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Comparative Study of Natural Antimicrobial Activity on Fruits and Vegetables Against *Pseudomonas monteilii*, *Escherichia coli* ATCC 25922 and *Bacillus subtilis* ATCC 19659

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Abstract

Generally, many of the fruits and vegetables skin we cannot consume but they have also health benefits activity. Some fruits and vegetables are rich in nutrients as flavoid and essential oil that can use for anticancer and antimicrobial activity. Isolation of 3 bacterial strains from soil and water. Perform gram's staining and biochemical test of isolates. The aim of the study was to evaluate comparative natural antimicrobial activity of Vegetables and fruit juices on different isolate bacterial strains B1, B2 and B3. 16s rRNA Sequencing of isolates.

Keywords
Antimicrobial activity

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INTRODUCTION:

Change in time drug resistance bacterial infections had led to the need for new therapies. Finding agents which killed the bacteria but not gave harmful effect to humans is a fierce task. Fruits and vegetables are considered as an important natural good diet. Likewise, they are known to reduce risk of chronic diseases. Fruits and vegetables contain significant amounts of phytoconstituents which are negatively associated with the morbidity and mortality from certain types of cancer like cerebrovascular and cardiovascular [1, 2, 3]. Fruits and vegetables are

cooked in indian food, different components have activities like antioxidant, antimicrobial, anti-inflammatory and antiproliferation etc [4, 5]. Fruits and vegetables have a enormous potential serve as source of newer, better, safe and effective antioxidant and antimicrobial agents [6]. In our current study we have studied comparative natural antimicrobial activities of different fruits and vegetables against isolated bacterial species B1, B2 and B3.

Materials and Methods:

Sample Collection:

Figure 1 Sample collection site

Soil sample were collected from garden of Ganpat university Kherva, [Latitude: 23.0396 and Longitude: 72.566] Gujarat India. Soil Samples were collected from 10cm depth from soil surface in a sterile polythene bag with a sterile spatula. Water sample were collected from well water from Ganpat University Kherva Mehsana Gujarat. Collect samples (min.200 mL) in sterile bottles which are appropriate for isolation of *E. coli*. Sample was stored at 4^{o}C until used.[7]

Enrichment and Isolation of bacteria: Enrichment of bacteria was done using different media such as nutrient broth. For isolation of bacteria, a loop full culture from nutrient broth (Hi-media) as streaked on nutrient agar media by four sector method at 37^{o}C for 24-48hrs in incubator [7,8]. Isolates were preserved in respective medium at 4˚C for further study.

Morphological and Biochemical characterization of Isolates:

Selected isolates were purified by four sector method. Morphologically characterized by Gram's staining, Capsules staining by Hiss method and Colony is characterized by color, form, size, shape etc. and biochemical tests were done for the identification of bacteria.

Extraction method of fruit juices:

Fruits of various plants were collected from one of the well-known cities of Gujarat Mehsana on 17th of July 2019. Most importantly, Juice from the fruits was taken out with the help of electrical equipment, Juicer [9]. The juices were undoubtedly utilized for the test of antimicrobial effect. For check natural antimicrobial activity solution were prepared in sterile distilled water. Different concentration of juices dissolved in distilled water [10]. Agar well diffusion method used for antimicrobial activity. Prepare agar plate and Spread isolated pure culture of B1, B2 and B3 in different petri plates after spreading prepare well in agar plate by using cup borer. 6.5mm diameter of cup borer which is used for preparing well in agar plate. Add sample in well and incubate all the plates at 37^{o}C for 24-48hrs in incubator. Zone of inhibition observed around well after incubation of 24hr. measure the zone by using Scientific zone scale-C in millimeter [11]. The present study describes the antibacterial activity of fruit juices [pomegranate, Apple, Grapes, Orange, Banana, Mango, Guava and Lemon] against isolated bacterial strains B1, B2 and B3. Antimicrobial activity of fruit juices was tested by well diffusion assay by zone of inhibition surrounding the well.[12] Inhibition zone was arranged around the disc was certainly calculated in millimeter.

Extraction method of Vegetables:

Vegetables of various plants were collected from one of the well-known cities of Gujarat Mehsana on 29th of Sep. 2019. The freshly collected samples were brought to the laboratory within two hours and they were washed with sterile water so as to prolong the shelf-life of the fruits and vegetables [12]. Most importantly, Juice from the vegetables was taken out with the help of electrical equipment, Juicer. The juices were undoubtedly utilized for the test of antimicrobial effect. For check natural antimicrobial activity solution was prepared in sterile distilled water. Perform Agar well diffusion method prepares a well by using Cup borer. Incubate all plates at 37˚C for 24-48hrs [13]. Zone of inhibition were measured by using Scientific Zone Scale-C in millimeter. The present study describes the antibacterial activity of fruit juices [pomegranate, Apple, Grapes, Orange, Banana, Mango, Guava and Lemon] on pathogenic bacterial strains. Antimicrobial activity of vegetables juices was tested by well diffusion assay by zone of inhibition surrounding the well.[14]

