

# Evaluation of the Antimicrobial Effect of *Raphanus Sativus*, *Beta Vulgaris* and *Allium Cepa* on Pathogenic Bacteria Isolated from Street-Side Cut Fruits

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## ABSTRACT

Street-side cut fruits are consumed more now-a-days due to the busy lives of people. Due to this people tend to have more ready-made, apparently healthy food. Street-side vended fruits and fruit salads consist of peeled, sliced, shredded, trimmed and washed fruits that are ready to eat without any need to cut, peel or rinse as it has been already done by the vendors. Street vendors are unlicensed or local hawkers who usually have limited knowledge about food hygiene. The present research work was undertaken to isolate potentially pathogenic food borne microorganisms from the street-side cut fruits. *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter sp*, *Klebsiella sp*, *Vibrio sp* and *Salmonella* were isolated from different fruit samples analyzed in this study. These organisms are known to cause food spoilage or diseases in consumers. Antibiotics can be given in case of infection. However, if these bacteria are resistant to those antibiotics, then the treatment would not be successful, which we confirmed to be true from our antibiotic sensitivity tests. Since most of the isolated organisms showed resistance to antibiotics, vegetable extracts were used as an alternative to antibiotic therapy. The next objective of this study was to evaluate the effectiveness of *Raphanus sativus* (Radish), *Allium cepa* (Onion), *Beta vulgaris* (Beetroot) on the food pathogens isolated from street vended cut fruits. Extracts of these three vegetables were used to check the antibacterial effects on the isolated pathogenic bacteria from street-side cut fruits. It was found that onion and beetroot extracts were more effective on all organisms than radish extract. Hence, instead of depending on antibiotic treatment vegetable extracts can be used for treatment of cut-fruits before consuming the fruits, thus reducing the chances of infection or food poisoning. Also, the fact that these vegetable extracts are safely consumable and their ease of use makes them promising candidates for counteracting common pathogenic microorganisms.

**Keywords:** Street foods, Foodborne pathogens, Antibiotic resistance, Radish extract, Onion extract, Beetroot extract

## INTRODUCTION

Fruits are highly nutritious and widely recommended for their health promoting properties. They are included in dietary guidelines as they have high concentrations of vitamins, mostly vitamin A and C,

minerals, electrolytes and phytochemicals especially antioxidants. (1-2) Fresh cut fruits are commonly sold in markets, which include mango, jackfruit, papaya, and guava, melons like muskmelon, watermelon and pineapple. (1,3-4) They are sold in streets, malls and open

stalls. These days there are many online grocery shops which delivery the cut fruits to home. Due to busy life of people living in cities, ready to eat foods like street vended fruits and chats - are preferred as an alternative to homemade food. The involvement of unsafe food handling by food handlers due to lack of experience and training can cause several food-borne diseases. (5, 6-8) To determine whether such roadside cut fruits contain potentially disease-causing and food spoilage causing bacteria, nine different fruit samples (three each of watermelon, papaya and guava) were obtained from street-side vendors from different areas in Bangalore, India. Following biochemical tests on the organisms from watermelon and papaya samples which were obtained from street side cut fruits, six different pathogenic organisms were identified: *Staphylococcus aureus*, *Enterobacter spp*, *Escherichia coli*, *Vibrio spp*, *Klebsiella spp* and *Salmonella*.

To know whether all the organisms are sensitive to antibiotics or are there any resistant bacteria which are resistant to any of the antibiotics used, antibiotic sensitivity test was done. There are a number of antibiotics available today and the research to find novel antibiotics is on-going. The antibiotics used are Ampicillin (9), Ampicillin-cloxacillin (10), Amoxicillin (11-12), Kanamycin (9-10), Methicillin (13-14), Penicillin-G (13) and Tetracycline. (14) Our study shows that all bacteria analyzed here are resistant to one or more antibiotics, which led us to look for alternatives like vegetable juice with antimicrobial properties to inhibit the growth of pathogenic organisms. Due to increase in lot of discoveries have been made from few years about the plant based antimicrobials but not many researches are done on their usage. Medicinal plants have few pharmacological properties which are attributed to the presence of important chemical constituents which are responsible for few important physiological functions in living organisms. (15)

