% fruit juices concentrations. Six sets of four test tube containing 9ml nutrient broth were prepared for six test microorganisms. Then 1ml of each concentration of fruit juices was added into the respective test tube. After this step 0.1ml test pathogen suspension were inoculated into respective labelled test tube. After inoculation, the test tubes were kept in a shaker incubator for overnight at 37°C and results were observed in the form of turbidity and O.D. were observed at 600nm on U.V. Spectrophotometer. However, as some shortcomings attached to the use of disc, agar well diffusion methods, the use of spectrophotometer to detect the Minimum Inhibitory Concentration (MIC) has some advantages over the others.

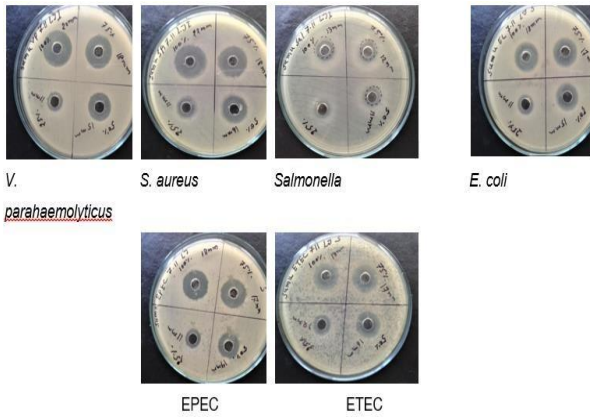
### Statistical Analysis

The yields of extracts were calculated in percentage and the mean and standard deviation of the three replication of antibacterial susceptibility assay was calculated using statistical analysis software Statistix 10 (USA), version 10.

### Results

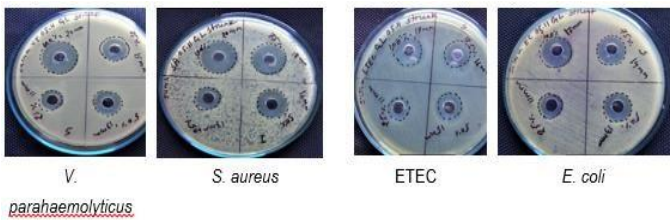
Fruit juices are valuable and readily available resources for primary health care and complementary health care system, undoubtedly fruit juices containing substances of medicinal value that have yet to be discovered, though large number of fruit juices are constantly being screened for their antimicrobial effect, these fruits may prove to be a rich source of compounds with possible antimicrobial activities. In the present study agar well diffusion method was used here in order to get antimicrobial activity of fresh citrus and non-citrus fruit juice against six bacterial strains among one is Gram-positive *S. aureus* and others are Gram-negative *E. coli*, Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), *V. parahaemolyticus*, *Salmonella* spp. Also MIC measurement was done to know the minimum inhibitory concentration of fruit juices by OD measurement using UV. Spectrophotometer. The non-citrus fruit include banana (*Musa acuminata*), pomegranate (*Punicagranatum*), sapodilla (*Manilkarazapota*), apple (*Malusdomestica*), pineapple (*Ananascomosus*), cucumber (*Cucumissativus*), and guava (*Psidiumguajava*) whereas, only pineapple can show antimicrobial activity. The citrus fruit include lime (*Citrus aurantiifolia*), pomelo (*Citrus maxima*), kaffir lime (*Citrus hystrix*) whereas, all showed antimicrobial activity. Results of our study showed that juices of four fruits exhibited inhibitory actions against the Gram-positive *S. aureus* and other is Gram-negative *E. coli*, Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), *V. parahaemolyticus*, *Salmonella* spp. Table no.1-4 represented the antimicrobial activity of various concentrations (100%, 75%, 50%, and 25 %.) of fresh Lime, Kaffir lime, Pineapple and Pomelo fruit juices respectively. The minimum inhibitory concentration (MIC) of various concentration of Lime, Kaffir lime, Pineapple and Pomelo juices based on OD measurement (mean±sd) represented in Table 5-8. The zones of inhibition against various selected pathogens were measured in mm (mean±sd). Zone of inhibition were seen against all species confirming the antimicrobial activity of fresh Lime, Kaffir lime, Pineapple and Pomelo juices (Figures 1-4). The fresh crude lime fruit juice produced the highest antimicrobial activity against *S. aureus* (21.33±1.155mm) followed by *V. parahaemolyticus* (20.33±0.577mm), *E. coli* (18.00±1.00mm), EPEC (18.333±0.577 mm), ETEC (17.667±0.577mm), & *Salmonella* spp. (14.00±1.00 mm) (Table 1). The fresh crude kaffir lime fruit juice produced the highest antimicrobial activity against EPEC (23.00±1.00mm) followed by *V. parahaemolyticus*

(19.33±1.154mm), ETEC (16.667±0.577mm), *S. aureus* (19.67±0.577mm) & *E.coli* (16.667±1.528 mm) (Table 2).