RESULTS:

Colony Characterization of Isolates:

Isolates	Size	Shape	Margin	Elevation	Surface Texture	Consistency	Opacity	Pigmentation
B1	Medium	Round	Entire	Raised	Smooth	Moist	Transparent	Green
B2	Medium	Round	Entire	Convex	Smooth	Moist	Translucent	Cream
B3	Large	Round	Entire	Flat	Smooth	Moist	Opaque	White

Table 1 Colony characteristic of selected isolates B1, B2 and B3

- **Morphological and Biochemical characterization:**

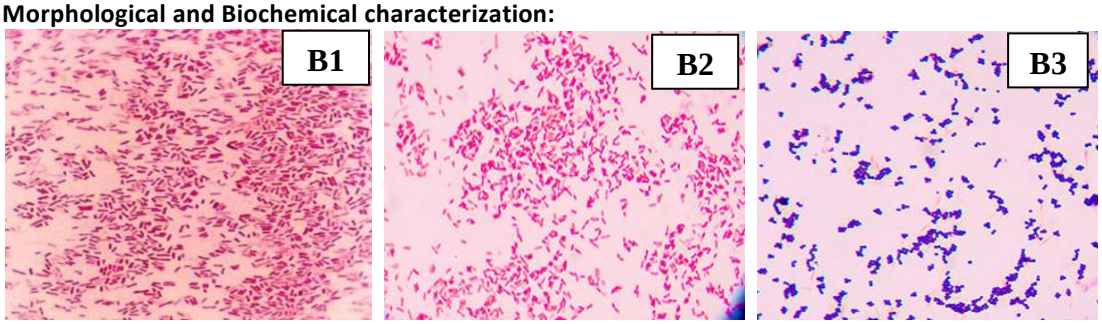

Figure 2 Gram 's staining of selected isolates B1, B2 and B3

Morphological characterization of Isolates:

Isolates	Size	Shape	Arrangement	Gram's reaction	Capsule staining
B1	Short	Rod shape	Single, in chain	Gram's negative	-
B2	Short	Rod shape	Single, in chain	Gram's negative	-
B3	Big	Rod shape	Single, in chain	Gram's positive	+

Table 3 Morphological characteristic of selected isolates B1, B2 and B3

Biochemical characterization of Isolates:

Sr.no	Name of test	B1	B2	B3
1	Methyl red test	-	+	+
2	Voges – Prauskar test	-	-	-
3	Indole production test	-	+	-
4	H_2S gas production test	-	-	+
5	Nitrate Reduction test	+	+	+
6	Urea hydrolysis test	-	-	+
7	Phenyl alanine de amines	-	+	+
8	Starch hydrolysis test	-	-	+
9	Catalase test	+	+	+
	Fermentation test			
	Glucose	-	+	+
	Mannitol	+	+	-
	Fructose	+	+	+
	Maltose	-	+	-
	Sucrose	-	+	+

(+) indicates positive results (–) indicates negative results

Table 4 Biochemical characteristic of selected isolates B1, B2 and B3

Figure 3 Antimicrobial activity of fruits

ANTIMICROBIAL ACTIVITY OF FRUITS

S.r no	Test sample	B1	B2	B3
1.	pomegranate	15mm	19mm	16mm
2.	Apple	14mm	18mm	16mm
3.	Grapes	18mm	20mm	23mm
4.	Orange	19mm	19mm	22mm
5.	Banana	0mm	12mm	0mm
6.	Guava	15mm	16mm	11mm
7.	Lemon	20mm	20mm	23mm
8.	Mango	19mm	16mm	18mm