Radish, *Raphanus sativus L.* has long been grown as a food crop has high medicinal

value. *R. sativus* seeds and leaves contain raphanin which possesses antibacterial and antifungal activities. Research reveals that *R. sativus* root juice contains various substances like alkaloids, flavonoids, tannins, glycosides, proteins and sterols exhibits considerable antimicrobial activity (15 -16) against *Klebsiella pneumoniae*, *Pseudomonas pyocyaneus*, *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus*, *streptococci*, *Pneumococci*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli*(8). Onion, *Allium cepa*. *L* was traditionally used by folk healers to prevent infections and disorders. Onions or onion extracts are said to act against diseases like cough, sore throat, common cold. They have high protein, carbohydrate, potassium, phosphorous, sodium components. Secondary metabolites like alkaloids, Terepenoids, tannins and flavonoids are present in onions which contain antimicrobial properties. Onion extract containing phytochemicals and their derivatives which include Allicin are said to be antitumor, antioxidant, antimicrobial, antiallergic and Antidiabetic properties and also anti-hyperlipidemic, anti-inflammatory, antithrombic activities. (17-19). Aqueous extract of onion is said to be effective against organisms like *Cholerae*, *E. coli*, *Cereus*, *Salmonella*, *Shigella*, *Pseudomonas*, *S.pyogens*. (20,18,21,19) and Yeasts. Beetroot, *Beta vulgaris* contains Beta lain, a natural colorant/ pigment used in food industry. Beta lain is effective against many diseases as it can act as a scavenger of free radicals, ROS species and also effective against oxidative stress. Beetroot is said to possess antimicrobial, anti-inflammatory, hepatoprotective activities (22). Beetroot peel contains L-tryptophan, p-coumari and ferulic acids which are effective against bacteria like *Staphylococcus*, *Streptococcus*, *E.coli*, and *Bacillus*. (23). Extract of *Beta vulgaris* has antibacterial activity against *S. aureus*, *B.cereus*, and few other Gram negative bacteria. (23)

In an attempt to inhibit the growth of pathogenic bacteria, filtered vegetable juices

were analyzed for their antibacterial potential. Juices of vegetables like radish, beetroot and onion were extracted and filtered. It was observed that onion is the most effective among the three vegetable juices analyzed. Our results corroborate previous studies that have shown the antibacterial properties of these three vegetables. (21, 23, 24) We have now shown that these vegetable juices can be used to treat roadside cut fruits so as to counteract potential pathogens.

Thus, this study would aid in furthering our knowledge about microorganisms associated with street vended cut fruits pathogens and effect of vegetable extracts in controlling these organisms.

## **MATERIALS & METHODS**

### **SAMPLE COLLECTION**

Street side cut fruits samples of Watermelon, papaya and guava were collected from different areas of Bangalore (Mallechwaram, Yeshwanthpur and Mathikere) in sterile pouches made up of aluminum foil.

### **ISOLATION AND CHARACTERIZATION**

The samples were inoculated on different differential media like Baird Parker agar, EMB agar, DCA, SS agar and TCBS agar. Sample inoculated plates were incubated at 37°C for 24 hours after which results were noted. Different Colonies from each of the plates were picked and streaked on to agar slants. Isolated organisms were identified and characterized by Gram staining, catalase test, Oxidase test, Mannitol salt agar test (confirmatory test done for *S. aureus*) Lactose fermentation test, Indole test (for lactose positive gram negative rods), Citrate utilization test, MR-VP test, Glucose fermentation test, Motility test by hanging drop method, H<sub>2</sub>S production, Na<sup>+</sup> enrichment test (required for growth) Urease test and Lysine decarboxylase test were performed following standard protocol to confirm the isolated organisms .