**Figure 1: Growth inhibition of some pathogenic bacterial strains caused by lime juice.**

(The antimicrobial activity of lime juices on selected bacterial pathogen such as *S. aureus*, *E. coli*, Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), *V. parahaemolyticus*, *Salmonella*.)



**Figure 2: Growth inhibition of some pathogenic bacterial strains caused by Kaffir lime juice.**

(The antimicrobial activity of kaffir lime juices on selected bacterial pathogen such as *S. aureus*, *E. coli*, Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), and *V. parahaemolyticus*.)



**Figure 3: Growth inhibition of some pathogenic bacterial strains caused by pineapple juice.**

(The antimicrobial analysis of pineapple juices on selected bacterial pathogen such as *E. coli*, Enteropathogenic *E. coli* (EPEC) and Enterotoxigenic *E. coli* (ETEC).)



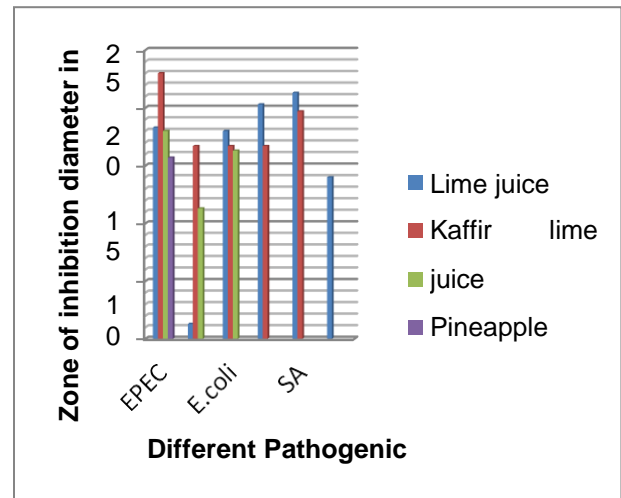
**Figure 4: Growth inhibition of pathogenic bacterial strains caused by Pomelo juice.**

(The antimicrobial activity of pomelo juices on selected bacterial pathogen such as Enteropathogenic *E. coli* (EPEC).)



Antimicrobial activity of lime juice and standard antibiotic (Penicillin) against *E. coli*. Antimicrobial activity of lime juice and standard antibiotic (Erythromycin) against EPEC.

**Figure 5: Growth inhibition of pathogenic bacterial strains (*E. coli* and EPEC) caused by lime juice and standard antibiotic (Penicillin and Erythromycin). (The lime juice shows maximum antimicrobial activity than antibiotic, here the negative control is distilled water that is not showed antimicrobial activity.)**



**Figure 6: Optimum microbial activity (Zone of inhibition) of four fruit juices against different pathogenic bacteria.**

The fresh crude pineapple fruit juice produced the highest antimicrobial activity against EPEC (18.00±1.00 mm) followed by *E. coli* (16.33±0.577 mm), & ETEC (11.33±1.527 mm) (Table 3). The fresh crude pomelo fruit juice produced the highest antimicrobial activity against EPEC (15.667±0.577mm) (Table-4). Table 1-4 show the diameters (in mm) of zones of inhibitions

(ZOI) created in the agar plate by crude (100%) and diluted (75%), (50%), (25%) juices of three Citrus and one non Citrus fruits respectively. It appeared from our study that one Citrus fruit namely –lime exhibited inhibitory actions against all the bacteria tested in our study. Pineapple also exhibited inhibitory action against as many as three bacterial species except for *S. aureus*, *V. parahaemolyticus*, *Salmonella* spp. Kaffir lime, another Citrus fruit exhibited its inhibitory activities against as many as five bacterial species except against *Salmonella*. The fresh citrus fruit juices & pineapple juices were subjected to get the MIC against selected pathogens.

*parahaemolyticus*, *Salmonella* spp. Kaffir lime, another Citrus fruit exhibited its inhibitory activities against as many as five bacterial species except against *Salmonella*. The fresh citrus fruit juices & pineapple juices were subjected to get the MIC against selected pathogens.

