

Diversity of Bacteria isolated from fresh fruit Juices sold by road side vendors of Durg district, Chhattisgarh

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Abstract- Fruits are good source of minerals, vitamins and hold nutritive values all over the world. Millions of people everyday consumes fresh fruit juices sold at market, but they are frequently associated with microorganisms due to improper handling and unhygienic conditions. This causative agent causing spoilage in fruit and fruit juices are microbes. The bacteriological evaluation of fruit juices will help to aware people for unhygienic conditions, safety and quality of juices which may prevent them from various foods borne disease. Fruit juices; 4 types were collected from vendors and cultured on Mannitol salt agar and MacConkey agar plates, relevant morphological and biochemical tests were performed and isolate was confirmed. The microbial count measured and drug resistance tested by the Kirby Muller method. *Escherichia coli*, *Staphylococcus*, *Streptococcus* and *Micrococcus* were confirmed through morphological and biochemical test and antibiotic sensitivity showed by all the isolates. The contamination arises from poor hygiene, contaminated water, dust and poor handling, causing the spoilage of fruit juice resulting in diseases to consumers.

Key Words: Juice, Contamination, Hygiene, Health, Precaution,

I. INTRODUCTION

Fruits are a part of our daily consumptions. The global fruit juice market reached a volume of 45.3 Billion litres in 2019. This, coupled with the refreshing taste and longer shelf-life of fruit juice, makes it one of most widely consumed beverages across the globe. Fruit juices contain antioxidants, vitamins, and minerals that are essential for human being and they play important role in the prevention of heart diseases, cancer, and diabetes. Fruit juices contain essential nutrients which support the growth of acid tolerant bacteria, yeasts, and moulds.

A structural part of a plant is fruit which contains seeds, normally fleshy, sweet and edible in the raw states that include: mangoes, oranges, grapes, litchis, and pomegranates etc. They are ripe ovaries or carpels that contain seed (McGee, 2004). Similar in composition to vegetables, fruits contain various phytochemical

compounds and a high percentage of water averaging 85%, also in small amount fat; protein and carbohydrate (cellulose and starch) are present (Ihekoronye&Ngoddy, 1985). Most fruits are eaten as desserts and they can be processed into liquid product which includes fruit juices, wines and other preserves like; marmalade, jams, jellies etc.

The aqueous liquid, puree of the edible portions is juice, or any concentrates of such liquid or puree from one or more fruits or vegetables can be juice either. Fruit juices are mainly used for their nutritional value, refreshing nature also for their medicinal importance. In detoxification of human body and in improvement of blood lipid profile in patients of hypercholesterolemia fruit and vegetable juices play great roles. Fruit juices are nutritious drinks with great taste and health benefits (Suaads & Hamed, 2008). Fruit juices are important sources of nutrients and contain several important therapeutic 3 properties that may reduce the risk

of various diseases. They contain large amounts of antioxidants, vitamins C and E.

Health benefits of different fruit juices: Juices are mostly consumed for their perceived health benefits. For example, orange juice is rich in vitamin C, folic acid, potassium, which are an excellent source of bio available antioxidant phytochemicals and in people affected with hypercholesterolemia it significantly improves blood lipid profiles. Pomegranate juice reduce dangerous LDL-cholesterol in blood, improve blood flow to the heart in patients with coronary artery disease, reduce thickening of the arteries that supply blood to the brain, lower the level of systolic blood pressure also an antioxidant-rich fruit. This fruit may also be able to help fight cancer which researchers have been looking for. Mango juices are perfect to replenish salts, vitamins and energy after physical exercise. In gall bladder cancer a protective effect of mangoes consumes has been proven. Mango juice also contains a lot of tryptophan, the precursors of serotonin. Litchi juice contains high amount of antioxidants which is effective to prevent early ageing also effective to protect from asthma and a rich source of nutrient that is required for the production of blood. It provides manganese, magnesium, copper, iron and folate that are required for the formation of RBC. Sweet lime juice helps in combating infections, treating ulcer and wounds, improves blood circulation and boost the immune system and fights cancer cell formation. Pineapple juice may suppress inflammation, boost immunity, helps in digestion.