### **ANTIBIOTIC SENSITIVITY TEST**

Antibiotic sensitivity test was done by disc diffusion method. Ampicillin (HiMedia-SD002-10mcg/disc), Ampicillin-cloxacillin (HiMedia-SD113-10mcg/disc), Amoxicillin (HiMedia – SD063-30mcg/disc), Kanamycin (HiMedia–SD223–5mcg/disc), Penicillin (HiMedia–SD028–10units/disc), Tetracycline (HiMedia–SD037–30mcg/disc), Methicillin (HiMedia–SD019–5mcg/disc) were tested against isolated organisms like *Staphylococcus aureus*, *Enterobacter spp*, *Escherichia coli*, *Vibrio spp*, *Klebsiella spp* and *Salmonella*. Nutrient agar medium was used for antibiotic test. After incubation, Zone of inhibition in diameter were measured and tabulated.

### **ANTIBACTERIAL ACTIVITY OF VEGETABLE EXTRACTS**

Nutrient agar media was prepared, autoclaved and poured on sterile petriplates to solidify. Antibacterial activity was determined by well-diffusion method. Test organisms were swabbed on the Nutrient agar plates using sterile cotton swabs and wells were made on it. Juices were extracted from Radish, beetroot and onion and filtered using Whatman No. 1 filter paper. Filtered juice of radish, beetroot and onion was pipetted in to the wells and control plates for each organism were kept without the juice. All the plates were incubated for 24-48 hours at 37°C. After incubation plates were checked for zone of inhibition, the diameter of zone of inhibition was measured and tabulated.

## **RESULT AND DISCUSSION**

### **The roadside cut fruits studied were found to be contaminated with potentially disease causing pathogenic bacteria**

To determine whether roadside cut fruits contain potentially disease causing and food spoilage causing bacteria, nine fruit samples (three each of watermelon, papaya and guava) were obtained from street-side vendors of Mallechwaram, Yeshwanthpur and Mathikere in Bangalore, India. These fruits were purchased in previously made

sterile aluminum foil pouches. The organisms were isolated on different selective media: Baird Parker agar, DCA, EMB agar, SS agar and TCBS agar. All the media were prepared, autoclaved and poured on sterilized petri plates in aseptic conditions. Once the media has solidified, the fruit samples bought in sterile aluminum foil pouches was inoculated. Three partitions were made on each petri plate for all the three fruits. All the plates were labeled and incubated at 37°C for 24 hours. After incubation plates were checked for growth and colony morphology was done. It was found that guava did not have any growth and thus it was eliminated from further tests. According to Jasmine Ara Farahana (2) guava contains antimicrobial properties and this could be the reason for it to not to give any microbial growth. Watermelon and papaya gave growth and those plates were considered for further studies. Agar slants

were prepared and the isolated organisms were sub cultured.

### Six different potentially pathogenic bacteria were isolated from two road side cut fruits

The isolation of the bacteria was followed by Gram's staining and biochemical tests like, catalase, oxidase, Mannitol salt agar test, lactose fermentation test, glucose fermentation test, Indole, citrate utilization, sodium salt required for growth, MR-VP, H<sub>2</sub>S production, motility by hanging drop, Urease and lysine decarboxylase test. The various differential and selective media used for the isolation are Baird Parker agar, DCA, EMB agar, SS agar and TCBS agar. Based on these tests, the potentially pathogenic organisms identified were *Staphylococcus aureus*, *Enterobacter sp*, *Escherichia coli*, *Vibrio sp*, *Klebsiella sp* and *Salmonella*. (Table 1)

Isolated bacterium	Source	Gram staining	Oxidase	Catalase	Lactose	Indole	Citrate	MR	VP	H <sub>2</sub> S	Mannitol	Glucose	Motility	Urease	Na <sup>+</sup> for growth	Lysine
<i>Staphylococcus aureus</i>	W,P	+ cocci		+							+					
<i>Enterobacter sp</i>	W,P	- rods			+	-		-	+							-
<i>Escherichia coli</i>	W,P	- rods			+	+	-									
<i>Vibrio sp</i>	W,P	- rods	+									+			+	
<i>Klebsiella sp</i>	W,P	- rods			+	-		+	-							
<i>Salmonella</i>	W,P	- rods	-		-	-				+			+	-		

Table 1. Characterization of isolated bacteria (W-watermelon; P-papaya)