**Table 1: Antimicrobial Activity of Lime Juice on Bacterial Pathogens.**

Conc. Of LJ (%)	Antimicrobial Activity of Lime juice on Bacterial Pathogens. Zone of inhibition diameter in mm (Mean±SD). Data are means of three replicates (n = 3) ± standard deviation					
	Organism					
	<i>E. coli</i>	EPEC	ETEC	VP	SA	<i>Salmonella</i> spp.
100	18.00±1.00	18.333±0.577	17.667±0.577	20.33±0.577	21.33±1.155	14.00±1.00
75	16.667±0.577	16.333±0.577	16.667±0.577	16.67±1.527	17.33±0.577	10.67±0.577
50	14.333±1.154	13.00±1.00	14.667±1.154	15.00±1.00	15.67±0.577	9.67±0.577
25	10.33±1.154	9.33±1.527	11.33±0.577	10.00±1.00	10.33±1.154	-
*LJ-Lime juice *EPEC- Enteropathogenic <i>E. coli</i> *ETEC- Enterotoxigenic <i>E. coli</i> *SA- <i>Staphylococcus. aureus</i> * VP- <i>V. parahaemolyticus</i>						

**Table 2: Antimicrobial Activity of kaffir lime extract on Bacterial Pathogens.**

Conc. Of KJ (%)	Antimicrobial Activity of Kaffir juice on Bacterial Pathogens. Zone of inhibition diameter in mm (Mean ±SD). Data are means of three replicates (n = 3) ± standard deviation				
	Organism				
	<i>E. coli</i>	EPEC	ETEC	VP	SA
100	16.667±1.528	23.00±1.00	16.667±0.577	19.33±1.154	19.67±0.577
75	13.667±0.577	19.667±0.577	15.667±0.577	18.33±1.154	18.00±1.00
50	12.00±1.00	16.667±1.527	13.667±1.527	14.33±0.577	14.667±1.527
25	9.66±0.577	12.00±1.00	10.33±0.577	12.17±0.577	11.667±1.154
*KJ-Kaffir juice *EPEC- E nteropathogenic <i>E. coli</i> *ETEC- Enterotoxigenic <i>E. coli</i> *SA- <i>Staphylococcus. aureus</i> * VP- <i>V. parahaemolyticus</i>					

**Table 3: Antimicrobial Activity of Pineapple Juice on Bacterial Pathogens.**

Conc. Of PJ (%)	Antimicrobial Activity of pineapple juice on Bacterial Pathogens. Zone of inhibition diameter in mm (Mean±SD). Data are means of three replicates (n = 3) ± standard deviation		
	Organism		
	<i>E. coli</i>	EPEC	ETEC
100	16.33±0.577	18.00±1.00	11.33±1.527
75	10.33±0.577	15.00±1.00	9.33±0.577
50	9.66±1.154	12.33±0.577	-
25	6.00±1.00	10.33±0.577	-
*PJ-Pineapple juice *EPEC- E nteropathogenic <i>E. coli</i> *ETEC- Enterotoxigenic <i>E. coli</i> *			

**Table 4: Antimicrobial Activity of Pomelo Juice on Bacterial Pathogens.**

Conc. Of PJ (%)	Antimicrobial Activity of Pomelo juice on Bacterial Pathogens. Zone of inhibition diameter in mm (Mean ±SD). Data are means of three replicates (n = 3) ± standard deviation
	Organism
	EPEC

100	15.667±0.577
75	9.667±1.527
50	-
25	-

**Table 5:** MIC of various concentration of Lime juice against different pathogenic bacteria.

Conc. of LJ (%)	Minimum inhibitory concentration (MIC) of lime juice against different pathogenic bacteria. Based on OD. Measurement (Mean ±SD). Data are means of three replicates (n = 3) ± standard deviation					
	Organism					
	<i>E. coli</i>	EPEC	ETEC	VP	SA	<i>Salmonella</i> spp.
100	0.1197±0.0162	0.238±0.01661	0.144±0.0142	0.2177±0.0186	0.2137±0.0137	0.3747±0.025
75	0.083±0.0159	0.0447±0.0136	0.077±0.0182	0.181±0.0108	0.169±0.0145	0.2530±0.0135
50	0.114±0.013	0.3463±0.0131	0.0553±0.0138	0.372±0.0150	0.3720±0.0170	0.459±0.0135
25	0.1453±0.0129	0.2737±0.014	0.3473±0.0147	0.4517±0.0155	0.4517±0.0155	0.4617±0.016

\*LJ-Lime juice \*EPEC- Enteropathogenic *E. coli* \*ETEC- Enterotoxigenic *E. coli* \*SA- *S. aureus*\* VP- *V. parahaemolyticus*