Deterioration of fruits results from so many factors such as physical factors, fruits' own enzyme action microbial action or combination of all these. Four types of factors determine the colonization of fresh-cut fruits and derivatives by microorganisms such as intrinsic factors, which are dependent on food composition, such as wateractivity, pH, nutrients, structures, and antimicrobial agents; technological treatments, which can modify the initial microbiota; extrinsic factors or environmental conditions of the medium such as temperature, relative humidity, and atmosphere; implicit factors, which depend on the developing microbiota (Tambekar *et al*, 2009, Almas *et al*, 2010, , Ukwo *et al*, 2011).

The causative agents of microbiological spoilage in fruits and fruit juices can be bacteria, as well as yeasts and molds. The main spoilage agents can be considered as due to the low pH of most fruits. Some bacteria such as *Campylobacter spp.*, *E. coli*, *Salmonella spp.*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Shigella spp.*, *Erwinia spp.*, *Enterobacter spp.*, *Alicyclobacillus spp.*, *Propionibacterium cyclohexanicum*. *Pseudomonas spp.*, and lactic acid bacteria can cause spoilage in fruit and fruit juices (Walker and Phillips, 2008). Certain common molds such as *Penicillium spp.*, *Aspergillus spp.*, *Eurotium spp.*, *Alternaria spp.*,

Cladosporium spp., *Paecilomyces spp.*, and *Botrytis spp.* have been shown to be involved in the spoilage of fresh fruits (Lund and Snowdon, 2000, A.Tilarmare *et al*, 2009).

II. MATERIALS AND METHODS

Study place:

The laboratory works of this project was done in the laboratory of the Department of Microbiology of Swami Shri Swaroopanand Saraswati College, Bhilai Chhattisgarh.

Study period:

This research work was carried out from 3 June to 3 September, 2019.

Media:

Different types of media were used for selective growth, enrichment culture. Media preparation and sterilization were done according to the protocol and standard recipe.

Biochemical test media -

Different specific biochemical mediums were prepared for different biochemical tests. (Reiner, K. 2010, Cappuccino *et al*, 2005)

Antibiotic disc -

7 different antibiotic discs were used for identifying antibiotic sensitive and resistant bacteria.

Methods:

Sample collection:

Juice samples of mango, pomegranate, sweet lime and pineapple were collected in sterile bottles from different area of Durg viz; Station road, Bus stand, Gurudwara road, Indira market. Four juice samples were collected from local vendors shop.

Sample processing:

After collecting the samples, pH was measured with the help of pH meter, serial dilutions were done from the samples and spread plate method was used to isolate and count microorganisms. All plates were then incubated at 37°C for 24-48 hours. Finally, the plate's showing colonies were counted and purification of microorganisms was done. After incubation, the growth patterns of the bacteria were evaluated for pigmentation, form, margin, elevation and texture. Gram staining was done to differentiate between two principal groups of bacteria: gram positive and gram negative (D.P. Mahale *et al*, 2008 & R. Khade, 2018).

Biochemical analysis characterization of the bacteria:

Several biochemical tests were carried out. Most of the methods were done according to the microbiology laboratory manual. The biochemical tests performed were Triple sugar iron agar test (Cappuccino & Sherman, 2005), IMVIC test (Indole production test (MacWilliams, 2009),

Methyl red test, Voges- Proskauer test, Citrate utilization test), Catalase test(Reiner, 2010).

Antimicrobial resistance and susceptibility analysis :

In clinical microbiology laboratory it is an important task to check the performance of antimicrobial susceptibility testing of significant bacterial isolates. The aim of this test is to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. The disk diffusion test, or agar diffusion test, or Kirby-Bauer test is the effectiveness of antibiotics on a specific microorganism. This method is used to determine the best antibiotic to use against a new or drug-resistance pathogen.

III. RESULTS

Although fruit juices are very common and potential for human health, but over their hygiene, safety and quality much concern have been raised. In present study, four juice samples such as mango juice, sweet lime juice, pomegranate juice, pineapple juices were examined for microbial analysis.

pH measurement of juice samples:

Fruit juices are comparatively rich in organic acids so they have a low PH. The minimum PH values allowing the growth of lactic acid bacteria pH 2.9-3.5, acetic acid bacteria 3.0-4.5, and enteric bacteria pH 3.6-4.5. The pH range of juices are shown in table 1. In this study, the highest 3.7 was found in the pineapple juices and the lowest pH 2.2 was found in the sweet lime juices.