### Bacteria which were isolated from roadside cut fruits were resistant to one or more antibiotics

To know whether all the organisms are sensitive to antibiotics or are there any resistant bacteria which are resistant to any of the antibiotics used, antibiotic sensitivity test was done. The procedure used for this is called antibiotic disc diffusion method. Seven different antibiotics were used here against all the organisms. The antibiotics used are Ampicillin, Ampicillin-cloxacillin, Amoxicillin, Kanamycin, Methicillin, Penicillin-G and Tetracycline. If the

organism is sensitive to a particular antibiotic zone of inhibition can be seen, if the organism is resistant, no zone would be seen. The test plates with zone are separated and the length of the zone of inhibition is measured and tabulated. Based on HiMedia guidelines, whether the organism is sensitive, resistant or intermediate was determined. The organism resistant to a particular antibiotic is represented as R, the one which is sensitive is represented as S and intermediate sensitivity is represented by I (Table 2). *E.coli* (C) was found resistant to all the antibiotics, *Enterobacter* (B) was

found resistant to all except tetracycline, *Staphylococcus aureus* (A) was resistant to Ampicillin, Kanamycin, Methicillin, penicillin-G and tetracycline, *Vibrio* (D) is resistant to Ampicillin, Ampicillin-cloxacillin, Amoxicillin, Methicillin and

Penicillin-G, *Klebsiella* (E) is resistant to Ampicillin, Ampicillin-Cloxacillin, Amoxicillin and Kanamycin and *Salmonella* (F) is resistant to Ampicillin, Ampicillin-Cloxacillin, Methicillin and penicillin-G (Figure 1).

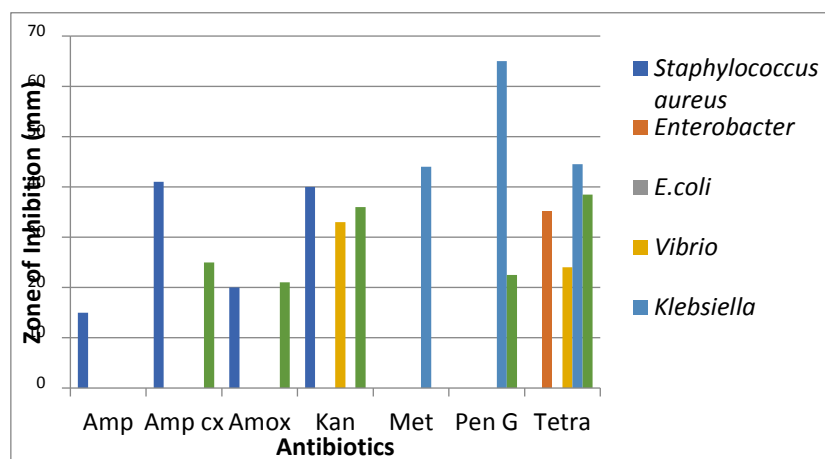


Figure 1. Graph showing antimicrobial sensitivity of the isolated bacteria against the indicated antibiotics (Amp- Ampicillin, Amp cx- Ampicillin-Cloxacillin, Amox- Amoxicillin, Kan- Kanamycin, Met- Methicillin, Pen G- Penicillin, Tetra- Tetracycline)

Thus, antibiotics are not capable of inhibit growth of all bacteria or in other words, all bacteria are resistant to one or more antibiotics, which lead us to use an alternative like vegetable juice with antimicrobial properties to inhibit the growth

of pathogenic organisms. Thus, instead of antibiotics, natural means like vegetable extracts which have antibacterial properties could be used to kill the pathogenic organisms.

Organisms	Ampicillin 10mcg/disc	Ampicillin-Cloxacillin 10mcg/disc	Amoxicillin 30mcg/disc	Kanamycin 5mcg/disc	Methicillin 5mcg/disc	Penicillin 10units/disc	Tetracycline 30mcg/disc
<i>Staphylococcus aureus</i>	R (15mm)	S (41mm)	S (20mm)	S (40mm)	R (0)	R (0)	R (0)
<i>Enterobacter sp</i>	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	S (35mm)
<i>Escherichia coli</i>	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
<i>Vibrio sp</i>	R (0)	R (0)	R (0)	S (35mm)	R (0)	R (0)	S (24mm)
<i>Klebsiella sp</i>	R (0)	R (0)	R (0)	R (0)	I (44mm)	S (65mm)	S (44.5mm)
<i>Salmonella</i>	R (0)	S (25mm)	S (21mm)	S (36mm)	R (0)	R (22.5mm)	S (38.5mm)

Table 2. Antibiotic sensitivity test of isolated bacteria from street-side cut fruits against the indicated antibiotics along with zone of inhibition in diameter (mm).