Table 5 Antimicrobial activity of fruits

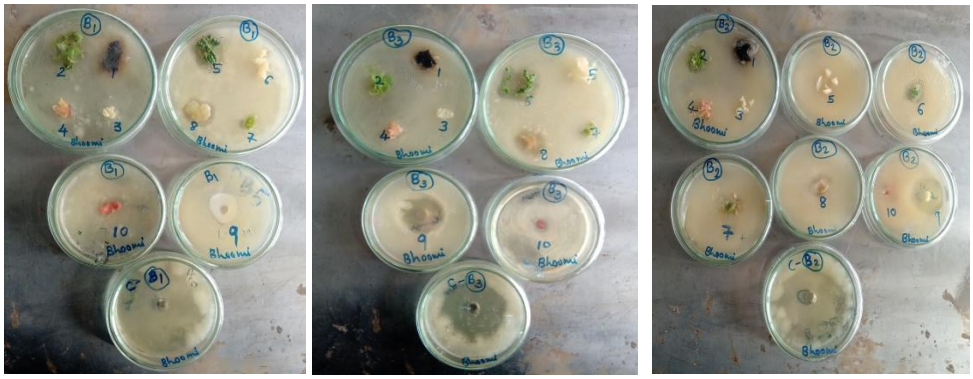

Figure 4 Antimicrobial activity of vegetables

ANTIMICROBIAL ACTIVITY OF VEGETABLES

Sr. no	Test sample	B1	B2	B3
1.	Onion	14mm	13mm	13mm
2.	Cauliflower	0mm	0mm	0mm
3.	Cabbage	0mm	11mm	0mm
4.	Bitter guard	17mm	19mm	16mm
5.	Garlic	20mm	21mm	23mm
6.	Carrot	0mm	0mm	0mm
7.	Chili	0mm	0mm	0mm
8.	Tomato	14mm	15mm	12mm