**Table 6:** MIC of various concentration of kaffir Lime juice against different pathogenic bacteria.

Conc. Of KJ (%)	Minimum inhibitory concentration (MIC) of Kaffir juice against different pathogenic bacteria. Based on OD. Measurement (Mean ±SD). Data are means of three replicates (n = 3) ± standard deviation				
	Organism				
	<i>E. coli</i>	EPEC	ETEC	VP	SA
100	0.2400±0.0145	0.1733±0.0155	0.2377±0.0166	0.2853±0.011	0.2420±0.0207
75	0.3147±0.0162	0.0957±0.0156	0.153±0.018	0.1570±0.0125	0.1410±0.0167
50	0.064±0.0125	0.2193±0.0196	0.0643±0.014	0.3457±0.0102	0.0760±0.0207
25	0.081±0.0150	0.1590±0.0128	0.0303±0.0182	0.1740±0.0145	0.350±0.0161

\*KJ-Kaffir juice \*EPEC- Enteropathogenic *E. coli* \*ETEC- Enterotoxigenic *E. coli* \*SA- *S. aureus*\* VP- *V. parahaemolyticus*

**Table 7:** MIC of various concentration of Pineapple juice against different pathogenic bacteria.

Conc. Of PJ (%)	Minimum inhibitory concentration (MIC) of Pineapple juice against different pathogenic bacteria. Based on OD. Measurement (Mean ±SD). Data are means of three replicates (n = 3) ± standard deviation		
	Organism		
	<i>E. coli</i>	EPEC	ETEC
100	0.113±0.0127	0.4160±0.0115	0.330±0.0187
75	0.0247±0.0181	0.3157±0.017	0.3467±0.0123
50	0.1167±0.0112	0.4133±0.0156	0.2503±0.0165
25	0.2730±0.0125	0.2453±0.0145	0.3767±0.0153

\*PJ-Pineapple juice \*EPEC- Enteropathogenic *E. coli* \*ETEC- Enterotoxigenic *E. coli* \*SA- *S. aureus*\* VP- *V. parahaemolyticus*

**Table 8:** MIC of various concentration of Pomelo juice against different pathogenic bacteria.

Conc. Of PJ (%)	Minimum inhibitory concentration (MIC) of Pomelo juice against different pathogenic bacteria. Based on OD. Measurement (Mean ±SD). Data are means of three replicates (n = 3) ± standard deviation	
	Organism	
	EPEC	
100	0.311±0.0105	
75	0.2410±0.0105	
50	0.3620±0.0106	
25	0.3763±0.0203	

The MIC observed by OD measurement, whereas 75% concentration of lime juice showed as minimum inhibitory concentration (MIC) against *E. coli* (0.083±0.0159), EPEC (0.0447±0.0136), *V. parahaemolyticus* (0.181±0.0108), *S. aureus* (0.169±0.0145), *Salmonella* spp. (0.2530±0.0135), and 50% concentration of lime juice against ETEC (0.0553±0.0138) respectively (Table 5). The MIC observed at 75% concentration of pomelo juice against EPEC (0.2410±0.0105) (Table 8). The MIC observed at 25% concentration of kaffir lime juice against ETEC (0.0303±0.0182), 50% concentration of kaffir lime juice against *E. coli* (0.064±0.0125), and *S. aureus* (0.0760±0.0207), 75% concentration of kaffir lime juice against EPEC (0.0957±0.0156), and *V. parahaemolyticus* (0.1570±0.0125) (Table 6). The MIC observed at 25% concentration of pineapple juice against EPEC (0.2453±0.0145), 50% concentration of pineapple juice against ETEC (0.2503±0.0165), 75% concentration of pineapple juice against *E. coli* (0.0247±0.0181) (Tables 7,8). From the above observation we can see that, Pineapple juice, showed appreciable inhibitory potential both in crude and in diluted form against *E. coli*, EPEC & ETEC but failed to inhibit other three bacteria such as *V. parahaemolyticus*, *Salmonella* spp. & *S. aureus*. Lime juice, however, showed appreciable inhibitory potential both in crude and in diluted form against *E. coli*, EPEC, ETEC, *V. parahaemolyticus*, *Salmonella* spp. & *S. aureus*. Whereas, Pomelo juice, showed appreciable inhibitory potential both in crude and in diluted form against EPEC only. Kaffir lime juice; however, showed appreciable inhibitory potential both in crude and in diluted form against *E. coli*, EPEC, ETEC, *V. parahaemolyticus* & *S. aureus* but failed to inhibit *Salmonella* spp. (Figures 5-6).