(R: resistant, S: sensitive, I: intermediate) The inhibition zone size (diameter in mm) interpretation was based on HiMedia instruction sheet (the following values are upper and lower cut-off lines for R and S, respectively): 13 and 17 (for *Enterobacteriaceae*), and 28 and 29 (for others) for Ampicillin; 16 and 22 (for *E.coli*),

and 35 and 37 (for *S. aureus*) for Ampicillin cloxacillin; 19 and 20 for Amoxicillin; 13 and 18 for Kanamycin; 17 and 22 for Methicillin; 26 and 47 (for *Enterobacteriaceae*), and 19 and 28 (for others) for Penicillin; 11 and 15 for Streptomycin; 14 and 19 for Tetracycline (25)

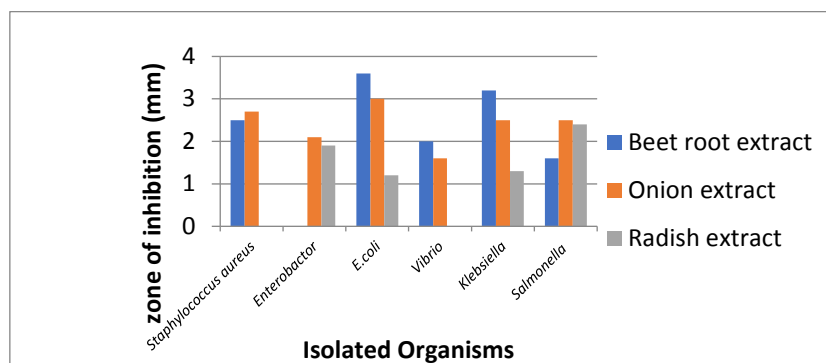


Figure 2. Graph showing antimicrobial activity of Beetroot (*Beta vulgaris*), Onion (*Allium cepa*) and Radish (*Raphanus sativus*) extracts against the indicated bacteria

### Filtered juices of radish, onion and beetroot showed antibacterial activity against the isolated pathogens

In an attempt to inhibit the growth of pathogenic bacteria, filtered vegetable juices were analyzed for their antibacterial potential. Juices of vegetables like radish, beetroot and onion were extracted and filtered. These filtrates were added in to the wells which were previously made on agar plates and swabbed with test organisms. After incubation zone of inhibition was seen which were measured and tabulated. It was observed that onion is the most effective among the three and it showed zones for all the organisms followed by beetroot which inhibited all organisms except *Enterobacter* and finally radish which inhibited all organisms except *S. aureus* and *Vibrio*. The graphs of vegetable extracts and zone of inhibition were plotted for all organisms (Figure 2). These results corroborate previous studies that have shown the antibacterial properties of these three vegetables (1, 5, 10). We have now shown that these vegetable juices can be used to treat roadside cut fruits so as to counteract potential pathogens. Further work will be required to determine the whether and how these vegetable juices can be used to increase the shelf life of such cut fruits. Also, it would be interesting to determine the best ways to treat these fruits so as to minimize the aftertaste of these vegetables. Thus, our study has shown that these vegetable juices could be used as an alternate source to antibiotics<sup>(26)</sup> to inhibit the pathogenic

bacteria present of cut fruits sold at street-side vendors.

### CONCLUSION

In conclusion, we have shown that cut fruits sold on the street-side harbour a wide variety of potentially pathogenic bacteria. These bacteria are generally resistant to multiple antibiotics. As such cut fruits are regularly consumed by many people; the chance of infection is also very high. Hence, instead of depending on antibiotic treatment vegetable extracts can be used for treatment of cut-fruits before consuming the fruits, thus reducing the chances of infection or food poisoning. Also, the fact that these vegetable extracts are safely consumable makes them promising candidates for counteracting common pathogenic microorganisms.

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