Table 6 Antimicrobial activity of vegetables

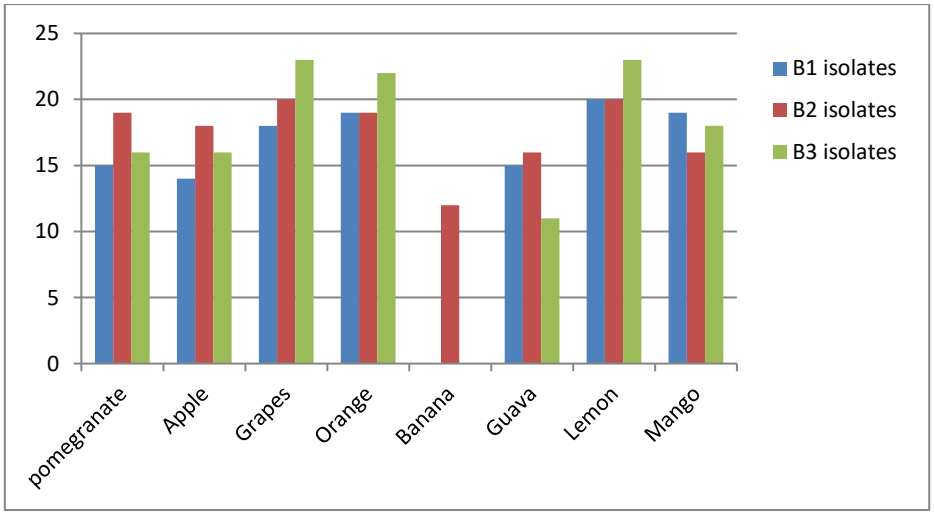

Figure 5 Antimicrobial activities against fruits

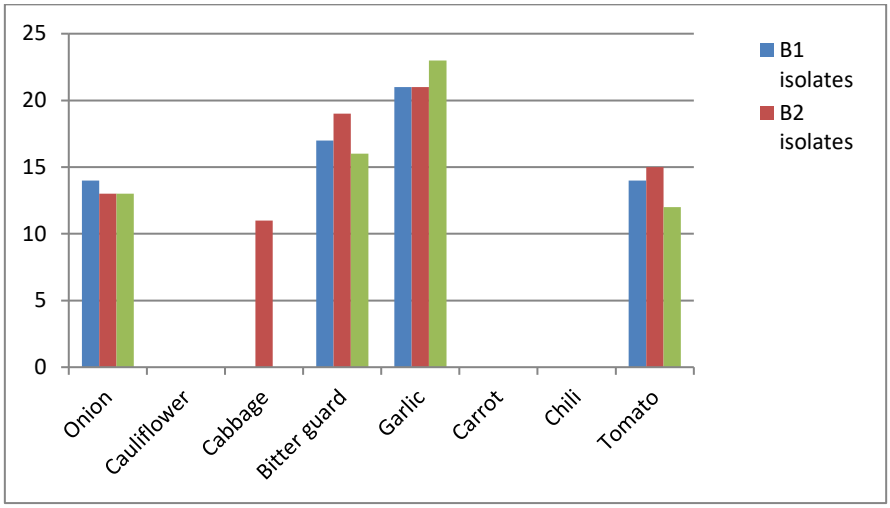

Figure 6 Antimicrobial activities against vegetables

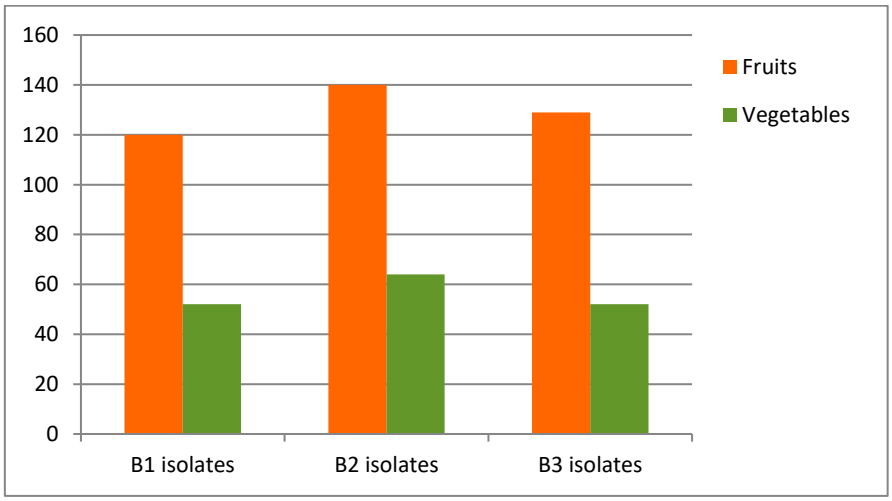

Figure 7 Comparative antimicrobial activity of fruits and vegetables against B1, B2 and B3 isolates

DISCUSSION AND CONCLUSION:

In the present study isolation of bacteria from soil and water samples. Soil sample were collected from garden of Ganpat university Kherva, Gujarat India. Isolation of bacteria on nutrient agar by four sector method incubated all plates at 37^{o}C for 24-48hrs in incubator. Isolate bacteria B1, B2 and B3 used for antimicrobial activity against different fruits and vegetables sample. Morphological identification are shown in (Table-) and biochemical identification shown in (Table-). Agar well diffusion method used for the antimicrobial activity of samples. Antimicrobial activity was measured by zone of inhibition on nutrient agar plate. The well containing different vegetables and fruits sample. Zone of inhibition is measured by scientific zone scale-C in millimeter. Antimicrobial activity of vegetables against B1, B2 and B3. Resulted in garlic and bitter guard gave best antimicrobial activity in which garlic gave 20mm zone against B1, 21 mm zone against B2 and 23mm zone against B3 isolates. Bitter guard gave 17mm zone against B1, 19 mm zone against B2 and 16mm zone against B3 isolates. Onion, tomato and cabbage have low antimicrobial activity against isolates. There is no any antimicrobial activity given by cauliflower, carrot and chili against isolate B1, B2, and B3 respectively. Antimicrobial activity of fruits against B1, B2 and B3. Resulted in lemon, orange, grapes and mango gave best antimicrobial activity against in which lemon gave 20mm zone against B1, 20 mm zone against B2 and 23mm zone against B3 isolates. Orange gave 19mm zone against B1, 19 mm zone against B2 and 22mm zone against B3 isolates. Grapes gave 18mm zone against B1, 20 mm zone against B2 and 23mm zone against B3 isolates. Mango 19mm zone against B1, 16 mm zone against B2 and 18mm zone against B3 isolates. While pomegranate, apple, banana and guava have low antimicrobial activity against B1, B2 and B3. Comparison of antimicrobial activity between

vegetables and fruits, resulted in fruits have high antimicrobial activity comparision to vegetables.

Molecular identification of the isolate B1, B2, B3 were carried out by 16S r-RNA sequencing. It was sent for sequencing PCR amplified 16S r-RNA gene at Chromous Biotech, Bangalore analyzed by Sanger's method and the sequence obtained. 16S rRNA Isolate B1 is Gram-negative, rod-shaped, motile, non-spore-forming bacterial strain, CMST, isolated from soil was characterized using phenotypic and molecular taxonomic methods. 16S rRNA gene sequence analysis revealed that the organism belongs phylogenetically to the genus Pseudomonas. Pseudomonas monteilii, P. plecoglossicida and P. mosselii were the most closely related species, with 16S rRNA gene sequence similarities to the respective type strains of Pseudomonas monteilii 99.79%, P. plecoglossicida 99.73% and P. mosselii 99.59 %. Isolate B2 is 100% similarity with Escherichia coli ATCC 25922 recommended and widely used as a control Gram-negative bacterium for various laboratory experiments, especially for antibiotic susceptibility assays, was found to be antagonistic against another E. coli strain which is uniquely susceptible to fusidic acid, an antibiotic mainly active against Gram-positive bacteria. This antagonistic property appears to be selective, since it was not found against other E. coli or Staphylococcus aureus strains. Isolate B3 is Gram's positive, aerobic, is 100% similar to Bacillus subtilis ATCC 19659 which is used as Testing antimicrobial hand washing formulations and Testing disinfectants.

Authors Contribution statement:

Bhoomi N. Patel, Vaidehi S. Patel, Masi S. Mahammad, Twinkle D. Kalwani, Nisha Khanchandani conceptualize and gathered the data with practical work experience. This work was performed in Microbiology laboratory Mehsana Urban Institute of Sciences, Ganpat University, Kherva, Gujarat.

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