Table 1: pH values of juice samples

Juice Samples	pH values
Mango juice	2.6
Pomegranate juice	3.5
Sweet lime juice	2.2
Pineapple juice	3.7

Total bacterial count of collected juice samples:

Microbial count of different fruit juices is shown in the table 2. From the result it is clear that total viable count (microbial load) showed the presence of bacteria in juice samples except the sweet lime juice. The other three samples of juices contained higher load of microbes. The highest load of bacterial count found in mango and the lowest bacterial count found in pomegranate. No bacteria found in sweet lime juice plate.

Table 2: Total bacterial count in fruit juices

Types of juices	Media used	Colonies appeared	Codes	Total viable count (TVC) cfu/ml
Mango juice	NAM	34	MJNA	3.4×10 ⁴
	MacConkey	26	MJMCC	2.6×10 ³
Pomegranate juice	NAM	30	PJNA	3.0×10 ³
	EMB	10	PJEMB	1.0×10 ²
Pineapple juice	NAM	28	PAJNAM	2.8×10 ⁴
	EMB	12	PAJEMB	1.2×10 ⁴

Gram reaction and colony morphology:

Gram staining and colony morphology of different isolates collected from the different samples are tabulated in Table 3. Colour of the colonies, forms of them, margin reactions, elevations, gram reactions of those isolates were analysed in the following table.

Table 3: Morphological characteristics of bacterial colonies and gram reactions

Bacterial isolates	Colour	Shape	Margin	Elevation	Gram reaction	Organism identified
MJNA M	Off white	Irregular	Undulate	Flat	Negative	<i>Staphylococcus spp.</i>
	Off white	Circular	Entire	Flat	Positive	<i>Micrococcus spp.</i>
	Cream	Irregular	Entire	Raised	Negative	<i>Staphylococcus spp.</i>
	Bright yellow	Irregular	Lobate	Raised	Positive	<i>Micrococcus spp.</i>
PJNA M	White	Circular	Entire	Flat	Positive	<i>Streptobacillus spp.</i>
	White	Circular	Undulate	Flat	Positive	<i>Streptobacillus spp.</i>
	Off white	Irregular	Entire	Raised	Negative	<i>Staphylococcus spp.</i>
PJMCC	Pink	Circular	Entire	Convex	Negative	<i>Escherichia coli.</i>
	Pink	Circular	Entire	Convex	Negative	<i>Escherichia coli.</i>
PANA M	Off white	Circular	Undulate	Flat	Positive	<i>Streptobacillus spp.</i>
	Off white	Irregular	Entire	Raised	Negative	<i>Staphylococcus spp.</i>
	Off white	Irregular	Entire	raised	Positive	<i>Streptobacillus spp.</i>
PAMC C	Off white	Circular	Lobate	Flat	Positive	<i>Streptobacillus spp.</i>
	Pink	Circular	Entire	Convex	Negative	<i>Escherichia coli.</i>

Biochemical characteristics of bacterial isolates of different juice samples

Microorganisms collected from different culture media according to their growth pattern, morphology, appearance are compared with the morphology of suspected microorganism. After comparing with suspected organism the isolates were then sub-cultured and some specific biochemical test were done for identification and after the biochemical test, it was found that out of four juices samples *Escherichia coli*, and *Streptobacillus sp.* were found in pomegranate juice and pineapple juice. *Staphylococcus sp* were found in pomegranate juice, pineapple juice and mango juice. *Micrococcus spp.* was found in mango juice only. Different biochemical test were done for the identification of bacteria isolates from the juice samples.(Table-4)

Antibiotic sensitivity: Bacterial isolates showed varied response for all the 7 antibiotics. Some organism showed resistance to Cloxacillin and *Streptobacillus* showed resistance to amoxicillin and ciprofloxacin. The organisms showed clear zone to the antibiotics which meant they are sensitive and it can be said that it varies from organism to organism.(Table-5)