### Discussion

Herbal medicines are a valuable and readily available resources for primary health care and complementary health care system, undoubtedly the plant kingdom still holds many species of plants containing substances of medicinal value that have yet to be discovered, though large number of plants and their fruits are

constantly being screened for their antimicrobial effect, these may prove to be a rich source of compounds with possible antimicrobial activities, but more pharmacological investigations are necessary. Overall, in terms of extent of the increasing ZOI, it appears that Citrus fruits exhibited better inhibitory activities against pathogens than non-Citrus fruits. The non-citrus fruit include banana (*Musa acuminata*), pomegranate (*Punicagranatum*), sapodilla (*Manilkarazapota*), apple (*Malusdomestica*), pineapple (*Ananascomosus*), cucumber (*Cucumissativus*), guava (*Psidiumguajava*) whereas, only pineapple showed antimicrobial activity [14]. The citrus fruit include lime (*Citrus aurantiifolia*), pomelo (*Citrus maxima*), kaffir lime (*Citrus hystrix*) whereas, all showed antimicrobial activity. Enteropathogenic *E. coli* (EPEC) is only organism that shows sensitive activity against four fruit juices such as three citrus fruits including lime, kaffir lime, pomelo & one non citrus fruit juice including pineapple. The fresh crude lime fruit juice produced the highest antimicrobial activity against *S. aureus* (21.33±1.155 mm), followed by *V. parahaemolyticus* (20.33±0.577 mm), *E. coli* (18.00±1.00 mm), EPEC (18.333±0.577mm), ETEC (17.667±0.577mm), & *Salmonella* spp. (14.00±1.00mm) [15]. The fresh crude kaffir lime fruit juice produced the highest antimicrobial activity against EPEC (23.00±1.00mm) followed by *V. parahaemolyticus* (19.33±1.154mm) ETEC (16.667±0.577mm), *S. aureus* (19.67±0.577mm) & *E. coli* (16.667±1.528mm) [16,17]. The fresh crude pineapple fruit juice produced the highest antimicrobial activity against different pathogenic bacteria including EPEC (18.00±1.00mm) followed by *E. coli* (16.33±0.577mm) & ETEC (11.33±1.527mm). The fresh crude pomelo fruit juice produced the highest antimicrobial activity against EPEC (15.667±0.577mm) only. This study supports the use of these fresh fruit juices of Pineapple, Pomelo, Lime, and Kaffir lime not only as dietary supplements but also as the agent to prevent or control the bacterial infections. Investigation of the antimicrobial activity of lime juice has been investigated and lime juice has been found to have high antimicrobial activity [18]. In this study it is observed that the potency of lime fruit is enhanced by the type of solvent used

indicating that there are some active ingredients in lime which have high antimicrobial effect. Lime juice was found very effective against *V. parahaemolyticus*. Similar results have been reported by (WHO, 2002), tested four strains of *V. parahaemolyticus*, *E. coli*, *Salmonella* spp. The result of antimicrobial susceptibility assay and the MIC assays showed promising evidence for the antimicrobial activity of fresh Pineapple, Pomelo, Lime, and Kaffir lime juices against bacterial pathogens. The fresh citrus fruit juices & pineapple juices were subjected to get the MIC against selected pathogens. The MIC observed at 75% concentration of lime juice against *E. coli*, EPEC, *V. parahaemolyticus*, *S. aureus* and *Salmonella* spp. 50% concentration of lime juice against ETEC [19]. The MIC observed at 75% concentration of pomelo juice against EPEC. The MIC observed at 50% concentration of kaffir lime juice against *E. coli*, 75% concentration of kaffir lime juice against EPEC, 25% concentration of kaffir lime juice against ETEC, 75% concentration of kaffir lime juice against *V. parahaemolyticus* and 50% concentration of kaffir lime juice against *S. aureus*. The MIC observed at 75% concentration of concentration of pineapple juice against ETEC. The antimicrobial activity of lime juice and standard antibiotic (Erythromycin) against EPEC whereas, the lime juice showed maximum antimicrobial activity than antibiotic, here the negative control was distilled water, which showed no antimicrobial activity. Hereafter, same findings found whereas, lime juice showed most potential antimicrobial activity even than penicillin against *E. coli*. Therefore, these fruit juices could be seen as a good source for useful alternative medicine.

## Conclusion

The present study suggested that, the citrus fruits and pineapple fruit juices have great potential as antimicrobial agents against selected pathogenic bacteria and it also indicate that by intake these type of common fruits, mass people of developing country like Bangladesh might protect and cure them from different kinds of pathogen raised diseases.

## Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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