Table: 4 Biochemical tests of bacterial isolates from fruit juice

S.No.	Biochemical test	<i>Staphylococcus sp.</i>	<i>Micrococcus sp.</i>	<i>Escherichia coli</i>	<i>Streptobacillus sp.</i>
1.	Catalase rest	+	-	+	-
2.	Indole	-	+	+	+
3.	Methyl red	+	+	-	+
4.	Vogesproskauer	+	-	-	+
5.	Simmon's citrate	+	+	+	+
6.	TSIA	+	-	+	+
7.	Slant/Butt	Y/B	R/R	Y/B	Y/Y
8.	Glucose	+	-	+	+
9.	Lactose	+	-	+	+
10.	Sucrose	+	-	+	+
11.	H ₂ S production	+	-	+	-
12.	Gas production	-	+	+	-

'+'=POSITIVE, '-'= NEGATIVE, 'Y'= YELLOW, 'R'= RED, 'B'= BLACK

Table 5: Antibiotic susceptibility test of bacterial isolates from juice samples

Antibiotic	<i>Staphylococcus sp.</i>		<i>Micrococcus sp.</i>		<i>Escherichia coli.</i>		<i>Streptobacillus sp.</i>	
	ZS	INP	ZS	INP	ZS	INP	ZS	INP
Amoxicillin	7	S	12	S	4	S	NI L	R
Amikacin	6	S	NI L	R	11	S	6	S
Vancomycin	5	S	7	S	10	S	7	S

Rifampicin	4	S	5	S	7	S	9	S
Ciprofloxacin	4	S	NI L	R	7	S	NI L	R
Cloxacillin	NI L	R	NI L	R	NI L	R	8	S
Chloramphenicol	NI L	R	NI L	R	4	S	7	S

ZS = Zone size in mm, INP = Interpretation, S = Sensitive, R = resistance

DISCUSSION:

In India, millions of people are widely consuming fresh fruit juices in every season as it provides an affordable source of nutrients to them and availability at right time. However street juices are frequently associated with diarrheal diseases due to their improper handling and serving practices (Basar *et al*, 2008; Bagchi & Temiz *et al*, 2011). The testing of fruit juices helps to aware people of unhygienic conditions in the freshly prepared juices and also helps to analyse the presence of pathogens to prevent various health problems caused due to contamination (Joy *et al*, 2006).

The Total Viable Count (TVC) or colony forming unit (cfu) which is the number of bacteria per ml of dilution. CFU of fresh juices collected from different shops of different locations were highest in mango juice and lowest in pomegranate juice. No bacterial colony found in sweet lime juice may be due to low PH value i.e. 2.2. From different medium after incubation of 24 hours, some different morphological characteristics showing colonies from nutrient agar, cream, white off-white colony, circular and irregular, pink colonies from MacConkey agar (considered as *E.coli*) and the other bacteria were found, observed and identified by gram reaction. Based on the biochemical characteristics, isolates were confirmed as *Escherichia coli*, *Staphylococcus*, *Micrococcus* and *Streptobacillus*. Antibiotics susceptibility test were done in which almost all the bacteria showed their sensitivity to the antibiotics. They showed resistance to some of the antibiotics.

IV. CONCLUSION

From the data presented in the current study, it could be hard to claim that, consumption of fresh fruit juices are safe because the juice samples collected from different area of Durg were not so satisfactory except the sweet lime juice collected from the street road. *Escherichia coli*, *Staphylococcus sp.*, *Micrococcus* and *Streptobacillus sp.* were found from the juice samples. The contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing utensils, contaminated water, ice, poor personal and domestic hygiene, peeling of fruits before, shops in crowded place, dust particles, heavy vehicular traffic or by the side of the waste disposal system seems to add to the contamination (Ahmed *et al*, 2009). On the other hand, sweet lime juice samples collected three times from the

same shop doesn't shown any contamination of bacterial colony, it may be because the shop vendor keeps their machines clean and before taking out the juice they wash machine as well as the fruit, cut the fruit fresh when required. Glasses used are of paper cups which lessens the infection of microorganism (Jedah & Robinson, 2002). The practice of consuming fresh fruit juices cannot be stopped on unhygienic grounds and not the juice vendors prohibited from the selling such items, as such activities provide them with a source of livelihood but if they take the precautive measures, the juices will become more healthy and beneficial as they will be free from microbes (Raybaudi *et al*, 2009).

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